Review Article

The tree shrew as a model for infectious diseases research

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Abstract: Despite major advances in medicine, infectious diseases still pose a significant threat to humanity. Mammalian models of disease have proved extremely useful in adding to the understanding of infectious diseases and the development of prophylactic and/or therapeutic interventions. Arguably the most important considerations of any animal model are (I) the similarity of the model to humans with respect to anatomy, physiology, immunology and disease progression, and (II) the expense of conducting experiments using the model organism. Often the choice of a model represents a compromise between these factors. Here we review the Northern Tree shrew (*Tupaia belangeri*), or tupaia, as a useful model for the study of infectious diseases. Tupaias are non-human primates similar in size to squirrels that are indigenous to Asia. Their genome has been sequenced and, overall, shows relatively high similarity to humans. There is also a close homology of many aspects of tupaia biology with human biology. Importantly, from an infectious diseases viewpoint, tupaias are susceptible to infection with unadapted human pathogens and manifest clinical signs akin to human infections. Overall, the relatively small size of the tupaia, their homology to humans and their susceptibility to human pathogens make them a useful model for the study of infectious diseases.

Keywords: Tree shrew; respiratory viruses; infectious disease

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Introduction

Infectious diseases are still a threat to humanity, accounting for approximately 23% of annual human mortality globally (1). Limited interventions and the propensity for genetic diversity and adaptation inherent in many pathogens increase the complexity and difficulty of this problem. Mammalian models have proven invaluable in the study of infectious diseases and the development of interventions. The utility of mammalian models of human disease rests on several factors. Arguably the most important of these are (I) the similarity of the model to humans with respect to anatomy, physiology and immunology and disease progression, and (II) the expense of conducting experiments using the model organism. Often the choice of a model represents a compromise between these factors. For example, the mouse model is a commonly used mammalian model due to the comparatively low costs associated with it and the wide availability of reagents and techniques available. However, not all aspects of the murine immune system are similar to those of humans (2). Further, the mouse is not suitable for all infectious diseases research due to altered susceptibilities compared to humans (3,4).

Another commonly used animal model is the ferret, particularly in the study of respiratory viruses such as influenza virus. This is because the types of sialic acids, the glycans that influenza viruses bind to initiate infection, are similar in humans and ferrets. Further, ferrets experience similar symptoms of influenza to humans. However, ferrets are significantly more expensive than mice and there is poor availability of reagents to study the immune response (5). Of all animal models, non-human primates are, overall, the closest to humans biologically and have proved to be a useful, accurate model of several infectious diseases, including influenza, hepatitis, metapneumovirus, yellow fever and dengue hemorrhagic fever (6). However, high costs limit the routine use of larger non-human primates. Smaller non-human primates share much of their biology with larger primates, including humans, but their smaller size means that they are less expensive to use. As such, they represent a useful animal model.

One of the smaller non-human primates is the Northern Tree shrew *(Tupaia belangeri)*, or tupaia. These are small squirrel-like mammals indigenous to southwest Asia (7). The tupaia genome has been sequenced and, overall, shows higher similarity to humans than rodents and there is also close homology of much of their biology with humans (8). Importantly, from an infectious diseases viewpoint, tupaias are susceptible to infection with unadapted human pathogens and manifest clinical signs akin to human infections (9,10). Here we review the anatomic features and immune system of tupaias, focusing on human infectious diseases caused by bacterial and viral pathogens, for which tupaias have served as models in recent years.

The tupaia anatomy

Overall the tupaia anatomy is similar to that of other non-human primates, with some differences. The tupaia lung has three lobes on the left side termed left cranial, left middle and left caudal lobes, and four on the right termed right cranial, right middle, right caudal and right accessory lobes, whereas human have three right lobes and two left lobes (11). Compared with humans and monkeys, tupaias have longer respiratory bronchioles, richer mucosal folds in terminal bronchioles and thinner blood-air barriers, suggesting active pulmonary gas exchange (12). The tupaia liver is divided into left, central, right and caudate lobes, with the gallbladder sitting on the central lobe (13). Human livers also have four lobes but the gallbladder sits beneath the right lobe of the liver. The central nervous system of tupaias show a rudimentary gyrus and the tupaias cerebrum are well developed, particularly the posterior lobe, which is more advanced compared to an infant monkey (14). The tupaia gastrointestinal tract is somewhat simpler compared to a human, as it lacks a pyloric canal and the boundaries between the duodenum, jejunum, and ileum are vague (13). The tupaia colon is slightly larger than other insectivores and connoted with a primitive small cecum (15,16). Overall, the types, distribution and relative frequencies of gut endocrine cells in the tupaia are similar to humans and other mammalian species (17).

