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REVIEW ARTICLE

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The wide utility of rabbits as models of human diseases

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Abstract

Studies using the European rabbit *Oryctolagus cuniculus* contributed to elucidating numerous fundamental aspects of antibody structure and diversification mechanisms and continue to be valuable for the development and testing of therapeutic humanized polyclonal and monoclonal antibodies. Additionally, during the last two decades, the use of the European rabbit as an animal model has been increasingly extended to many human diseases. This review documents the continuing wide utility of the rabbit as a reliable disease model for development of therapeutics and vaccines and studies of the cellular and molecular mechanisms underlying many human diseases. Examples include syphilis, tuberculosis, HIV-AIDS, acute hepatic failure and diseases caused by noroviruses, ocular herpes, and papillomaviruses. The use of rabbits for vaccine development studies, which began with Louis Pasteur's rabies vaccine in 1881, continues today with targets that include the potentially blinding HSV-1 virus infection and HIV-AIDS. Additionally, two highly fatal viral diseases, rabbit hemorrhagic disease and myxomatosis, affect the European rabbit and provide unique models to understand co-evolution between a vertebrate host and viral pathogens.

Introduction

Small laboratory animals, such as mice, rats, guinea pigs, and European rabbits, have long been used as models to improve our understanding of several human maladies. The primary goal of developing animal models for research is to create an experimental system in which the conditions occurring in humans are phenocopied as

accurately as possible in the laboratory animal. The rabbit was the first animal model used in several immunological studies and was crucial, for example, for the development of the rabies vaccine by Louis Pasteur in 1881¹. The pioneering studies of rabies and syphilis conducted in rabbits continued to advance our understanding of these and other infectious diseases. Furthermore, the study of rabbit immunoglobulins established much of what is known about the structure, function and regulated expression of antibodies [reviewed in refs.²⁻⁴]. Although, rabbit was a major animal model used for the study of molecular immunology in the late 1980s, rabbits were increasingly replaced by rodents in the subsequent years⁵. Among the reasons for the increasing use of rodents, such as mice, instead of rabbits are reduced maintenance costs,

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small size, availability of inbred strains, ease of breeding, short reproductive cycle, high numbers of progeny, wide availability of commercial immunological reagents, and availability of many knockout (KO) and transgenic models^{6,7}. However, rabbits have the advantage of an intermediate size between rodents and larger, more costly animal models, such as primates. The size of rabbits permits the ready sampling of blood and greater access to many cells and tissues from a single animal. Additionally, rabbits have a longer life span than that of rodents, and the immune system genes of rabbits are apparently more similar to those of the human immune system than are rodent genes^{8–10}. Rabbits are also carriers or reservoirs of several pathogens that can cause zoonotic diseases. Some studies in mice found a lack of disease symptoms mimicking those of human infection. Additionally, the low-success rates in the translation of findings from some mouse studies to human diseases suggest that other animal models, such as rabbit, may often be more appropriate^{11,12}. The rabbit is actively used as a laboratory model for several non-infectious conditions, including atherosclerosis^{13,14}, intestinal immunity¹⁵, reproduction¹⁶, lupus¹⁷, arthritis¹⁸, cancer¹⁹, and Alzheimer's disease²⁰.

The rabbit has also been increasingly used during the last two decades as a reliable animal model for many infectious diseases. In this essay, several examples, including viral, bacterial, and parasitic infectious diseases, are described in which rabbits provide a more reliable model for host-pathogen interactions than rodents for their human disease counterparts.

