

Primate Protection

Nirmala Bhogal and Michelle Hudson of the Fund for the Replacement of Animals in Medical Experiments (FRAME) discuss the use of primates for medical research and the subsequent implications for animal welfare and the clinic

Between 100,000 and 200,000 non-human primates (NHPs) are used worldwide each year for preclinical research and testing (1,2). Around two thirds of these animals are Old World monkeys (mainly macaques) and around 10 per cent are apes that are used in live experiments or as a source of research tissues. A further 15 per cent are New World species, predominantly marmosets, with the remainder of NHPs being lower order primates. The US, UK, France, Germany and Japan account for the use of most of these animals, and this aligns closely with the level of pharmaceutical research and vaccine development in these countries. This level of NHP usage is disconcerting from an animal welfare perspective. A number of recent reports suggest primate experimentation also raises false hopes and assurances as to the efficacy and safety of many treatments and prophylactics developed as a consequence of using these species during the preclinical phase. Here, the perceptions and reality of primate experimentation are contrasted, and questions are raised as to whether primate experimentation is in keeping with the current state of knowledge and the evolving healthcare market.

PRIMATE EXPERIMENTATION: PERCEPTION VERSUS REALITY

Within the EU, around 80 per cent of the NHPs used in experiments are used for the evaluation of medicines and vaccines or to understand the mechanisms of human disease and infection. The general perception remains that NHPs, and in particular Old World species and apes, are the most relevant models of human physiology and pathophysiology because they are phylogenetically the most closely related of all the laboratory species. The outcomes of a number of genome projects have reinforced the perception that regulators will insist on primate experimentation and are closed to dialogue to the contrary. However, even though our closest evolutionary relations – chimpanzees and bonobos – are 99.1 per cent identical to us when the protein-encoding genomes are

compared to that of humans, studies suggest that the slight genetic differences not only influence the physical distinction between humans and other primates, but also greatly reduce the physiological significance of NHPs as models of humans. For instance, the DNA sequence differences, while small, alter the sequence of protein-encoding genes and patterns of gene regulation and cause differences in DNA structure such that the patterns of DNA expression within specific tissues can be dramatically different in chimpanzees and humans (3). Furthermore, DNA re-arrangements, insertions and deletions (indels) can alter epigenetic regulation of genes significantly, such that dramatic differences in spatial expression of key functional and structural proteins are inevitable.

These findings are of particular significance in neurobiology. A recent study identified differences in the expression levels of 169 proteins from a broad range of functional classes within the cerebral cortex of chimpanzees and humans. Since humans





appear to express higher levels of many brain-specific proteins, such variations could form the basis for the enhanced neural activity and networking, and cerebral physiology and function seen in humans in contrast to that seen in chimpanzees and other primates (4). Indeed, the expression of genes involved in aerobic respiration and neuronal function is higher in humans than in other primates, including (to a limited extent) chimpanzees (5). Hence, differences between humans and other primates are substantially greater than originally suggested, to the extent that using primates for fundamental behavioural, learning, association, memory and other cognitive function studies provides information which cannot always be readily extrapolated to humans.

NHPs are used in some areas of research and have been considered a source of transplantable organs because of anatomical similarities with humans. For instance, human and NHPs possess forward-facing eyes and chromatic vision, unlike other vertebrate species. This has culminated in the use of several species of NHPs in vision research. Yet, over the years, it has unfolded that there are important differences between the function and neuronal organisation within the visual centres of the brains of humans and NHPs that suggest the use of primates in vision research is not scientifically justified. These include the fact that New World monkeys only display dichromatic vision, rather than trichromatic vision.

Primates are also used in the development of centrally active therapies. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) safety guideline S6 states that preclinical trials of a pharmaceutical should be performed in a species that expresses homologues of human proteins, and possesses tissues that show a similar cross-reactivity profile to that of humans. This lends itself to the thinking that NHPs are superior to other, less developed organisms. However, similarity alone does not form a sufficient basis for assuming that primates are the most suitable models for humans. Single amino acid differences in protein sequences, small changes in expression levels, or slight differences in biochemical pathways relating to drug action or distribution, can have dramatic consequences in terms of pharmacology. This is widely recognised by researchers within the fields of cell surface receptor and channels research and by those engaged in understanding the role of metabolism in determining drug efficacy and safety. For instance, the resistance of the squirrel monkey to glucocorticoids is caused by site-specific alterations in the amino acid sequence of its equivalent human glucocorticoid receptor, and similar factors account for the differences in chimpanzee and human responses to the hallucinogen phencyclidine (PCP). It is perhaps then not surprising that key reasons for drug attrition have changed very little over

the last 30 years or so (6). After all, we are still essentially using the same animal models as we were when modern medicine came to fruition some 50 years ago. In fact, CNS medicine is one of two areas (the second being oncology) where animal models are notoriously unproductive of patient outcomes.