The tupaia immune system

The immune cell populations present in human blood all have homologues in the peripheral blood of tupaia, with overall percentages of macrophages, granulocytes, lymphocytes and monocytes similar between humans and tupaia (18).

Lymphocytes

Two important T lymphocyte differentiation molecules, CD4 and CD3ε, have been identified in the tupaia. Tian *et al.* cloned the full-length coding sequence of the tupaia CD4 (tCD4) and demonstrated that the tCD4 amino acid sequence shared high similarity to human and macaque (19). Whilst similar, differences in negative charges and N-glycosylation sites in the CD4 extracellular domain D1, thought to contain the antibody binding site, are evident between human and tupaia (19). Therefore, there is no cross-species reactivity of mouse anti-human CD4 antibody to tCD4 (19). Similarly, the potential glycosylation sites in tupaia CD3ε (tCD3ε) differ from human CD3ε and surface charges in the extracellular domain are also different, meaning antibodies against human or mouse CD3ε are not reactive against tCD3ε (20). In the cytoplasmic domain, the tyrosine protein kinase-binding residues (KKTCQC) in CD4 play a pivotal role in TCR-mediated signal transduction and activation (19). This region is conserved between tupaias and humans. The tupaia CD3ε cytoplasmic domain required for TCR/CD3 complex-mediated signal transduction also shares a similar structure in to its human counterpart (20).

Major histocompatibility complex (MHC)

MHC class I and class II molecules expressed by nucleated cells play an important role in the presentation of peptides

(e.g., viral proteins in virus-infected cells) at the cell surface to $CD8^+$ and $CD4^+$ T cells, respectively (21). The tupaia encompasses a unique MHC class I locus and a MHC class II region homologous to all human class II genes (8). The MHC class III is conserved in human and tupaias except for the C4 region, of which humans and mice have two copies whereas tupaias have one (8).

Pattern recognition receptors (PRRs)

Recognition of invading pathogens by germ line-encoded PRRs is the first step in the activation of an antimicrobial immune response. Toll-like receptors (TLRs) are membrane bound receptors that sense lipids and nucleic acids of pathogenic microorganisms and play a key role in detection of distinct evolutionarily conserved structures on pathogens, termed pathogen associated molecular patterns (PAMPs). Differences in TLR composition among human, rodents and tupaias have been reported. For instance, there are 10 functional TLRs (*TLR1-TLR10*) in humans and 12 functional TLRs (*TLR1-TLR9* and *TLR11-TLR13*) in mice (22,23). Thirteen tupaia TLRs (*tTLR1-tTLR13*) have been identified, although *tTLR10* appears to be a pseudogene, as it is in rodents. These observations indicate that tupaias have closer relationship to rodents than humans in TLR composition (23). However, after hepatitis C virus (HCV) infection of tupaias, mRNA transcripts of *tTLR2*, *tTLR3*, *tTLR4* and *tTLR8* in tupaia primary liver-derived cells were significantly altered, with a pattern consistent to that of human hepatocytes (23). However, the downstream cytokines and chemokine profile associated with tTLR recognition remain unknown. Retinoid acid-inducible gene I (RIG-I)-like receptors (RLRs), including RIG-I and melanoma differentiation-associated gene 5 (MAD5), are key cytosolic sensors of PAMPs and are responsible for type I interferon (IFNs) induction. The tupaia has a defective RIG-I and alternatively uses MAD5 and LGP2 to sense Sendai virus for inducing type I IFNs by interacting with the RIG-I binding adaptor tMITA (24).

In chicken, it is well proved that the absence of RIG-I weakens IFN response contributing to severe disease after influenza virus infection (25). These results provided additional knowledge on evolutionary adaptation and functional diversity of antiviral activity in vertebrates. Mitochondrial antiviral signaling protein (MAVS, also known as IPS-1, Cardif or VISA), another component coupled to the activation of RIG-I and MDA5, are expressed variously in tissues of tupaias and share close homology to those in primates (26). The function of tupaia MAVS shows homology to other mammals, as it can potentiate the virally triggered activation of IFN regulatory transcription factor 3 (IRF3), nuclear factor-κB (NF-κB) and IFN-β (26).

Cytokines

After a high quality tree shrew genome was released, Fan *et al.* created the tupaia database, facilitating understanding of mRNA expression of a number of cytokines and chemokines (27). Based on the Tupaia genome, Li *et al.* predicted the presence of IFNs in the tree shrew (tIFNs), identifying five subtypes of type I IFN (α, β, ω, κ, ε and δ), one type II IFN (γ) and two type III IFNs (λ1, λ2/3) (28,29). Except for small differences in cysteine positions and N-glycosylation sites, the predicted structures of tIFNα and β are close to their human counterparts (28). The structure of the tIFNλ2/3, and tIFN3 receptor subunits, tIFNλR1 and tIL10R2, also share similar features to their human counterparts (28).