Overview of the rabbit immune system, therapeutics, and co-evolution between host and viral pathogens

The pre-immune antibody repertoire in young rabbits develops in two stages: first, with antibody heavy chain-encoding gene segments V (variable), D (diversity), and J (joining) rearrangements in bone marrow and, second, with immunoglobulin (Ig) gene diversification in gut-associated lymphoid tissue (GALT)^{21,22}. In the bone marrow, one VH gene segment, VH1, is preferentially used for V(D)J gene rearrangements, whereas numerous kappa or lambda variable gene segments are used in VJ gene rearrangements of the light chain. After leaving the bone marrow and migrating to GALT, the B cells undergo proliferation and diversify their Ig genes by somatic hypermutation and gene conversion. Specific commensal microbes drive this B-cell expansion and Ig gene diversification through a mechanism that is not yet fully elucidated. Understanding how commensal bacteria drive these processes will provide insight into the host-microbial interactions that shape the pre-immune antibody repertoire.

The rabbit mucosal IgA system is highly unusual. Thirteen IgA C α genes are found in rabbits²³, in contrast to most mammals, which have only one (e.g., mice) or two (e.g., hominoids) IgA subclasses. Of the 13 C α genes, most have allelic forms, and 11 are expressed as different IgA subclasses in mucosal tissues. Concomitantly, rabbits have lost the IgA-specific FcR and Fc α RI²⁴, and the IgA rabbit genes have been evolving under strong positive selection^{24,25}. How the rabbit mucosal immune system has adapted to the many IgA subclasses and which FcRs bind these IgA subclasses remain a mystery; studies of this system may open new therapeutic possibilities.

In addition to the diversification of rearranged heavy and light chain genes that occurs in the GALT of young rabbits during pre-immune repertoire development, rabbits respond to infections or experimental immunizations in peripheral sites, including the spleen and lymph nodes, by producing highly specific antibodies of high affinity through diversification of rearranged heavy and light chain genes via both gene conversion and somatic hypermutation. For this reason, rabbits are a major source of polyclonal and monoclonal antibodies (mAbs) used for research and therapeutics.

A fully rabbit polyclonal anti-thymocyte globulin, approved by the FDA in 1998, remains in use in immunosuppressed patients to arrest acute rejection of kidney transplants²⁶. For non-immunosuppressed patients, human polyclonals produced in rabbits with humanized immunoglobulin genes are under development²⁷. Humanized rabbit mAbs that are currently being developed and tested include anti-human CD40, anti-vascular endothelial growth factor (VEGF), and a smaller single-chain fragment anti-VEGF (Fv) mAb (Brolucizumab)²⁸. Strategies for humanization of rabbit mAbs are reviewed in refs. ^{28,29}.

Additionally, rabbits have been used for studies of therapeutics destined for treatment of human inhalational anthrax. Tests of the human mAb Raxibacumab³⁰ and the chimeric mouse/human mAb Obiltoximab were conducted in many rabbits and fewer monkeys before approval for treatment of inhalational anthrax under the United States Food and Drug Administration (FDA) animal efficacy rule. Animal-to-human dose translation for Obiltoximab was also investigated in rabbits [reviewed in ref. ³¹].

Chemokines and their receptors play a crucial role during immune responses. Because of gene conversion with the CCR2 gene^{32,33}, the CCR5 gene of the European rabbit underwent a change at the region corresponding to the CCL8 docking site in humans^{34,35}. The evolutionary study of this CCR5 ligand in Lagomorphs indicated an adaptive pseudogenization process of the CCL8 gene^{36,37}. This makes the European rabbit a suitable model for

studies of the pathways of receptor and ligand interaction during inflammation-driven pathogenicity.

The European rabbit is also a good model to understand the co-evolution between vertebrate hosts and viral pathogens. In the last 60 years, wild and domestic rabbit populations suffered a sharp decline due to hunting, habitat destruction and the emergence of two viral diseases, namely, myxomatosis in the 1950s and rabbit hemorrhagic disease (RHD) in the 1980s. These diseases led to a contraction of rabbit populations with serious economic and ecological consequences [reviewed in ref. ³⁸]. In Australia, the dynamic observed between the genetic resistance of natural European rabbit populations against myxomatosis and the virulence grades of its causative agent, the myxoma virus (MYXV), is one of the best textbook examples of virus/host co-evolution [reviewed in refs. ^{39,40}]. RHD, caused by the rabbit hemorrhagic disease virus (RHDV), was responsible for a dramatic decline in natural rabbit populations at the end of the 1980s. Although the populations subsequently recovered, the emergence, in 2010, of a new RHDV variant, genetically and antigenically highly distinct from the previously described strains, led to increased mortalities⁴¹. The new variant remains highly lethal and has high levels of recombination⁴², which provides an opportunity to understand how the vertebrate host and the virus will mutually co-evolve.

