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Rodents and Their Role in Ecology, Medicine and Agriculture

Edited by Mohammad Manjur Shah

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Published in London, United Kingdom

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http://dx.doi.org/10.5772/intechopen.105295
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Contributors
Tomoko Fujiwara, Rieko Nakata, Masanori Ono, Yumi Adachi, Hiroaki Yoshikawa, Takashi Hosono, Hiroshi Fujiwara, Takiko Daikoku, Hitoshi Ando, Sihem Mbarek, Oumeima Hammami, Oumeima Achour, Rafika Ben Chaoucha-Chekir, Rishika Jana, Souvik Karmakar, Bishal Hazra, Subhadeep Roy, Jayasri Das Sarma, Naveed Akhtar, Sara Hayee, Muhammad Idnan, Faheem Nawaz, Sadaf BiBi, Vinod Goyal, Mahalakshmi Bandari

First published in London, United Kingdom, 2023 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Rodents and Their Role in Ecology, Medicine and Agriculture
Edited by Mohammad Manjur Shah
p. cm.
Print ISBN 978-1-83769-625-3
Online ISBN 978-1-83769-624-6
eBook (PDF) ISBN 978-1-83769-626-0

Meet the editor

Dr. Mohammad Manjur Shah obtained his Ph.D. from Aligarh Muslim University, India, in 2003. He is a pioneer in the field of insect parasitic nematodes. He has presented his findings at several conferences and published articles in various reputed international journals. He completed two post-doctoral fellowships under the Ministry of Science and Technology, Government of India before joining Yusuf Maitama Sule University, Nigeria, in 2015. He has edited seven books as well as served as a reviewer of several scientific journals. He is currently an Associate Professor of Biology, the director of Research Innovation and Development, and chairman of the Research Ethics Committee, all at Yusuf Maitama Sule University.

Contents

Preface

Rodents are mammals with extra-large, continuously growing incisors in both the upper and lower jaws. They belong to the order Rodentia, which includes more than 2000 living species. Rats and mice are known to spread many diseases worldwide, while various other rodents are beneficial, for example, by serving as food sources or models for biomedical and genetic research. This book presents an overview of rodents and the various roles they play in experimental disease models. Chapters discuss the importance of rodents in human eye research, gynecologic disorders, zoonotic diseases, and drug discovery. This volume is an up-to-date and comprehensive resource that adds to the understanding of disease mechanisms.

Dr. Mohammad Manjur Shah
Associate Professor,
Department of Biological Sciences,
Yusuf Maitama Sule University,
Kano, Nigeria

Section 1

Rodents as Experimental Model

Chapter 1

Dietary Habit-Induced Gynecologic Disorders in Young Female Students – Lessons from Rodent Experiments

Tomoko Fujiwara, Rieko Nakata, Masanori Ono, Yumi Adachi, Hiroaki Yoshikawa, Takashi Hosono, Hiroshi Fujiwara, Takiko Daikoku and Hitoshi Ando

Abstract

Currently, dieting and breakfast skipping is increasing among young women in Japan. We found that breakfast skipping among female students was accompanied by menstrual disorders, while students who had dieted in the past experienced deterioration in menstrual pains, warning that abnormal eating in young women may induce obstetric and gynecological disorders in the future. We named this concept "adolescent dietary habit-induced obstetric and gynecologic disease (ADHOGD)". A questionnaire survey showed that pregnant women who had menstrual pain in their youth were at high risk of hypertensive disorders during pregnancy. In rodents, ovulation was suppressed in young female rats whose feeding was limited to the non-active (light) phase. In female mice, feeding stimulation directly regulated the uterine clock gene rhythm. Furthermore, in conditional knockout mice of uterine *Bmal1,* the fetuses died before delivery, indicating that abnormal uterine clock function cannot maintain fetal development. These findings suggest a mechanism of ADHOGD, in which hunger stress due to inappropriate eating habits during adolescence and young adulthood affects uterine function via clock gene abnormalities, causing placental dysfunction and fetal growth failure during pregnancy. Thus, valid and appropriate rodent experiments are effective to analyze ADHOGD, especially from the aspect of circadian rhythms.

Keywords: ADHOGD, breakfast skipping, dietary habit, dysmenorrhea, uterine dysfunction

1. Introduction

Currently, both dieting and breakfast skipping are increasing among young women in Japan. We found that breakfast skipping among female students was accompanied by menstrual disorders, while students who had dieted in the past

experienced deterioration in menstrual pains after recovery [1, 2], warning that abnormal eating rhythm in young women may induce obstetric and gynecological disorders in the future [3, 4]. Subsequently, a correlation between menstrual pain and breakfast skipping in young women was confirmed worldwide [5, 6]. Therefore, we considered menstrual pain associated with this eating rhythm abnormality as a universal risk for obstetric and gynecologic diseases across races and proposed the concept that abnormal eating habits in youth can induce obstetric and gynecologic diseases [7].

The concept of developmental origins of health and disease (DOHaD) is currently becoming a great concern in the world [8]. This hypothesis was initially called 'fetal origins of adult disease' [9] and proposed that exposure to a poor nutritional environment during critical periods of development and growth may determine the later onset of human diseases in adulthood [10]. In DOHaD theory, prenatal and perinatal stages were proposed as the responsible windows when predictive adaptation to environmental influences can occur [11]. On the other hand, reproductive organs markedly develop and mature during adolescence and young adulthood. Consequently, we proposed that inadequate dietary habits such as dieting and breakfast skipping during adolescence and young adulthood impair the development and maturation of reproductive functions, which induces the latent progression of obstetrics and gynecologic disorders and leads to the latter onset of obstetrics and gynecologic diseases [3, 12]. Recently, we named this concept "adolescent dietary habit-induced obstetric and gynecologic disease (ADHOGD)" (**Figure 1**) [7].

Although it has been known that abnormalities in the light–dark cycle, such as dieting and night work, suppress the hypothalamic system in humans, the effects of feeding rhythms on reproductive function were unknown. Therefore, when young female rats were restricted to feeding only during the active (dark) or inactive (light) phase, suppression of ovulation was observed in the inactive feeding group [13]. In addition to the above, the following important discoveries regarding the mechanism of ADHOGD and its effects on the uterus have been made by the authors. First, Ono et al. showed in a questionnaire survey of pregnant women that women who had menstrual pain in their youth were at high risk of developing hypertensive disorders of pregnancy (HDP) even if their pain improved [14]. Gestational hypertension is believed to be caused by the inadequate reconstruction of maternal blood vessels during placentation, and these results indicate that the uterus may "remember" the abnormal uterine function associated with menstrual pain in young adulthood. On the other hand, Ando et al. observed that feeding stimulation can directly regulate the uterine clock gene expression rhythm in mice. This indicates that abnormal feeding rhythm may directly disrupt uterine function without involving the ovary [15]. Furthermore, Daikoku et al. created mice deficient in the uterus-specific clock gene Bmal1 and observed that the fetuses died before delivery, indicating that abnormal uterine clock function cannot maintain fetal development. The cause of this phenomenon was elucidated to be abnormal placentation accompanied by a failure in the construction of maternal blood vessels, indicating the possibility that reproductive and perinatal diseases are induced by abnormal uterine clock [16].

Based on this background, in this chapter, we precisely introduce a new concept of dietary habit-induced gynecologic disorders in female students and the rodent experiments that provided current evidence to support this concept from the aspect of circadian rhythms.

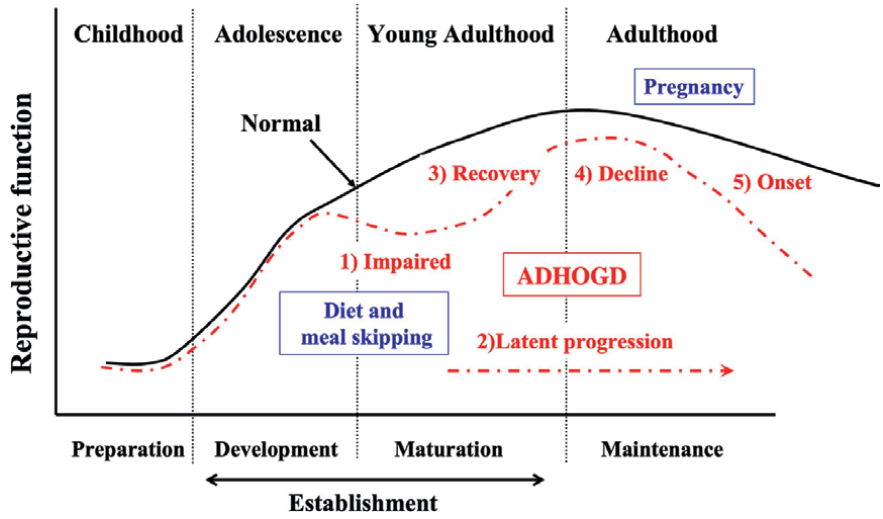

Figure 1.
Concept of ADHOGD. 1) Adverse dietary habits such as diet and meal skipping during adolescence and young adulthood impair the development and maturation of the reproductive function, 2) which induces the latent progression of obstetric and gynecologic disorders. Although 3) recovery is achieved after correcting adverse eating habits, 4) the reproductive function is precociously declined, 5) which later leads to the onset of obstetrics and gynecologic diseases, especially during pregnancy.

2. Mechanism of dietary habit-induced gynecological disorders

2.1 Relationship between inadequate dietary habits and gynecological disorders

Skipping meals and dieting for beauty purposes are spreading among young Japanese women. In parallel with this, the increase in gynecological diseases such as endometriosis, which causes dysmenorrhea, has become a big problem. Although there has been little evidence about the relationship between menstrual pain and eating habits, we previously reported that breakfast deprivation among female students was accompanied by menstrual cramps. For female students, menstrual pain is important medical information because it is a symptom suggesting organic gynecological diseases.

Since there is no appropriate medical terminology for females (ages 18-22) who are in the process of completing their reproductive functions after puberty, preventive dietary guidance and indicators that target this generation were not sufficient. Therefore, we position this period as the "maturation age of reproductive function," and have conducted a fact-finding survey of female students for about 20 years with the aim of clarifying the relationship between dietary habits and reproductive dysfunction. As a result, we found that students who have dieted in the past have strong menstrual cramps, and this led to a warning of the danger of “development of organic gynecological diseases after ending the diet” [2, 4].

On the other hand, skipping breakfast was associated with menstrual pain in female students, although the total daily food intake did not decrease [1]. Later, a large-scale Palestinian study confirmed that skipping breakfast is associated with menstrual pain [5]. Recently, it was proposed that the disruption of the circadian rhythm can cause poor reproductive outcomes [17]. Focusing on the fact that breakfast corresponds to the start of the circadian rhythm, we proposed the hypothesis

that "skipping breakfast interferes with the circadian rhythm and adversely affects reproductive function" [3, 7].

2.2 The effects of meal timing during the circadian cycle on female rat reproductive function

To investigate the effects of meal timing during the circadian cycle on ovarian function, we explored this issue using young female rats [13]. Considering that rats are active at night, 8-week-old female Wistar rats were classified into three groups: day-only feeding group (inactive phase), night-only feeding group (active phase), and control group (no time or calorie restriction). During a 4-week feeding restriction, body weight in each group was measured by weighing scales and ovulatory frequency was assessed by vaginal smears. After the dietary restriction, ovaries were removed and the numbers of growing follicles and corpus luteum were evaluated by examining hematoxylin- and eosin-stained tissue sections.

As a result, in the daytime feeding group, the ovulatory number and frequency were significantly reduced compared to the control group. At the same time, the amount of daily food intake was reduced by approximately 20% and body weight gain was suppressed. In contrast, in the night-time-fed group, there were no differences in the frequency of ovulation as compared with the control group, indicating that the defect of food intake in the non-active phase did not affect the estrus cycle. These findings led us to conclude that feeding defect in the active phase is critical for young female rats and that the timing of food intake during the active or non-active phase is a crucial factor that influences female reproductive function [13].

Furthermore, even when 8-week-old female rats were fed only at night for 4 weeks with 20% less food in the control group (no restriction), their estrus cyclicity did not change despite significant reductions in weight gain and food intake as compared with the control group. These findings also support the above speculation that reproductive dysfunction was more intensely induced by the differences in the timing of food intake during the active or non-active phase than by insufficient calorie intake [13].

In humans, it was well known that shift workers increase the incidence of reproductive disorders such as menstrual troubles, endometriosis, infertility, miscarriage, or pre-term delivery [18–20]. Food intake is one of the important regulators that can reset the rhythm of the central clock in the brain and peripheral clocks in the digestive organs [21]. It should be noted that in shift workers, the timing of food intake is synchronous with their active behaviors. In this regard, the rat model in which the feeding is inhibited during the active phase may be more stressful than shift workers. Our findings showed that feeding defect in the active phase of the post-pubertal female rat impairs ovarian function, indicating that the timing of feeding during the circadian cycle can interfere with female reproductive function. However, in adult mice, it was reported that although the birth of the first litter was significantly delayed and total reproductive output was significantly reduced in the light-fed group, estrous cycling and pregnancy maintenance did not differ between the light-fed and dark-fed groups, concluding that mistimed feeding inhibits reproduction in mice by reducing successful mating behavior [22].

2.3 Feeding directly regulates the uterine clock rhythm in mice

To examine the relationship between food intake and the uterine clock rhythm, Ando et al. investigated the effects of the first meal occasion in the active phase on

the uterine clock expression [15]. In this study, Zeitgeber time (ZT) was defined as ZT 0 (8:45) with lights on and ZT 12 (20:45) with lights off. Eight-week-aged young female mice were divided into three groups: group I (ad-libitum feeding), group II (time-restricted feeding during ZT12-16, initial 4 hours of the active period), and group III (time-restricted feeding during ZT20-24, last 4 hours of the active period, a breakfast-skipping model). After two weeks of dietary restriction, mice in each group were sacrificed at 4-hour intervals and the expression profiles of uterine clock genes, *Bmal1, Per1, Per2, and Cry1*, were examined.

In accordance with previous reports that the rat uterus has circadian rhythms of clock genes [23, 24], both mRNA and protein expressions of Bmal1, Per1, Per2, and Cry1 in the murine uterus were demonstrated to create a circadian clock rhythm. In addition, immunohistochemical analysis showed that Bmal1 protein expression was synchronized among the endometrium and myometrium. Importantly, in groups I and II, both mRNA and protein expressions of Bmal1 were elevated after ZT12 at the start of the active phase, whereas Bmal1 expression was elevated just after ZT20 in group III, indicating that the uterine clock rhythm had shifted 8 hours backward. A similarly delayed time lag was also observed for Cry1 mRNA expression (**Figure 2**). This indicates that time-restricted feeding can reset the circadian rhythm of the uterine clock gene expressions and suggests that the uterine peripheral clock is more intensely regulated by diet than by the light/dark cycle, which is a main regulator of the central clock [25, 26].

Dietary intake was reported to regulate the rhythm of the hepatic circadian clock in the liver [27]. However, in contrast to these vital organs for energy metabolism, the physiological significance of dietary regulation of the uterine peripheral clock is unclear. In pregnant mice, clock gene oscillation was reported to be present in the uterus, placenta, and fetal membranes [28]. For the fetus that cannot receive light stimulation in the uterus, transplacental glucose transport from maternal blood after food intake can be a direct signal to detect the maternal circadian rhythm during pregnancy [29]. A recent study reported that the trophoblast controls transport across the placenta in mice during pregnancy by regulating the circadian expression and activity of the xenobiotic efflux pump, ATP-binding cassette sub-family B member 1 (ABCB1) [30]. Accordingly, we speculate that the uterus is prepared to synchronize its function with food intake to provide an adequate environment for the fetus.

As we demonstrated in the above section, ovulations were impaired in daytime-fed young female rats, indicating that the timing of food intake is an important factor regulating the hypothalamic-pituitary-ovarian axis [13]. Both dietary and light/dark cycles mainly regulate circadian rhythms in the brain [25, 26]. Recent studies suggest that reproductive rhythms are disrupted by circadian rhythms [20, 31]. Since meal skipping at the start of the active phase can interfere with the central clock system [32], it is speculated that breakfast-skipping influences reproductive rhythms [3, 13]. In addition, taking into consideration that the timing of food intake can reset the uterine clock rhythm, we can elicit another mechanism that meal skipping directly disturbs the circadian rhythm of the uterine clock system and causes uterine dysfunction [7].

2.4 Uterine deletion of Bmal1 induces intrauterine fetal death in mice

Systemic *Bmal1* knockout (KO) female mice were reported to be infertile, showing multiple organ disorders [33]. However, despite the circadian expression of uterine clock genes such as *Per1-3, Cry1-2, Bmal1, and Clock*, their precise roles in

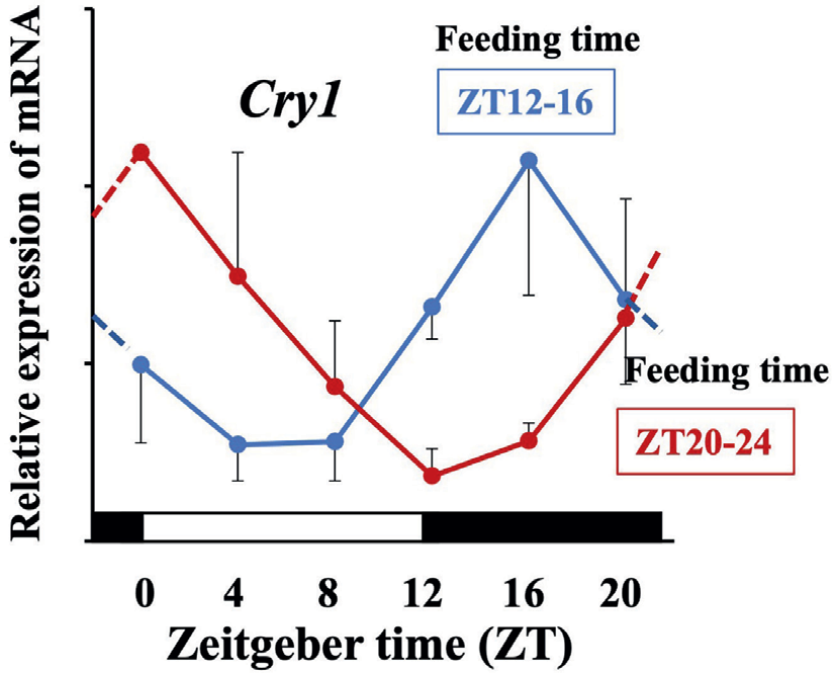

Figure 2.
Circadian rhythms of uterine clock gene Cry1. The mRNA expressions of Cry1 in the uterus showed circadian cycles. Relative expressions are presented as fold units per minimal values. Cry1 expression in the group (feeding during ZT12-16) was elevated on ZT12 at the start of the active phase and food intake. In contrast, its expression in the group (feeding during ZT20-24) was elevated on ZT20 at the start of food intake, showing an 8-hour backward shift in circadian rhythms. This suggests that the uterine peripheral clock is more strongly regulated by diet than by the light/dark cycle.

reproductive functions remain unclear [34, 35]. Later, Ls et al. reported that the uterine expression of *BMAL1* was decreased in women suffering from recurrent spontaneous abortion [36]. They also showed that *BMAL1*-depleted decidual cells inhibited trophoblast invasion, proposing the important role of the uterine clock in the establishment of human pregnancy [36]. A recent report reported that mRNA expression of Clock was increased in the human placenta during preeclampsia, suggesting the involvement of Clock in the pathogenesis of preeclampsia [37].

Based on this background, to investigate the pathological roles of uterine clock genes during pregnancy, we produced conditional deletion of uterine *Bmal1* (cKO) mice [16] using *Bmal1*-loxP [38] and progesterone receptor-cre mice as reported previously [39, 40]. As a result, cKO mice could receive embryo implantation, but not sustain the pregnancy. By histological examinations, abnormal structures of the placenta were observed in cKO mice. The main feature of placental abnormality in cKO mice was the poor development of maternal vascularity. In cKO mice, it was speculated this structural abnormality caused the narrowing of the maternal vascular spaces and the reduction of maternal blood flow into the intervillous spaces in the labyrinth layer, leading to impaired maternal-fetal nutritional exchanges through this layer.

Next, we performed microarray analysis to detect the changes in gene expression in the uterine tissues in cKO mice. Gene ontology analysis of microarray revealed that several genes related to immune response were down-regulated in the cKO uterus,

suggesting that immune dysfunction is involved in the poor formation of placenta. It is well known that the cellular immune responses by regulatory T cells, effector T cells, NK cells, and monocytes are related to the pathogenesis of gestational hypertension [41]. Among the down-regulated genes, we paid an attention to GO:0045954 "positive regulation of natural killer cell-mediated cytotoxicity", because NK cells were shown to directly interact with trophoblast and regulate their invasion and vascular reconstruction [42]. Consequently, we examined the distribution of subtypes of uterine NK (uNK) in the embryo implantation sites and the placenta from early pregnancy to mid-pregnancy.

In cKO mice, the numbers of NK cells expressing PAS(+)/DAB(-) were decreased in the spongiotrophoblast layer of the placenta. In addition, uNK cells in cKO mice hardly expressed CD161 which is an immunosuppressive molecule [43], indicating that their subtypes of uNK cells are different from those in WT mice. This also suggests that the deletion of clock genes induced functional changes in uNK cells, which directly interact with fetal trophoblast. Since CD161 is an immunosuppressive NK marker [43], the decrease of uNK cells in the spongiotrophoblast layer may interrupt trophoblast function to sustain maternal-fetal immune tolerance. From these findings, we concluded that the murine uterine clock system is critical for the placental function to maintain pregnancy after embryo implantation and proposed that the disorder of a uterine clock system can be a candidate to induce uterine dysfunction during pregnancy in ADHOGD.

Intriguingly, progesterone supplementation recovered pregnancy outcomes in cKO mice, showing that some cKO mice sustained pregnancy until the term [16]. Although the structural abnormalities in the placenta were not improved, the number of CD161-positive NK cells in the spongiotrophoblast layer was increased in cKO mice. Clinically, it has been accepted that progesterone supplement prevents miscarriage in women with recurrent miscarriage of unclear etiology [44] and protects against the onset of HDP [45]. Since a recent study reported that progesterone supplement therapy also improves the functional failure of the placenta with structural abnormalities [46], this cKO model will provide supporting experimental evidence of the role of progesterone in the treatment of perinatal complications.

2.5 HDP is associated with dysmenorrhea in early adulthood

During human placentation, the trophoblast invades the maternal decidua and reconstructs maternal spiral arteries. This remodeling reduces arterial contractility and supplies adequate maternal blood flow into the intervillous spaces [47]. If this process is disrupted, the poor blood supply to the intervillous space will induce placental dysfunction and various pregnancy complications, such as HDP, fetal growth restriction, and stillbirth in the late stage of pregnancy [42]. The impaired vascular reconstruction associated with abnormal placental formation observed in our cKO mouse model may be corresponding to the pathogenesis of human placental dysfunction in HDP.

Recently, by a prospective cohort questionnaire study, we found that pregnant women who experienced dysmenorrhea at a younger age in the past had a higher incidence of developing HDP [14]. We recruited 193 pregnant participants and collected valid data from 190 women concerning characteristics, menstrual abnormalities, and lifestyle factors. We also used medical records to examine the relationship between menstrual abnormalities and the onset of HDP. As a result, a total of 26 patients developed HDP, 10 had early onset and 16 had late onset. The HDP group

was significantly older than the non-HDP group. Although no significant association was observed between HDP and dysmenorrhea just prior to pregnancy, there was a significant increase in the incidence of HDP when patients had dysmenorrhea around the age of 20 years.

Since the reproductive organs develop and become matured during young adulthood, this period is important for establishing female reproductive functions. As described in the above section, we reported that the incidence of dysmenorrhea is high in female college students with a history of dieting and proposed that dysmenorrhea and poor diet in young adulthood induce the later development of perinatal and gynecologic diseases [7]. Consequently, this cohort study suggests one of the progressing processes of ADHOGD whereby although dysmenorrhea in young adulthood is improved, it will become manifested as ADHOGD when physical stresses such as pregnancy are imposed (**Figure 1**).

3. Conclusion

The epidemiological data and animal experimental findings suggest that irregular competition between light and feeding rhythm can induce uterine clock dysfunction. These findings also suggest a mechanism of ADHOGD, in which hunger stress due to inappropriate eating habits during adolescence and young adulthood affects uterine function via clock gene abnormalities and other factors, causing placental dysfunction and fetal growth failure during pregnancy, which in turn affects the next generation. Thus, rodent experiments are needed to analyze the new concept of dietary habit-induced gynecologic disorders, especially from the aspect of circadian rhythms, and valid and appropriate experimental models will provide current evidence in support of this concept.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (nos. 19H01617, 19K09776, 21K18297, and 22K09556) and a Health and Labour Sciences Research Grant (no. 19216689).