PRIMATES: THE DEFAULT SPECIES?

One of the most rapidly growing areas of NHP use is in the study of infectious diseases towards the management of both perceived and real pandemics. Perhaps the biggest challenge presented in the last century was the Aids pandemic. Here, we see that a large body of research that has involved the use of NHPs has been largely fruitless. Remarkably, this has not entirely deterred the use of these animals, and neither have the enormous logistical problems of caring for infected animals. In fact, rhesus and cynomolgus macaques have been used in Aids research for over 20 years, even though they cannot be infected with the HIV virus. Instead, these animals are used to study the mechanisms of SIV infection towards understanding the mechanisms of HIV transmission and Aids development in humans. There is clearly a fundamental flaw to this approach, since not only do the immune systems of macaques and humans differ, but also because HIV-1 and SIV share less than 60 per cent genetic identity, and this has already been shown to influence how the two viruses interact with the macaque and human immune systems (7-9). It is perhaps not surprising then that DNA-based vaccines that have produced encouraging results in NHPs have not yet been successful in humans (10). More disturbingly, chimpanzees continue to be used in Aids research, even though it is now recognised that the reason why several vaccines that appeared effective at preventing Aids in chimpanzees were unsuccessful in humans stemmed from the fact that there are crucial differences between the immune systems of the two species; these differences slow viral replication in chimpanzees but not in humans (this also has a bearing on the development of immunomodulatory therapeutic entities as explained below). The use of these animals in this way cannot be justified when *in vitro* studies of human cells and tissues have enabled the investigation of the immunogenicity of potential vaccines and the analysis of HIV transmission (11-13).

Within Europe, Directive/96/609/EEC, which sets out the framework that guides the use of animals in experiments in Europe, has been under discussion and subject to revisions. The final decision as to the content of an updated Directive that replaces Directive 86/609/EEC is currently taking place at Commission level. Of widespread concern is a specific provision within the revisions that allows Great Apes to be used in the future to preserve the species in question, or human life, should there be an unexpected outbreak of a life

threatening or debilitating clinical condition (the so-called Safeguard Clause). Severe acute respiratory syndrome (Sars) was assumed to satisfy these criteria and was at one time considered the first emerging epidemic of the 21st century. The genetic code of the coronavirus (CoV) responsible for Sars has been cracked and used to guide vaccine development. In 2003, NHPs were reported to be susceptible to Sars CoV infection (14,15). Indeed, the lungs of infected cynomolgus and rhesus macaques exhibit lesions consistent with infection, and also present antibodies and cytotoxic T cells towards Sars CoV. Yet these animals do not develop clinical signs of infection, nor do they transmit the infection. This reactionary work resulted in many macaques potentially carrying the virus and spurred on research which has culminated in NHPs being used as default species for Sars research. In fact, it has been proven that ferrets might provide the most accurate model of human Sars infection and transmission between humans. Furthermore, various Sars CoV strains can be propagated in Vero cells (16). The question then remains as to whether it is scientifically valid or ethically acceptable if the Safeguard Clause means that NHPs are the acceptable default when it comes to studying and developing treatments to manage other suspected pandemics. Sadly, Aids and Sars are not the only pandemics that have resulted in the kneejerk response of using primates. In June 2009, the World Health Organization declared that a new strain of influenza A (H1N1) as the causal agent of a new emerging pandemic. Within weeks, researchers in Japan and the US had demonstrated infection in macaques towards development of a treatment. Again, other species, including the ferret, and more importantly, *in vitro* models, are available to study swine flu infections (17). Once again, the perceived pandemic might not be a pandemic at all, given that the UK Chief Medical Officer Sir Liam Donaldson made a statement on the 11 December 2009 that suggests swine flu isn't as deadly as once thought.