Myxomatosis and oncolytic activity

The natural history and biomedical relevance of the European rabbit cannot be dissociated from one of the most preponderant infectious agents of the species, the myxoma virus (MYXV). MYXV is a rabbit-specific poxvirus (family *Poxviridae*, genus *Leporipoxvirus*), and similar to many other poxviruses, its genes can be divided into two classes: “essential” genes (i.e., required for virus propagation in culture) encoding proteins involved in viral replication, gene expression and virion assembly and “non-essential” genes (i.e., those that can be deleted with continued virus propagation in culture) encoding factors required for host-range, immunomodulation, and virulence^{39,40}. The host-range genes evolved in the natural *Sylvilagus* hosts of MYXV, the South American tapeti and the North American brush rabbit, in which the virus causes a localized cutaneous fibroma. However, when MYXV infected the naive European rabbit as a new host, the virus caused an outbreak of the novel and lethal systemic disease myxomatosis^{39,40}.

The study of MYXV and European rabbit interaction makes an important contribution to the field of emerging infectious diseases. It provides an outstanding model to study dynamic host-pathogen interactions and makes myxomatosis in the European rabbit an exceptional example of host-virus co-evolution. The selective tropism

of MYXV toward the European rabbit was fundamental to studies of the MYXV host-range and immunomodulatory genes and their encoded proteins, which continue to evolve in the new host. Targeted knockouts of these viral genes frequently resulted in virus attenuation in the rabbit host and consequently allowed a deeper understanding of the molecular basis for MYXV pathogenesis in the European rabbit³⁹. Such knowledge has also been important for the ongoing development of MYXV as a potential oncolytic virotherapeutic for the treatment of a variety of human cancers by exploiting the ability of the virus to productively infect a wide diversity of non-rabbit cancer cells^{43,44}. For example, the M135R-knockout MYXV cannot induce myxomatosis in European rabbits and is non-pathogenic for all known vertebrate hosts; however, this knockout is fully oncolytic in human cancer cells and is currently being developed as a clinical candidate for oncolytic immunotherapy to treat human hematological malignancies^{44,45}.

Rabbit hemorrhagic disease virus as a model of human noroviruses and hepatic fulminant diseases

Initial studies on RHDV showed that it attached to glycans of the histo-blood group antigens (HBGAs)⁴⁶. Because this virus belongs to the family *Caliciviridae*, these results provided the impetus to search for glycans potentially used by human caliciviruses, such as noroviruses, which constitute a major cause of gastroenteritis worldwide⁴⁷. Unlike mouse strains, human strains of noroviruses, although also attaching to HBGAs, do not all present the same pattern of recognition⁴⁸. Because of the genetic polymorphism of HBGAs, not all humans are equally susceptible to individual strains of noroviruses, suggesting a past and possibly ongoing co-evolution between humans and noroviruses⁴⁹. Using the European rabbit as a model, a survey of the frequency of their HBGA polymorphisms in wild populations affected by the virus conducted in parallel with a survey of the evolution of the specificity for HBGA recognition of the virus itself allowed documentation of the co-evolution between host and pathogen at a molecular level, underscoring the importance of the rabbit as a model for relevant human gastrointestinal pathogens^{50,51}.