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ADHOGD	adolescent dietary habit-induced obstetric and gynecologic disease
DOHaD	developmental origins of health and disease
HDP	hypertensive disorders of pregnancy
KO	knockout
uNK	uterine NK
ZT	Zeitgeber time

Author details

Tomoko Fujiwara[1*], Rieko Nakata[2], Masanori Ono[3], Yumi Adachi[4], Hiroaki Yoshikawa[4], Takashi Hosono[5], Hiroshi Fujiwara[5], Takiko Daikoku[6] and Hitoshi Ando[7]

1 Department of Social Work and Life Design, Kyoto Notre Dame University, Kyoto, Japan

2 Department of Food Science and Nutrition, Nara Women's University, Nara, Japan

3 Department of Obstetrics and Gynecology, Tokyo Medical University, Tokyo, Japan.

4 Health Service Center, Kanazawa University, Kanazawa, Japan

5 Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

6 Division of Animal Disease Model, Research Center for Experimental Modeling of Human Disease, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

7 Department of Cellular and Molecular Function Analysis, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

*Address all correspondence to: fujiwara@notredame.ac.jp and tomokof@bd5.so-net.ne.jp

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References

[1] Fujiwara T. Skipping breakfast is associated with dysmenorrhea in young women in Japan. International Journal of Food Sciences and Nutrition. 2003;**54**(6):505-509

[2] Fujiwara T, Nakata R. Young Japanese college students with dysmenorrhea have high frequency of irregular menstruation and premenstrual symptoms. The Open Medical Informatics Journal. 2007;**1**:8-11

[3] Fujiwara T, Nakata R. Skipping breakfast is associated with reproductive dysfunction in post-adolescent female college students. Appetite. 2010;**55**(3):714-717

[4] Fujiwara T, Ono M, Iizuka T, Sekizuka-Kagami N, Maida Y, Adachi Y, et al. Breakfast skipping in female college students is a potential and preventable predictor of gynecologic disorders at Health Service Centers. Diagnostics (Basel). 2020;**10**(7)

[5] Abu Helwa HA, Mitaeb AA, Al-Hamshri S, Sweileh WM. Prevalence of dysmenorrhea and predictors of its pain intensity among Palestinian female university students. BMC Women's Health. 2018;**18**(1):18

[6] Hu Z, Tang L, Chen L, Kaminga AC, Xu H. Prevalence and risk factors associated with primary dysmenorrhea among Chinese Female University Students: A Cross-sectional Study. Journal of Pediatric and Adolescent Gynecology. 2020;**33**(1):15-22

[7] Fujiwara T, Ono M, Mieda M, Yoshikawa H, Nakata R, Daikoku T, et al. Adolescent Dietary Habit-induced Obstetric and Gynecologic Disease (ADHOGD) as a new hypothesis-possible involvement of clock system. Nutrients. 2 May 2020;**12**(5):1294

[8] Mandy M, Nyirenda M. Developmental Origins of Health and Disease: the relevance to developing nations. International Health. 2018;**10**(2):66-70

[9] Barker DJ. The origins of the developmental origins theory. Journal of Internal Medicine. 2007;**261**(5):412-417

[10] Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ. 1991;**303**(6809):1019-1022

[11] Gluckman PD, Hanson MA, Spencer HG. Predictive adaptive responses and human evolution. Trends in Ecology & Evolution. 2005;**20**(10):527-533

[12] Fujiwara T. Diet during adolescence is a trigger for subsequent development of dysmenorrhea in young women. International Journal of Food Sciences and Nutrition. 2007;**58**(6):437-444

[13] Fujiwara T, Nakata R, Ono M, Mieda M, Ando H, Daikoku T, et al. Time restriction of food intake during the circadian cycle is a possible regulator of reproductive function in postadolescent female rats. Current Development Nutrition. 2019;**3**(4):nzy093

[14] Nakayama M, Ono M, Iizuka T, Kagami K, Fujiwara T, Sekizuka-Kagami N, et al. Hypertensive disorders of pregnancy are associated with dysmenorrhea in early adulthood: A cohort study. The Journal of Obstetrics and Gynaecology Research. Nov 2020;**46**(11):2292-2297

[15] Hosono T, Ono M, Daikoku T, Mieda M, Nomura S, Kagami K, et al. Time-restricted feeding regulates circadian rhythm of murine uterine clock. Current Development Nutrition. 2021;**5**(5):nzab064

[16] Ono M, Toyoda N, Kagami K, Hosono T, Matsumoto T, Horike SI, et al. Uterine deletion of bmal1 impairs placental vascularization and induces intrauterine fetal death in mice. International Journal of Molecular Science. 11 Jul 2022;**23**(14):7637

[17] Sciarra F, Franceschini E, Campolo F, Gianfrilli D, Pallotti F, Paoli D, et al. Disruption of circadian rhythms: A crucial factor in the etiology of infertility. International Journal of Molecular Science. 30 May 2020;**21**(11):3943

[18] Labyak S, Lava S, Turek F, Zee P. Effects of shiftwork on sleep and menstrual function in nurses. Health Care for Women International. 2002;**23**(6-7):703-714

[19] Gamble KL, Resuehr D, Johnson CH. Shift work and circadian dysregulation of reproduction. Frontiers in Endocrinology (Lausanne). 2013;**4**:92

[20] Mills J, Kuohung W. Impact of circadian rhythms on female reproduction and infertility treatment success. Current Opinion in Endocrinology, Diabetes, and Obesity. 2019;**26**(6):317-321

[21] Mendoza J. Circadian clocks: setting time by food. Journal of Neuroendocrinology. 2007;**19**(2):127-137

[22] Swamy S, Xie X, Kukino A, Calcagno HE, Lasarev MR, Park JH, et al. Circadian disruption of food availability significantly reduces reproductive success in mice. Hormones and Behavior. 2018;**105**:177-184

[23] Johnston JD, Ordovas JM, Scheer FA, Turek FW. Circadian Rhythms, Metabolism, and Chrononutrition in Rodents and Humans. Advances in Nutrition. 2016;**7**(2):399-406

[24] Fukuhara C, Tosini G. Peripheral circadian oscillators and their rhythmic regulation. Frontiers in Bioscience. 2003;**8**:d642-d651

[25] Mieda M, Sakurai T. Bmal1 in the nervous system is essential for normal adaptation of circadian locomotor activity and food intake to periodic feeding. The Journal of Neuroscience. 2011;**31**(43):15391-15396

[26] Mieda M. The central circadian clock of the suprachiasmatic nucleus as an ensemble of multiple oscillatory neurons. Neuroscience Research. Jul 2020;**156**:24-31

[27] Ando H, Ushijima K, Shimba S, Fujimura A. Daily fasting blood glucose rhythm in male mice: A role of the Circadian Clock in the Liver. Endocrinology. 2016;**157**(2):463-469

[28] Ratajczak CK, Herzog ED, Muglia LJ. Clock gene expression in gravid uterus and extra-embryonic tissues during late gestation in the mouse. Reproduction, Fertility, and Development. 2010;**22**(5):743-750

[29] Gaccioli F, Lager S. Placental nutrient transport and intrauterine growth restriction. Frontiers in Physiology. 2016;**7**:40

[30] Demarez C, De Assis LVM, Krohn M, Ramella N, Schwaninger M, Oster H, et al. The trophoblast clock controls transport across placenta in mice. Development. 15 Apr 2021;**148**(8):dev197673

[31] Ono M, Ando H, Daikoku T, Fujiwara T, Mieda M, Mizumoto Y, et al.

The Circadian Clock, nutritional signals and reproduction: A Close Relationship. International Journal of Molecular Science. 12 Jan 2023;**24**(2):1545

[32] Mieda M, Ono D, Hasegawa E, Okamoto H, Honma K, Honma S, et al. Cellular clocks in AVP neurons of the SCN are critical for interneuronal coupling regulating circadian behavior rhythm. Neuron. 2015;**85**(5):1103-1116

[33] Boden MJ, Varcoe TJ, Voultsios A, Kennaway DJ. Reproductive biology of female Bmal1 null mice. Reproduction. 2010;**139**(6):1077-1090

[34] Johnson MH, Lim A, Fernando D, Day ML. Circadian clockwork genes are expressed in the reproductive tract and conceptus of the early pregnant mouse. Reproductive Biomedicine Online. 2002;**4**(2):140-145

[35] Nakamura TJ, Moriya T, Inoue S, Shimazoe T, Watanabe S, Ebihara S, et al. Estrogen differentially regulates expression of Per1 and Per2 genes between central and peripheral clocks and between reproductive and nonreproductive tissues in female rats. Journal of Neuroscience Research. 2005;**82**(5):622-630

[36] Lv S, Wang N, Ma J, Li WP, Chen ZJ, Zhang C. Impaired decidualization caused by downregulation of circadian clock gene BMAL1 contributes to human recurrent miscarriagedagger. Biology of Reproduction. 2019;**101**(1):138-147

[37] Li Y, Li J, Hou Y, Huang L, Bian Y, Song G, et al. Circadian clock gene Clock is involved in the pathogenesis of preeclampsia through hypoxia. Life Sciences. 2020;**247**:117441

[38] Storch KF, Paz C, Signorovitch J, Raviola E, Pawlyk B, Li T, et al. Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. Cell. 2007;**130**(4):730-741

[39] Daikoku T, Dey SK. Two faces of PTEN. Nature Medicine. 2008;**14**(11):1192-1193

[40] Daikoku T, Hirota Y, Tranguch S, Joshi AR, DeMayo FJ, Lydon JP, et al. Conditional loss of uterine Pten unfailingly and rapidly induces endometrial cancer in mice. Cancer Research. 2008;**68**(14):5619-5627

[41] Miller D, Motomura K, Galaz J, Gershater M, Lee ED, Romero R, et al. Cellular immune responses in the pathophysiology of preeclampsia. Journal of Leukocyte Biology. 2022;**111**(1):237-260

[42] Pollheimer J, Vondra S, Baltayeva J, Beristain AG, Knofler M. Regulation of placental extravillous trophoblasts by the maternal uterine environment. Frontiers in Immunology. 2018;**9**:2597

[43] Buckle I, Guillerey C. Inhibitory receptors and immune checkpoints regulating natural killer cell responses to cancer. Cancers (Basel). 24 Aug 2021;**13**(17):4263

[44] Haas DM, Hathaway TJ, Ramsey PS. Progestogen for preventing miscarriage in women with recurrent miscarriage of unclear etiology. Cochrane Database System Review. 8 Oct 2018;**10**(10):CD003511

[45] Wu H, Zhang S, Lin X, He J, Wang S, Zhou P. Pregnancy-related complications and perinatal outcomes following progesterone supplementation before 20 weeks of pregnancy in spontaneously achieved singleton pregnancies: a systematic review and meta-analysis. Reproductive Biology and Endocrinology. 2021;**19**(1):165

[46] Barinov SV, Tirskaya YI, Shamina IV, Ledovskikh IO, Atamanenko OJ. Placental blood flow and pregnancy outcomes in women with abnormal placental localization and absence of placental "migration". The Journal of Maternal-Fetal & Neonatal Medicine. 2021;**34**(21):3496-3502

[47] Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biology of Reproduction. 2003;**69**(1):1-7

Chapter 2

Gerbil, Psammomys Obesus, a Human-like Rodent Model of Eye Research

Sihem Mbarek, Oumeima Hammami, Oumeima Achour and Rafika Ben Chaoucha-Chekir

Abstract

The purpose of this chapter is to illustrate the use of rodents other than mice and rats as relevant models of nutritionally human eye diseases. The sand rat or Psammomys obesus (*P. obesus*), is a desert rodent from the subfamily Gerbillinae, which has been widely used as an excellent animal model of diet-induced diabetes and metabolic syndrome. In previous studies, we showed that *P. obesus* develops type II diabetes when exposed to a high-calorie diet under laboratory conditions, resulting in diabetic retinopathy with similar visual disorders to that observed in humans. In this chapter, we will explore the notable similarities and differences between the human and rodent visual systems and the pertinence of using *P. obesus* as animal model of eye research. Retinal function, particularly that mediated by cone, will also be illustrated.

Keywords: *Psammomys obesus*, diurnal vision, human ocular research, cones, nocturnal vision

1. Introduction

Research using animal models in ophthalmology has enabled significant progress to be made in understanding the mechanisms of ocular diseases and in evaluating the efficacy of new therapeutic approaches [1]. However, commonly used models, such as mice, rats, and most of rodents, are not equivalent in terms of similarity to human vision and present major anatomical and functional differences [2]. Indeed, rodents differ in their visual adaptation according to their predominant activity during the day or night. The majority of these models have a visual system adapted to a nocturnal lifestyle, whereas the human visual system has evolved for a daytime habitat illuminated by light. In this chapter, we will explore the notable similarities and differences between the human and rodent visual systems to allow us to understand human eye disease.

2. Comparison of night and day vision adaptions in rodent and human

The most of rodent eyes are specifically adapted to their nocturnal lifestyle. Their eyes are generally larger than their heads [3]. In addition, their pupils can dilate more than those of humans. Nocturnal rodents have relatively larger crystalline lenses than diurnal rodents, completely filling the eyeball. This particular lens enables the transmission of ultraviolet light, which is required for UV-sensitive photoreceptors present in the mouse retina. This unique configuration allows them to capture and focus more light [4]. On the other hand, humans eyes are adapted to daytime vision, with a retina dominated by cones for color detection and accurate daylight vision. However, humans also have rods in their retina, enabling them to see in low light conditions, although less efficiently than rodents [5]. Nevertheless, it has been well established that rodents possess superior night vision compared to humans, primarily due to their greater density of rods in the retina.

In humans, the retinal photoreceptors encompass two distinct sensory types: cones and rods. These specialized cells contain the visual pigment [6]. Cones, located primarily in the fovea, enable us to perceive intricate details and exhibit sensitivity to colors. They are less light-sensitive compared to rods, thereby primarily contributing to daytime or photopic vision. The trichromatic model of human vision elucidates this phenomenon, where color sensations are attained by modulating the excitations of three cone types, each exhibiting distinct spectral sensitivities to various regions of the color spectrum. These cone types include those more responsive to blue light (S cones), green light (M cones), and red light (L cones) [7]. Consequently, a combination of light wavelengths corresponding to red, green, and blue can generate all perceivable color sensations. In contrast to humans, rodents possess dichromatic color vision, relying solely on cones sensitive to blue and green light. This distinction arises from the presence of two distinct types of opsin: S opsin cones (short or blue) and M opsin cones (medium or green) [8]. In the mouse retina, there exists a notable arrangement where cones predominantly expressing opsin S exhibit heightened contrast sensitivity and are situated closer to the ventral retina, preferentially surveying the upper portion of the visual field [9]. Conversely, the expression of M opsin progressively increases toward the dorsal retina, encoding the lower visual fields. This distinctive organization of the retina is believed to be a product of selective evolutionary adaptation aimed at efficiently recognizing images and natural scenes.

Anatomically, the organization of the rodent retina is similar to that of humans, with well-defined typical cellular and plexiform layers, comprising the outer retina containing the photoreceptor cell bodies and outer segments, and the inner retina, consisting of bipolar cells, amacrine cells, and the retinal ganglion cells (RGCs) [10], as recognized by Ramon and Cajal over 100 years ago [11]. However, there are notable differences in the arrangement and number of photoreceptor cells [6]. Rodents also have specific morphological features, such as more numerous ganglion cells and amacrine cells, which are involved in the processing of visual information [12, 13]. The topographic distribution of retinal ganglion cells throughout the retina has been studied in different animals, and cells are found to be densely distributed in areas of the retina where images are in focus [10].

Curcio and his colleagues [5] measured the density of photoreceptors in retinas from human eyes taken postmortem and analyzed histologically. They showed that the human retina contains a relatively high number of cones photoreceptors with an average of 4.6 million cones (4.08–5.29 million). However, they represent only 5–10% of all photoreceptors and are mainly concentrated in the fovea, in the center of the

retina, along the optical axis. The peak cone density in the fovea is around 199,000 cones per mm^2 with significant inter-individual variability. The fovea is devoid of rods over a diameter of 600 microns. Beyond this, the rods appear progressively and their density becomes equal to that of the cones at a distance of 400 to 500 microns from the center of the fovea. The retina contains 95% of total photoreceptors with an average of 92 million rods (77.9 to 107.3 million). The fovea has long been recognized as the site of maximal visual acuity [14]. However, it contains 0.3% of the total cones and 25% of the ganglion cells, illustrating its importance in primate vision [5, 15]. Rod density is the highest along an elliptical perimeter located at the same distance as the optic nerve from the fovea. Rod density appears to be the highest in the superior retina. The cell density per unit in nocturnal rodents in retinas, such as rats and mice, is ~3–4 times higher in the central retina than in the human macula, and therefore, the phagocytic load per RPE cell is higher in mice than in humans. Rods are very sensitive to light, but their density and their connections with other retinal cells (several rods for a bipolar cell) mean that they are unable to discern fine details in an image. Similar to other mammals, the retina of nocturnal rodents is predominantly composed of rod photoreceptors. In fact, they have approximately 6.4 million rods, which make up around 97% of the total photoreceptor population [16]. In addition, nocturnal rodents such as rats and mice have a retina that is low in the cones, responsible for daytime vision, which differs considerably from the foveolar region in humans, which is composed exclusively of cones. There are only around 200,000 cones, accounting for just 3% of the photoreceptors [6, 13]. As a result, these rodents are poorly suited to studying the morpho-functional changes in this population of cells. Interestingly, diurnal rodents have higher proportions of cone photoreceptors than nocturnal rodents, which facilitates the study of cone pathophysiology [2].

The most striking adaptation was mentioned by German researchers, in 2009 17] when they observed on histological sections of mouse retina that rod nuclei have an inverted structure compared with any other cell, with a dark center and a bright periphery corresponding to euchromatin toward the outside while heterochromatin is compact in the middle [17]. This distribution is found only in nocturnal species such as the mouse. In contrast, the rod nuclei of diurnal mammals such as horses, squirrels, and humans have a typical, universal organization (euchromatin in the center, heterochromatin at the periphery). Nocturnal rodents, like the 40 species of nocturnal mammals studied, have a unique architecture.

It is, therefore, essential to have a suitable animal model that faithfully reproduces the anatomical, physiological, and pathological characteristics specific to eye diseases in humans. In our studies, we proposed, the Tunisian gerbil *P. obesus* as a promising model for eye research, offering notable anatomical and functional similarities with the human eye [18, 19]. In this chapter, we explore the use of the gerbil *P. obesus* as a rodent model for eye research, highlighting its similarities to the human eye and its potential in the study of eye diseases.

3. Importance of *P. obesus* in vision research

3.1 General characteristics

P. obesus, commonly known as the Sand Rat, is a species of gerbil first described by P. Cretzschmar in 1828 (**Figure 1**). Belonging to the subfamily Gerbillinae, this rodent species is found in the arid desert regions of North Africa and the Middle East,

Figure 1.
Psammomys obesus. Obesus, or the sand rat Cretzschmar in (1828).

extending from Morocco to the eastern parts of Arabia [20]. Studies carried out since the 1960s showed that when sand rats were maintained under laboratory conditions and fed a standard rodent diet, they developed type 2 diabetes (T2D) [21, 22]. Diabetic features observed in *Psammomys* cover persistent hyperglycemia accompanied by hyperinsulinemia, dyslipidemia, to more advanced stages characterized by hypoinsulinemia, ketoacidosis, and diabetic retinopathy [18, 23–25]. These unique features have aroused great interest among researchers, making *Psammomys* a valuable and novel animal model for studying the mechanisms underlying (T2D) induced by nutritional manipulation. Therefore, *P. obesus* is well recognized as an interesting model for studying the relationships between diet, metabolism, and diabetes.

In 2017, Hargreaves and his colleagues conducted genome sequencing of the sand rat, leading to the discovery of a remarkable unusual chromosomal region, characterized by an abundance of G and C nucleotides [26]. Within this region, the Pdx1 homeobox gene, a transcriptional activator of insulin, exhibited significant sequence alterations, which has undergone massive sequence changes, probably contributing to diabetes and adaptation to low caloric intake. The main findings of this study imply that mutation rates vary within the same genome and that parts of the genome with high mutation rates may influence adaptation and ecological constraints. This unique genomic structure contributes to substantial divergence in the Pdx1 sequence. In addition, the authors warn that divergent regions may be missed by conventional short-read sequencing approaches, a consideration for current and future genome sequencing projects.

3.2 Interest of *P. obesus* in ophthalmological research

The desert-dwelling rodent *P. obesus* exhibits a sophisticated visual system and possesses distinctive attributes associated with its adaptation to arid habitats.

Notably, it demonstrates striking parallels to the human eye, particularly concerning retinal structure and the signaling cascades implicated in human ocular pathologies [18, 19]. Furthermore, *P. obesus*, characterized as a diurnal species, resembles the human retina in multiple aspects. The relatively larger size of its eyes (~8 mm in diameter) compared to rats (4–5 mm) potentially augments its visual capabilities and facilitates manipulations during experimental investigations [2].

The histology of the whole eye of *Psammomys* was done by Ref. [27]. However, the first study to determine the retinal phenotype was carried out by our team and was published in 2011 [18], as mentioned by Ref. [27]. The work highlighted the similarity in the organization of the different layers of the retina between gerbils and humans, making it easier to transpose research results to the human scale. We have shown that the *P. obesus* retina has a typical stratified structure (**Figure 2A** and **B**), with cones accounting for an average of 41% of total photoreceptor cells. Short and medium wavelength cones are present in typical relative proportions. Among diurnal rodents, *P. obesus* showed a higher percentage of cones related to rods, in comparison with the Mongolian gerbil (*Meriones unguiculatus*) whose retinal photoreceptors comprise 13% cones and the Arvicanthis species, *Arvicanthis ansorgei* (33%), *and Arvicanthis niloticus* (35%), which still considering pertinent models of the study of cone pathophysiology [28–30].

The importance of the cone system in our proposed model was confirmed by functional electrophysiological measurements in *P. obesus* (**Figure 2C**), done with ISCEV Standard for clinical ERG testing [19, 31]. The results were compared with those obtained in human subjects and Wistar rats. ERG measurements showed that the amplitudes of scotopic responses in *P. obesus* were quite similar to those in human subjects. However, under photopic conditions, ERG measurements share several characteristics with human ERG responses, while being quite different from those of the rat, for more details please see [19].

A strong cone-driven retinal response is indicated by the amplitude of the photopic a-wave, very similar to that observed in humans and six times higher than in the

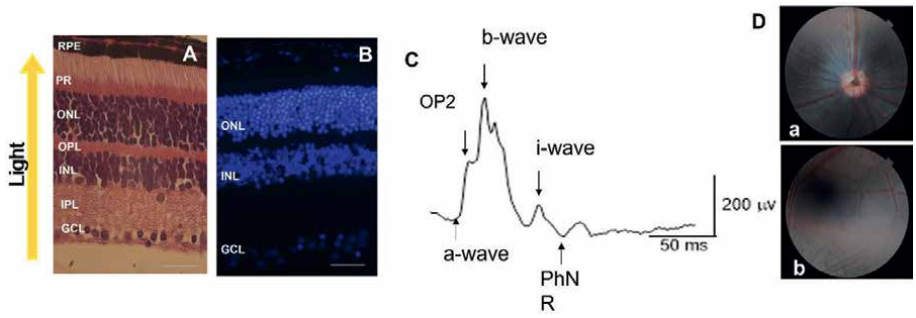

Figure 2.
Structural, neuronal, functional, and vascular exploration of the healthy P. obesus retina. A) Light micrograph of a vertical section through P. obesus retina (Hematoxylin and eosin staining). The basic retinal structure of P. obesus is arranged in different layers of cells, from retinal pigment epithelium (RPE), photoreceptor layer (PR), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), scale bar 50 µm. B) Dapi immunostaining of different neuronal retinal cells observed by fluorescence microscopy, scale bar 50 µm. C) Typical ERG response of a P. obesus healthy retina to a white flash under photopic condition using 3 cd.s/m² light intensity stimulation, a-wave mixed response elicited at 3 cd.s/m², b-wave mixed response in the dark adapted eye. D) Eye fundus vasculature (a) in the optic nerve and (b) in the visual streak.

albino rat. While the b-wave is significantly larger in *P. obesus* than in man and rat, the photopic b−/a ratio is lower than in rats and closer to the value in man, showing another surprising difference with nocturnal rodents.

The amplitudes of the photopic oscillatory (OP) and flicker potentials at 30 Hz were all significantly larger in *P. obesus* than in human and Wistar rats. Furthermore, like the human photopic ERG, the photopic ERG of *P. obesus* also includes prominent post-b wave components (i.e., i and d waves), whereas the ERG of Wistar rats does not show it. Specifically, another striking feature shared by *P. obesus* concerns the photopic ERG responses after the b-wave: the i-wave and the negative photopic response (PhNR). These response patterns are commonly observed in human photopic electroretinograms (ERGs), but does not exist in nocturnal rodents [32]. Similarly suggesting that gerbils may represent a valuable complement to mice models, particularly for investigating retinal cone function and studying ocular diseases associated with cones.

In addition, *P. obesus* has emerged as a novel animal model for research on diabetic retinopathy [25, 33, 34]. One remarkable feature of *P. obesus* is its prominent visual streak, which exhibits remarkably high densities of cones and ganglion cells (**Figure 2D**). Additionally, this species possesses specialized vasculature in the visual streak, characterized by the absence of major vessels in this area. The anatomical resemblance of *P. obesus* visual streak to the human fovea makes it a potentially valuable model for studying foveal pathologies associated with DR, such as diabetic macular edema.

4. Conclusion

In conclusion, the gerbil *P. obesus* provides a valuable model for studying the physiology and pathology of the human retina, complementing traditional nocturnal models. Its diurnal activity cycle and cone-rich retina make it particularly useful for studying cone and color processing cell function, and a powerful tool for research into ocular diseases and the development of new therapeutic strategies between different rodent species.

Acknowledgements

This research was funded by the Ministry of Higher Education and Scientific Research (MHESR) through the project: PAQ-Collabora (PAR&I-Tk), period 2020–2022.

DOI: http://dx.doi.org/10.5772/intechopen.1002183

Author details

Sihem Mbarek*, Oumeima Hammami, Oumeima Achour
and Rafika Ben Chaoucha-Chekir
Laboratory of Physiopathology, Food and Biomolecules, Higher Institute of
Biotechnology Sidi Thabet, BiotechPole, University of Manouba, Ariana, Tunisia

*Address all correspondence to: sihemmbarek@gmail.com

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References

[1] Iwata T, Tomarev S. In: Conn PM, editor. Animal Models for Eye Diseases and Therapeutics, in Sourcebook of Models for Biomedical Research. Totowa, NJ: Humana Press; 2008. pp. 279-287. DOI: 10.1007/978-1-59745-285-4_31

[2] Verra DM, Sajdak BS, Merriman DK, Hicks D. Diurnal rodents as pertinent animal models of human retinal physiology and pathology. Progress in Retinal and Eye Research. 2020;**74**:100776. DOI: 10.1016/j.preteyeres.2019.100776

[3] Schmitz L, Motani R. Morphological differences between the eyeballs of nocturnal and diurnal amniotes revisited from optical perspectives of visual environments. Vision Research. 2010;**50**(10):936-946. DOI: 10.1016/j.visres.2010.03.009

[4] Douglas RH, Jeffery G. The spectral transmission of ocular media suggests ultraviolet sensitivity is widespread among mammals. Proceedings of the Royal Society B: Biological Sciences. 2014;**281**(1780):20132995. DOI: 10.1098/rspb.2013.2995

[5] Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. The Journal of Comparative Neurology. 1990;**292**(4):497-523. DOI: 10.1002/cne.902920402

[6] Carter-Dawson LD, Lavail MM. Rods and cones in the mouse retina. I. Structural analysis using light and electron microscopy. The Journal of Comparative Neurology. 1979;**188**(2):245-262. DOI: 10.1002/cne.901880204

[7] Stockman A, Sharpe LT. Human cone spectral sensitivities: A progress report. Vision Research. 1998;**38**(21):3193-3206. DOI: 10.1016/S0042-6989(98)00060-1

[8] Szél Á, Röhlich P. Two cone types of rat retina detected by anti-visual pigment antibodies. Experimental Eye Research. 1992;**55**(1):47-52. DOI: 10.1016/0014-4835(92)90090-F

[9] Nadal-Nicolás FM, Kunze VP, Ball JM, Peng BT, Krishnan A, Zhou G, et al. True S-cones are concentrated in the ventral mouse retina and wired for color detection in the upper visual field. eLife. 28 May 2020;**9**:e56840. DOI: 10.7554/eLife.56840. PMID: 32463363. PMCID: PMC7308094

[10] Miyazaki T, Naritsuka Y, Yagami M, Kobayashi S, Kawamura K. Anatomy and histology of the eye of the nutria Myocastor coypus: Evidence of adaptation to a semi-aquatic life. Zoological Studies. 2022;**61**:e18. DOI: 10.6620/ZS.2022.61-18

[11] Behar-Cohen F, Gelizé E, Jonet L, Lassiaz P. Anatomie de la rétine, médecine/sciences. 2020;**36**(6-7):594-599. DOI: 10.1051/medsci/2020094

[12] Hendrickson A. Organization of the Adult Primate Fovea. In: Penfold PL, Provis JM, editors. Macular Degeneration. Berlin, Heidelberg: Springer; 2005. pp. 1-23. DOI: 10.1007/3-540-26977-0_1

[13] Jeon C-J, Strettoi E, Masland RH. The major cell populations of the mouse retina. The Journal of Neuroscience. 1998;**18**(21):8936-8946. DOI: 10.1523/JNEUROSCI.18-21-08936.1998

[14] Helmholtz H. Treatise On Physiological Optics, Vol. 2 OCR – PDF, Scribd. 1924. Available from: https://de.scribd.com/document/458457749/Helmholtz-H-1924-Treatise-on-Physiological-Optics-Vol-2-OCR (consulté le 12 juin 2023)

[15] Curcio CA, Allen KA. Topography of ganglion cells in human retina. The Journal of Comparative Neurology. 1990;**300**(1):5-25. DOI: 10.1002/cne.903000103

[16] Leamey CA, Protti DA, Dreher B. Comparative survey of the mammalian visual system with reference to the mouse. In: Chalupa LM, Williams RW, editors. Eye, Retina and Visual System of the Mouse. Cambridge: MIT Press; 2008

[17] Solovei I, Kreysing M, Lanctôt C, Kösem S, Peichl L, Cremer T, et al. Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. Cell. 2009;**137**(2):356-368. DOI: 10.1016/j.cell.2009.01.052

[18] Saïdi T, Mbarek S, Omri S, Behar-Cohen F, Chaouacha-Chekir RB, Hicks D. The sand rat, Psammomys obesus, develops type 2 diabetic retinopathy similar to humans. Investigative Ophthalmology & Visual Science. 2011;**52**(12):8993-9004. DOI: 10.1167/iovs.11-8423

[19] Dellaa A, Polosa A, Mbarek S, Hammoum I, Messaoud R, Amara S, et al. Characterizing the retinal function of Psammomys obesus: A diurnal rodent model to study human retinal function. Current Eye Research. 2017;**42**(1):79-87. DOI: 10.3109/02713683.2016.1141963

[20] Musser GG, Carleton MD. Superfamily Muroidea. In: Wilson, D.E. and Reeder, D.M., editors. Mammal Species of the World: A Taxonomic and Geographic Reference, Baltimore: The Johns Hopkins University Press; 2005. p. 2142

[21] Schmidt-Nielsen K, Haines HB, Hackel DB. Diabetes mellitus in the sand rat induced by standard laboratory diets. Science. 1964;**143**(3607):689-690. DOI: 10.1126/science.143.3607.689