There are occasions when a human pathogen will only infect NHPs and no other species. In these instances, there is a reluctance to consider alternatives to using primates founded on the assumption that no other model will suffice. For instance, NHPs are the only experimental species that can be successfully infected with the poliomyelitis virus. This has resulted in the extensive use of macaques for batch testing and vervet monkeys for production of polio vaccines (18). The oral polio vaccine (OPV) is currently tested in primates to ensure that it has not regained neurovirulence with a single batch of vaccine requiring the use of around 110 monkeys. In 1999, the WHO Recommendations for OPV testing were revised so that transgenic mice can replace primate-based neurovirulence testing for type 3 OPV and mutant analysis by PCR and restriction enzyme cleavage (MAPREC) can be used for DNA analysis to determine the molecular consistency of live viruses isolated from cell cultures. Yet, despite obvious cost savings and a dramatic reduction in the number of primates required for neurovirulence testing, primates are still considered to be the gold standard OPV model.

TGN1412: A LESSON LEARNED?

In 2005, six otherwise healthy male volunteers were administered a monoclonal antibody, TGN1412, during a first-in-man study. Within minutes, all six volunteers displayed severe adverse reactions consistent with a massive cytokine storm. TGN1412 is a superagonistic T cell stimulatory antibody designed to by-pass the inhibitory control of T cell activation. When tested in two different species of macaques, this antibody did not result in a cytokine release profile that could have predicted what would happen in humans. An extensive enquiry culminated in a number of findings, amongst which was the acceptance that a smaller T cell niche in macaques and other NHPs was almost certainly responsible for the much lower potency of TGN1412 in NHPs than in humans (19). Thus, receptor occupation and downstream activation appear as important as the presence of an equivalent target epitope (20). The culmination of these events has resulted in the drafting of a new ICH guideline for the conduct of clinical trials for biotherapeutics with novel mechanisms of action (21).

The TGN1412 case also highlights that, regardless of similarity, NHPs are not infallible models of the human immune system. This presents a major challenge for the development of biotherapeutics, with their inherently greater propensity towards triggering immunogenicity. Alternatives are emerging; several groups are already working on either developing complex interaction models capable of capturing elements of lymph and peripheral immune responses. One such model (Vaxdesign's MIMIC™ system) is currently being used to examine TGN1412 and determine whether a human blood cell-based system that is already capable of capturing population differences in immunogenicity of vaccines would have predicted the clinical trials outcome in a way that was not possible using macaques. *In vitro* systems of this kind hold enormous promise in that they not only allow a mechanistic understanding of disease processes and therapeutic interventions, but they will almost certainly assist with the development and qualification of markers that can assist with stratification of Phase II clinical trials in patients – the stage at which the greatest levels of preclinical attrition are reported for therapeutic agents and vaccines (22).

There was another very important lesson to be derived from the unfortunate TGN1412 incident, namely that the design of first-in-man studies must take into account how much (or little) we know about the mechanism of action of a new therapeutic entity and be designed with the precautionary principle firmly in mind. The rapid sequential administration of TGN1412 to all six volunteers failed to take into account the delay time between administration and elevation of key pro-inflammatory cytokines as seen during the macaque study. Hence, while in some cases primate studies might be warranted, it is as important to pay attention to the detail and ensure that clinical trials are designed to take into account delayed onset of action.

The value of hindsight should not be underestimated and it is encouraging to see that pharmaceutical companies are beginning to make their clinical data available. However, unless we understand the limitations of the models that are used to develop new prophylactics and treatments, the prospects for improving the predictivity of preclinical studies are bleak. A failure to revisit and revise existing drug development paradigms will not only result in higher costs, but also in many unnecessary animal studies. It is already recognised that we are entrenched in a process of therapeutics development that is fraught with problems, and that this is an unsustainable long-term approach. There are alternatives to using NHPs; only once we begin to explore these alternatives can we hope to reduce drug attrition rates, overcome barriers to vaccine development and deliver the healthcare that has been promised.