Acute hepatic failure (AHF) is a severe liver injury accompanied by encephalopathy that causes multi-organ failure with an extremely high-mortality rate. Severe AHF continues to be one of the most challenging problems in clinical medicine. Treatment has been limited by the lack of satisfactory animal models, particularly for acute viral hepatitis, a frequent cause of this condition. RHDV inoculation in rabbits is an excellent model for AHF of viral origin, displaying biochemical/histological characteristics, presence of encephalopathy, and clinical

features that resemble those in humans⁵². Additional advantages in comparison with rodent models are the larger size of rabbits and the sufficient time window before death, which renders testing of new liver support systems possible and permits sufficient samples of blood and tissue to be taken during treatments. Increased insight was gained into the physiologic derangements of virus-induced AHF⁵³ using the RHDV model, and beneficial effects of experimental treatment with different drugs^{54,55} and antioxidants^{56–58} were identified. The emerging new RHDV2 variant, which differs from RHDV in terms of duration of induced disease, mortality rates and higher occurrence of subacute/chronic forms⁵⁹, opens the possibility to establish rabbit models for chronic liver diseases.

Syphilis

Since, the identification and isolation of *Treponema pallidum* subsp. *pallidum* (*T. pallidum*) as the causative agent of syphilis⁶⁰, the rabbit has been the model of choice for the study of the infection and for propagation of this uncultivable bacterium. Rabbits can be readily infected with *T. pallidum*⁶¹, which is perhaps related to their susceptibility in nature to a very closely related bacterium, *Treponema paraluis-cuniculi*, which is sexually transmitted. The clinical, histological, and immunological similarities between syphilis infection in rabbit and human hosts are striking. Intradermal inoculation of rabbits with *T. pallidum* results in the development of lesions that strongly resemble human primary chancres both clinically and histologically^{62,63}, with CD4+ and CD8+ T lymphocytes and macrophage infiltration. As observed in humans, the primary chancres in rabbits resolve spontaneously, with subsequent development of a disseminated secondary stage rash and early invasion of the central nervous system. Rabbits mount an immune response similar to that developed by humans during natural infection⁶³, with recognition of the same subset of antigens and the same mechanisms of bacterial clearance. As in humans, following long-term infection, rabbits develop immunity to reinfection⁶². Rabbit size facilitates the study of dissemination of the bacteria to distant tissue sites⁶⁴, and rabbits provide models of congenital and neurosyphilis^{65–67}. Because *T. pallidum* cannot be cultured, rabbits are also critical for the isolation of new *T. pallidum* strains from clinical samples. Although some other animal species can be infected with *T. pallidum* (non-human primates [NHP], hamsters, guinea pigs, and mice^{62,68–70}), only rabbits and NHP develop clinical disease similar to that in humans. Despite the lack of inbred rabbit strains and the dearth of immunological reagents, the rabbit model continues to be the most widely used by syphilis investigators to deepen our understanding of syphilis pathogenesis⁷¹, evaluate the efficacy of new

therapies⁷², and test the protective ability of novel vaccine candidates⁷³.

Tuberculosis

Rabbits were used in Robert Koch's original experiments to establish *Mycobacterium tuberculosis* (MTB) as the causative agent of human tuberculosis (TB)⁷⁴ and extensively utilized thereafter. Experiments of both MTB infection, to which rabbits are relatively resistant, and *Mycobacterium bovis* (MBO) infection, which is much more virulent in rabbits, were pursued in great detail by Lurie in a cohort of 'resistant' and 'susceptible' rabbits⁷⁵. These seminal studies laid the foundation for future research on the host genetic predisposition to mycobacterial infections. The New Zealand White (NZW) partially inbred European rabbit strain developed by J. Thorbecke was also susceptible to MTB infection⁷⁶; this breed was used for the rabbit genome sequencing project (OryCun2.0). After the unexpected extinction of these rabbits, outbred New Zealand White rabbits were the most commonly used breed for TB research to model the human pathology of pulmonary active/cavitary TB (PTB), non-progressive latent MTB infection (LTBI), spinal TB and tuberculous meningitis (TBM)^{77–81}. Intrathecal or intracisternal infections of rabbits with virulent MTB or MBO cause progressive disease pathology in the brain, ultimately resulting in encephalopathy and paralysis characteristic of TBM^{78,82}.