[22] Marquié G, Duhault J, Jacotot B. Diabetes mellitus in sand rats (Psammomys obesus). Metabolic pattern during development of the diabetic syndrome. Diabetes. 1984;**33**(5):438-443. DOI: 10.2337/diab.33.5.438

[23] Haines H, Hackel DB, Schmidt-Nielsen K. Experimental diabetes mellitus induced by diet in the sand rat. American Journal of Physiology-Legacy Content. 1965;**208**(2):297-300. DOI: 10.1152/ajplegacy.1965.208.2.297

[24] Chrigui S, Hadj Taieb S, Jemai H, Mbarek S, Benlarbi M, Feki M, et al. Anti-obesity and anti-Dyslipidemic effects of Salicornia arabica decocted extract in Tunisian Psammomys obesus fed a high-calorie diet. Foods (Basel, Switzerland). 2023;**12**(6):1185. DOI: 10.3390/foods12061185

[25] Dellaa A, Mbarek S, Kahloun R, Dogui M, Khairallah M, Hammoum I, et al. Functional alterations of retinal neurons and vascular involvement progress simultaneously in the Psammomys obesus model of diabetic retinopathy. The Journal of Comparative Neurology. 2021;**529**(10):2620-2635. DOI: 10.1002/cne.25114

[26] Hargreaves AD, Zhou L, Christensen J, Marlétaz F, Liu S, Li F, et al. Genome sequence of a diabetes-prone rodent reveals a mutation hotspot around the ParaHox gene cluster. Proceedings of the National Academy of Sciences. 2017;**114**(29):7677-7682. DOI: 10.1073/pnas.1702930114

[27] Saadi-Brenkia O, Hanniche N, Lounis S. Microscopic anatomy of ocular globe in diurnal desert rodent Psammomys obesus (Cretzschmar, 1828). The Journal of Basic and Applied Zoology. 2018;**79**(1):43. DOI: 10.1186/s41936-018-0056-0

[28] Bobu C, Craft CM, Masson-Pevet M, Hicks D. Photoreceptor organization and rhythmic phagocytosis in the Nile rat Arvicanthis Ansorgei: A novel diurnal rodent model for the study of cone pathophysiology. Investigative Ophthalmology & Visual Science. 2006;**47**(7):3109-3118. DOI: 10.1167/iovs.05-1397

[29] Gilmour GS, Gaillard F, Watson J, Kuny S, Mema SC, Bonfield S, et al. The electroretinogram (ERG) of a diurnal cone-rich laboratory rodent, the Nile grass rat (Arvicanthis niloticus). Vision Research. 2008;**48**(27):2723-2731. DOI: 10.1016/j.visres.2008.09.004

[30] Yang S, Luo X, Xiong G, So K-F, Yang H, Xu Y. The electroretinogram of Mongolian gerbil (Meriones unguiculatus): Comparison to mouse. Neuroscience Letters. 2015;**589**:7-12. DOI: 10.1016/j.neulet.2015.01.018

[31] Robson AG, Frishman LJ, Grigg J, Hamilton R, Jeffrey BG, Kondo M, et al. ISCEV standard for full-field clinical electroretinography (2022 update). Documenta ophthalmologica. Advances in Ophthalmology. 2022;**144**(3):165-177. DOI: 10.1007/s10633-022-09872-0

[32] Rosolen SG, Rigaudière F, LeGargasson JF, Chalier C, Rufiange M, Racine J, et al. Comparing the photopic ERG i-wave in different species. Veterinary Ophthalmology. 2004;7(3):189-192. DOI: 10.1111/j.1463-5224.2004.04022.x

[33] Benlarbi-Ben Khedher M, Hajri K, Dellaa A, Baccouche B, Hammoum I, Boudhrioua-Mihoubi N, et al. Astaxanthin inhibits aldose reductase activity in Psammomys obesus, a model of type 2 diabetes and diabetic retinopathy. Food Science & Nutrition. 2019;7(12):3979-3985. DOI: 10.1002/fsn3.1259

[34] Saïdi T, Mbarek S, Chaouacha-Chekir RB, Hicks D. Diurnal rodents as animal models of human central vision: Characterisation of the retina of the sand rat Psammomys obsesus. Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht von Graefes Archiv für klinische und experimentelle Ophthalmologie. 2011;**249**(7):1029-1037. DOI: 10.1007/s00417-011-1641-9

Chapter 3

Mice as an Experimental Model to Understand the Pathobiology of Diseases

Rishika Jana, Souvik Karmakar, Bishal Hazra, Subhadeep Roy and Jayasri Das Sarma

Abstract

Murine models are widely used in scientific research because they share many genetic similarities with humans, making them a valuable tool for studying various diseases. C57BL/6 is an experimental mouse model to study the demyelination and inflammation aetiology of multiple sclerosis (MS). Intracranial inoculation of neurotropic murine β-coronavirus strain of mouse hepatitis virus in C57BL/6 mice induces demyelination with or without axonal loss, providing many insights regarding the mechanism of MS as well as SARS-CoV-2-mediated pulmonary and neuropathology in humans. By selectively using knockout mice in the wild-type C57BL/6 background, researchers can gain insights into the immunomodulatory nexus and can identify pathways involved in immune regulation which further can be efficiently studied with $CD4^{-/-}$, $CD40^{-/-}$, and $CD40L^{-/-}$ mice. In addition, C57BL/6 mice can also be used to generate syngeneic mouse models to investigate the aetiology and mechanism of various cancers, including ovarian cancer. Similarly, along with C57BL/6 mice, different immunocompromised mice models, such as nude mice, SCID mice, and NOD/SCID mice, can be used to study the aetiology, host-tumour interaction, function of the microenvironment, and tumour heterogeneity in tumour metastasis.

Keywords: experimental mouse model, murine β-coronavirus, mouse hepatitis virus, multiple sclerosis (MS), SARS-CoV-2, knockout mice, syngeneic mouse model, immunocompromised mice, cancer

1. Introduction

Mice are frequently employed as experimental models for various ailments, including cancer [1], diabetes [2], cardiovascular disease [3], and neurological disorders [4]. Because of several reasons like short generation time, low cost and adequate availability, being able to manipulate their genetics, ethical considerations, etc., mice are a preferred model organism in experimental research. Most importantly, genomic investigations have underlined the strong genetic similarities between humans and mice [5]. The ongoing pandemic of COVID-19, caused by the SARS-CoV-2, has affected millions

of individuals across the globe. Researchers have been examining various animal models that replicate the human disease to better understand this virus's pathogenesis and develop effective remedies and vaccines, among which mice models are noteworthy. Different mouse genotypes have been utilized to investigate SARS-CoV-2 infection. These mouse models developed using certain coronaviruses, such as mouse hepatitis virus (MHV), have provided valuable insights into the SARS-CoV-2 infection mechanism and have been used to test potential therapeutics and vaccines. Multiple sclerosis (MS), the most prevalent neurological condition, affects the central nervous system (CNS) and frequently causes severe physical or cognitive incapacity and neurological difficulties in young adults [6]. Although the exact cause of MS is unknown, infiltrating leukocytes and macrophages into the CNS and preexisting environmental and genetic factors may contribute to the disease's development [7, 8]. Several mouse models have been developed to investigate MS pathologies [9, 10]. Researchers can thoroughly understand the intricate interaction between the immune system and nervous system by using these animal models to comprehend the underlying mechanism of MS pathogenesis. In the 1980s, several research teams created methods to introduce exogenous coding DNA sequences into the mouse genome and impart Mendelian inheritable features [11]. In the second half of the 1980s, a novel technology was developed that made it possible to create mice models with gene-specific null mutations and/or models with gene-specific alterations at the endogenous chromosomal location of a particular gene [11, 12]. Numerous mechanistic studies utilizing these models have shown distinct cellular and molecular pathways and the independent and/or overlapping characteristics of these pathways in mammals. Studying complex interactions within biological systems, also known as systems biology, has become increasingly important in understanding the underlying causes of many human diseases. This is partly due to the emergence of new research fields such as genomics, proteomics, and metabolomics, collectively known as 'omics' [13]. One of the key contributors to this advancement has been the development of mice through gene targeting methods, enabling researchers to study the role of specific genes in disease development. Additionally, using gene-knockout mice in conjunction with transgenic mouse lines to produce 'humanized' mouse models as part of drug discovery methodologies has proven incredibly beneficial [14]. On the other hand, researchers can study the biology of cancers in complex, dynamic physiological systems by using various murine tumour models [1]. Since the introduction of gene targeting in mice, cancer researchers have been among the most frequent users of transgenic mouse models, significantly advancing the understanding of the origins and progression of cancer. Mice are used in more than 95% of *in vivo* cancer investigations [15]. However, translational research is constrained and hindered by various biological factors, such as animal behaviour and species distinctions, that may cause results to be misinterpreted. Throughout this chapter, we will be discussing the role of murine models in understanding infectious diseases and non-infectious diseases like cancer, as they provide valuable insights, allowing researchers to understand the underlying mechanisms of disease development better and to develop new therapies and treatments.

2. Versatility of mouse models in scientific research: an overview of common strains and their contributions

Different strains of mice are commonly used in scientific research, each with unique characteristics and advantages. C57BL/6 mice, BALB/c mice, SJL/J mice, nude mice, SCID mice, and NSG mice are some of the most commonly used experimental

mice strains [16, 17]. C57BL/6 mice is an immunocompetent mouse model that can be utilised to identify the key players in several human diseases, including liver disease, kidney disease, neurodegenerative diseases such as multiple sclerosis (MS), and cancer. It is a versatile model that can be used for physiology, immunology, oncology, and genetics research. In addition, to study the function of specific genes and their role in diseases, a revolutionised method in the field of biology is creating gene-knockout mice [11, 12]. On the other hand, various immunocompromised mice models (such as Nude, SCID, and NSG mice) are used to investigate the fundamental mechanisms behind tumour formation and assess the effectiveness of anti-cancer therapies. Throughout this chapter, we will discuss the various mice models and their significant contributions to the respective field of research.

3. C57BL/6 mice: an experimental mouse model to study neuroinflammatory demyelination and axonal pathology of murine β-coronavirus infection, which mimics specific pathologies of human neurological disease MS

White matter injury, consisting of the loss of axons, myelin sheath, and oligodendrocytes, is prevalent in various neurological conditions. Multiple sclerosis (MS) is a neurodegenerative and chronic inflammatory central nervous system (CNS) disease characterised by inflammation, demyelination, and axonal pathology [18]. Despite substantial research efforts, the aetiology and the exact mechanisms underlying MS pathogenesis still need to be better understood. Experimental animal models of multiple sclerosis could assist in advancing the understanding of the disease progression and identifying potential treatments. C57BL/6 mice are commonly used experimental models for investigating various diseases, including infectious and autoimmune disorders, due to their well-defined genetics, immunology, and ease of breeding and maintenance. The infection of C57BL/6 mice with murine β-coronavirus is one model that shares many resemblances of the pathological characteristics of human MS, including inflammation, demyelination, and CNS axonal damage. Implementation of this model has revealed insightful information into the pathogenesis of MS and assisted researchers in discovering potential therapeutic. Many studies have been performed to know the detailed pathobiology of mouse hepatitis virus (MHV)-induced demyelination in C57BL/6 mice, and numerous possible mechanisms have been hypothesised to explain the demyelination observed. One such hypothesis is that MHV induces an immune response [19] in the CNS where oligodendrocyte and/or myelin sheath are the main targets of immune system-mediated destruction, as seen in MS. New research has revealed that axonal damage is also present and is expected to be a significant factor contributing to the long-term disability observed in MS (**Figure 1**).

Furthermore, different MHV strains may cause demyelination through unique mechanisms. One such study revealed that a neurovirulent JHM strain of MHV-induced demyelination could be eliminated in RAG-deficient mice that are unable to produce functional T and B cells [22]. In contrast, another study indicates that MHV-A59-induced demyelination is feasible in the absence of B and T cells [23]. Based on the aforementioned observation, it is probable that certain strains of MHV, when the immune system is not functioning effectively, can induce demyelination in the CNS by direct injury to oligodendrocytes (OLGs) that could be due to direct glial dystrophy. Thus, C57BL/6 mice infected with MHV can also serve as an experimental

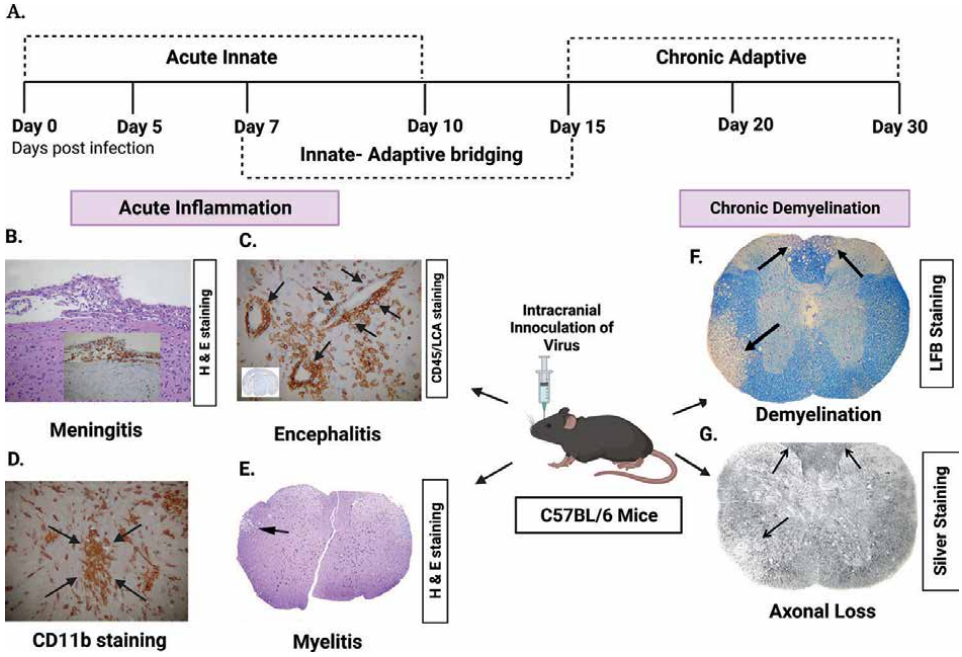

Figure 1.
Disease progression and pathological effects of intracranial inoculation with murine β-coronavirus (MHV-A59/RSA59) in C57BL/6 mice, a model to understand viral-induced demyelination concurrent with axonal loss. Coronal sections of MHV-A59 infected mice, taken on the 5th day after infection, stained using haematoxylin and eosin (H&E) or immunostained with antibodies for CD45 (a marker for inflammatory cells, also known as leukocyte common antigen) (LCA) and CD11b (a marker for microglia and macrophages). (A) Illustration of the timeframe of the infection, namely acute and chronic stages, along with the associated immune system. (B) H&E staining showing the presence of acute meningitis, and the inset displaying the existence of inflammatory cells that are positive for LCA. (C) Coronal sections of the brain stained for LCA indicating acute encephalitis, characterised by the formation of perivascular cuffing (arrows indicate perivascular cuffing). The inset indicates inflammatory infiltrates throughout the brain parenchyma. (D) In the MHV-A59-infected mice brain parenchyma, most inflammatory cells are positive for CD11b (arrows indicate microglial nodules). (E) Inflammatory lesion is observed in virus-infected mouse spinal cord at day 7p.i., stained with H & E (arrow indicates lesion areas in white matter). (F) LFB-stained sections showing loss of myelin in RSA59-infected mouse spinal cord at day 30p.i. (arrow indicates demyelinated area). (G) Bielchowsky silver impregnation showing loss of axons in RSA59-infected mouse spinal cord at day 30p.i. (arrows indicate the area of axonal loss). [adapted from [20, 21]] [created with BioRender.com].

animal model to understand non-immune-mediated mechanisms of demyelination that specifically affect oligodendrocytes. MHV can induce inflammation within the CNS by directly infecting neural cells, and the associated responses of glial cells play a crucial role in demyelination and axonal dystrophy. Astrocytes and microglia release cytokines and chemokines [24] that aid the host immune system in combating with the MHV pathogenesis and eradicating the virus from CNS. During the acute phase of infection, specific cytokines and chemokines, such as IL-6, IL-2, IL-1α, and IL-1β, tumour necrosis factor-α (TNF-α), interferon α, β, and γ, and CXCL10 are produced [25], while demyelination is observed during the chronic phase of infection. Activation of glial cells in the CNS typically initiates an innate immune response, releasing proinflammatory cytokines and chemokines known as cytokine storm. This response is intended to combat intruding pathogens but can also damage CNS tissues. The influx of anti-inflammatory cytokines is vital for reducing inflammation, and the peripheral immune system plays a crucial role in this process. CD40-expressed microglia/macrophage (innate immune cell) interacts with CD40L-expressed $CD4^+$

T cell (adaptive immune cell) through CD40-CD40L. This intricate interaction helps eliminate viruses and restore homeostasis by secreting anti-inflammatory milieu. A recent study has shown that RSA59 (an isogenic recombinant strain of MHVA59)-infected CD40L knockout mice developed severe demyelination concomitant with axonal loss, indicating that CD40-CD40L interaction is a crucial host defence mechanism against virus-induced demyelination [26]. Likewise, CD4 knockout mice infected with RSA59 exhibited increased susceptibility to axonal degeneration in the CNS, as well as poliomyelitis and bulbar vacuolation observed, indicating that the CD4 gene is protective against immunopathological disorders [27]. However, interferon-induced tetratricopeptide repeats 2 (Ifit2), a protein strongly induced by interferons, plays a significant role in providing antiviral immunity and the function of Ifit2 has been studied thoroughly in mounting host defence by creating Ifit2 knockout mice [28]. Thus, researchers can gain knowledge about the specific function of a knocked-out gene in different physiological conditions by creating gene-specific knockout mice. Later in this chapter, we will discuss the knockout mice in greater depth. Now, it is evident that the MHV infection model in C57BL/6 mice provides a valuable tool for studying the fundamental process of demyelination and axonal loss regardless of the particular mechanism involved in the progression of the disease. A comparative study was conducted between natural and recombinant strains of MHV that cause demyelinating (DM) and non-demyelinating (NDM) diseases to comprehend the mechanisms of demyelination and axonal degeneration. The object was to identify the differences between the two strains [29, 30] and better understand how they contribute to the diseases where a specific strain of MHV-induced C57BL/6 mice was used as an experimental model. This model assisted researchers to pinpoint therapeutic targets. For example, research has shown that blocking the activity of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α can reduce inflammation and demyelination in the CNS [31]. Moreover, treatments that promote remyelination, such as oligodendrocyte progenitor cells or drugs [32] that facilitate myelin repair, demonstrated encouraging results. In conclusion, the murine β-coronavirus-induced demyelinating disease model is a beneficial tool for investigating the pathogenesis of MS and identifying potential therapies. Nevertheless, caution should be exercised when extrapolating results from animal models to human disease, and additional research is required to explain the complexities of MS pathogenesis fully.

4. C57BL/6 mice: an experimental mouse model to study the disease progression and pathogenesis of EAE

Experimental autoimmune encephalomyelitis (EAE) is a common animal model for understanding the autoimmune pathobiology of MS [10]. Among the animal models, C57BL/6 mice are a popular experimental mouse model for investigating autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE), because they exhibit a uniform and stable disease course and clinical and pathological characteristics similar to those of human MS. Furthermore, these rodents have been exhaustively characterised, and their genetic and immunological backgrounds are well understood, making them an advantageous model for examining the pathogenesis of MS. This model has been extensively used to comprehend the disease progression and pathogenesis of MS, and it has yielded crucial insights into the immunological mechanisms underlying this disease. EAE could be induced by three different methods such as MBP-PLP fusion protein MP4, MOG peptide 35–55, and PLP peptide 178–191 in C57BL/6 mice, which

display distinct pathologies in CNS. These three models differ in three aspects: tract specificity, motor neuron involvement, and demyelination kinetics. MP4, MOG 35–55, and PLP peptide 178–191 induced three different models of EAE showing differential spinal cord tract pathology [33]. Mainly, three tracts of the spinal cord (pyramidal tract responsible for motor function, dorsal tract for fine touch, and anterolateral tract responsible for pain and crude touch) get affected differentially after immunisation with three different methods. In the acute and chronic phase of EAE, all mice displayed damage of anterolateral tracts in all three models. On the other hand, MP4 and MOG 35–55 models showed a resemblance to each other in the dorsal tract in the acute phase, while the PLP peptide model did not. In MP4- and MOG-induced models, motor neuron alterations were prominent, but in the PLP model, no such alteration in motor neurons was observed. Differential demyelination patterns have been noticed in three distinct EAE models. Demyelination was evident in the acute stage of the MP4 model, while it was transient in the MOG 35–55-induced EAE model and absent in the PLP peptide model. So, the use of MP4, MOG peptide 35–55 and PLP peptide 178–191 induced EAE models in C57BL/6 background could provide a reasonable strategy for reproducing distinct features of CNS pathology to comprehend the complex mechanism of MS.

Four stages of disease progression are depicted in **Figure 2** following subcutaneous infection with MOG 35–55 peptide, complete Freund's adjuvant (CFA), and pertussis toxin in C57BL/6 mice [35].

Mice immunised with MOG display waddling gait, tail limpness, hind limb paralysis, and ascending paralysis [36]. The pathogenesis of EAE in C57BL/6 mice involves activating autoreactive $CD4^+$ T cells, which recognise myelin antigens and become activated in the periphery [37]. The upregulation of integrins, such as the lymphocyte function-associated antigen (LFA-1) and the very late antigen-4 (VLA-4), allows these activated T cells to cross the blood-brain barrier (BBB). Autoreactive T cells reencountering antigens in the CNS become reactivated and produce inflammatory cytokines. These cytokines stimulate nearby immune and neural cells, attracting more inflammatory cells to the central nervous system (CNS). This process was seen in macrophages, believed to cause damage to the CNS either directly or indirectly [38]. Activated $CD4^+$ T cells may differentiate into T helper 1 (Th1) and T helper 17 (Th17) cells [39], which are the primary effector cells responsible for developing EAE, with IFN-γ and IL-17A being the key cytokines produced by these cells, respectively. IFN-γ, IL-2, and TNF are inflammatory cytokines produced by $CD4^+$ Th1 cells. In multiple sclerosis (MS) patients, the severity of the disease was linked to the levels of IFN-γ and IL-12 expression in both the cerebrospinal fluid (CSF) and CNS [40]. Th17 cells release proinflammatory cytokines, particularly IL-17 and IL-22 [41].

Additionally, these cells stimulate other types of cells to produce proinflammatory factors, such as granulocyte/macrophage colony-stimulating factor (GM-CSF), cytokines such as IL-6, and several chemokines like CXCL8, indicating that Th17 cells play an essential role in promoting inflammation in the CNS [42]. This model's consistency and reliability, which permits the standardisation of experimental conditions and the comparison of results within studies, is one of its considerable advantages. In addition, the recurring and predictable disease course of EAE in C57BL/6 mice and the similarities to human MS in terms of clinical and pathological features make this model a useful tool for elucidating the underlying mechanisms of MS in order to develop novel therapies. This model has been used to test therapeutic targets for over 3–4 decades. Further studies utilising this model are required to clarify the complex immunological and pathological processes involved in MS and identify new therapeutic intervention targets. EAE can also be induced in SJL mice by immunisation with proteolipid protein (PLP), myelin basic

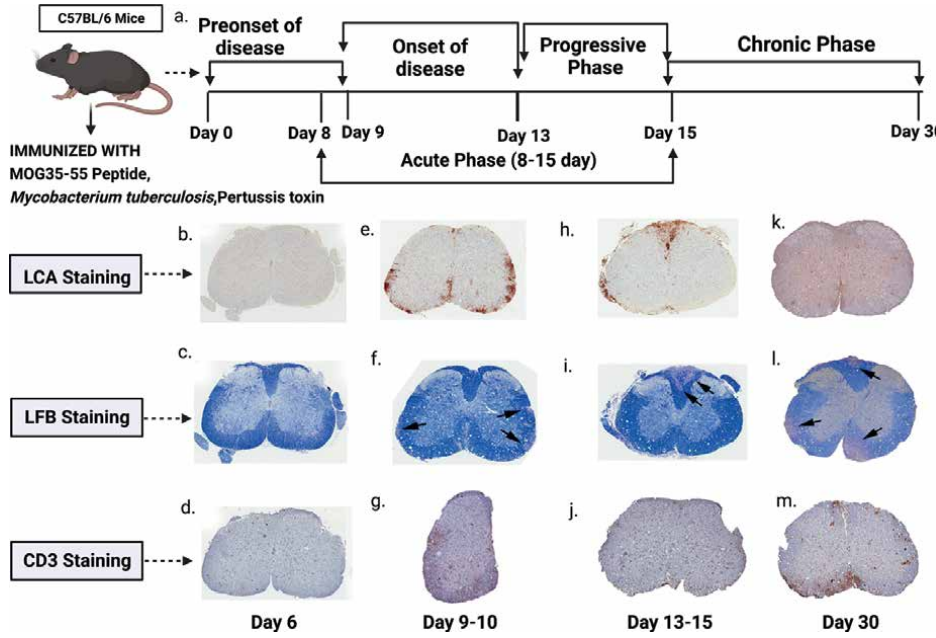

Figure 2.
Disease progression and pathological effects of C57BL/6 mice after immunisation with MOG 35–55. Representative sections (5 μm thick) of spinal cords from mice with EAE are taken at different time points after immunisation and stained with Luxol fast blue (LFB) for myelin, as well as histopathology for CD45 (LCA, to identify inflammatory cells) and CD3 (to identify T cells). (a) Demonstrating the timeline of disease progression of C57BL/6 mice after being immunised with MOG 35–55. A hemicord is depicted in (g). [(b)–(d)] Before EAE onset (day 6 after immunisation). [(e)–(g)] EAE onset (day 9–10 after immunisation). [(h)–(j)] Progressive phase (day 13–15 after immunisation), [(k)–(m)] chronic phase (day 30 after immunisation) [(b), (e), (h), (k)] LCA stain. [(c), (f), (i), (l) Luxol fast blue. [(d), (g), (j), (m)] CD3. Spinal cords of days 9–10 of EAE mice show an increase in LCA and CD3 positive cells in the white matter compared to spinal cords of day 6. The Luxol fast blue stain signifies the presence of early demyelinating plaques in the white matter. During the progressive phase, the size of demyelinating plaques increased (arrows denote locations of demyelinating plaques), and ongoing LCA immunoreactivity was observed, while CD3 immunoreactivity decreased. In the chronic phase, LCA immunoreactivity decreased, while CD3 immunoreactivity increased. The number and size of demyelinating plaques continued to increase during this phase. [adapted from [34]] [created with BioRender.com].

protein (MBP), or peptides corresponding to the immunodominant epitopes of MBP, although here in this book chapter, we have only discussed about C57BL/6 mice as an experimental mouse model to understand the pathogenesis of EAE. While C57BL/6 mice are widely used to understand the inflammatory demyelinating disease as discussed in length, it can also serve as a neuroinflammatory model to understand the pathobiology of other coronaviruses like SARS-CoV-2, which is now a significant threat to human health. So, in the next section of this chapter, we will focus on how MHV-infected C57BL/6 mouse model could assist researchers in better understanding the pathobiology of SARS-CoV-2.

5. MHV-infected C57BL/6 mice as a model to understand SARS-CoV-2 pathobiology

While C57BL/6 mice proved to be a very efficient model for studying neurodegenerative disorders like MS, researchers have even extensively used mouse models to gain insights into various infectious diseases. The ongoing COVID-19 pandemic caused

by SARS-CoV-2, a β-coronavirus, is keeping the human race in trauma. Significant drawbacks in SARS-CoV-2 research are the unavailability of BSL-3 facilities and limited patient data, which hinders research on SARS-CoV-2. In the peripheral systems, SARS-CoV-2 can primarily cause upper respiratory tract infection, followed by inflammation in the lungs leading to severe pneumonia and enteritis. Moreover, if it enters the CNS, it can cause meningitis and encephalitis. In some patients, it is reported to cause myelin loss too. It can also affect the peripheral nervous system (PNS), causing Guillain-Barré syndrome. There are reports of SARS-CoV-2 manifestation in the CNS as viral RNA could be found in the cerebrospinal fluid [43]. As human data are limited, in this context, mouse hepatitis virus (MHV) can be considered an excellent model to draw the parallel to understand the pathogenesis, as it is a β-coronavirus belonging to the same genus as SARS-CoV-2. Infection of mice with MHV shows varied severity depending on the strain of the virus used and the age of the mice. On the other hand, intracranial inoculation of MHV in C57BL/6 mice exhibits similar pathologies to SARS-CoV-2 with respect to CNS manifestations, such as meningitis, encephalitis, and myelin loss [20, 44]. Hence, MHV-infected C57BL/6 mice can be used as an experimental model to understand the pathobiology of moderate and severe COVID-19 cases, as both viruses exhibit similar pathogenesis, organ tropism, and immune response (**Figure 3**).