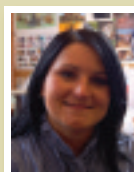
References

1. Carlsson HE, Schapiro SJ, Farah I and Hau J, Use of Primates in Research: A Global Overview, *American Journal of Primatology* 63: pp225-237, 2004
2. Hagelin J, The Use of Live Apes in Research in the Twenty-first Century, *ATLA* 33: pp111-118, 2005
3. Watanabe H *et al*, DNA sequence and comparative analysis of chimpanzee chromosome 22, *Nature* 429: pp382-388, 2004
4. Caceres M, Lachuer J, Zapala MA, Redmond JC, Kudo L, Geschwind DH, Lockhart DJ, Preuss TM and Barlow C, Elevated gene expression levels distinguish human from non-human primate brains, *Proceedings of the National Academy of Sciences USA* 100: pp13,030-13,035, 2003
5. Uddin M, Wildman DE, Liu GZ, Xu WB, Johnson RM, Hof PR, Kapatos G, Grossman LI and Goodman M, Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles, *Proceedings of the National Academy of Sciences USA* 101: pp2,957-2,962, 2004
6. Reynolds PD, Pittler SJ and Scammell JG, Cloning and Expression of the Glucocorticoid Receptor from the Squirrel Monkey (*Saimiri boliviensis boliviensis*), a Glucocorticoid-Resistant Primate, *Journal of Clinical Endocrinology and Metabolism* 82: pp465-472, 1997
7. Bende S and Johnston MI, Update: Search for an AIDS vaccine, *AIDS Reader* 10: pp526-538, 2000
8. Nabel GJ, Challenges and opportunities for development of an AIDS vaccine, *Nature Medicine* 4: pp1,002-1,007, 2001
9. Langley G, *Phasing Out Primate Use in Belgian Laboratories*, GAIA, Brussels, Belgium: p85, 2002
10. Peters BS, HIV immunotherapeutic vaccines, *Antiviral Chemistry and Chemotherapy* 11: pp311-320, 2000
11. Zheng L, Huang XL, Fan Z, Borowski L, Wilson CC and Rinaldo CR, Delivery of liposome encapsulated HIV type 1 proteins to human dendritic cells for stimulation of HIV type 1-specific memory of cytotoxic T lymphocyte responses. *AIDS Research and Human Retroviruses* 15: pp1,011-1,020, 1999
12. Kawamura T, Cohen CC, Borris DL, Aquilino EA, Glushakova S, Margolis LB, Orenstein JM, Offord RE,

About the authors



Nirmala Bhogal graduated with a BSc (Hons) in Biochemistry and Pharmacology, PhD Biochemistry and Molecular Biology and a postgraduate degree in Law. Her expertise is in the preclinical development of small chemical drugs and protein hormone mimetics. Nirmala joined FRAME in 2004 and currently oversees the FRAME Research Programme, working closely with scientists within the FRAME Alternatives Laboratory based at the University of Nottingham. Her area of research focuses on the evaluation of medicinal products.
Email: nirmala@frame.org.uk



Michelle Hudson graduated from the University of Sheffield with a Masters degree in Zoology. Her main research interests are the application of methods to reduce the number of animals used in experiments, the refinement and regulation of animal procedures and the replacement of laboratory non-human primates. **Email:** michelle@frame.org.uk

- Neurath AR and Blauvelt A, Candidate microbiocides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants, *Journal of Experimental Medicine* 192: pp1,491-1,500, 2000
13. Collins KB, Patterson BK, Naus GJ, Landers DV and Gupta P, Development of a new *in vitro* organ culture model to study transmission of HIV-1 in the female genital tract, *Nature Medicine* 6: pp475-479, 2000
14. Anonymous (2003) *Report of WHO Consultation on Needs and Opportunities for SARS Vaccine Research and Development* at: http://www.who.int/vaccine_research/diseases/sars/events/2003/11/en/report.pdf
15. Anonymous (2004) *International Workshop on Development of Vaccines for SARS and New Human Influenza Vaccines* at: http://www.who.int/vaccine_research/diseases/sars/events/2004/03/en/summary_conclusions.pdf
16. Martina BEE, Haagmans BL, Kuiken T, Fouchier RAM, Rimmelzwaan GF, van Amerongen G, Peiris JSM, Lim W and Osterhaus ADME, SARS virus infection of cats and ferrets, *Nature* 425: p915, 2003
17. Itoh Y *et al*, *In vitro* and *in vivo* characterization of new swine-origin H1N1 influenza viruses, *Nature* 460: pp1,021-1,025, 2009
18. European Commission, *The Welfare of Non-Human Primates Used in Research*, p135, 2002
19. Report available from: www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_063117
20. Schellekens H and Casadevall N, Immunogenicity of recombinant human proteins causes and consequences, *Journal of Neurology* 251 (s2): pp114-119, 2004
21. Available from: www.emea.europa.eu/pdfs/human/swap/2836707en.pdf
22. Bhogal N, Combes R and Balls M, Preclinical Drug Development Planning, In: *Preclinical Development Handbook*, pp1-63, 2008