The primary advantage of rabbit models over the mouse model of TB is the maturation of inflammatory leukocytic foci into organized granulomas following aerosol infection. These lesions often undergo caseating necrosis, the pathologic hallmark of TB, and can develop fibrosis and/or cavitation, in addition to mineralization depending on the bacterial strain used for infection^{77,81,83,84}. In contrast to most mouse models, necrotic granulomas in rabbits also develop hypoxic microenvironments⁸⁵, which can serve as models for drug exposure studies.

The outcome of pulmonary infection in outbred NZW rabbits is also dependent on the nature of the infecting MTB strain. Whereas infection with hypervirulent MTB strains of the W-Beijing lineage cause cavitary TB, other strains, such as the hyperimmunogenic CDC1551, cause a range of disease presentation, including LTBI, which can reactivate upon immune suppression treatment^{81,86}. The ability of rabbits to control MTB infection, establish LTBI and reactivate to active disease upon immunosuppression provides a unique model for studies of the regulation of latency, reactivation, and immune reconstitution syndromes similar to those observed in human immunodeficiency virus infection^{77,79,81}.

The rabbit model has helped to elucidate the pharmacologic properties of standard and new/novel anti-MTB compounds, demonstrating a similar drug distribution

and pharmacokinetic/pharmacodynamic properties as in studies in humans undergoing lung resection surgery^{87–91}. Immune-modulating host-directed adjunctive therapy has emerged as a novel approach to improve TB treatment. Several proof-of-concept studies conducted in rabbit models of PTB and TBM demonstrated that these drugs not only improve bacterial killing but also minimize the disease pathology and restore organ and vasculature function^{92,93}. Rabbit models have also been used to test the efficacy of TB vaccines, including BCG, *M. vaccae*, *M. microti* and MTB fusion proteins in protecting against MTB challenge^{82,94,95}.

In summary, the rabbit model of TB has great histopathologic similarity to human disease. Therefore, this model will continue to play a vital role in deciphering the intricate pathogenesis of various forms of human TB and in devising better intervention strategies.

Human papillomaviruses (HPV)