A strain of MHV, MHV-A59-infected C57BL/6 mouse model can be considered an experimental model for mild-moderate cases of SARS-CoV-2. MHV-A59 infection starts with activating the brain resident astrocytes and microglia that help in the up-regulation of various cytokines and chemokines such as IL-6, IL-1β, IL-12, IL-15, TNF-α, and IFN-γ resulting in the infiltration of different peripheral innate immune cells such as neutrophils and monocytes/macrophages [45] as discussed earlier.

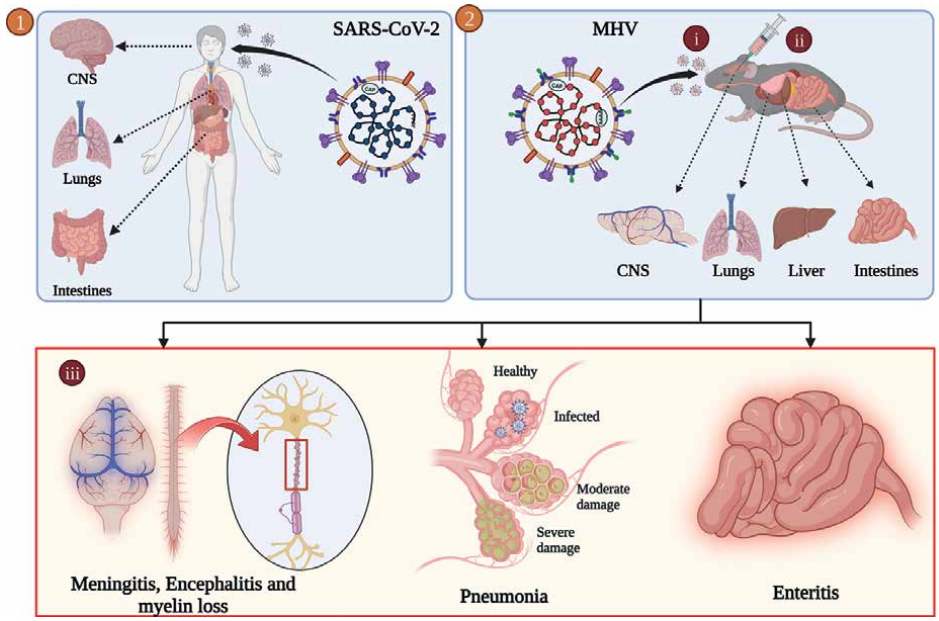

Figure 3.
Upon intranasal/intracranial inoculation (2-i and 2-ii), MHV infects the brain, lungs, and intestine, respectively, and similar organ tropism has also been found in SARS-CoV-2 infection [1]. Various infectious pathological conditions, such as meningitis, encephalitis, and myelin loss, are observed when intracranially introduced, and severe pneumonia and enteritis can be efficiently studied when peripherally introduced (2-iii) [created with BioRender.com].

Microglia/macrophages play an essential role in limiting viral replication and spread by recruiting several immune players of adaptive immunity, including $CD4^+$ T cells, $CD8^+$ T cells, and NK T cells into the CNS. Activation of the adaptive immune cells causes the secretion of even more cytokines. The release of these cytokines results in a cytokine storm, a phenomenon similar to SARS-CoV-2. Generally, infectious viral particles are cleared within 10 days p.i.; however, viral mRNA persists beyond day 10p.i. [20]. This persistent infection in mice gives rise to demyelination, which can be histopathologically confirmed by LFB staining [46]. This condition is similar in SARS-CoV-2 infection, where myelin loss is caused due to the activation of glial cells brought on by the proinflammatory state generated by the cytokine storm, primarily sustained by IL1, IL-6, and TNF-α [47]. To summarise, MHV-A59 causes a persistent infection with moderate neuroinflammation in C57BL/6 mice.

On the other hand, MHV-JHM.SD-infected C57BL/6 mice can be considered a model for severe COVID-19 cases due to vigorous viral replication, suboptimal CD4/CD8 T cell response, and the occurrence of cytokine storm [48]. These events result in severe pathologies of the disease and even death. MHV-JHM.SD infection in mice resulted in a longer innate immune response with upregulation of proinflammatory cytokines and chemokines with enhanced chemotaxis of innate immune cells such as macrophages, neutrophils, and NK cells into the CNS, leading to the destruction of the brain parenchyma. JHM.SD exhibits low T cell responses and IFN-γ levels [49]. Due to the high mortality rate of JHM.SD, an attenuated strain of MHV (2.2-V-1) is used to study the T cell response. So, to conclude, JHM.SD replicates severe pathogenesis as displayed by COVID-19 compared to MHV-A59, which mimics the mild-moderate pathogenesis of the SARS-CoV-2.

6. Gene-knockout mice and their role in studying neurodegenerative disease biology

Gene-knockout mice are genetically modified mice with a specific gene or genes deliberately removed or 'knocked out' to study the function of that gene. This is usually done by gene targeting, which involves introducing a modified version of the gene into embryonic stem cells, which are then used to create mice missing the targeted gene.

The use of gene-knockout mice has revolutionised the study of genetics and has provided valuable insights into the function of genes and their role in various diseases. They have been used to study multiple diseases, including cancer [50], Alzheimer's disease [51], Parkinson's disease [52], diabetes [53], and many others. For example, researchers have used knockout mice to study the role of the BRCA1 gene in breast cancer development [54, 55], the role of the amyloid precursor protein gene in Alzheimer's disease, and the role of the insulin receptor gene in diabetes. Though these are the common knockout mice used by researchers, in this chapter, we will discuss the knockout of immunomodulatory molecules to understand their individual roles in the viral-induced demyelination model.

Intracranial inoculation of RSA59 (an isogenic recombinant strain of MHV-A59) results in sequential recruitment and activation of several immune players in the CNS. The outcome of this infection is determined by how RSA59 interacts with the innate immune system. When the virus replication is initially controlled, it triggers a robust immune response that includes proinflammatory and type I interferon responses and promotes an anti-inflammatory response. Maintaining a delicate

balance between these opposing but mutually supportive immunity states is crucial for restoring tissue homeostasis. If the balance tips towards either extreme, it can be harmful and lead to immune-related health issues and tissue damage. The shift from innate to the adaptive immune response is controlled by a very important immune checkpoint regulator: CD40-CD40L dyad. Knocking out several immune system checkpoint genes such as CD4 [27], CD40R, CD40L [26], gave us an excellent idea about the consequences of disease biology (like neurodemyelinating diseases) in their absence.

Overall, knockout mice are a powerful tool for understanding the genetic basis of disease and developing new treatments and therapies. However, it is important to note that knockout mice do not perfectly replicate diseases in humans. Additional research is required to properly comprehend the implications of genetic changes in animal models for human health.

By studying the phenotype (observable traits) of mice that have had a specific gene knocked out, researchers can learn about the function of that gene and the consequences of its absence. This can help identify potential targets for drug development and develop new genetic disease treatments.

7. How are knockout mice made?

Gene knockout mice are created through gene targeting or gene disruption. This process involves embryonic stem cells (ES cells), pluripotent cells capable of developing into any cell in the body.

Researchers design a DNA construct containing a modified version of the gene of interest to create a gene-knockout mouse. This construct typically includes a selectable marker gene and a homology region for the target gene. The DNA construct is then introduced into embryonic stem cells, replacing the endogenous gene through homologous recombination.

The modified ES cells are then selected using the selectable marker gene, and those that have undergone successful homologous recombination are injected into mouse blastocysts. After that, these blastocysts are implanted into the uterus of a surrogate mother mouse.

If successful, the injected ES cells will give rise to chimeric mice with both regular and modified cells. The chimeric mice are then bred with wild-type mice to produce offspring carrying the altered gene in all their cells. These offspring are then screened to confirm that the targeted gene has been successfully disrupted [11, 56].

The resulting mice with the targeted gene disruption can then be studied to understand the gene's function and role in disease or normal physiology. The detailed mechanism of creating a gene-knockout mouse using Neomycin cassette mutagenesis is described in **Figure 4**.

Another method is the creation of conditional mutant mice. This can be achieved by The Cre-loxP system [58], a genetic engineering technique commonly used in molecular biology research to manipulate and modify genes in living organisms, including bacteria, plants, and animals. It uses two components: Cre recombinase and loxP DNA sequences. By using Cre recombinase to selectively delete or modify specific genes in a tissue-specific or temporal-specific manner, researchers can investigate the role of these genes in different physiological processes. In a tissue-specific way, the absence of that particular gene can be studied conditionally.

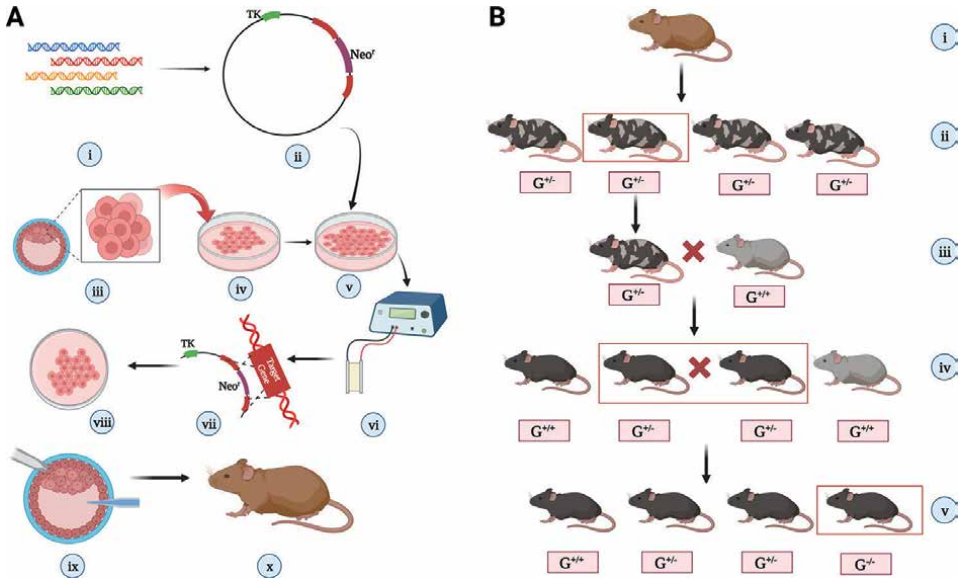

Figure 4.
Procedure of making gene-knockout mice using neomycin resistance cassette: (A) implantation of a particular gene-knockout blastocyst in a foster mother [(i) the gene which is to be knocked out is isolated from gene library; (ii) a cloning vector is designed in such a manner that it has a Neo^r gene and in its either side, there are terminal sequences of the target gene, and there even should be a thymidine kinase gene; (iii), (iv) embryonic stem cells are isolated from a donor mouse blastocyst and cultured in vitro; (v), (vi) the plasmid and the cells are mixed, followed by electroporation; (vii) by homologous recombination, there will be an exchange of gene segments in some cells; (viii) transformed cells are selected using a media containing neomycin and ganciclovir; (ix), (x) the positive cells are isolated and inserted in a donor mouse's blastocyst by microinjection and then implanted inside a foster mother], (B) crossing and obtaining the gene-knockout mice [(i) pregnant foster mother; (ii) newborn mice will be chimera; (iii) chimera having gonads derived from knockout gene are crossbred with a wild-type mice; (iv) inbreeding is performed in the offsprings having one copy of knockout gene in all cells and are heterozygous,; (v) probability of getting complete gene knockout is 25 per cent] [part B adapted and modified from [57]] [created with BioRender.com].

8. Understanding the immunomodulatory nexus between CD4$^+$ T cells and microglia/macrophages with the help of gene-knockout mice

During the neuroinflammatory process of virus-induced demyelination, communication between innate immune cells, such as microglia/macrophages (expressing CD40R), and adaptive immune cells, like activated CD4$^+$ T cells (expressing CD40L), plays a significant role that serves crucial functions in fighting the body against infections and regulating tissue homeostasis. On one hand, CD4$^+$ T cells can activate microglia and macrophages to enhance their phagocytic and antigen-presenting capabilities, allowing them to better clear pathogens and damaged cells. On the other hand, microglia and macrophages can also modulate the activity of CD4$^+$ T cells by presenting antigens and secreting cytokines that either promote or suppress CD4$^+$ T cell activation and differentiation. Depending on the cytokine environment and the type of virus, CD4$^+$ T cells can differentiate into different subsets, including Th1, Th2, Th17, and Tfh cells.

Microglia are the cellular immunological occupants of the CNS, defending against invasive infections and damaged cells as the first line of defence. Conversely, macrophages are immune cells that can be recruited to the CNS from the blood in response to infection or injury.

Overall, the interplay between $CD4^+$ T cells and microglia/macrophages is critical for maintaining immune homeostasis in the CNS and protecting against infections and injuries. Dysregulation of this interplay can lead to neuroinflammatory and neurodegenerative diseases in mice, highlighting the fact that the absence of any genes individually (like CD4, CD40, or CD40R) responsible for the proper functioning of this complex interaction is critical.

When the CD4 gene in C57BL/6 mice was functionally knocked down by deleting exon 5 (among the 11 exons) and then intracranially inoculated with the neurotropic virus RSA59 to study the demyelination and inflammatory aetiology of MS, the results were quite astonishing. In the absence of CD4, there was a significant decrease in the number of $CD11b^+$ microglia/macrophages in the acute phase. At the same time, viral replication was observed with the presence of viral RNA transcripts even 30 days after infection. These infected CD4 knockout mice displayed symptoms such as poliomyelitis, bulbar vacuolation, and axonal degeneration and were more susceptible to chronic phase encephalitis and demyelination. However, during the chronic phase, $CD11b^+$ phagocytic macrophages were present throughout the inflamed regions (including white and grey matter) of both the brain and spinal cord in the CD4 knockout mice suggesting a protective nature of the CD4 gene, that is, they help in reducing immunopathological disease and related morbidity and even aid in the clearance of the pathogen (**Figure 5**) [27].

Similarly, functionally knocking out the CD40L gene (expressed primarily by activated T cells) of C57BL/6 mice by replacing exons 3 and 4 of the gene on the X chromosome with a PGK-Neo cassette, and intracranially inoculating with neurotropic virus RSA59 showed increased disease severity by dampening

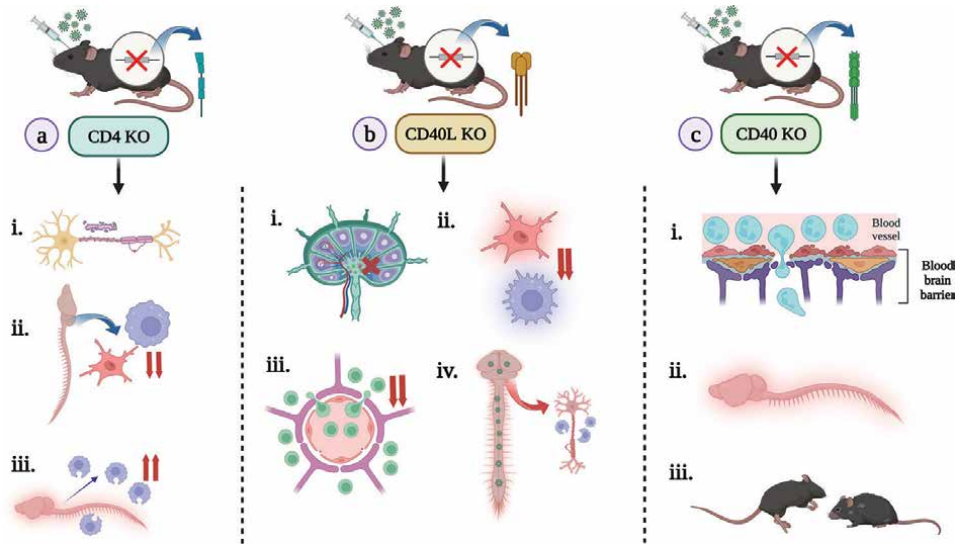

Figure 5.
Consequences of knocking out a particular gene and exploring its necessity in its absence, post-infection: (a) intracranial inoculation of RSA59 in a CD4 knockout (KO) mice [resulted in (i) axonal degeneration, bulbar vacuolation, poliomyelitis; (ii) significant decrease in $CD11b^+$ microglia/macrophages during the acute phase; (iii) presence of phagocytic macrophages in the inflamed regions during chronic phase], (b) intracranial inoculation of RSA59 in a CD40L knockout (KO) mice [(i) impaired $CD4^+$ T cell activation and priming in CLN; (ii) during the acute phase of the disease, microglia/macrophage activation got dampened; (iii) decreased infiltration of $CD4^+$ T cells; (iv) myelin stripping by phagocytic macrophages during chronic phase], (c) intracranial inoculation of RSA59 in a CD40 knockout (KO) mice [(i) increased neutrophil infiltration in the acute phase; (ii) increased inflammation of CNS; (iii) increased clinical scores and morbidity] [created with BioRender.com].

microglia/macrophage activation during the acute phase; reduced infiltration of T cells in the CNS and even impairing priming of T cells in the cervical lymph node. In the chronic phase, due to extensive replication of the virus in the CNS, severe demyelination and axonopathy were observed due to the presence of phagocytic microglia/macrophages (whose population got skewed due to the absence of the CD40L gene) (**Figure 5**) [26].

The CD40R is expressed by various haematopoietic cell types such as B cells, dendritic cells (DCs), monocytes, platelets, and macrophages and by non-haematopoietic cells such as myofibroblasts, fibroblasts, epithelial, and endothelial cells [59]. Because of its widespread prevalence, its absence will have detrimental effects on an individual. When this gene in C57BL/6 mice was functionally knocked out by replacing exon 3 (among the 9 exons) with neo-cassette and then infected with RSA59 intracranially, increased acute phase encephalitis was seen in the infected subjects as compared to the mock-infected ones, and the mice even showed high mortality rate. This is because, during the acute phase following the RSA59 infection, there was an increase in infiltrating neutrophils that led to heightened bystander neuroinflammation (unpublished data, JDS lab, IISER Kolkata, West Bengal, India) (**Figure 5**).

These studies suggest that the immunomodulatory nexus between $CD4^+$ T cells and microglia/macrophages is highly interconnected and regulated by a complex network of genes and signalling pathways. And, if researchers are interested in understanding the role of a particular gene in the immune response to a specific pathogen (like viruses), they could create a knockout mouse in which that gene is disabled. They could then expose both the knockout mouse and a normal mouse to the pathogen and observe how their immune systems respond differently. Further research using gene-knockout mice will be necessary for elucidating the specific mechanisms governing this interaction and developing new therapies to modulate immune responses in various disease states. Mice have even played a critical role in advancing our understanding of cancer and developing new cancer treatments, and they will likely continue to be an essential tool in cancer research for many years to come. Hence, in the next section, we will discuss how different mice models can be efficiently used to study cancer in detail as they share many biological similarities with humans, making them excellent models for studying cancer development, progression, and treatment.

9. Mice as an experimental model in cancer research

Mice are frequently utilised as experimental models in cancer research due to their genetic and physiological similarities with humans. Various rodent models that mimic human cancers have been established to gain insight into the key mechanisms behind tumour formation and assess the effectiveness of anti-cancer therapies. The essential phases of tumour development and progression start with normal cells, and their development into aggressive tumours is recapitulated in these models. These models have helped identify the elements contributing to tumour metastasis, recurrence, and therapeutic resistance [60].

Tumour development is monitored in genetically engineered mice (GEM) models in which mutations, deletions, or overexpression of genes with links to human cancers have been introduced. This model allows researchers to examine tumour development and chemotherapy responses in a functional immune system context. However, human cancer xenografts in immunocompromised mice are another extensively used model because mouse tumour cells might not entirely replicate the tumorigenic

mechanism in humans. These models use human tumour samples, cell lines, or cancer stem cells to develop tumours in various tissues. However, the roles of the tumour microenvironment, stroma, and conventional immune surveillance in tumour xenograft studies have not been investigated. This has led to several novel strains of humanised mouse models. In general, immunocompetent and immunocompromised mice models are the two main types of murine models used in cancer research [61].

9.1 Immunocompetent mice models

Immunocompetent mice have an immune system capable of mounting a normal immunological response to infections and foreign substances. This implies they have a fully working immune system, which allows researchers to explore the relationship between the immune system and various diseases. The use of immunocompetent mice is especially significant in cancer research because it provides a more realistic investigation of the complicated interactions between the immune system and tumours. Multiple immunocompetent murine models can be generated, which are discussed below.

9.1.1 Spontaneous tumour models

Spontaneous tumour models involve the genetic modification of mice to carry on mutations that imitate natural disease conditions in humans. The most therapeutically relevant tumour microenvironment for studying immunotherapeutic mechanisms can be found in these tumour models. Given the wide range of genes and cell targets that can be exploited, it has been discussed how generating mice with a targeted genetic mutation that causes spontaneous pancreatic cancer can be a helpful research tool. It was further reported that mice harbouring the mutant oncogene (G12D) spontaneously generate non-metastatic pancreatic cancers. Nevertheless, metastatic pancreatic tumours are produced when the p53 tumour suppressor gene is simultaneously mutated [62]. Another study summarised how this mouse model could examine the impact of oestrogens on mammary development and carcinogenesis. Further, they discussed the role of oestrogen receptors in controlling the proliferation, apoptosis, and differentiation of mammary epithelial cells. They have also looked into the bidirectional coordination between the epithelium and stroma in maintaining cancer cell stemness [63].

9.1.2 Genetically modified mouse models

The capacity to engineer mice to test the tumour progression is astounding, given the availability of the whole mouse genome sequence, genome manipulation technology, well-defined inbred strains, and thorough knowledge of the polymorphisms within strains. The function of a gene can now be altered, lost, mutated, under-expressed, or overexpressed in suitable cell types *in vivo*, making experiments to evaluate the results more straightforward. As discussed earlier, scientists started using homologous recombination in murine embryonic stem cells (ES cells) to knock out or knock in genes to create a more reproducible disease model, including cancer. In immunocompetent animals, these genetically engineered mice (GEM) models facilitate cancer researchers to explore how, when, where, and in what combinations of specific gene mutations may lead to cancer progression.

To offer a systemic or tissue-specific expression of oncogenes, such as MYC and KRAS in breast cancer [64] or deletion of tumour suppressor genes, such as TP53 and

PTEN in prostate cancer [65], GEM models are typically developed using transgenic technologies. The two major types of GEM models are germline and non-germline GEM models [66]. Germline GEM models contain mutations that result in the spontaneous development of malignant tumours. For instance, it has been found that a variety of solid and haematological tumours manifest in mice with a TP53 gene mutation [67]. Germline GEM models have enabled the investigation of mechanisms underlying tumour formation and progression. Still, it does not permit control over the time and location of tumour development [68]. On the other hand, non-germline GEM models offer spatiotemporal control of the development of tumours. Several mechanisms, such as the tamoxifen-inducible Cre-loxP system, can induce somatic mutations at a specific time and in a particular tissue. Hence any gene flanked by loxP sites can be deleted once the Cre-recombinase is activated by tamoxifen [69]. Furthermore, CRISPR/Cas9 technology has lately been extensively exploited for editing oncogenes, resulting in the development of models of lung cancer, hepatocellular carcinoma, and breast cancer [70–72].

9.1.3 Syngeneic mouse models

Traditionally, human tumour xenograft models were established by injecting cancer cell lines or transferring tissues from patients into immunodeficient mice. However, this model needed to be modified to comprehend the function of tumours and host immune factors in malignant transformation and metastasis. Thus, a syngeneic transplanted model has been established by introducing homologous cell lines into immunocompetent mice, which generates tumours rapidly while avoiding host rejection in an immunocompetent milieu [73]. C57BL/6 and BALB/c mice are suitable hosts for transplanting spontaneous, carcinogenic, or transgenic tumour cell lines [74, 75]. Cells implanted subcutaneously or intravenously multiply in the mice within a few weeks. Hence, the development of these kinds of models is rapid [76].

Researchers have used C57BL/6 mice to generate immunocompetent syngeneic allograft mouse models of paediatric diffuse midline glioma (DMG), a highly malignant and incurable brain tumour [77]. They created three genetically unique transplant models of the histone 3 wild-type (H3WT) and the K27M-mutant DMG (H3.3K27M and H3.1K27M). The histopathologic phenotype of these transplanted models matched that of their human counterparts. In the year 2000, potential syngeneic mouse models were developed for events related to ovarian cancer [78]. In a recent study, mouse epithelial ovarian cancer cell lines, ID8 and ID8-VEGF (overexpressing VEGF), were intraperitoneally injected in C57BL/6 female mice to generate a syngeneic ovarian cancer mouse model. It was observed that ID8-VEGF cells had a greater capacity to induce aggressive tumour growth as compared to ID8 cells, having basal levels of VEGF expression. On the other hand, *in vitro* studies found that these highly tumorigenic ID8-VEGF cells had reduced gap junctional intracellular communications compared to ID8 cells. Overall, the researchers utilised the syngeneic mice model to explain the reduction in gap junctions mediated intracellular communication in the tumorigenic ovarian cancer cell lines [79].

The effect of immunomodulating treatment is generally developed gradually and is measured by an increased survival rate [80]. Thus, the short time period provided by the rapid kinetics of tumour growth in syngeneic models is often insufficient to determine its efficacy. Moreover, the efficacy of immunotherapeutic medicines at earlier stages of tumour growth cannot be evaluated in syngeneic models either [81]. Now, we will discuss the various immunodeficient mice models used in cancer research.

9.2 Immunocompromised mice models

To determine whether a patient's tumour will respond to a specific therapeutic agent, the anti-cancer response to a human tumour, rather than a mouse tumour, must be examined. This is where a human tumour xenograft on nude mice, SCID mice, or NOD/SCID humanised mice can be helpful. Particularly when *in vitro* findings are needed to be confirmed *in vivo*, these models have been utilised extensively to examine the molecular mechanism of the antitumor impact of therapeutic drugs, combination therapies, metastasis and invasion, epithelial-mesenchymal transition, and cancer stem cells (CSCs) [82]. Immunocompromised or immunodeficient mice lack specific genes leading to reduced or no expression in certain cells. This allows for establishing humanised mice and *in vivo* simulation of the human environment. Immunodeficient mice evolved from nude mice deficient in T cells to NOD/SCID rg^{null} mice deficient in T, B, and NK cells.

Over time, various immunocompromised mice models have been developed (**Figure 6**). These include nude mice that lack T lymphocytes due to abnormal thymus formation. However, nude mice remain restricted in their application to many diseases due to their relatively low levels of immunodeficiency. Mice with a mutated Prkdc gene constitute the severe combined immunodeficiency (SCID) models.