Interleukin-7 (IL-7) plays a role in modulating T cell homeostasis and overcoming chronic viral infections (30,31). The splicing region of transcripts tIL7-sv2, tIL7-sv4 and tIL7-sv5 are homologous to human IL7δ5, IL7δ3/4 and IL7δ3/4/5, respectively (32). However, the tupaia has seven other transcripts (tIL7-sv1, tIL7-sv3, tIL7-sv6, tIL7-sv7, tIL7-sv8, tIL7-sv9, tIL7-sv10) of which human homologues are not present (32). The tTNF-α gene shows a relatively high similarity of 84.8% to the human homologue, and it was also demonstrated that tTNF-α was able to suppress HBV replication intermediates, which reflect observations in mice (33).

Use of tupaias in infectious disease research

Bacteria

Current researches into bacterial infections using the tupaia model are limited. Li *et al.* developed a burnt skin infection model of tupaia using *Staphylococcus aureus* and a dacron graft infection model of *Pseudomonas aeruginosa* (34). These bacteria cause a persistent infection in tupaia of up to 6 to 7 days, whilst *P. aeruginosa* inoculation does not produce symptoms in mice (34). Therefore, the tupaia appears to be a much better animal model compared to mice in this instance.

Influenza viruses

Influenza viruses are important respiratory pathogens. Several animal models have been used in various aspects of influenza virus research. Those most commonly used are mice and ferrets, however as mentioned in the introduction each of these models has their drawbacks. The suitability of tupaia as a model of human influenza was investigated using subtype H1N1 and H3N2 viruses. These studies showed that influenza virus-inoculated tupaias exhibited slight fevers, shed virus and seroconverted by day 21 post inoculation (35). They symptomology of the tupaia proved to be different to that of the ferret. Whilst ferrets become sluggish and display similar respiratory symptoms to humans, influenza virus-inoculated tupaias did not show symptoms and behaved comparably to uninfected tupaias (35). The ferret model is useful as, unlike mice, it can be used to study influenza virus transmission. As yet, influenza transmission studies have not been conducted using the tupaia. However, lectin histochemistry studies of the tupaia respiratory tract have revealed that the distribution and types of sialic acid linkages are similar to humans. Influenza viruses bind to sialic acids to initiate infections and it has been shown that they type of linkage is an important species barrier to influenza viruses, as avian viruses prefer binding to avian-type α2,3-linked sialic acids whilst mammalian adapted viruses prefer binding to mammalian-type α2,6-linked sialic acids. In humans and tupaias, α2,3-linkages are only found in the deep lung, whilst α2,6-linkages are more predominant in the upper respiratory tract (35).

Zoonotic transmission of influenza viruses is incompletely understood, however, molecular markers of adaptation for replication in mammals have been identified. One such marker is 627K in the polymerase basic 2 (PB2) gene of influenza viruses, which confers enhanced replication in mammalian cells. Studies on tupaias using duck-origin H9N2 influenza viruses containing or lacking PB2 627K revealed that the 627K mutation led to increased viral replication and disease severity in tupaias compared to viruses lacking 627K. The 627K mutation also resulted in greater pathology in the upper respiratory tract and increased expression of pro-inflammatory cytokines. These results further show that the tupaia is an accurate and useful model of influenza virus infection.

Adenoviruses

Adenoviruses are common infectious pathogens in both children and adults. They cause clinical manifestations including gastroenteritis, hepatitis, keratoconjunctivitis, meningoencephalitis, cystitis, upper and lower respiratory tract infections and myocarditis. They can spread among broad range of vertebrate host species and cause acute respiratory diseases in humans (36). The tupaia adenoviruses (TAdV) have been identified and shown to infect *Tupaiidae* spp. TAdV has been identified as belonging to a separate lineage within genus *Mastadenovirus* (36,37). Therefore, tupaia appear to be a useful model for adenovirus research.