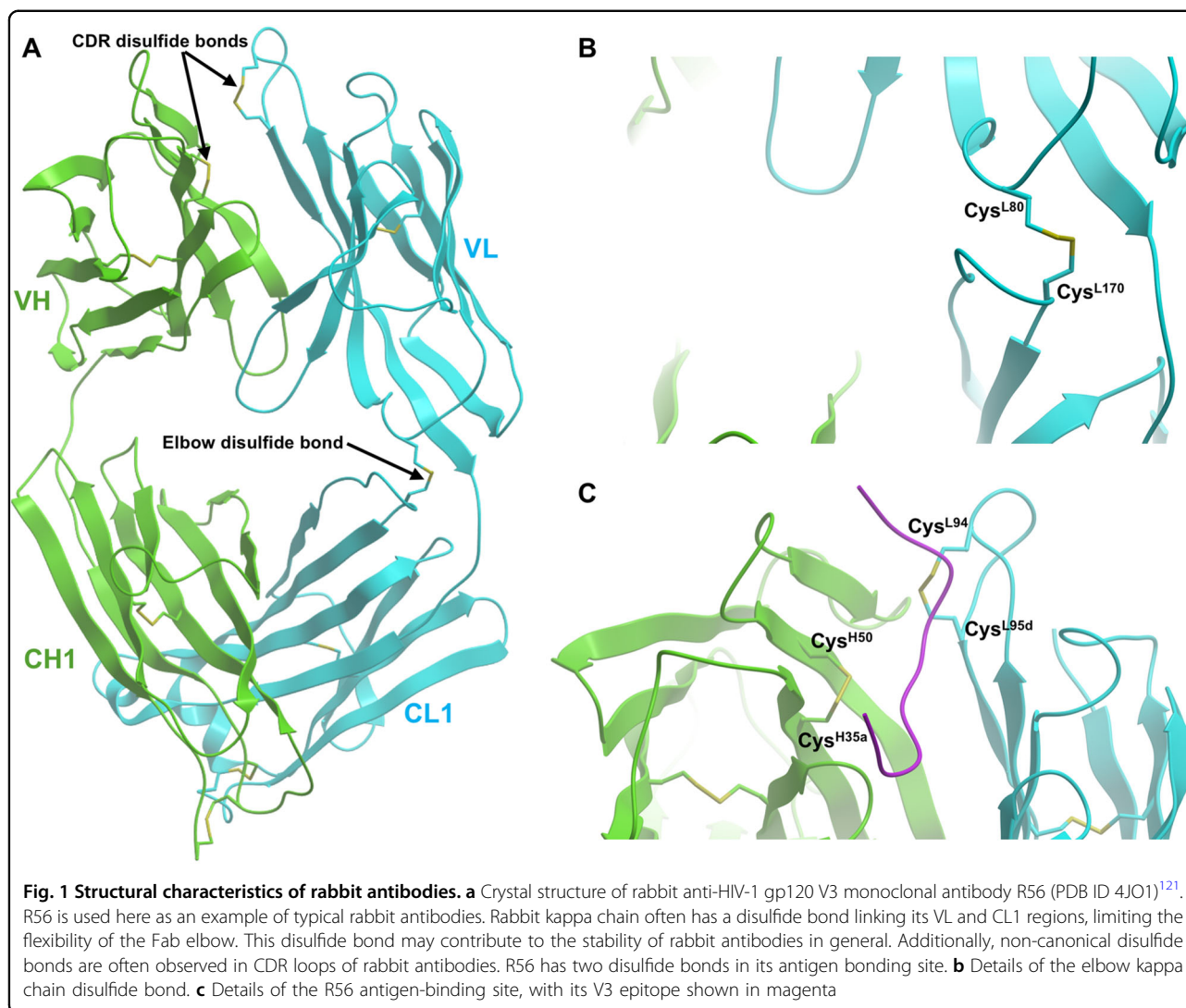
HPV are important viral pathogens that cause a variety of mucosal infections of the anogenital and oral mucosa. These infections can lead to epithelial cancers at these mucosal sites. Papillomaviruses (PVs) are species-restricted such that HPVs cannot be directly studied in pre-clinical laboratory animal models. Two rabbit papillomavirus models have been used extensively to study various aspects of papillomavirus biology, including vaccine testing⁹⁶, anti-viral treatments⁹⁷, papillomavirus biology⁹⁸, and latent viral infections⁹⁹. The viruses include cottontail rabbit papillomavirus (CRPV), which is a cutaneous-tropic virus whose lesions spontaneously progress to cancer, and rabbit oral papillomavirus (ROPV), which is a mucosa-tropic virus that induces oral infections. Numerous viral mutant genomes⁹⁸ and a unique HLA.A2.1 transgenic rabbit line¹⁰⁰ have been developed to study host immune responses to viral infection, therapeutic T-cell-based vaccines and host anti-CD8 immunity to viral proteins.

Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV-1) is the causative agent of AIDS. The development of a permissive, readily available, immunocompetent small animal model for the study of HIV-1 transmission and pathogenesis and the testing of antiviral strategies has been hampered by the inability of HIV-1 to infect non-human cells productively. Over the last 20 years, multiple species-specific barriers to HIV-1 replication have been identified in mouse, rat, and rabbit cells^{101–107}, with some caused by non-functional cellular cofactors or potent antiviral restriction factors that directly target the incoming virus (e.g., HIV-1 capsid by rabbit TRIM5-alpha¹⁰⁶) or cannot be antagonized by the accessory proteins of HIV-1 (e.g.,