Natural killer (NK) cells are present in SCID mice despite the absence of T and B lymphocytes. Further, introducing the SCID mutation into NK cell-deficient non-obese diabetic (NOD) mice, NOD/SCID mice developed. However, these animals have a short lifespan, impaired NK cell function, and a high rate of spontaneous thymic

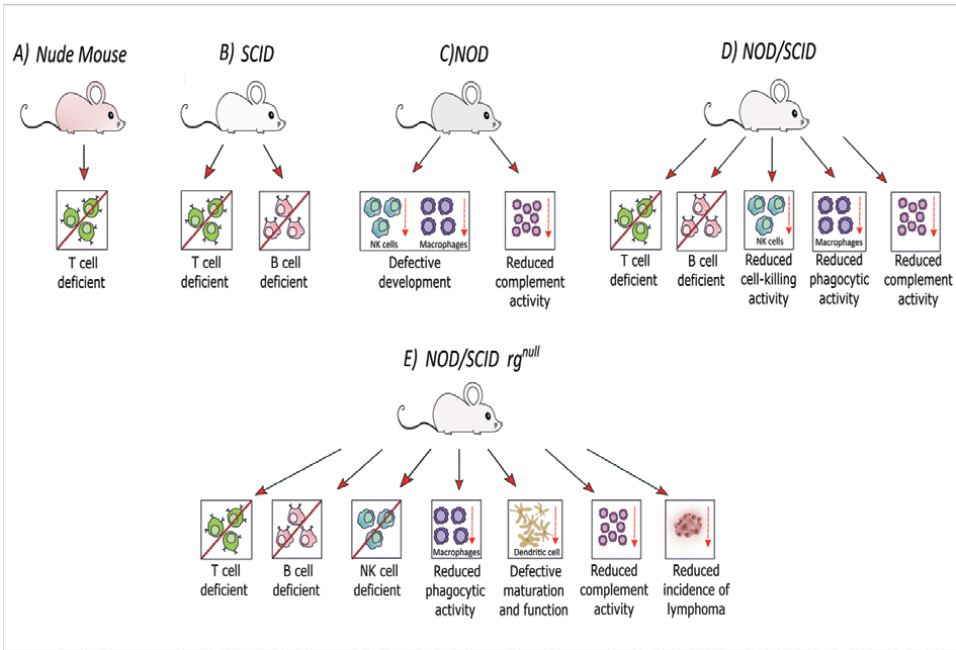

Figure 6.
Different types of immunocompromised mice models: (A) T cell-deficient nude mice; (B) T cell and B cell-deficient SCID mice; (C) NOD mice with defective development of NK cells and macrophages along with reduced complement activity; (D) NOD/SCID mice lacking T and B cells along with defective development of NK cells and macrophages and reduced complement activity; (E) T cell, B cell, and NK cell-deficient NOD/SCID rg^{null} mice with reduced phagocytic activities of macrophages, defective maturation and function of dendritic cells, reduced complement activity, and incidence of lymphoma [created with Inkscape.org].

lymphoma. As a result, they are not used frequently as humanised animal models. To improve this situation, the IL-2 receptor gamma chain was knocked out in the NOD/SCID mice to generate the NOD/SCID rg^{null} mice [16]. These mice had a higher implantation rate of human cells without spontaneous thymomas and are presently the global standard immunocompromised mouse model.

9.2.1 Nude mice models

The earliest immunodeficient mouse model is nude mice, which Flanagan first described in 1966 [83]. Due to a Foxn1 gene malfunction, a mutation on chromosome 11 hinders the proper development of the thymus, which results in the reduced formation of mature T lymphocytes. IgM is the most abundant immunoglobulin in these mice, with very little or no IgA [84]. As a result, they do not show signs of rejecting allogeneic tissue. The most often used strains, Swiss-nu, BALB/c-nu, NIH-nu, and NC-nu, are extensively employed in researching immunological disorders and malignancies. They cannot, however, fully accept the engraftment of human immune cells since they still have B cells and NK cells, making them unsuitable for use as an ideal humanised mouse model [16].

9.2.2 SCID and NOD/SCID mice models

Inbred CB-17 mice with recessive mutations in a single gene on chromosome 16 were discovered in 1983. This mutation altered the recombination enzyme activity of the sequence encoding the mouse lymphocyte antigen receptor gene, making it challenging to produce IgG, T, and B lymphocyte receptors [85]. Because of this mutation, T and B cell receptors have more difficulty repairing and recombining. The capacity of these cells to differentiate and mature is similarly hindered. There are thus fewer mature T and B lymphocytes and fewer immunoglobulins in the blood and lymphoid tissues of SCID mice. However, NK cells and macrophages functioned normally in SCID animals [86]. Additionally, 'leakage' was seen, meaning that T and B cells returned in some mice as they aged [87].

Non-obese diabetic (NOD) mice were developed for the first time by scientists in 1980 through inbreeding and selective breeding [88]. Similar pathology alterations and symptoms to those seen in humans with diabetes were seen in these animals. Low NK cell and macrophage activity and no complement activity in the blood are symptoms of a weakened innate immune system in NOD mice. It was hypothesised that crossing NOD mice with animals carrying the SCID mutation would result in a strain of mice with compromised adaptive and innate immune systems known as NOD/SCID mice [89]. Therefore, NOD/SCID mice developed a deficiency in T and B lymphocytes and other immune cells, including NK cells. As a result, their already-weak immune systems became even more compromised. Recently, NOD/SCID mice have not been frequently utilised as humanised mice models due to the larger amount of haematopoietic stem cells required for engraftment. Instead, the more advanced and effective NOD/SCID rg^{null} mice are being used now.

9.2.3 NOD/SCID rg^{null} mice

The interleukin-2 (IL-2) receptor gamma chain is one of the significant components of the receptors for cytokines such as IL-2, IL-4, IL-7. These cytokines are essential for the development and maturation of T, B, and NK cells. Thus, NOD/SCID rg^{null} mice lacking the gamma chain of IL-2 receptors are significantly compromised

in their innate and adaptive immune responses [90]. These mice have reduced phagocytic activities of macrophages along with defective maturation and function of dendritic cells. NOD/SCID rg^{null} mice are classified as either NOG or NSG mice based on the type of mutation in the IL-2 gamma chain. The IL-2 gamma chain targeted mutation is completely null in NSG mice leading to no expression of the IL-2 gamma chain. The IL-2 gamma chain mutation in NOG mice results in an expressed protein that binds cytokines but cannot transduce the signal. Transplantation success was more significant in NOG and NSG mice than in SCID or NOD/SCID mice, making these mouse strains the best models for human cell and tissue transplantation [91].

9.3 Generation of xenograft and allograft transplantation models

Transplanted tumours are most commonly used to test the therapeutic efficacy of a wide range of anti-cancer agents. Different laboratories currently use two major tumour transplantation models: allograft transplants and xenograft transplants (**Figure 7**).

Cancer cells from one mouse are transplanted into another mouse with a desired genetic feature in allograft (or syngeneic) transplantation models. Mice with functional immune systems accept the transplant when the cancer cells and the recipient share a common origin. This allows us to study the effects of therapeutic interventions in a setting very similar to that in which the tumour develops in an immunocompetent host. However, there is a possibility that the complexities of human malignancies will not be adequately represented by transplanted mouse tissue. Problems with GEM models, such as convoluted breeding plans and ineffective chemotherapy drugs, can be sidestepped with this transplanting approach [60].

To prevent the rejection of human cells, athymic nude mice or SCID mice are used to transplant human tumour cells subcutaneously or into the organ type (orthotopic)

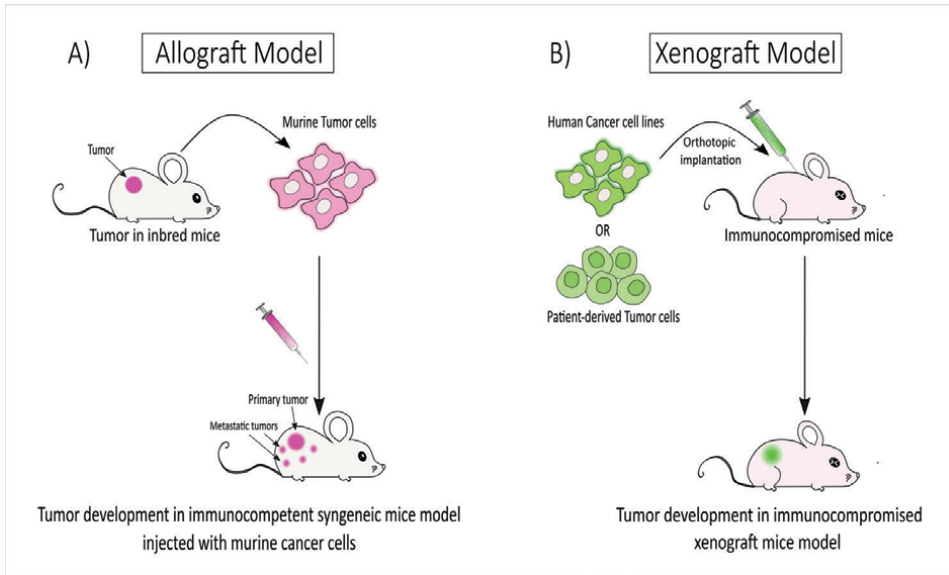

Figure 7.
Generation of allograft and xenograft transplantation models: (A) allograft transplant: murine tumour cells isolated from inbred donor mice are injected into immunocompetent mice resulting in tumour development. (B) Xenograft transplant: human cancer cells or patient-derived tumour cells are implanted into immunocompromised mice, resulting in tumour development [created with Inkscape.org].

from where the tumour originated. This model involves implanting human cancerous cells or solid tumours into a mouse. The mice's immune systems have been damaged to prevent cellular rejection by the host's immune system. Orthotopic transplants place the tumour in its natural location on the host, while subcutaneous transplants place it just under the recipient's skin. The human origin of the xenograft means that it shares some of the characteristics and complexities of human cancer. Once the tumour has reached a sufficient size, its response to therapy regimens can be performed according to the quantity of injected cells. As a result, these two transplantation models can be employed for a wide range of cancer-related experiments [60].

10. Conclusion

Human diseases have long been studied using mouse models. While these models have limitations and do not always accurately replicate actual disease, they have considerably aided in understanding disease mechanisms and developing new treatments. Mice are commonly used in medical research as study subjects due to their adaptability in replicating various human diseases and conditions in the lab. Mice are preferred models due to several factors, including their relative ease of handling, low cost, wide availability, and potential for genetic manipulation. Most importantly, genomic investigations have underlined the strong genetic similarities between humans and mice. Understanding complex human diseases might be challenging; hence, knockout mice can be used to understand the role of genes in complex diseases. Apart from knockout mice, immunocompromised mice can be efficiently used as models for cancer, immunology study as well as can be used for infectious diseases. Different types of immunocompromised mice exist, such as nude mice, NOD mice, SCID mice, and NOD/SCID mice. By virtue of their compromised immunity, these mice can be ideally used for xenograft mice models. Due to their smaller size and well-known genetic system, mice can be an appropriate model to study several complex diseases and disorders ranging from neurodegeneration to cancer, as discussed in this chapter.

The laboratory mouse: a heroic figure in the field of science

On the grounds of Novosibirsk's Institute of Cytology and Genetics in southwestern Siberia, there lies a monument that serves as a tribute to the mice who were utilised in genetic research to gain insights into biological and physiological processes that could lead to the development of new medications and treatments for various illnesses [92].

Acknowledgements

The authors would like to express their sincere gratitude to the Indian Institute of Science Education and Research, Kolkata, for their valuable support and assistance. Neurotropic m-CoV research in an experimental animal model has provided a platform to write this book chapter. This work has been a collaborative effort of the Indian Institute of Science Education and Research Kolkata (IISER-K), India; the Indian Institute of Sciences (IISc), India; the University of Pennsylvania, USA; Thomas Jefferson University, USA; and the University of Colorado Denver, USA, for the past two decades. The bench research to study M-CoV biology was generously supported

by the Department of Biotechnology (DBT) of India, the Council for Scientific and Industrial Research (CSIR) of India, the National Multiple Sclerosis Society, USA, the M.E. Groff Surgical Medical Research and Education Charitable Trust, and the Lindback Foundation Career Enhancement Award, USA. Indo-U.S. Science and Technology Forum (IUSSTF) also supported this work. The authors would also like to show their sincere gratitude to the System Medicine Cluster grant supported by the Department of Biotechnology (DBT) for cancer research. Finally, the success of this work has largely depended on the contribution and devotion of many researchers whose names are listed in the references.

Conflict of interest

The authors stated that they had no potential conflicts of interest with respect to the research, writing, and/or publication of this book chapter.

Author details

Rishika Jana[1†], Souvik Karmakar[1†], Bishal Hazra[1†], Subhadeep Roy[1†] and Jayasri Das Sarma[1,2*]

1 Department of Biological Sciences, Indian Institute of Science Education and Research, Mohanpur, India

2 Department of Ophthalmology, University of Pennsylvania, Philadelphia, USA

*Address all correspondence to: dassarmaj@iiserkol.ac.in

† All four authors have equally contributed to writing this book chapter.

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References

[1] Zhang W, Moore L, Ji P. Mouse models for cancer research. Chinese Journal of Cancer. 2011;**30**(3):149-152

[2] King AJ. The use of animal models in diabetes research. British Journal of Pharmacology. 2012;**166**(3):877-894

[3] Jia T, Wang C, Han Z, Wang X, Ding M, Wang Q. Experimental rodent models of cardiovascular diseases. Frontiers in Cardiovascular Medicine. 2020;**7**:588075

[4] Hafezparast M, Ahmad-Annuar A, Wood NW, Tabrizi SJ, Fisher EM. Mouse models for neurological disease. The Lancet Neurology. 2002;**1**(4):215-224

[5] Emes RD, Goodstadt L, Winter EE, Ponting CP. Comparison of the genomes of human and mouse lays the foundation of genome zoology. Human Molecular Genetics. 2003;**12**(7):701-709

[6] Compston A, Coles A. Multiple sclerosis. Lancet (London, England). 2008;**372**(9648):1502-1517

[7] Gourraud PA, Harbo HF, Hauser SL, Baranzini SE. The genetics of multiple sclerosis: An up-to-date review. Immunological Reviews. 2012;**248**(1):87-103

[8] Holmøy T, Hestvik AL. Multiple sclerosis: Immunopathogenesis and controversies in defining the cause. Current Opinion in Infectious Diseases. 2008;**21**(3):271-278

[9] Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD. Theiler's virus infection: A model for multiple sclerosis. Clinical Microbiology Reviews. 2004;**17**(1):174-207

[10] Robinson AP, Harp CT, Noronha A, Miller SD. The experimental autoimmune encephalomyelitis (EAE) model of MS: Utility for understanding disease pathophysiology and treatment. Handbook of Clinical Neurology. 2014;**122**:173-189

[11] Doyle A, McGarry MP, Lee NA, Lee JJ. The construction of transgenic and gene knockout/knockin mouse models of human disease. Transgenic Research. 2012;**21**(2):327-349

[12] Menke DB. Engineering subtle targeted mutations into the mouse genome. Genesis (New York, NY: 2000). 2013;**51**(9):605-618

[13] Chen BS, Wu CC. Systems biology as an integrated platform for bioinformatics, systems synthetic biology, and systems metabolic engineering. Cell. 2013;**2**(4):635-688

[14] Nair RR, Corrochano S, Gasco S, Tibbit C, Thompson D, Maduro C, et al. Uses for humanised mouse models in precision medicine for neurodegenerative disease. Mammalian Genome : Official Journal of the International Mammalian Genome Society. 2019;**30**(7-8):173-191

[15] Onaciu A, Munteanu R, Munteanu VC, Gulei D, Raduly L, Feder RI, et al. Spontaneous and induced animal models for cancer research. Diagnostics (Basel). 31 Aug 2020;**10**(9):660

[16] Chen J, Liao S, Xiao Z, Pan Q, Wang X, Shen K, et al. The development and improvement of immunodeficient mice and humanized immune system mouse models. Frontiers in Immunology. 2022;**13**:1007579

[17] Croxford AL, Kurschus FC, Waisman A. Mouse models for multiple

sclerosis: Historical facts and future implications. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2011;**1812**(2):177-183

[18] Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. The New England Journal of Medicine. 2000;**343**(13):938-952

[19] Wang F-I, Stohlman SA, Fleming J. Demyelination induced by murine hepatitis virus JHM strian (MHV-4) is immunologically mediated. Journal of Neuroimmunology. 1990;**30**:31-41

[20] Das SJ. A mechanism of virus-induced demyelination. Interdisciplinary Perspectives on Infectious Diseases. 2010;**2010**:109239

[21] Shindler KS, Kenyon LC, Dutt M, Hingley ST, Das Sarma J. Experimental optic neuritis induced by a demyelinating strain of mouse hepatitis virus. Journal of Virology. 2008;**82**(17):8882-8886

[22] Wu GF, Dandekar AA, Pewe L, Perlman S. CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. Journal of Immunology (Baltimore, Md : 1950). 2000;**165**(4):2278-2286

[23] Matthews AE, Lavi E, Weiss SR, Paterson Y. Neither B cells nor T cells are required for CNS demyelination in mice persistently infected with MHV-A59. Journal of Neurovirology. 2002;**8**(3):257-264

[24] Li Y, Fu L, Gonzales DM, Lavi E. Coronavirus neurovirulence correlates with the ability of the virus to induce proinflammatory cytokine signals from astrocytes and microglia. Journal of Virology. 2004;**78**(7):3398-3406

[25] Buchmeier MJ, Lane TE. Viral-induced neurodegenerative disease. Current Opinion in Microbiology. 1999;**2**(4):398-402

[26] Saadi F, Chakravarty D, Kumar S, Kamble M, Saha B, Shindler KS, et al. CD40L protects against mouse hepatitis virus-induced neuroinflammatory demyelination. PLOS Pathogens. 2021;**17**(12):e1010059

[27] Chakravarty D, Saadi F, Kundu S, et al. CD4 deficiency causes poliomyelitis and axonal Blebbing in murine coronavirus-induced neuroinflammation. Journal of Virology. 2020;**94**(14):e00548-20

[28] Das Sarma J, Burrows A, Rayman P, Hwang M-H, Kundu S, Sharma N, et al. Ifit2 deficiency restricts microglial activation and leukocyte migration following murine coronavirus (m-CoV) CNS infection. PLOS Pathogens. 2020;**16**(11):e1009034

[29] Kenyon LC, Biswas K, Shindler KS, Nabar M, Stout M, Hingley ST, et al. Gliopathy of demyelinating and non-demyelinating strains of mouse hepatitis virus. Frontiers in Cellular Neuroscience. 2015;**9**:488

[30] Biswas K, Das SJ. Effect of microtubule disruption on neuronal spread and replication of demyelinating and nondemyelinating strains of mouse hepatitis virus in vitro. Journal of Virology. 2014;**88**(5):3043-3047

[31] Hart BAT, Brok HPM, Remarque E, Benson J, Treacy G, Amor S, et al. Suppression of ongoing disease in a nonhuman primate model of multiple sclerosis by a human-anti-human IL-12p40 Antibody1. The Journal of Immunology. 2005;**175**(7):4761-4768

[32] Sariol A, Mackin S, Allred MG, Ma C, Zhou Y, Zhang Q, et al. Microglia

depletion exacerbates demyelination and impairs remyelination in a neurotropic coronavirus infection. Proceedings of the National Academy of Sciences of the United States of America. 2020;**117**(39):24464-24474

[33] Kuerten S, Gruppe TL, Laurentius LM, Kirch C, Tary-Lehmann M, Lehmann PV, et al. Differential patterns of spinal cord pathology induced by MP4, MOG peptide 35-55, and PLP peptide 178-191 in C57BL/6 mice. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 2011;**119**(6):336-346

[34] Kishore A, Kanaujia A, Nag S, Rostami AM, Kenyon LC, Shindler KS, et al. Different mechanisms of inflammation induced in virus and autoimmune-mediated models of multiple sclerosis in C57BL6 mice. BioMed Research International. 2013;**2013**:589048

[35] Giralt M, Molinero A, Hidalgo J. Active induction of experimental autoimmune encephalomyelitis (EAE) with MOG (35-55) in the mouse. Methods in Molecular Biology (Clifton, NJ). 2018;**1791**:227-232

[36] Sarma JD, Ciric B, Marek R, Sadhukhan S, Caruso ML, Shafagh J, et al. Functional interleukin-17 receptor A is expressed in central nervous system glia and upregulated in experimental autoimmune encephalomyelitis. Journal of Neuroinflammation. 2009;**6**(1):14

[37] Keller CW, Sina C, Kotur MB, Ramelli G, Mundt S, Quast I, et al. ATG-dependent phagocytosis in dendritic cells drives myelin-specific CD4 (+) T cell pathogenicity during CNS inflammation. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(52):E11228-E11e37

[38] Takeshita Y, Ransohoff RM. Inflammatory cell trafficking across the blood-brain barrier: Chemokine regulation and in vitro models. Immunological Reviews. 2012;**248**(1):228-239

[39] Lovett-Racke AE, Yang Y, Racke MK. Th1 versus Th17: Are T cell cytokines relevant in multiple sclerosis? Biochimica et Biophysica Acta. 2011;**1812**(2):246-251

[40] Gutcher I, Becher B. APC-derived cytokines and T cell polarization in autoimmune inflammation. The Journal of Clinical Investigation. 2007;**117**(5):1119-1127

[41] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. Annual Review of Immunology. 2009;**27**:485-517

[42] Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nature Medicine. 2002;**8**(5):500-508

[43] Viszlayová D, Sojka M, Dobrodenková S, Szabó S, Bilec O, Turzová M, et al. SARS-CoV-2 RNA in the cerebrospinal fluid of a patient with long COVID. Therapeutic Advances in Infectious Disease. 2021;**8**:20499361211048572

[44] Zanin L, Saraceno G, Panciani PP, Renisi G, Signorini L, Migliorati K, et al. SARS-CoV-2 can induce brain and spine demyelinating lesions. Acta Neurochirurgica. 2020;**162**(7):1491-1494

[45] Yu D, Zhu H, Liu Y, Cao J, Zhang X. Regulation of proinflammatory cytokine expression in primary mouse astrocytes by coronavirus infection. Journal of Virology. 2009;**83**(23):12204-12214

[46] Das Sarma J, Kenyon LC, Hingley ST, Shindler KS. Mechanisms of primary axonal damage in a viral model of multiple sclerosis. The Journal of

Neuroscience: The Official Journal of the Society for Neuroscience. 2009;**29**(33):10272-10280

[47] Shabani Z. Demyelination as a result of an immune response in patients with COVID-19. Acta Neurologica Belgica. 2021;**121**(4):859-866

[48] Bender SJ, Weiss SR. Pathogenesis of murine coronavirus in the central nervous system. Journal of Neuroimmune Pharmacology : the Official Journal of the Society on NeuroImmune Pharmacology. 2010;**5**(3):336-354

[49] Rempel JD, Murray SJ, Meisner J, Buchmeier MJ. Differential regulation of innate and adaptive immune responses in viral encephalitis. Virology. 2004;**318**(1):381-392

[50] Jiang Y, Yu Y. Transgenic and gene knockout mice in gastric cancer research. Oncotarget. 2017;**8**(2):3696-3710

[51] Heber S, Herms J, Gajic V, Hainfellner J, Aguzzi A, Rülicke T, et al. Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience. 2000;**20**(21):7951-7963

[52] Lee Y, Dawson VL, Dawson TM. Animal models of Parkinson's disease: Vertebrate genetics. Cold Spring Harbor Perspectives in Medicine. 2012;**2**(10):a009324

[53] Bunner AE, Chandrasekera PC, Barnard ND. Knockout mouse models of insulin signaling: Relevance past and future. World Journal of Diabetes. 2014;**5**(2):146-159

[54] Diaz-Cruz ES, Cabrera MC, Nakles R, Rutstein BH, Furth PA. BRCA1 deficient mouse models to study pathogenesis and therapy of triple negative breast cancer. Breast Disease. 2010;**32**(1-2):85-97

[55] Shapiro AM, Miller-Pinsler L, Wells PG. Breast cancer 1 (BRCA1)-deficient embryos develop normally but are more susceptible to ethanol-initiated DNA damage and embryopathies. Redox Biology. 2016;**7**:30-38

[56] Hall B, Limaye A, Kulkarni AB. Overview: Generation of gene knockout mice. Current Protocols in Cell Biology. Sep 2009;Chapter 19:Unit 19.12 19.12.1-17

[57] Ney A, Canciani G, Hsuan JJ, Pereira SP. Modelling pancreatic neuroendocrine cancer: From bench side to clinic. Cancers (Basel). 28 Oct 2020;**12**(11):3170

[58] Kim H, Kim M, Im SK, Fang S. Mouse Cre-LoxP system: General principles to determine tissue-specific roles of target genes. Laboratory Animal Research. 2018;**34**(4):147-159

[59] Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunological Reviews. 2009;**229**(1):152-172

[60] Datta A, Mondal D. Chapter 5 - development of mouse models for cancer research. In: Verma AS, Singh A, editors. Animal Biotechnology. San Diego: Academic Press; 2014. pp. 73-94

[61] Walrath JC, Hawes JJ, Van Dyke T, Reilly KM. Genetically engineered mouse models in cancer research. Advances in Cancer Research. 2010;**106**:113-164

[62] Ding Y, Cravero JD, Adrian K, Grippo P. Modeling pancreatic cancer in vivo: From xenograft and

carcinogen-induced systems to genetically engineered mice. Pancreas. 2010;**39**(3):283-292

[63] Pelekanou V, Leclercq G. Recent insights into the effect of natural and environmental estrogens on mammary development and carcinogenesis. The International Journal of Developmental Biology. 2011;**55**(7-8-9):869-878

[64] Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P. Coexpression of MMTV/v-ha-ras and MMTV/c-myc genes in transgenic mice: Synergistic action of oncogenes in vivo. Cell. 1987;**49**(4):465-475

[65] Dotan ZA, Niki M, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature. 2005;**436**(7051):725-730

[66] Heyer J, Kwong LN, Lowe SW, Chin L. Non-germline genetically engineered mouse models for translational cancer research. Nature Reviews Cancer. 2010;**10**(7):470-480

[67] Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature. 1992;**356**(6366):215-221

[68] Kersten K, de Visser KE, van Miltenburg MH, Jonkers J. Genetically engineered mouse models in oncology research and cancer medicine. EMBO Molecular Medicine. 2017;**9**(2):137-153

[69] Shibata H, Toyama K, Shioya H, Ito M, Hirota M, Hasegawa S, et al. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. Science (New York, NY). 1997;**278**(5335):120-123

[70] Weber J, Öllinger R, Friedrich M, Ehmer U, Barenboim M, Steiger K, et al. CRISPR/Cas9 somatic multiplex-mutagenesis for high-throughput functional cancer genomics in mice. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**(45):13982-13987

[71] Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell. 2014;**159**(2):440-455

[72] Annunziato S, Kas SM, Nethe M, Yücel H, Del Bravo J, Pritchard C, et al. Modeling invasive lobular breast carcinoma by CRISPR/Cas9-mediated somatic genome editing of the mammary gland. Genes & Development. 2016;**30**(12):1470-1480

[73] Wong DY, Feinberg SE. Incidence of growth of syngeneic oral squamous cell carcinoma in C57B1 bg/bg beige mice. Zhonghua yi xue za zhi =Chinese medical journal; Free China ed. 1990;**45**(1):53-59

[74] Zhu H, Kauffman ME, Trush MA, Jia Z, Li YR. A simple bioluminescence imaging method for studying cancer cell growth and metastasis after subcutaneous injection of Lewis lung carcinoma cells in syngeneic C57BL/6 mice. Reactive Oxygen Species (Apex, NC). 2018;**5**(14):118-125

[75] Jungwirth U, van Weverwijk A, Melake MJ, Chambers AF, Gao Q, Fivaz M, et al. Generation and characterisation of two D2A1 mammary cancer sublines to model spontaneous and experimental metastasis in a syngeneic BALB/c host. Disease Models & Mechanisms. 2018;**11**(1)

[76] Ngiow SF, Loi S, Thomas D, Smyth MJ. Mouse models of tumor immunotherapy. 2016;**130**:1-24

[77] du Chatinier A, Meel MH, Das AI, Metselaar DS, Waranecki P, Bugiani M, et al. Generation of immunocompetent syngeneic allograft mouse models for pediatric diffuse midline glioma. Neuro-Oncology Advances. 2022;**4**(1):vdac079

[78] Roby KF, Taylor CC, Sweetwood JP, Cheng Y, Pace JL, Tawfik O, et al. Development of a syngeneic mouse model for events related to ovarian cancer. Carcinogenesis. 2000;**21**(4):585-591

[79] Mulchandani V, Banerjee A, Vadlamannati AV, Kumar S, Das SJ. Connexin 43 trafficking and regulation of gap junctional intercellular communication alters ovarian cancer cell migration and tumorigenesis. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2023;**159**:114296

[80] Olson B, Li Y, Lin Y, Liu ET, Patnaik A. Mouse models for cancer immunotherapy research. Cancer Discovery. 2018;**8**(11):1358-1365

[81] Gulley JL, Drake CG. Immunotherapy for prostate cancer: Recent advances, lessons learned, and areas for further research. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2011;**17**(12):3884-3891

[82] Lei ZG, Ren XH, Wang SS, Liang XH, Tang YL. Immunocompromised and immunocompetent mouse models for head and neck squamous cell carcinoma. OncoTargets and Therapy. 2016;**9**:545-555

[83] Flanagan SP. 'Nude', a new hairless gene with pleiotropic effects in the mouse. Genetical Research. 1966;**8**(3):295-309

[84] Tani N, Kuchiba K, Osada T, Watanabe Y, Umemoto T. Effect of T-cell deficiency on the formation of periapical lesions in mice: Histological comparison between periapical lesion formation in BALB/c and BALB/c nu/nu mice. Journal of Endodontics. 1995;**21**(4):195-199

[85] Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983;**301**(5900):527-530

[86] Zhang B, Duan Z, Zhao Y. Mouse models with human immunity and their application in biomedical research. Journal of Cellular and Molecular Medicine. 2009;**13**(6):1043-1058

[87] Bosma GC, Fried M, Custer RP, Carroll A, Gibson DM, Bosma MJ. Evidence of functional lymphocytes in some (leaky) scid mice. The Journal of Experimental Medicine. 1988;**167**(3):1016-1033

[88] Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese, diabetic strain of mice. Jikken dobutsu Experimental animals. 1980;**29**(1):1-13

[89] Hesselton RM, Greiner DL, Mordes JP, Rajan TV, Sullivan JL, Shultz LD. High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to human immunodeficiency virus type 1 infection in NOD/LtSz-scid/scid mice. The Journal of Infectious Diseases. 1995;**172**(4):974-982

[90] Takahashi T, Katano I, Ito R, Goto M, Abe H, Mizuno S, et al. Enhanced antibody responses in a novel NOG transgenic mouse with restored lymph node organogenesis. Frontiers in Immunology. 2017;**8**:2017

[91] Brooks DG, Kitchen SG, Kitchen CM, Scripture-Adams DD,

Zack JA. Generation of HIV latency during thymopoiesis. Nature Medicine. 2001;7(4):459-464

[92] This Russian Mouse Honors The Humble Lab Mouse [Internet]. 2017. Available from: https://www.smithsonianmag.com/smart-news/russian-statue-honoring-laboratory-mice-gains-renewed-popularity-180964570/ [Accessed: 2023-04-23]

Section 2

Rodents in Drug Discovery and Human Zoonotic Pathogen Transmission

Chapter 4

Rodents Human Zoonotic Pathogens Transmission: Historical Background and Future Prospects

Naveed Akhtar, Sara Hayee, Muhammad Idnan, Faheem Nawaz and Sadaf BiBi

Abstract

Rodents (Order Rodentia) are one of the most speciose and diversified groups of terrestrial mammals with several beneficial roles in nature. They constitute 2277 known species which make up 42% of total mammal fauna. They are reported to inhabit in all continents except Antarctica and several small islands. They perform several beneficial roles in the environment. Despite of several beneficial roles, rodents are also a source of zoonotic pathogens. Rodents are important reservoirs of evolving zoonotic diseases because they come into close contact with livestock in the agricultural background and humans in urban zones. Almost 10% of the rodent population is either a carrier or reservoir of zoonotic pathogens of public health significance. Rapid development of agricultural and industrial change throughout the globe, has led to a significant increase in zoonotic borne disease of rodents. Rodents transfer pathogenic agents to humans through direct contact, and animals or via contamination of human food, water with rodents' stool, or through urine. Arthropod vectors on the skin of several rodents are also able to carry zoonotic pathogens. Several factors regulate human rodent pathogen transmission like rodent population handling, human socio-economic lifestyle, and even war. Human activities such as animal trade, migration, urbanization, and large-scale traveling are facilitating factors in rodent-pathogens transfer.