Hepatitis viruses

Hepatitis viruses (HBV and HCV) cause acute and chronic hepatitis, which is one of the major health problems in the world. Chronic infection develops in most patients and can progress to serious liver disease (38-40). Primary tupaia hepatocytes (PTHs) are susceptible to HBV infection *in vitro* and in chimeric mice transplanted with PTH (41-43). The clinical courses of acute and chronic infections in tupaias are quite similar to those in humans (44,45). Persisting serum hepatitis B surface antigen (HBsAg) and piecemeal necrosis that underlie chronic HBV infection in humans are also observed in tupaia. Histopathological findings in the liver of infected tupaias showed similar pathological changes to those observed in humans. These included megalocytosis, which is associated with the risk of hepatocellular carcinoma, and 'ground-glass hepatocytes', which are HBsAg positive hepatocytes whose numbers are inversely proportional to the activity of hepatitis virus overall (45).

The disease course of chronic HCV in the tupaia is similar to that in humans, in that it is manifested by intermittent or persistent viraemia and lymphocyte infiltration in the liver (46,47). Long term studies of tupaia HCV infections of up to three years in length revealed histological liver disorders, including steatosis, fibrosis, and cirrhosis. However, HCV viraemia in tupaia was of lesser magnitude and duration compared to human cases, which was likely due to the somewhat more restricted replication of HCV in tupaia compared to humans (46,47).

Host factors associated with HCV susceptibility have also been studied in tupaia. The essential receptors for HCV entry are present in tupaia and show high sequence homology (87% to 96%) to human homologues. These receptors are CD81, scavenger receptor class B type I (SR-BI), claudin 1 (CLDN1), and occludin (OCLN) (45). Further, the four residues in the large extracellular loops

(LEL) of CD81 responsible for binding with HCV E2 protein, namely Ile182, Phe186, Asn184, and Leu162, are conserved between human and tupaia (48). HCV pseudoparticle replication are greater in tupaia CD81-expressing HepG2 cells compared to human CD81-expressing cells, likely due to differences outside the LEL (48). SR-BI, CLDN1 and OCLN have also been shown to be involved in HCV entry, as blockade of these receptors prevents HCV infection (48). Moreover, two miRNAs expressed in the liver have been shown to be 100% homologous between tupaia and humans. These miRNAs, miR-122 and Let-7 facilitate and inhibit HCV infection, respectively.

Herpes simplex virus (HSVs)

HSVs can infect the nervous system and result in lifelong chronic infections (49,50). Intravenous, intraperitoneal, or subcutaneous inoculation of HSV-1 or HSV-2 can be fatal in juvenile (28 to 45 days old) tupaias but not in adult tupaias, which also did not experience chronic infections (51). However fatal disease was reported both in juvenile and adult tupaias following intrahepatic transfection of HSV DNA (52). Intraocular inoculation of HSV-1 resulted in limited or undetectable replication in cells of the trigeminal ganglion region (53). This replication was much less than that observed in mice, indicating that tupaias could be a useful model for the study of HSV latency (53). Interestingly, inoculated tupaia showed robust expression of latency-associated transcript (LAT) genes, whilst LAT transcripts in mice were low by comparison. In this respect, tupaias are closer to humans than mice (53).

Viruses causing gastrointestinal disease

Enterovirus type 71 (EV71), coxsackie virus group A type16 (CA16), rotavirus and foamy virus all cause significant disease burden in many populations. EV71 and CA16 are the major causative agents of hand-foot-mouth disease (HFMD) worldwide (54). The natural route of infection of tupaia, oral and intranasal inoculation, with EV71 resulted in a disease course similar to that in humans and rhesus monkeys (55). The immune response and clinical course seen in inoculated tupaias were also close to that seen in humans and neonatal rhesus monkeys, including fever and lymphocyte-related inflammatory responses (55-58). However, HFMD symptoms were absent in tupaia. Pathological changes were observed in the brain, heart, lung, spleen and kidney of inoculated tupaias and high viral loads were found in multiple organs, including the central nervous system, and also in feces (57). The disease course following rotavirus inoculation in tupaias also shared many aspects of the disease course in human infants, including diarrhea, malaise, anorexia, dehydration and intestinal lesions (59-61). These are similar to those seen in infant mice and in contrast to adult mice, which lack diarrhea or lesions (59-61). Therefore, the tupaia appears a useful model for the study of viral gastrointestinal diseases.

Conclusions

Overall, every animal model of human disease has its strengths and weakness – there is no such thing as a perfect model. This is also true for the tupaia. However, the tupaia has proved to be a useful model for a diverse range of infectious diseases and exhibits disease courses similar to those seen in humans. Their small size and relative genetic closeness to humans means that they represent a good balance between cost and human similarity. The availability of the tupaia genome and the apparent cross reactivity of currently available reagents to other animal models further add to the usefulness of tupaias. Further investigations on the divergent functions of the immune system, including MHC, PRRs and other key classes of molecules, will likely add to the current understanding of host restriction factors and pathogenicity of infectious agents.

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Footnote

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