rat and mouse CD317/tetherin by HIV-1 Vpu¹⁰⁸ or rabbit APOBEC1 by HIV-1 Vif¹⁰⁹). This knowledge has fueled strategies to generate genetically modified small animals in which transgenesis, knock-in, and knockout approaches combined with limited modifications to HIV-1 may allow the virus to overcome species-specific limitations and render the rodent or lagomorph host fully permissive to infection by this pathogenic human lentivirus. In contrast to several thus far unresolved replication defects in the late phase in mice¹¹⁰ and, to a lesser extent, in rats¹⁰⁴, primary T cells and macrophages from rabbits impose only three apparent barriers to HIV-1 replication: virion entry, which can be overcome by coexpression of the HIV-1 receptor complex composed of human CD4 and CCR5^{107,111}; reverse transcription, which can be ameliorated by depletion of TRIM5-alpha or modifications of HIV-1 *gag*; and a cell type-specific infectivity defect of HIV-1 virions released from macrophages, the cause of which is still unclear¹⁰⁷. This knowledge combined with recent advances in knock-in and knockout technologies^{112,113} and the overall suitability for vaccine and drug studies makes the rabbit species an attractive candidate for the generation of a fully permissive animal model of HIV-1 infection.

Additionally, the rabbit model has been used extensively in developing an HIV vaccine. In contrast to other small animal models, such as mouse or rat, the rabbit model provides several advantages that include ease of induction of high-titer, high-affinity antigen-specific antibody responses to almost any type of antigen and very low non-specific responses. Consequently, rabbit immune sera can be used for a wide range of assays, including ELISA, western blots, and tests for functional antibody responses such as neutralizing antibodies^{114–120}. The rabbit model was used first to study polyclonal antibody responses and established the immunogenicity of DNA immunization as a novel immunization method¹²¹. Subsequently, a DNA prime-protein boost approach was developed¹²², and it was established that this approach elicited neutralizing antibodies against a difficult virus¹²³, improved induction of antibodies against key neutralizing epitopes, and improved activity and avidity^{124–126}. More recently, a panel of novel rabbit monoclonal antibodies (mAbs) against HIV-1 Env antigen was produced, and the crystal structures for some of these rabbit mAbs were obtained (Fig. 1)^{127–129}. These studies also demonstrated that the overall structures of rabbit mAbs are very similar to those of human mAbs against the same epitopes. This is highly significant considering that the mechanisms to diversify these two types of mAbs are very different. Overall, rabbits have been an excellent animal model to study the immunogenicity of HIV-1 DNA vaccines, including the production and analysis of HIV-1 specific mAbs¹³⁰.



Ocular herpes infection and immunity

Most of the potentially blinding, recurrent herpes stromal keratitis (rHSK) in latently infected humans occurs following spontaneous reactivation of HSV-1 from latently infected sensory neurons and virus shedding in tear film^{131–135}. However, unlike in latently infected humans, spontaneous HSV-1 reactivation and virus shedding in tears occurs at very low levels or not at all in latently infected mice^{132,135,136}. Despite this fact, most pre-clinical animal studies investigating the cellular and molecular mechanisms that orchestrate rHSK have used a mouse model of primary acute infection¹³⁶. To avoid inherent drawbacks in the mouse model of primary acute infection, an alternative HLA transgenic rabbit (HLA Tg rabbit) model in which HSV-1 reactivation and virus shedding in tear film occur spontaneously was developed¹³⁷. One major component of the immune system in these HLA Tg rabbits is replaced by the identical