Keywords: zoonotic transmission, public health, medical importance, rodent borne pathogens, risk factors

1. Introduction

The term "rodent" is derived from the Latin word "rodere" which means "to gnaw". Rodents (Mammalia; Rodentia) are one of the largest and diversified groups of mammals characterized by peculiar dentation consisting of single pair of continuously growing incisors in both jaws and a set of chewing teeth [1]. With 2277 discovered species from 33 different families, they comprise more than 42% of global mammalian biodiversity [2–4]. Most living rodents are thermally sensitive and have a compact bodies. They inhibit in all regions of the earth from the tropics to Polar Regions and from sea level to high mountain climate except Antarctica and some isolated islands [3, 5].

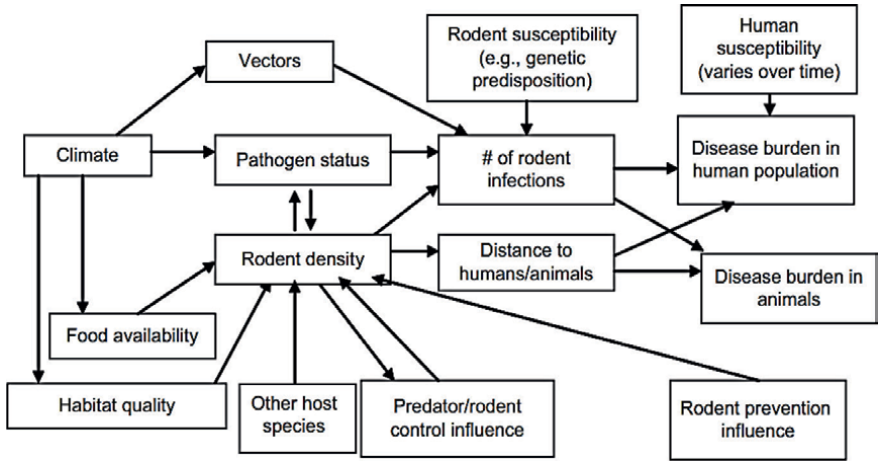

Figure 1.
A simplified model of rodent diseases model [13].

They range in size from the tiny pygmy mouse (*Mus minutoides*) which weighs almost seven grams to 50 kg of American capybara (*Hydrochoerus hydrochaeris*) [6]. Rodents are important herbivores and various seed predators. The high compatibility makes rodents one of the best-suited mammals for living in various territories [2].

Rodents perform several beneficial roles in the ecosystem including soil digging and mixing, facilitation of biotic recovery, insect control, dispersal of seed and spores, vegetative succession, pollination, and regulation of the nutrient cycle [7, 8]. They are important in maintaining the health of grassland and forest [10]. Humans consume more than 71 1genera and 89 species of rodents in their diet especially in the tropical world [10]. For example, Cambodia exports almost two tons of wild rats to Vietnam per day during the peak season of rats [11].

Despite of several beneficial roles in the ecosystem, rodents are reported to play many disservices through their role as pests of cropland. They cause massive damage to standing crops and stored grains. About 1% of the cereal crops of the world are destroyed by rodents annually [12]. Additionally, rodents are important reservoirs of zoonotic diseases. They are a carrier of contagious diseases and hosts of many infectious parasites. They also cause significant economic losses by feeding on stored grains, spoiling food supplies, and transferring infectious pathogens to human beings. Rodents host a great diversity of zoonotic pathogens than any other mammalian order, and together with bats and other primates, they harbor the majority of zoonotic viruses. Rodents are vectors, reservoirs and carry a diversity of pathogens including helminthes, bacteria, viruses, and zooanthroponoses [8]. They are considered the most important hosts of infectious diseases and are responsible for more than 80 diseases including plague, leptospirosis, and hemorrhagic fever (**Figure 1**) [14].

2. Historical background of rodents borne zoonosis

A zoonotic disease is a disease or infection that can be transferred from vertebrate animals to human beings or from human beings to vertebrate animals. Most of the diseases affecting human beings are of animal origin [15]. It is estimated that 60% of human

emerging infections are zoonotic and more than 70% of these pathogens are originated from wildlife species [16]. In recent decades, the newly emerged human diseases were animal in origin and were directly associated with animal-based food [17, 18]. Among the human pathogens, about 61% are zoonotic in nature [19]. Zoonoses can be categorized into different groups viz.; bacterial zoonoses, viral zoonoses, parasitic zoonoses, fungal zoonoses, chlamydial zoonoses, and protozoan zoonoses [15].

Rodents are among the most important hosts of infectious diseases globally [20, 21]. Rodents are known to be the reservoir of at least 60 zoonotic diseases. They are known to play an important role in the transmission and spread of diseases [13, 22–24]. They can act as definite hosts as well as intermediate hosts of zoonotic and vector-borne diseases. Rodents-borne zoonotic diseases can be divided into two main categories i.e., directly or indirectly transmitted diseases. The first category includes those zoonotic diseases which are directly transmitted by biting or inhaling germs present in the fecal matter of the rodents, whereas in the second category, humans are infected by rodent-contaminated water or urine and through consuming rodents as food [7]. Rodents could also amplify and transfer diseases transmitted by arthropod vectors. Also, rodents accidentally eaten by the livestock, could mediate disease transmission to humans if livestock products were not properly processed prior to consumption (**Figures 2** and **3**) [24].

The history of rodent-borne zoonotic diseases is linked with the plague, a bacterial infection caused by *Yersinia pestis which is transmitted to humans through the bite of a flea. The plague is carried by small rodents such as rats and mice which have lived among human and their food supplies for centuries.* Although the presence of the plague has been noted throughout human history, there have been three major epidemics that have been particularly devastating to the human population [25].

Major rodent-borne zoonotic diseases include plague, leptospirosis, hemorrhage fever with renal syndrome, and HPS. Rodents that are captured from wild populations or domesticated outdoors may carry zoonotic pathogens. They are reported to be reservoir hosts for at least 80 zoonotic diseases that represent a serious threat to

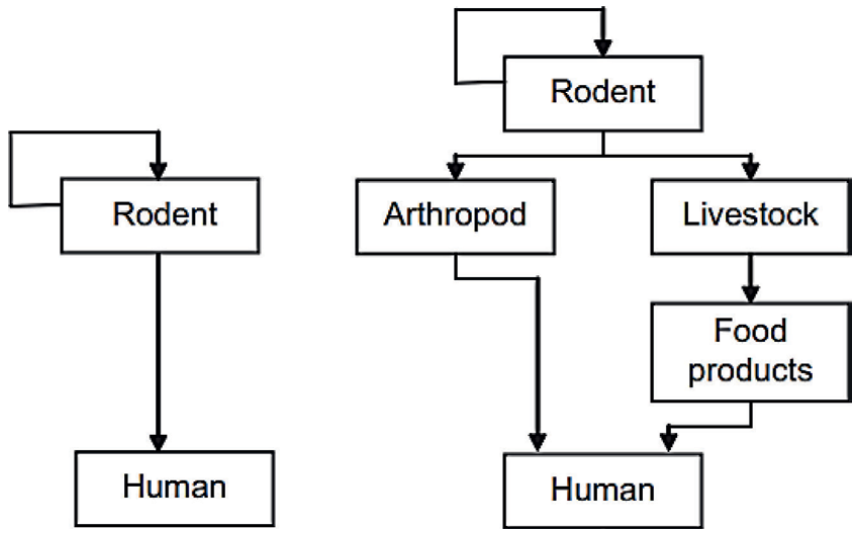

Figure 2.
Two different pathogens transmission pathways through rodents [13].

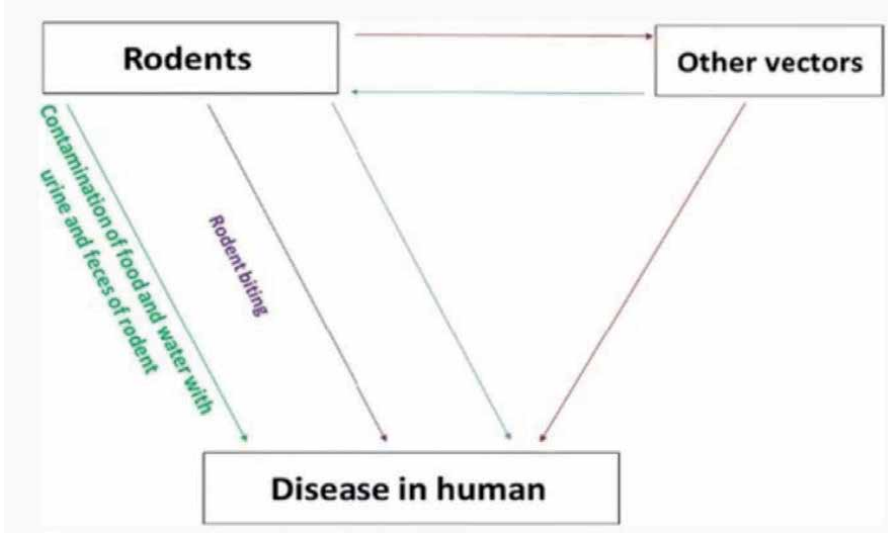

Figure 3.
The ways of transmitting disease from rodents to humans.

public health [13, 26]. They play an important role in the transmission of diseases in different ways [27]. In terms of public health, rodents zoonotic transmission includes salmonellosis, plague, leptospirosis, leishmaniasis, toxoplasmosis, rat-bit fever, taeniasis-like *Capillaria hepatica*, zoonotic babesiosis, Lassa fever, hemorrhagic fever with renal syndrome (HFRS), and the hantavirus cardiopulmonary syndrome (HCPS), both caused by *Hantavirus*. In addition, other Arenaviruses are responsible for South American Hemorrhagic Fevers (SAHF) [22]. Rodents may also harbor different complex bacteria like *Mycobacterium tuberculosis*, *Mycobacterium microti*, and *Escherichia coli* [28]. They are also good reservoirs of different disease-causing

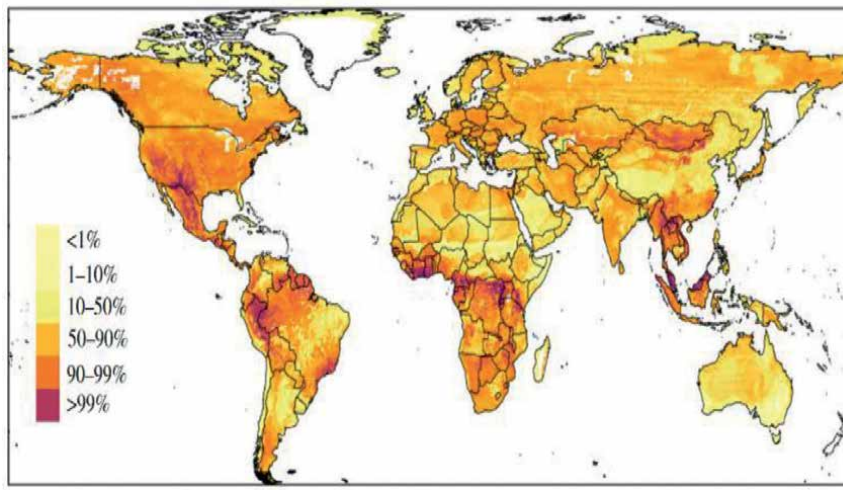

Figure 4.
Prediction of the hotspot of rodent-borne zoonotic diseases based on sustainable development land use change scenario [29].

pathogens including agents of tularemia, tick-born relapsing fever, Lyme disease, ehrlichiosis, bartonellosis, listeriosis, and Q fever (**Figure 4**) [7].

3. Current status of rodent borne zoonotic diseases

Most outbreaks of novel pathogens have been observed when infectious agents are spread from animals to humans [30–32]. It has been reported that over one billion people get affected by zoonotic diseases. Considering public health priority, zoonotic pathogens are wild reservoirs [33]. Many zoonotic diseases caused by viruses, bacteria, helminths, fungi, and protozoa are transmitted by 217 species of rodents which harbor about 66 zoonoses [31]. It is expected that zoonotic disease will prevail in the regions where humans are having more contact with wildlife [34]. Land use change is a current issue when the relationship is considered between rodents and humans. This results in a significant loss of biodiversity and degradation of the natural ecosystem; a problem worldwide now [35]. It has resulted in the emergence of zoonotic diseases [31]. Many studies have revealed that land use change caused increased interaction between wildlife, domestic and synanthropic animals, and humans. Since such types of interactions are favorable to cross-species pathogen transmission so disease emergence is automatically promoted [36]. In recent times, when humanity has faced the current COVID-19 pandemic, it is worth mentioning that zoonotic infections can bring disastrous effects on public health and the world economy [37, 38]. Now it is possible to find out the wild species of rodents which cause these diseases with remarkable accuracy using various tools and techniques. The current hotspots of rodent diversity are more common in Europe, Coast of South America, North America, Russia, and some parts of East and Central Asia [39]. Rodent-based zoonotic infections can be transmitted by two methods; direct method or indirect method.

3.1 Diseases directly spread by rodents

Rodents are capable of direct disease transmission in various ways. An infected rodent can transmit disease by biting. A person can become infected when breathing in contaminated air or eating contaminated food. Direct contact by touching an infected rodent's eyes, nose, or mouth brings the same results. Even touching of excreta and urine of infected rodents may cause transmission of a causative agent.

3.2 Disease indirectly spread by rodents

Rodents are important vertebrate hosts for many other organisms. They may be bitten by fleas, ticks, mosquitoes, and mites. Since these organisms feed on host blood, the rodent becomes infected easily. Diseases can be transmitted to man from infected rodents due to food consumption of any intermediate host like cockroaches and beetles [40].

4. Role of Peridomestic rodents as reservoirs for foodborne zoonotic pathogens

Peridomestic rodents are the rodent types living in and around human habitations. The role of this category of rodents is very important in zoonotic disease spread due to its close association with humans. Sadly, it is also linked to poverty, because

unprivileged house infrastructure and poor living conditions bring exposure to rodents [41]. When foodborne pathogenic human diseases are taken into consideration, the uniting factor comes to be associated with peridomestic rodents [42]. Peridomestic and commensal rodents are of special importance regarding global health initiatives. It is due to the fact native and regional invasive species of rodents like mice and rats get benefits from human activities especially due to agricultural practices. These rodents transmit diseases to poultry, livestock, and raw products by infecting the farm environment [43]. Since it has been reported that an infected rodent releases a huge number of viruses in its urine, the contamination of stored is also a major reason for disease transmission in humans through rodents. The pathogens are amplified in any environment because of the daily basis deposit of urine and fecal pellets. For example, a single rodent when gets a chance to live or enter in a farmland, can introduce up to 23 million of Salmonella bacteria within one day [44].

5. Factors determine rodent born zoonotic Spillover

Rodents are globally famous and abundant for extreme population fluctuations that manifest eruptive zoonotic outbreaks [45]. These fluctuations are heterogonous and include climatic conditions, density dependence, food availability, predation rates, and land use change [46]. Complexities of zoonotic transmission, including seasonality of rodent abundance, changes in land use creating artificial habitats, and diversity of transmission modes, cause many zoonotic disease systems to be considered as unique, with a need for control efforts tailored to the ecological nuances of each system. The ecological phenomenon which involves pathogenic transmission between different species with the crossing of the species barrier is known as zoonotic spillover, host jump, and zoonotic transfer [47, 48]. A spillover can also be defined as parasite cross-transmission in a host population not infected earlier [49]. Human history is full of epidemics and disease outbreaks. Some of the diseases reach different continents. About 60% of all human infectious diseases have zoonotic origin [50]. Animal handling, poaching, meat consumptions, and use of animal-derived products are some factors that are linked with spillover events [51]. In addition to food consumption, many animals are sold in markets as pets, for medicinal gains and cultural practices, etc. Pathogenic transmission occurs not only due to meat, blood, and biofluids but also due to contaminated surfaces and aerosols [52, 53]. Spillover is a multifactorial complex process. The increased risk of spillover is associated with prevalence and infection intensity in host reservoirs. The distribution and density of an infected are also determining factors of a spillover [48]. It is important to understand all the factors which bring about zoonotic spillover considering the host species, environment, and pathogen (**Figure 5**).

5.1 Land use change

Land use change is the conversion of natural vegetation to anthropogenic habitats. As discussed earlier, land use change promotes disease transmission. The real mechanism behind this is not known. Land use change results in the extinction of local species. It causes modification of host abundance and community structure. This brings a change in pathogen transmission dynamics [54]. The host and pathogen association is changed due to host communities. Ultimately, pathogenic prevalence is enhanced in a host which adapted to human-dominated landscapes. Changes in land use design

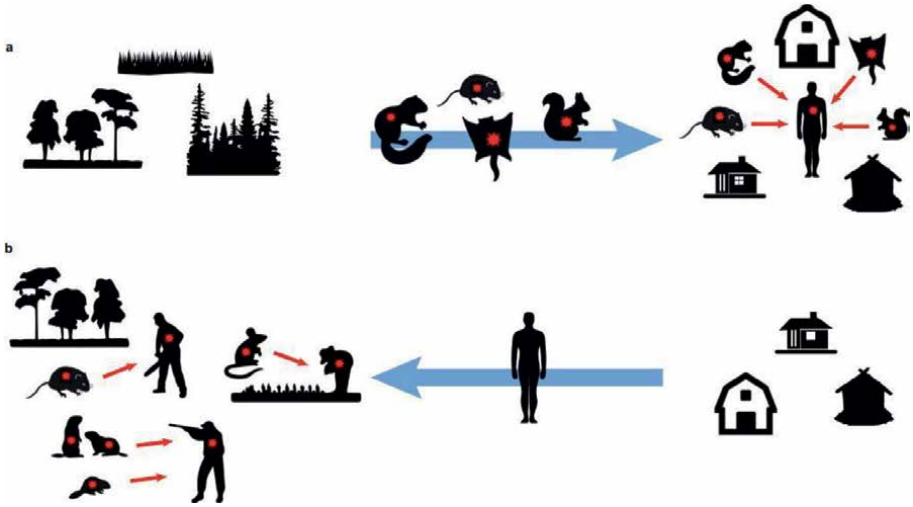

Figure 5.
Pathways increasing transmission from rodent-borne pathogens (a). Contact between rodents and humans increases transmission risk; either with rodents moving into human dwellings and environments or with (b). Humans moving into rodent habitats or using rodents as a natural resource [46].

modify ecological interactions between local communities. So, there is an increased possibility of zoonotic diseases emergence such as Nipah virus, Lyme disease, encephalitis, Hantavirus, and plague [55]. The whole world has recently evidenced this through the COVID-19 pandemic. Zoonotic diseases can have catastrophic effects on public health and the world economy [37]. The shared socioeconomic pathway narratives have captured future land use setups. It predicts about land change use of the future which is based on the high demand for energy and food by a growing population [56].

5.2 Environmental variations

Humans, animals, and the environment play a significant role in the emergence and transmission of different infectious diseases [57]. Environmental variations are also associated with species distribution, abundance, and richness. Due to the anthropogenic emission of greenhouse gases, there is an increase in temperature globally. This has an impact on the distribution of vectors. It also increases the risk of vector-borne zoonotic diseases [58]. The spread of Hantavirus in China hosted by rodents is also considered due to anthropogenic human activities [59]. Rodents make up about 42% of mammals. They damage our forests, farmlands, and agriculture and transmit diseases yet they are important in ecosystem maintenance [60]. Rodents are preyed upon by many other species. Because of their short life span and high reproductive potential, they are very responsive to global changes. Extrinsic and intrinsic factors influence the population abundance of small rodent species [61]. Many environmental factors like rainfall, photoperiod, and temperature affect the reproduction of rodents [62].

5.3 Types of pathogens transmitted by rodent

Various types of rodents transmitted by rodents include mainly bacteria, viruses, some protozoan parasites, and nematodes. We will discuss each category briefly.

5.3.1 Bacterial diseases

A number of rodents are host to bacteria. Many bacterial infections are being transmitted to humans by rodents [63]. Some of the important pathogenic bacteria are discussed here:

An important pathogenic bacterium is *Yersinia pestis* whose natural host is the rat. This bacterium may infect about 200 different species of rodents. It is a gram-negative facultative anaerobic bacterium that causes plague in humans. It causes three types of infections which include pneumonic, bubonic, and septicemic. It is transmitted to humans by biting infected fleas [64]. This bacterium is involved in rodent flea life cycle and causes plague. Moreover, some ectoparasites like ticks are also infected with this bacterium. Thus, infected rodents and vectors like ticks and fleas are everlasting sources of *Y. pestis*. This type of life cycle is called as enzootic cycle [65].

Leptospira is a pathogenic spirochete. This genus has about 22 known species [66]. It causes leptospirosis a worldwide major zoonotic disease [41, 67]. This bacterium may be transmitted by direct or indirect methods, for example, through dirty surroundings and infected hosts [68]. Among rodents, their main host is rats including two important species; *Rattus rattus* and *Rattus norvegicus*. This bacterium is particularly present in the proximal part of rat renal tubules [69]. Leptospirosis symptoms involve headache, chills, fever, conjunctivital suffusion, etc. [70].

Salmonella genus belongs to Enterobacteriaceae, a rod-shaped bacterium that is gram-negative. The two main species of Salmonella include *Salmonella typhi* and *Salmonella paratyphi*. When this pathogen lives in the gastrointestinal tract of its host, it remains asymptomatic for most time [71]. This pathogenic bacterium is a curse for farm animals. Infected rodents are a big threat to whole livestock. From here the infection leads to many food chains. So, it is very important to remove its infestation at an early stage [13]. Not only bacterial, but rodents are also capable of transmitting viral diseases.

5.3.2 Viral diseases

A number of viral diseases are spread by rodents. Some of these diseases are discussed here:

Hantaviruses are viruses that are transmitted by a number of rodents. It can cause hemorrhagic fever leading to hantavirus cardiopulmonary syndrome (HCPS) and renal syndrome (HFRS). Its first outbreak happened in 1993 in South West USA and resulted in a high number of deaths [72]. Hantavirus belongs to the family Hantaviridae and has four genera which include Mobatvirus, Thottimvirus, Loanvirus, and Orthohantavirus. Hantavirus can be transmitted directly to humans by rodent biting. But it affects lesser individuals. It is spread more commonly by food contamination with the infected host's saliva or urine [73].

Another important virus spread by these creatures is Rotavirus belonging to Reoviridae. This virus causes gastroenteritis especially affecting young children. It causes severe dehydrating diarrhea. It has been estimated that about 2 million people are hospitalized and outpatients which visit globally are about 25 million (WHO). Rotavirus causes diarrhea which is a leading cause of high mortality rate in children. Mice are the carrier agents of this virus. This virus is transmitted through the fecal-oral route and airborne infection [74].

Recently coronavirus shook the whole world. It is enveloped RNA-containing virus which mainly affects the respiratory system. From 2019 to 2022, it affected millions of people. Nearly three years passed and the whole world is still struggling to understand

the extent of the pandemic. Coronavirus also known as SARS-CoV-2 causes a sequence of pneumonia [75]. It has been reported that bats and rats are the primary resources of coronaviruses. The pathogenic behavior of this virus has been detected in cattle, mice, and rats [76]. Coronaviruses from rats are very contagious and spread through aerosols, fomites, and direct contact [77].

The Hepatitis E virus also known as HEV causes liver cirrhosis. It is transmitted through the GIT tract or from the fecal-oral route. This virus has become a serious problem where global public health is concerned [78]. This RNA-containing virus belongs to the family Hepeviridae. Its origin is not known. Now it has been detected that HEV is found in animals like rodents, deer, and rabbits. It is transferred in man by eating, meetings or any direct or indirect means [79]. In Germany, a research work

Pathogen	Type of organism	Rodent reservoir	Disease	References
Campylobacter spp.	Bacteria	Water vole, Wood Mouse, Rat	Stomach flue	[2, 80]
Clostridium spp.		Common vole, Black rat, Eurasian harvest mouse	Clostridial infection	[70, 81]
Escherichia coli		Chipmunk, vole, rat	Fever, diarrhea	[82]
Salmonella spp.		Rat, mouse	Salmonellosis	[13]
Borrelia burgdorferi		Mouse, vole, chipmunk, woodrat	Lyme Disease	[83]
Coxiella burnetii		Mouse, vole, chipmunk, rat, Squirrel	Q fever	[84]
Francisella tularensis		Beaver, Hamster, Squirrel, vole	Tularemia	[85]
Coronavirus	Virus	Rat, mouse	COVID-19,	[77]
Hantavirus		Rat	Pulmonary Syndrome	[86]
Lassa Virus		Rat	Lassa Fever	[86]
Tick-Borne Encephalitis Virus		Vole, mouse	Encephalitis	[87]
Rotavirus		Rat, mouse	Diarrhea	[88]
Hepatitis E Virus		Rat, mouse	Liver Cirrhosis	[89]
Arenavirus		Rat, mouse	Hemorrhagic Fever	[90]
Cowpox		Vole, rat, mouse	Blister Formation	[91]
Leishmania	Protozoan Parasite	Rat, mouse	Leishmaniasis	[92]
Babesia		Rat, mouse	Babesiosis	[93]
Cryptosporidium		Mouse and rat		[94]
Toxoplasma gondii		House mouse, rat	Toxoplasmosis	[95]
Giardia spp.		Rat	Giardiasis	[42]
Trichinella spp.	Nematode	Mouse, rat, Persian jird	Trichinosis	[96]

Table 1.
Pathogens transmitted by various rodents.

isolated this virus from *R. norvegicus*. Some studies show that some of the murine rodents are able to transfer this virus to man (**Table 1**) [97].