component taken from a human counterpart (i.e., HLA-A*0201 class I molecules)^{137–143}. This HLA Tg rabbit model is capable of mounting “human-like” CD8⁺ T-cell responses specific to human HLA-A*0201-restricted epitopes. The immunopathology of rHSK in the HLA Tg rabbit mimics the immunopathology of human rHSK that occurs after episodes of spontaneous HSV-1 reactivation (Fig. 2). Moreover, this HLA Tg rabbit model allows pre-clinical investigation of the role of HLA-restricted CD8⁺ T-cell responses specific to human epitopes in reducing spontaneous reactivation of HSV-1, and assessment of the immunotherapeutic efficacy of human CD8⁺ T-cell epitope-based vaccines against rHSK^{137–143}. Recently developed immunological reagents (e.g., mAbs specific for immune molecules and human tetramers) have allowed analysis of rabbit HSV-specific CD8⁺ T cells’ phenotype, function, and the localization of CD8⁺ T-cell infiltrates in infected cornea and trigeminal ganglia

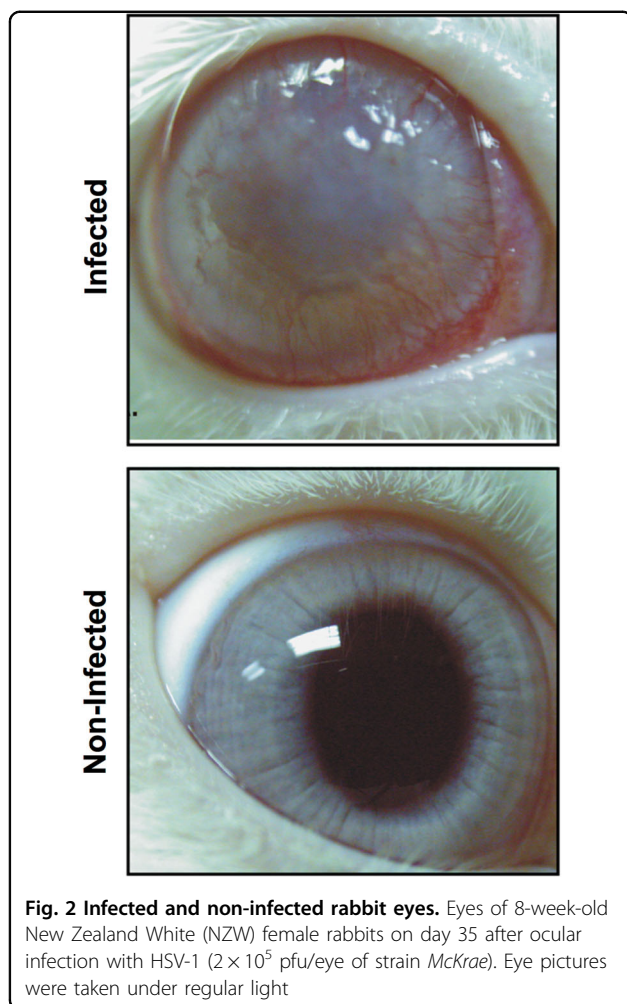


Fig. 2 Infected and non-infected rabbit eyes. Eyes of 8-week-old New Zealand White (NZW) female rabbits on day 35 after ocular infection with HSV-1 (2×10^5 pfu/eye of strain *Mckrae*). Eye pictures were taken under regular light

(the site of latency/reactivation cycles) of infected HLA Tg rabbits¹³⁰. The striking similarities between the HSV-1-infected HLA Tg rabbit model and HSV-1 seropositive humans in terms of ocular herpes infection and immunity make the HLA Tg rabbit a preferred model to study the role of CD8⁺ T cells in controlling spontaneous HSV-1 reactivation and recurrent HSK^{130–137}.

Conclusions

Studies using the European rabbit contributed to elucidating numerous fundamental aspects of antibody structure and diversification mechanisms and continue to be valuable for the development and testing of therapeutics. Rabbits have also served as important, reliable models for the understanding of human infectious and non-infectious diseases, including tuberculosis, syphilis, and papilloma-, herpes-, pox-, and norovirus infections, and have been adapted to advance the understanding of the immune response to HSV-1 and HIV-1 to inform immunotherapy and vaccine development.

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