Lassa fever is a disease spread by the Lassa virus. This virus belongs to the family Arenaviridae. A species of mouse *Mastomys natalensis* is the only known non-human host of this virus [98]. It has been reported that humans are infected with the Lassa virus when they come in contact with the feces, urine, and blood of the infected Mastomys rats [99]. Arenavirus and cowpox are also zoonotic viruses. Humans, cows, cats, and many other zoo animals are the hosts of the cowpox virus [100]. Each species of these viruses is linked to related species of rodents [101].

6. Preventive measures

Rodents live in close proximity with humans. These organisms are kept as pets. They are a constant threat because of gnawing damage to food storage, crops, and health. They are important competitors for food with humans [13]. It is necessary to look for preventive measures to minimize the economic loss as well as the transmission of diseases to humans which is a more important and serious issue. With the use of proper rodent control procedures, it is possible to minimize the cases of rodent-borne diseases. People should be guided when handling rodents as their pests. People working in forests, croplands, and farms should also be trained well not to handle any food directly. Rodent infestation is the main reason for disease spread in and around the home of any human. Good housing infrastructure and construction can help to minimize rodent invasion. This point is an integral part of the prevention strategy. Discourage rodent infestation by removing uncovered food items, water, and possible shelter. Do not touch rodent droppings or excreta directly. Removal of possible nesting sites from the property is also helpful. All the holes along the exterior of the home should be closed with the use of recommended rodent-proofing material. Garbage, debris, and clutter should be removed on daily basis. Rodents like rats and mice avoid the smell. Mint plants can be planted in gardens and can be kept at home for this purpose.

7. Future prospects

In the wake of the COVID-19 pandemic, there is a lot of attention needed to understand the role of rodents in emerging zoonotic diseases so that one health approach may be adopted to combat future pandemics. The rapid development of industry, agriculture, urbanization and the intensity of the interactions between people, rodents, and the environment is enhancing the chances of zoonotic spillover and challenge for the healthcare system. This situation is a bit severe in developing countries where changing the distribution and abundance of available hosts or reservoirs is impacting pathogen persistence. These variations in the behavior movement pattern and immune status of the host are altering the susceptibility and transmission prospects of rodent-borne zoonotic infections. Changes in the utility of the environment also increase the chance of human rodents interaction and subsequently enhance disease prevalence. In developing countries with poor sanitation, high-density housing and increased flooding are increasing human-rodent interactions and subsequent disease transmission.

8. Conclusions and recommendations

Rodents are the most diversified group of mammals and their proximity help in the transmission of various zoonotic diseases. They act as reservoirs hosts for many important zoonotic pathogens posing a serious health threat to human beings causing massive morbidity and mortality globally. Rodents' population fluctuate in abundance over both seasonal and multiannual time scales, their population dynamics affect infection dynamics of rodent-borne diseases.

Exposure to zoonoses from ectoparasites, blood, and urine while handling rodents to be used for food is another possible risk that deserves attention. Considering their extensive distribution globally, it is crucial to pay more attention to their role in the dispersion of infectious diseases for healthier control of the diseases. To better understand disease ecology and parasite transmission, it must be considered that not all hosts are equally involved in parasite transmission as some species (and individuals) can be responsible for a disproportionate number of transmission events (Paull et al.). There is severe need for understanding the drivers of rodent-borne zoonotic infections transmission. Surveillance of different pathogens in rodents at the different animal-human interfaces is highly recommended. To avoid multidrug resistance (MDR) in the different bacterial-borne rodent infections, continuous monitoring of the evolution of known pathogens and their detection using different in-vitro and in-vivo models is needed. There is the utmost need for the development of better treatment plans including vaccines for rodent-borne zoonotic diseases.

Conflict of interest

The authors declare no conflict of interest.

Author details

Naveed Akhtar[1]*, Sara Hayee[2], Muhammad Idnan[3], Faheem Nawaz[4] and Sadaf BiBi[5]

1 Department of Zoology, Government Graduate College Pattoki (Kasur), Punjab, Pakistan

2 Department of Zoology, Government Graduate College Samanabad, Lahore, Pakistan

3 Department of Wildlife and Ecology, University of Okara, Pakistan

4 Institute of Zoology, University of the Punjab, Lahore, Pakistan

5 Department of Zoology, Concordia College Khudian Khas (Kasur), Pakistan

*Address all correspondence to: naveed.akhtar430@gmail.com

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References

[1] Kryštufek B, Vohralík V. Mammals of Turkey and Cyprus, Volume 2, Rodentia I: Sciuridae, Dipodidae, Gliridae, Arvicolinae. Koper, Slovenia: University of Primorska, Science and Research Centre Koper; 2005. DOI: 10.1080/09397140.2010.10638406

[2] Stefoff R. The Rodent Order. New York: Marshall Cavendish; 2008

[3] Wilson DE. Reeder DM. editors. Mammal Species of the World: A Taxonomic and Geographic Reference. 3rd edition. Baltimore, Maryland: Johns Hopkins University Press; 2005:2142. ISBN: 0-8018-8221-4

[4] Burgin CJ, Colella JP, Kahn PL, Upham NS. How many species of mammals are there? Journal of Mammalogy. 2018;**99**:1-14. DOI: 10.1093/jmammal/gyx147

[5] Hart JS. Rodents. In Mammals, 1971. Academic Press. (pp. 1-149). 10.1016/B978-0-12-747602-5.50007-1

[6] Kay EH, Hoekstra HE. Rodents. Current Biology. 2008;**18**(10):R406-R410

[7] Rabiee MH, Mahmoudi A, Siahsarvie R, Kryštufek B, Mostafavi E. Rodent-borne diseases and their public health importance in Iran. PLoS Neglected Tropical Diseases. 2018;**12**(4):e0006256. DOI: 10.1371/journal.pntd.0006256

[8] Khaghani R. The Economic and Health Impact of Rodent in Urban Zone and Harbors and Their Control Methods. Health Science Research. 2007;**4**:1071-1078

[9] Witmer G. Rodents in agriculture: A broad perspective. Agronomy. 2022;**12**(6):1458

[10] Lynwood AF. Rodents as a food source. In Proceedings of the Fourteenth Vertebrate Pest Conference, 1990. Vertebrate Pest Conference Proceedings collection. USDA/APHIS/S&T, Denver Wildlife Research Center. Insect damage: Post-harvest Operations. WWW.icipe.Org. 1990. University of Nebraska – Lincoln. Available from: https://core.ac.uk/download/pdf/188056897.pdf

[11] Doyle K. Cambodian rat meat: A growing export market. 2014. Available from: http://www.bbc.com/news/world-asia-28863315

[12] The economic ill effects of rodent infestation. Available from: https://www.victorpest.com/articles/economic-effects-of-rodent-infestation

[13] Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. Critical Reviews in Microbiology. 2009;**35**(3):221-270

[14] Faust C. Risky rodent landscapes: Where will zoonotic infections become more common? 2021. Bug bitten. Available from: https://blogs.biomedcentral.com/bugbitten/2021/10/15/risky-rodent-landscapes-where-will-zoonotic-infections-become-more-common/

[15] Rahman MT, Sobur MA, Islam MS, Ievy S, Hossain MJ, El Zowalaty ME, et al. Zoonotic diseases: Etiology, impact, and control. Microorganisms. 2020;**8**(9):1405. DOI: 10.3390/microorganisms8091405

[16] Salyer SJ, Silver R, Simone K, Behravesh CB. Prioritizing zoonoses for global health capacity building—themes from One Health zoonotic disease workshops in 7 countries,

2014-2016. Emerging Infectious Diseases. 2017;**23**(Suppl. 1):S55. DOI: 10.3201/eid2313.170418

[17] Slingenbergh J. World Livestock 2013: Changing Disease Landscapes. Food and Agriculture Organization of the United Nations (FAO); 2013

[18] Akhtar N, Nawaz F, Bukhari F. Increasing zoonotic infectious diseases and COVID-19: Time to rethink wild food. Microbes and Infectious Diseases. 2020;**1**(2):43-48. DOI: 10.21608/MID.2020.34031.1028

[19] Nakoune E, Olliaro P. Waking up to monkey pox. BMJ. 2022;**2022**:377. DOI: 10.1098/rstb.2001.0888

[20] Han BA, Kramer AM, Drake JM. Global patterns of zoonotic disease in mammals. Trends in Parasitology. 2016;**32**(7):565-577

[21] Mollentze N, Streicker DG. Viral zoonotic risk is homogenous among taxonomic orders of mammalian and avian reservoir hosts. Proceedings of the National Academy of Sciences. 2020;**117**(17):9423-9430. DOI: 10.1073/pnas.1919176117

[22] Dahmana H, Granjon L, Diagne C, Davoust B, Fenollar F, Mediannikov O. Rodents as hosts of pathogens and related zoonotic disease risk. Pathogens. 2020;**9**(3):202. DOI: 10.3390/pathogens9030202

[23] Taylor PJ, Arntzen L, Hayter M, Iles M, Frean J, Belmain S. Understanding and managing sanitary risks due to rodent zoonoses in an African city: Beyond the Boston Model. Integrative Zoology. 2008;**3**(1):38-50

[24] Buckle A, Smith R. Rodent Pests and Their Control. Wallingford, UK: CABI; 2015

[25] Zoonosis History. Available from: https://www.news-medical.net/health/Zoonosis-History.aspx

[26] Luis AD, Hayman DT, O'Shea TJ, Cryan PM, Gilbert AT, Pulliam JR, et al. A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special? Proceedings of the Royal Society B: Biological Sciences. 2013;**280**(1756):20122753

[27] Hamidi K. How do rodents play role in transmission of foodborne diseases? Nutrition and Food Science International Journal. 2018;**6**(1)

[28] Van Soolingen D, Van Der Zanden AG, De Haas PE, Noordhoek GT, Kiers A, Foudraine NA, et al. Diagnosis of *Mycobacterium microti* infections among humans by using novel genetic markers. Journal of Clinical Microbiology. 1998;**36**(7):1840-1845

[29] García-Peña GE, Rubio AV, Mendoza H, Fernández M, Milholland MT, Aguirre AA, et al. Land-use change and rodent-borne diseases: Hazards on the shared socioeconomic pathways. Philosophical Transactions of the Royal Society B. 2021;**376**(1837):20200362

[30] Taylor LH, Latham SM, Woolhouse ME. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. 2001;**356**(1411):983-989

[31] Jones KE et al. Global trends in emerging infectious diseases. Nature. 2008;**451**:990-993

[32] Lloyd-Smith JO et al. Epidemic dynamics at the human-animal interface. Science. 2009;**326**:1362-1367

[33] Karesh WB et al. Ecology of zoonoses: Natural and unnatural histories. Lancet. 2012;**380**:1936-1945

[34] Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. Nature. 2007;**447**:279-283

[35] Newbold T et al. Global effects of land use on local terrestrial biodiversity. Nature. 2015;**520**:45-50

[36] Patz JA et al. Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. Environmental Health Perspectives. 2004;**112**:1092-1098

[37] Pike J, Bogich T, Elwood S, Finnoff DC, Daszak P. Economic optimization of a global strategy to address the pandemic threat. Proceedings of the National Academy Science USA. 2014;**111**:519-523

[38] Dobson AP et al. Ecology and economics for pandemic prevention. Science. 2020;**369**:379-381

[39] Barbara A. Han, et al., Rodent reservoirs of future zoonotic diseases. PNAS. 2015;**112**(22):7039-7044

[40] Webster J, Macdonald D. Parasites of wild brown rats (*Rattus norvegicus*) on UK farms. Parasitology. 1995;**111**:247-255

[41] Haake DA, Levett PN. Leptospirosis in humans. Leptospira and Leptospirosis. 2015;**14**:65-97

[42] Tan TK, Low VL, Ng WH, Ibrahim J, et al. Occurrence of zoonotic Cryptosporidium and Giardia duodenalis species/genotypes in urban rodents. Parasitology International. 2019;**69**:110-113

[43] Rodriguez A, Pangloli P, Richards HA, Mount JR, et al. Prevalence of Salmonella in diverse environmental farm samples. Journal of Food Protection. 2006;**69**:2576-2580

[44] Nusrat A, Lindsey LL, Kipp EJ, Reinschmidt A, Heins BJ, Runck AM, et al. Nanopore-based surveillance of zoonotic bacterial pathogensin farm-dwelling peridomestic rodents. Pathogens. 2021;**10**:1183-1186

[45] Krebs CJ, Myers JH. Population cycles in small mammals. Advances in Ecological Research. 1974;**8**:267-399

[46] Ecke F, Han BA, Hörnfeldt B, Khalil H, Magnusson M, Singh NJ, et al. Population fluctuations and synanthropy explain transmission risk in rodent-borne zoonoses. Nature Communications. 2022;**13**(1):7532

[47] Lu G, Wang Q, Gao GF. Bat-to-human: Spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends in Microbiology. 2015;**23**:468-478

[48] Plowright RK, Parrish CR, McCallum H, Hudson PJ, Ko AI, Graham AL, et al. Pathways to zoonotic spillover. Nature Reviews. Microbiology. 2017;**15**:502-510

[49] Wells K, Clark NJ. Host specificity in variable environments. Trends in Parasitology. 2019;**35**:452-465

[50] Woolhouse MEJ. Gowtage-Sequeria. Host range and emerging and reemerging pathogens. Emerging Infectious Diseases. 2005;**11**(12):1842-1847

[51] Kurpiers LA, Schulte-Herbrüggen B, Ejotre I, Reeder DM. Bushmeat and emerging infectious diseases: Lessons from Africa. In: Angelici F, editor. Problematic Wildlife: A Cross-Disciplinary Approach. Cham: Springer; 2016. pp. 507-551

[52] Brown C. Emerging zoonoses and pathogens of public health significance - an overview. Revue Scientifique et Technique. 2004;**23**:435-442

[53] Lo MY, Ngan WY, Tsun SM, Hsing HL, Lau KT, Hung HP, et al. A field study into Hong Kong's wet markets:

Raised questions into the hygienic maintenance of meat contact surfaces and the dissemination of microorganisms associated with nosocomial infections. Frontiers in Microbiology. 2019;**10**:2618

[54] Gottdenker NL, Streicker DG, Faust CL, Carroll CR. Anthropogenic land use change and infectious diseases: A review of the evidence. EcoHealth. 2014;**11**:619-632

[55] Mccauley DJ et al. Effects of land use on plague (Yersinia pestis) activity in rodents in Tanzania. American Journal of Tropical Medicine Hygiene. 2015;**92**:776-783

[56] Hosseini PR et al. Does the impact of biodiversity differ between emerging and endemic pathogens? The need to separate the concepts of hazard and risk. Philosophical Transactions on Royal Society B. 2017;**372**:160-169

[57] Thompson RA, Polley L. Parasitology and one health. International Journal for Parasitology: Parasites and Wildlife. 2014;**3**(3):A1

[58] Naicker PR. The impact of climate change and other factors on zoonotic diseases. Archives of Clinical Microbiology. 2011;**2**:1

[59] Tian H, Stenseth NC. The ecological dynamics of Hantavirus diseases: From environmental variability to disease prevention largely based on data from China. PLoS Neglected Tropical Diseases. 2019;**13**(2):e0006901

[60] Kaarlejarvi E, Eskelinen A, Olofsson J. Herbivores rescue diversity in warming tundra by modulating trait-dependent species losses and gains. Nature Communications. 2017;**8**:8

[61] Stenseth NC. Population cycles in voles and lemmings: Density dependence and phase dependence in a stochastic world. Oikos. 1999;**87**(3):427-461

[62] Muteka SP, Chimimba CT, Bennett NC. Reproductive photoresponsiveness in Aethomys ineptus and A. namaquensis (Rodentia: Muridae) from southern Africa. Journal of Zoology. 2006;**268**:225-231

[63] Gage KL, Kosoy MY. Natural history of plague: Perspectives from more than a century of research. Annual Review of Entomology. 2005;**50**:505-528

[64] Van Loghem J. The classification of the plague- bacillus. Antonie Van Leeuwenhoek. 1944;**10**:15-25

[65] Adeolu M, Alnajar S, Naushad S. S. Gupta R. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': Proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology. 2016;**66**(12):5575-5599

[66] Picardeau M. Virulence of the zoonotic agent of leptospirosis: Still terra incognita? Nature Reviews Microbiology. 2017;**15**:297-307

[67] Ellis WA. Animal leptospirosis. Leptospira and Leptospirosis pp. 2015:99-137

[68] Murray GL. The molecular basis of leptospiral pathogenesis. Leptospira and Leptospirosis. 2015;**43**:139-185

[69] Sterling CR, Thiermann AB. Urban rats as chronic carriers of leptospirosis: An ultrastructural investigation. Veterinary Pathology. 1981;**18**(5):628-637

[70] Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: A re-emerging virus. The Lancet. 2012;**379**:662-671

[71] Blaser MJ, Duncan DJ, Warren GH, Wang WL. Experimental Campylobacter jejuni infection of adult mice. Infection and Immunity. 1983;**39**:908-916

[72] Yusof FM, Ismail AIB, Ali NM. Effect of Predators on the Spread of Hantavirus Infection. Sains Malaysiana. 2014;**43**(7):1045-1051

[73] Himsworth CG, Parsons KL, Jardine C, Patrick DM. Rats, cities, people, and pathogens: A systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. Vector Borne and Zoonotic Diseases. 2013;**13**:349-359

[74] Vlasova AN, Amimo JO, Saif LJ. Porcine rotaviruses: Epidemiology, immune responses and control strategies. Viruses. 2017;**9**:48-54

[75] Wan Z, Yn Z, He Z, Liu J, Lan K, Hu Y, et al. A melting curve-based multiplex RT- qPCR assay for simultaneous detection of four human coronaviruses. International Journal of Molecular Sciences. 2016;**17**:1880-1885

[76] Monchatre-Leroy E, Boué F, Boucher JM, Renault C, Moutou F, Ar Gouilh M, et al. Identification of alpha and beta coronavirus in wildlife species in France: Bats, rodents, rabbits, and hedgehogs. Viruses. 2017;**9**:364-365

[77] Okba NM, Raj VS, Haagmans BL. Middle East respiratory syndrome coronavirus vaccines: Current status and novel approaches. Current Opinion in Virology. 2017;**23**:49-58

[78] Lee GH, Tan BH, Teo ECY, Lim SG, Dan YY, Wee A, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. Gastroenterology. 2016;**150**:355-357

[79] Abravanel F, Lhomme S, El Costa H, Schvartz B, Peron JM, Kamar N, et al. Rabbit hepatitis E virus infections in humans, France. Emerging Infectious Diseases. 2017;**23**:1191-1194

[80] Viswanathan M, Pearl DL, Taboada EN, Parmley EJ, Mutschall S, Jardine CM. Molecular and statistical analysis of Campylobacter spp. and antimicrobial-resistant Campylobacter carriage in wildlife and livestock from Ontario farms. Zoonoses and Public Health. 2017;**64**(3):194-203

[81] Jardine CM, Reid-Smith RJ, Rousseau J, Weese JS. Detection of Clostridium difficile in small and medium-sized wild mammals in Southern Ontario. Canada. Journal of Wildlife Diseases. 2013;**49**(2):418-421

[82] Kilonzo C, Li X, Vivas EJ, Jay-Russell MT, Fernandez KL, Atwill ER. Fecal shedding of zoonotic food-borne pathogens by wild rodents in a major agricultural region of the central California coast. Applied and Environmental Microbiology. 2013;**79**(20):6337-6344

[83] Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease. The Journal of clinical investigation. 2004;**113**(8):1093-1101

[84] Rozental T, Ferreira MS, Guterres A, Mares-Guia MA, Teixeira BR, Gonçalves J, et al. Zoonotic pathogens in Atlantic Forest wild rodents in Brazil: Bartonella and Coxiella infections. Acta Tropica. 2017;**168**:64-73

[85] Sharma N, Hotta A, Yamamoto Y, Uda A, Fujita O, Mizoguchi T, et al. Serosurveillance for Francisella tularensis among wild animals in Japan using a newly developed competitive enzyme-linked immunosorbent assay. Vector Borne and Zoonotic Diseases. 2014;**14**(4):234-239

[86] Peterson AT, Moses LM, Bausch DG. Mapping transmission risk of Lassa fever in West Africa: The importance of quality control, sampling bias, and error weighting. PLoS One. 2014;**9**(8):e100711

[87] Michelitsch A, Wernike K, Klaus C, Dobler G, Beer M. Exploring the reservoir hosts of tick-borne encephalitis virus. Viruses. 2019;**11**(7):669

[88] Morzunov SP, Rowe JE, Ksiazek TG, Peters CJ, St. Jeor SC, Nichol ST. Genetic analysis of the diversity and origin of hantaviruses in Peromyscus leucopus mice in North America. Journal of Virology. 1998;**72**(1):57-64

[89] Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology. 2012;**55**(4):988-997

[90] Howard CR, Fletcher NF. Emerging virus diseases: Can we ever expect the unexpected? Emerging Microbes & Infections. 2012;**1**(1):1-9

[91] Prieto-Granada CN, Lobo AZ, Mihm Jr MC. Skin infections. In: Diagnostic Pathology of Infectious Disease. Elsevier; 2018. pp. 542-647. DOI: 10.1016/B978-0-323-44585-6.00020-5

[92] Vojtkova B, Spitzova T, Votypka J, Lestinova T, Kominkova I, Hajkova M, et al. Central Asian rodents as model animals for Leishmania major and Leishmania donovani research. Microorganisms. 2020;**8**(9):1440

[93] Gray JS, Estrada-Peña A, Zintl A. Vectors of babesiosis. Annual Review of Entomology. 2019;**64**:149-165

[94] Zhao W, Zhou H, Huang Y, Xu L, Rao L, Wang S, et al. Cryptosporidium spp. in wild rats (Rattus spp.) from the Hainan Province, China: Molecular detection, species/genotype identification and implications for public health. International Journal for Parasitology: Parasites and Wildlife. 2019;**9**:317-321

[95] Brouat C, Diagne CA, Ismaïl K, Aroussi A, Dalecky A, Bâ K, et al. Seroprevalence of Toxoplasma gondii in commensal rodents sampled across Senegal. West Africa. Parasite. 2018;**2018**:25

[96] Ribicich M, Gamble HR, Bolpe J, Scialfa E, Krivokapich S, Cardillo N, et al. Trichinella infection in wild animals from endemic regions of Argentina. Parasitology Research. 2010;**107**:377-380

[97] Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, et al. Hepatitis E virus in blood components: A prevalence and transmission study in southeast England. The Lancet. 2014;**384**(9956):1766-1773

[98] Pyne NJ, Pyne S. Sphingosine kinase 1: A potential therapeutic target in pulmonary arterial hypertension? Trends in Molecular Medicine. 2017;**23**(9):786-798

[99] Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, et al. Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases. 2014;**8**(3):e2748

[100] Johnson RF, Yellayi S, Cann JA, Johnson A, Smith AL, Paragas J, et al. Cowpox virus infection of cynomolgus macaques as a model of hemorrhagic smallpox. Virology. 2011;**418**(2):102-112

[101] Murphy FA. Emerging zoonoses: The challenge for public health and biodefense. Preventive Veterinary Medicine. 2008;**86**(3-4):216-223

Chapter 5

Rodents in Drug Discovery

Vinod Goyal and Mahalakshmi Bandari

Abstract

Animals, especially rodents, are an integral part of any drug discovery and development program. Once initial *in silico* and *in vitro* experiments are completed, a new chemical is tested for its pharmacokinetic profile, efficacy, and safety in animals, rodents being the most commonly used animals. Millions of rodents (rats and mice) are being used annually to understand the properties of new chemicals. Apart from wild types, genetically modified rats and mice such as knock-out or knock-in animals are very popular nowadays in understanding the biology behind diseases. Though the emergence of advanced technologies undermines the use of rodents in research, replacing animal use in research now seems to be a dream.

Keywords: rodents, rat, mouse, drug discovery, pharmacokinetics, efficacy, safety

1. Introduction

Thinking of rodents, rat or mouse, chills run down anyone's spine. These small creatures can be a source of lots of nuisance in our daily life like damaging household items, spoiling food in granaries, spreading diseases like plague and so on. However, there is always another side of the coin; rodents are very useful animals, especially in biomedical research. One cannot think of bioresearch sans rodents. They are considered as an integral part of biomedical research [1]. Animal research and testing are behind almost every prescription medicine available today. Moreover, animal research saves animals, too. It has resulted in many remarkable lifesaving and life-extending treatments for cats, dogs, farm animals, wildlife, and endangered species [2].

Successful and efficient development of a new drug revolves around – chemistry, manufacturing and controls (CMC), non-clinical studies (distribution, metabolism, and pharmacokinetic [DMPK], pharmacology and toxicology), and clinical trials. The use of animal models in research aided in the advancement of knowledge about the pathobiology of several diseases of animals and humans, which led to the discovery and development of new therapies for the prevention and/or treatment of many diseases symptomatic or disease-modifying. Animal testing is a vital part of drug development. Animal testing is necessary for understanding the safety and proper dosages of new medicines and treatments. A new chemical is initially tested in isolated cells, tissue slices or organs. The next step is testing in living animals to show whether the chemical works the same way inside

the body as it did in the artificial environment of the laboratory. Animal testing also sheds light on how the chemical alters the interactions between different cells and organs of the body.

The significance of animal use in biomedical research is emphasized from time to time by various scientific groups around the globe. For example, in 1993, the NIH office released a position statement on the use of animals in research which stated, "The development of knowledge necessary for the improvement of the health and well-being of humans, as well as other animals, requires *in vivo* experimentation with a wide variety of animal species." "Whole animals are essential in research and testing because they best reflect the dynamic interactions between the various cells, tissues, and organs comprising the human body" [3].

Among animals, rodents play a crucial role in biomedical research. More than 95% of animals used in biomedical research are mice and rats. Their close resemblance with humans in term of physiology and genetic makeup makes them a natural choice for biomedical research. Though there are certain differences between people and rodents, the similarities outweigh them and provided researchers with an enormously powerful and versatile tool to investigate human diseases [4].

Why are rodents (rats and mice) most commonly used in biomedical research? The answer lies in the fact that they reproduce quickly, they are social, they are adaptable, and they are omnivores. Additionally, the rodent's diminutive size allows relatively easy storage in labs, and their shared evolutionary roots with humans mean the similarity of their genetic makeup to human DNA. Rodents have become the animal model of choice for biomedical researchers because their physiology and genetic makeup closely resemble that of people. **Table 1** depicts how much rats and mice genome is similar to the humans compared to other animals [5, 6]. With the evolution of genetic engineering, research started using genetically modified or humanized rodents for the identification of gene/s responsible for various diseases, exploring the mechanism of diseases, and understanding how to circumvent and find a solution/treatment for many diseases. Rodents are also used for testing the biocompatibility of medical devices used in humans. Therefore, the use of rodents becomes an integral/indispensable part of drug discovery [4, 7].

Species	Genetic similarity (%)
Humans and Humans	99.9
Humans and Chimpanzee	>99
Humans and Mice	> 98
Humans and Rats	≈ 97–99
Humans and Pigs	98
Humans and Dogs	94
Humans and Cats	90
Humans and Cows	80
Humans and Fruit flies	60

Table 1.
Genetic correlation between Humans and laboratory animals.

2. History of rodents use in biomedical research

There is no verifiable evidence available of when and where people research started using animals in biomedical research. Based on the writings of Aristotle, Diocles, Praxagoras, Erasistratus and Herophilusit, it can be estimated that the use of animals in biomedical research might have started as early as the third-century BC [8].

Mice and rats have been used for research since the seventeenth century, but with the rise of biological and genetic experiments in the 1900s, they have become the most widely used animal in research. The first publication using mice in research dates to 1902, with French biologist Lucien Cuénot. Early in the twentieth century, mouse breeding started scientifically, resulting in some of the first inbred mouse lines in biomedical research. In 1929, Clarence Cook Little (1888–1971) founded the Jackson Lab in Bar Harbor Maine, which became the first American institution to supply laboratory animals as a tool for genetics and medical research across the globe [9].

The first evidence of the use of Albino rats was dated back to 1828, when these rats were used to study the quality of proteins present in the body. Similar rats continued to be used until 1906, when an institute in Philadelphia created rats by selecting inbreeding to be used as a model for present-day biomedical research. Most present-day rats originated from that original colony maintained at Wistar institute [9].

During the last three decades, due to emergence of sequencing technologies and platforms led to significant advancements in the rat genome assembly. This has led to the discovery of genome variants in rats, which have been widely used to detect quantitative trait loci underlying complex phenotypes based on gene, haplotype, and sweep association analyses [10].

3. Usage of animals in research

Tens of millions animals are used in biomedical research worldwide; rodents outnumber others. The exact number is difficult to arrive as many organizations never disclose how many animals they use. In one report [11], about 1.1 million rodents were used for scientific procedures in Great Britain alone in the year 2021 [12]. In the United States of America, an estimated 100 million mice and rats were held captive in laboratories or used in experiments in 2019 [13].

4. Commonly used rodent strains in biomedical research

The most commonly used rodents strains include but are not limited to the following:

- Mice: C57BL/6, BALB/c, Swiss, CD-1
- Rat: Wistar, Sprague–Dawley, Brown Norway, Long-Evans, F344
- Hamster: Golden or Syrian hamster
- Gerbil: Mongolian Gerbil

5. Rodents in drug discovery

5.1 Process of drug discovery and development

A successful drug needs about 15 years and hundreds of millions of dollars, from the conception of the idea to marketing approval. For a chemical to be declared drug and launch in the market, several thousands of chemicals are tested for their efficacy and safety. It is estimated that 1 in 10,000 chemicals reach their final destination. Therefore, a major chunk of the research and development budget is spent developing candidate drugs that are not approved. Furthermore, there are instances where drugs are withdrawn from the market even after approval, mostly due to either lack of sufficient efficacy or unanticipated toxicity that appeared during post-market surveillance [14].

A typical drug discovery and development process consists of several stages. The preliminary basic research on clinical disease and biomarker identification leads to a better understanding of disease diagnosis, progression, and outcomes, followed by the involvement of chemists and biologists collaborating to develop candidate therapeutic agents. These are typically evaluated for efficacy in cell culture-based high throughput screening assays. For new drug development, small molecules, therapeutic proteins/antibodies, antisense oligonucleotides, and off-target effects of currently available pharmaceuticals are considered.

Animal experiments are a very important milestone of drug discovery which generally starts as soon as the *in-vitro* data is available and continues till phase 3 studies or even post-marketing. The major areas where animals are used include pharmacokinetics (the action of the body on the medicine), efficacy testing, and safety evaluation. Apart from the above said, animals are also used to prove the mechanism of action of the proposed drug.

Animal testing is necessary for understanding the safety and proper dosages of new medicines and treatments. The use of animals in research remains essential to understand the causes, diagnoses, and treatment of disease and suffering in humans and in animals [15]. Experimentation in humans generally begins once the researchers confirm that the drug is safe and effective based on animal studies. Prior to drug approval, human and animal testing is regulated by law, and regulatory agencies (like FDA, EMEA) mandate animal testing before any clinical trials in humans for safety reasons. Animal research and testing is a crucial step in drug discovery and development since testing drugs in humans before assuring their safety would be extremely dangerous and unethical.

There are arguments against use of animals in research. Often times it is believed that animal findings do not translate to humans. This is particularly more relevant to the research in the field of neuropsychiatry as animals do not manifest psychiatric symptoms. It is true that we may not be able to translate rodent or any other non-clinical data completely in humans which may be due to several factors, but still, animals serve as a valuable tool in biomedical research. Since there is no alternative that mimics complex functions of human physiology as of now, the use of animals cannot be substituted completely. Animals will continue to be used in drug discovery to identify new drugs for various unmet medical conditions both of humans and animals [15].

5.2 Pharmacokinetics (the action of body on the drug)

Any drug after administration (with the exception of topically administered drugs) must enter the bloodstream and get distributed to the site of action to show its

pharmacological response. The process of movement of drug from the site of administration to site of action is known as pharmacokinetics, which includes absorption, distribution, metabolism and elimination of drug [16].

- **Absorption:** *defined as passage of drug from its site of administration into the blood*
- **Distribution:** *defined as the delivery of the drug to the tissues*
- **Metabolism:** *defined as the breakdown of drug into metabolites (usually pharmacologically inactive or less active)*
- **Elimination:** *defined as excretion of drug from the body*

For assessment whether the drug is being absorbed from the intestine and reaches to the systemic circulation, rodents are primarily used for this kind of experiment early in the discovery phase. Similarly, comparative bioavailability of different routes of administration is also carried out in animals, again primarily in rodents. Other animals used in these experiments are dogs, monkeys, rabbits, and non-human primates. Once it is determined that systemic exposures in rodents are sufficient to exert therapeutic benefit, a drug moves to efficacy studies.

Other parts of pharmacokinetics like distribution, metabolism, and elimination of chemicals, are also evaluated later in the discovery phase in a timely manner. Metabolic profiling is a very important aspect of drug development. Initially, *in-vitro* studies are done to understand the extent of metabolism in different species like rodents, non-rodents and humans using either liver microsomes or primary hepatocytes. Once the extent of metabolism is done, the next step is to see whether there is any unique metabolite form in humans which is not form in any animal species checked. This exercise is important for the selection of the right species for subsequent safety studies.

Tissue distribution and elimination patterns are also a very important aspect of the discovery program. Initially, rats are used to determine the extent of tissue distribution and elimination pattern.

5.3 Pharmacodynamics (the action of drug on the body)

Pharmacodynamics is defined as the physiological and biochemical response of drugs and their mechanism of action at organ system, sub-cellular and molecular levels. It includes binding the drug to the molecular target and eliciting the desired response.

Pharmacology is defined as the branch of science that deals with the interaction of drugs administered with the living system to produce a biological response. It can be divided into the following three categories: [17, 18].

- primary pharmacology/pharmacodynamics
- secondary pharmacology/pharmacodynamics
- safety pharmacology.

5.3.1 Primary pharmacology

Primary pharmacology studies demonstrate the intentional drug-related effects on enzymes, receptors and other targets. The pharmacology of a drug influences pre-clinical species selection for some studies [19]. Efficacy evaluation is one of the most important aspects of drug discovery. The first step is to identify the right target and the right mechanism of the disease in question. Once it is determined, several new entities (either chemicals or biological) are tested for their potential interaction with the target using *in-vitro* techniques. Out of several entities, only few go to the next level of testing to determine their efficacy in reliable models, either *in vitro*, *ex vivo*, or *in vivo*, most often combination of all.

Different animal models are being used based on the target/disease in question, mostly rodents, to understand the efficacy of chemicals/drugs. The reason for this widespread use of rodents is their similarity with humans. Many genetically engineered mice/rats are developed to mimic human pharmacology/genomics to understand the pathology, efficacy and safety of new drugs, and they are sometimes called as humanized animals (wherein human genes are incorporated in animals).

There are hundreds of animal models developed so far to test chemicals for their efficacy in different diseases. In general, once the chemical proves to be efficacious in animal model/s, it moves to human testing only if considered to be safe to be administered in humans. Examples of some of the most commonly used rodent models are chemically induced diabetes models, diabetic wound models, cancer models, colitis models, etc. (**Table 2**). Apart from the model developed for many diseases in wild-type rodents, new research focuses on genetically engineered animals to mimic human pathology. The commonly used transgenic/genetically modified models are listed in **Table 3** [22].

5.3.2 Secondary pharmacology

Secondary pharmacology studies evaluate the potential off-target or unintentional effects of a drug. These studies are important in predicting potential toxicities and demonstrating safety [19]. This is a cost-effective approach used by many pharmaceutical companies as a safety screen early in drug development. Information on the potency of a drug for a given biological target can be used to determine structure–activity relationships, assess potential liability for off-target effects, and influence early clinical trial design, dose selection and patient monitoring [23].

5.3.3 Safety pharmacology

Safety pharmacology studies are defined as those studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above.

Animal models for Disease Conditions	
Mouse	Cancers and genetic diseases
Rats	Osteoporosis, inflammatory diseases, diabetes, obesity, cardiovascular dysfunctions, neurodegenerative diseases, cancers

Table 2.
Animal models for Disease conditions [20].

System/organ affected	Human genetic disease	Gene/genetically modified rats and mice
Cardiovascular	Pulmonary arterial hypertension	BMPR2/KO
	Primary pulmonary hypertension 4 (PPH4)	Kcnk3/KO
	Atrial fibrillation, familial, 18 (ATFB18)	Myl4/KO
	Familial hypertrophic cardiomyopathy and myocardial genetic diseases	Myh7b/KO
Nervous system	Epileptic encephalopathy, early infantile, 63 (EIEE63)	Cplx1/KO
	Dystonia 25 (DYT25)	Gnal/KO
	Schizophrenia	Drd2/KI reporter
	Amyotrophic lateral Sclerosis (ALS)	Fus/KI point mutation R521C
	Epileptic encephalopathy, early infantile, 24 (EIEE24)	Hcn1/KO
	Autism spectrum disorder	Cntnap2/KO Shank2/KO
	Parkinson's disease	Lrrk2/KO
Gastrointestinal	Hereditary tyrosinemia type I	Fah/KO
	Hirschsprung disease	Ednrb/KO
	Rotor syndrome	OATP1B2 /KO
	Atypical hereditary non-polyposis colorectal cancer	Msh6/KO
	familial colon cancer	Apc/KO
Muscle	Muscular dystrophy (Duchenne and Becker forms)	Dmd/KO and BigDel
	Myostatin-related muscle hypertrophy	Mstn/KO
Lung	Cystic fibrosis	Cftr/KO and DF508 CFTR/KI and G5551D
Endocrine	Glucocorticoid resistance	Nr3c1/cKO
	Estrogen resistance (ESTRR)	Esr1/KO and Esr2/KO
	Congenital hypothyroidism	Tshr/KO
	Allan-Herndon Dudley-syndrome	Mct8/KO
Metabolic	Congenital leptin deficiency	Lep/KO
	Leptin receptor deficiency	Lepr/KO
	Aceruloplasminemia	Cp/KO
	Diabetes mellitus, non-insulin dependent, 5 (NIDDM5)	AS160 (TBC1D4)/KO
	Dwarfism	Ghsr/Tg, Ghsr/KO
	Obesity	Mc3RMc4R/DKO
Nephrology	Focal segmental glomerulosclerosis 2 (FSGS2)	Trpc6/KO BigDel
	C3 glomerulopathy	C3/KO
	REN-related kidney disease	Ren/KO

Ophthalmology	**Autosomal dominant congenital stationary night blindness and retinitis pigmentosa**	**Rho s334ter/Tg**
	Retinitis pigmentosa 85 (RP85)	**Ahr/KO**
Cancer	Li-Fraumeni syndrome	Tp53
Immune and hematological systems	Hemophilia A	F8/KO
	SCID	Rag1/KO Rag2/KO Prkdc/KO
	X-linked SCID	Il2Rg/KO
Sleep disorders	Narcolepsy	Orexin KO mouse *Orexin/ataxin-3 transgenic mouse or rat*

Table 3.
Genetically modified rat and mice models of human genetic diseases (reproduced from [21]).

The main objectives of these studies are to identify undesirable pharmacodynamic properties of a substance that may have relevance to its human safety, to evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies, and to investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected.

In the first phase of safety pharmacology studies, functions of the most critical organs or systems such as the cardiovascular, respiratory and central nervous systems, are assessed. Subsequently, other organ systems, such as the renal or gastrointestinal system, the functions of which can be transiently disrupted by adverse pharmacodynamic effects without causing irreversible harm, are of less immediate investigative concern and can be evaluated later in development if the need arises [24, 25]. Functions of these organs/systems are evaluated using various techniques; some are well established, and many are emerging (**Table 4**).

5.4 Toxicology

The non-clinical safety assessment is one of the most important aspects for marketing approval of any drug; a set of studies need to be completed before filing a new drug application for market authorization, including general toxicity studies, genotoxicity, and reproduction and development toxicity studies. Other toxicity studies such as carcinogenicity, phototoxicity, immunotoxicity, juvenile animal toxicity and abuse liability need to be conducted on a case-by-case basis, based on the property of drug, outcome of other studies, intended length of use, target population and so on [27].

This requirement may vary in different countries, but overall several studies are required to assess the toxic potential of any drug. Similarly, timing to conduct these studies may also differ in different regions. For example, in the United States, assessment of embryo-fetal development can be deferred until before phase 3 for women of childbearing potential (WOCBP) using precautions to prevent pregnancy in clinical trials. In the EU and Japan definitive non-clinical developmental toxicity studies should be completed before exposure to WOCBP (with some exceptions).

The ultimate aim of safety assessment is characterization of toxic effects, identification of target organs, exposure-response relationship, and potential reversibility of

Safety Pharmacology Studies- Core Battery		
Organs/Systems	*Established*	*Emerging*
Central Nervous System	*Behaviour-* (modified) Irwin, FOB *Locomotion-* Photo Electric Beam interruption/rotarod *Nociception-* hot plate/tail flick/paw pressure *Seizure liability-* EEG	*Nociception-* video automated system *Seizure liability-* integrated video EEG, in-vitro hippocampal brain slice *Drug abuse-* lever chamber models *Drug dependence-* telemetry
Cardiovascular System	*In-vitro patch clamp-* hERG assay- QT prolongation *Telemetry-* blood presssure, heart rate, ECG *Isolated myocardial systems-* heart rate, ECG	*In-vitro* Automated high-throughput patch clamp External telemetry with high definition oscillometry *In-silico* computer modeling hESC-CM and hiPS-CM models
Respiratory System	Respiratory rate, tidal volume, O_2 saturation, airway resistance, compliance- Plethysmography, telemetry	Unrestrained video-assisted plethysmography *Biomarker-* VQM
Secondary/Follow-up Battery		
Gastrointestinal System	Gastric emptying and secretion, intestinal motility, ulcer index, histopathology	Capsule endoscopy, Telemetry, PBPK modeling, *Biomarkers-* Citruline, miR-194, Calprotectin
Renal System	Urine- volume, osmolality, pH, Na+, Cl-, K+, Urea, AST, ALT, LDH, GGT, ALP, β-NAG, Serum- Osmolality, BUN, Creatinine, Cystatin C. GFR, Clearance rate	*Biomarkers*: β2- Macroglobulin, KIM-1, CLU, TFF3, NGAL, α-GST, μ-GST, RPA-1

ALP- alkaline phosphatase; AST - aspartate aminotransferase; ALT - alanine aminotransferase, ; BUN - blood urea nitrogen; β-NAG - N-acetyl-β-D-glucosaminidase; CLU - clusterin, EEG -electroencephalography; ECG - electrocardiogram; FOB - Functional Observation Battery, GGT - γ-glutamyl transferase; GFR- glomerular filtration rate; GST - glutathione S transferase, hESC-CM- human embryonic stem cell derived cardiomyocytes; hiPS-CM- human inducible pluripotent stem cell derived cardiomyocytes, KIM-1 - kidney injury molecule-1; LDH - lactate dehydrogenase, miR - microRNA, RPA-1 - renal papillary antigen-1; NGAL - neutrophil gelatinase-associated lipocalin; PBPK - physiologically based pharmacokinetics; TFF3- trefoil factor 3VQM - ventilation (V)/perfusion (Q) mismatch (M).

Table 4.
Safety Pharmacology studies [26].

drug-induced adverse effects. The resultant information is helpful in deciding a dose that can be administered for human safety without any expected adverse events. The information from these studies is also helpful in finding out biomarkers that can be used for clinical monitoring of potential adverse events.

5.4.1 General Toxicity studies

5.4.1.1 Single or repeated dose studies

These studies range from single dose (acute toxicity) to repeated doses up to 12 months or more in duration in two animal species; one rodent and one non-rodent. Information on the acute toxicity of pharmaceutical agents could be useful in predicting the consequences of human overdose situations. The recommended duration of the repeated-dose toxicity studies is usually related to the duration, therapeutic indication, and scope of the proposed clinical trial.

Among rodents, most commonly used species is the rat. Rats are considered the most suitable species for the purpose of being small in size, having a limited life span of about 2 years, having high breeding capacity, having ease in handling, and having availability of robust historical data [28].

5.4.2 Reproductive and developmental toxicity studies

To identify hazard and characterize reproductive risk for human pharmaceuticals, animal testing is a regulatory requirement. The most commonly used species are rats and rabbits. Sometime NHPs are also used especially for biological.

As appropriate, observations through one complete life cycle (i.e., from conception in one generation through conception in the following generation) permit the detection of immediate and latent adverse effects. To evaluate all stages of life cycle, the following studies are generally conducted to support new drugs;

1. Fertility studies

2. Early embryonic development study

3. Embryo-fetal developmental studies

4. Pre and postnatal developmental studies

There are several combinations that can be used to evaluate the effect of any drug on a complete life cycle such as combining fertility studies with early embryonic development studies; combining EFD with prenatal developmental studies, etc. The ultimate goal is to estimate the effects of drug on all stages of human life. That is, pre-mating from conception to birth to sexual maturity [29].

5.4.3 Genotoxicity

The genotoxic potential of all drugs is evaluated early in drug development. Though the initial screening is carried out using *in vitro* tests like AMES, chromosomal aberration tests in cell lines, mouse lymphoma test, mammalian cell gene mutation test, and many more, at least one test is mandatory using animals. The most commonly used *in vivo* tests are the rodent micronucleus test, rodent comet assay, and bone marrow chromosomal aberration test in rodents [30].

5.4.4 Carcinogenicity

Carcinogenicity studies are generally required for the drugs that are expected to be used regularly over a substantial part of patient's life. These studies are generally performed in rodents, particularly rats and mice. The duration of these studies is lifetime exposure of the drug in these species to mimic possible human exposure (24 months for rats and 18 months for mice). Regulators require data from two species, that is, rat and mouse, to understand the potential carcinogenic effect as well as to assess human risk. With the invention of genetically modified mice, 18-month mice study can be replaced with 6-month study in genetically modified mice which is considered enough to explore the tumorigenic potential of drug.

Several factors need to be assessed before taking a call on the necessity of conducting a carcinogenicity study. These are duration of exposure (expected clinical use is continuous for at least 6 months or frequently in an intermittent manner in the treatment of chronic or recurrent conditions), evidence of genotoxicity, structure–activity relationship suggesting carcinogenic risk, evidence of pre-neoplastic lesions in repeated dose toxicity studies, previous demonstration of carcinogenic potential in the product class, etc. [31, 32]. A waiver request can be asked if the sponsor can justify that carcinogenicity studies are not required for drug approval. The requirement of carcinogenicity studies may be deferred to post-marketing with proper justification and in agreement with the regulators. This is generally possible when the drug is intended to be used in patients with severe diseases and very limited or no therapeutic options are available.

5.4.5 Phototoxicity

Phototoxicity is defined as a toxic response that is elicited after the initial exposure of skin to certain chemicals and subsequent exposure to light or that is induced by skin irradiation after systemic administration (oral, intravenous) of a chemical substance [33]. It is a kind of photosensitivity. In general, the phototoxic potential of any drug is evaluated by conducting two tests viz., phototoxicity, also known as photoirradiation and photoallergy. Phototoxicity studies evaluate the effect of an acute light-induced tissue response to a photo-reactive chemical, whereas photoallergy studies evaluate the effect of an immunologically mediated reaction to a chemical.

All drugs need not be evaluated for phototoxicity potential. Only drugs (chemical) that possess the following characteristics are required to undergo extensive evaluation-

- absorbs light within the range of natural sunlight (290–700 nm);
- generates a reactive species following absorption of UV/visible light;
- distributes sufficiently to light-exposed tissues (e.g., skin and eye).

If a chemical does not meet any of these conditions, then phototoxicity evaluation is not mandatory as these chemicals will not have a photo safety concern [34].

5.4.6 Special/mechanistic studies

5.4.6.1 Immunotoxicity studies

Assessment of potential adverse effects on the immune system is an important component of the overall evaluation of drug toxicity. The first assessment of immunotoxicity is generally observed during repeated-dose general toxicity studies where many aspects of the immune system are evaluated. In case of any adverse effect is observed, follow-up studies may be required to understand the toxic effect and its human relevance. In addition, immunosuppression should also be assessed for new drugs. Again, this is generally a part of repeat dose toxicity studies which include detailed clinical and anatomic pathology evaluation such as serum globulin levels, differential leukocytes count, gross pathology, weights of immune system-related organs (thymus and spleen) and their histological examination (thymus, spleen, lymph nodes, and bone marrow) [35].

5.4.7 Juvenile animal toxicity studies

When any drug is intended for pediatric population, depending on the therapeutic indication, and age of the pediatric population, non-clinical safety studies for sufficient duration to support pediatric trials in juvenile animals needs to be conducted. These studies are generally conducted in rodents [36].

5.4.8 Abuse potential studies

Any drug/pharmaceutical agent or its metabolite must be evaluated for abuse potential if it crosses the blood–brain barrier and reaches the brain. A set of studies determined that abuse potential needs to be assessed, which should be carried out to ascertain abuse potential and subsequent label updates. Rodents are the most commonly used animals for these studies (drug discrimination, physical dependence, and self-administration study). These studies are not always necessary to conduct for all chemicals, but the decision should be taken after a thorough review of the properties of chemicals/drugs and a detailed discussion with regulatory agencies about the need for these studies [37].

6. Conclusion

While *in vitro* and *in silico* methodologies play an integral role in drug discovery, animal models continue to prove their value. Although animal ethics groups impose many challenges against animal use in research, rodents, especially rats and mice, will continue to play a critical role in biopharmaceutical research. Whole animals are essential in research and testing because they best reflect the dynamic interactions between the various cells, tissues, and organs comprising the human body.

With the advancement of science and the genesis of humanized rodents, the role of rodents increased significantly in understanding disease processes in a better way and facilitating the transition of research from bench to bedside. Therefore, a complete removal of rodents from drug discovery, at present, is impossible, and they will be continued to provide deep insights during the drug discovery process.

Author details

Vinod Goyal* and Mahalakshmi Bandari
Suven Life Sciences Limited, Hyderabad, India

*Address all correspondence to: drvingoyal@gmail.com

References

[1] Smith JR, Bolton ER, Dwinell MR. The Rat: A Model Used in Biomedical Research. Methods in Molecular Biology. 2019;**2018**:1-41. DOI: 10.1007/978-1-4939-9581-3_1

[2] National Academies of Sciences, Engineering, and Medicine. 1991. Available from: https://fbresearch.org

[3] Position statement on the use of animals in research. NIH Guide. 26 Feb 1993;**22**(8)

[4] Bryda EC. The mighty mouse: The impact of rodents on advances of biomedical research. Missouri Medicine. 2013;**110**:3

[5] How genetically similar are we to other life forms. 2021. Available from: https://www.visualcapitalist.com/comparing-genetic-similarities-of-various-life-forms/

[6] Biological similarity of humans and other animals. Available from: https://speakingofresearch.com/facts/the-animal-model/

[7] Judith R, Wöhr HM, Alenina N. Comeback of the rat in Biomedical Research. ACS Chemical Neuroscience. 2017;**8**(5):900-903. DOI: 10.1021/acschemneuro.6b00415

[8] Ericsson AC et al. A brief history of animal modeling. 2013;**110**(3):201-205

[9] Mice and Rodents. Available from: https://ori.hhs.gov/education/products/ncstate/rodent.htm

[10] Zinski AL, Carrion S, Michal JJ, Gartstein MA, Quock RM, Davis JF, et al. Genome-to-phenome research in rats: Progress and perspectives. International Journal of Biological Sciences. 2021;**17**(1):119-133. DOI: 10.7150/ijbs.51628

[11] Annual Statistics of Scientific Procedures on Living Animals Great Britain. 2021

[12] Animal experiment statistics, House of Common's library by Georgina Sturge. 03 Aug 2022

[13] PETA. Available from: https://www.peta.org/issues/animals-used-for-experimentation/animals-used-experimentation-factsheets/animal-experiments-overview/

[14] Zuberi A, Lutz C. Mouse models for drug discovery. Can New Tools and Technology Improve Translational Power. 2016;**57**(2):178-185. DOI: 10.1093/ilar/ilw021

[15] Why animal research?. Available from: https://med.stanford.edu/animalresearch/why-animal-research.html

[16] Tracy TS. Drug Absorption and Distribution; Book- Modern Pharmacology with Clinical Applications by Craig CR and Stitzel RE. 5th Edition. 2003. Available from: https://www.academia.edu/43768448/Lippincott_Modern_Pharmacology_With_Clinical_Applications_6E

[17] Tripathi KD. General pharmacological principles. In: Essentials of Medical Pharmacology. 7th ed. New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd; 2013

[18] David RJ. Key Aspects of Non-clinical Pharmacology and Pharmacokinetics in Evaluation of Safety. Presentation - Session 1: Safeguarding Public Health. European Medicines Agency. Dec 13 2016

[19] Pharmacology. Available from: https://preclinicalgps.com/scientific-services/pharmacology/

[20] Animal models. Available from: https://toolbox.eupati.eu/resources/animal-models/ [Website assessed on 09 Feb 2023]

[21] Chenouard V, et al. Advances in genome editing and application to the generation of genetically modified rat models. 2021. DOI: 10.3389/fgene.2021.615491

[22] Mukherjee P, Roy S, Ghosh D, Nandi SK. In: Mukherjee et al., editors. Role of Animal Models in Biomedical Research: A Review. Vol. 38. Laboratory Animal Research; 2022. p. 18. DOI: 10.1186/s42826-022-00128-1

[23] Papoian T et al. Secondary pharmacology data to assess potential off-target activity of new drugs: A regulatory perspective. Nature Reviews Drug Discovery. Macmillan Publishers Limited; 2015. DOI: 10.1038/nrd3845-c1

[24] ICH S7A Guidance on Safety Pharmacology studies for Human Pharmaceuticals

[25] ICH S7B Guidance on Non-clinical evaluation of the potential for Delayed ventricular repolarization

[26] Hamdam J et al. Safety pharmacology – Current and emerging concepts. Toxicology and Applied Pharmacology. 2013;**273**(2):229-241. DOI: 10.1016/j.taap.2013.04.039

[27] ICH M3 (R2) guidance document

[28] ICH S4 Guideline on Duration of chronic toxicity testing in Animals

[29] ICH S5 (R3) Guidance on Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals

[30] ICH S2 (R1) Guidance on Genotoxicity testing and data interpretation for Pharmaceuticals intended for Human use

[31] ICH S1A Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals

[32] ICH S1B (R1) Testing for Carcinogenicity of Pharmaceuticals

[33] Phototoxicity. Available from: https://joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/alternative-methods-toxicity-testing/validated-test-methods-health-effects/phototoxicity_en#:~:text=Phototoxicity%20(photoirritation)%20is%20defined%20as,intravenous)%20of%20a%20chemical%20substance

[34] ICH S10 Guidance on Photosafety evaluation of pharmaceuticals

[35] ICH S8 Guidance on Immunotoxicity studies for Human Pharmaceuticals

[36] ICH S11 Guidance on Non-clinical safety testing in support of development of Paediatric Pharmaceuticals

[37] Assessment of Abuse Potential of Drugs, Guidance for Industry: Food and Drug Administration, Center for Drug Evaluation and Research (CDER). January 2017

Edited by Mohammad Manjur Shah

This book provides a comprehensive overview of rodents and their use in experimental studies of human diseases. It includes two sections on rodents in experimental models and rodents in drug discovery and human zoonotic transmission. Chapters examine rodent models of female reproductive abnormalities and human vision and their role in zoonotic pathogen transmission of diseases and drug discovery.

Published in London, UK

IntechOpen

ISBN 978-1-83769-626-0