A Brief Introduction to Human/Chimpanzee Biological Differences, Their Negative Impact on Research into Human Conditions, and Scientific Methods for Better and More Humane Research

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In addition to the important ethical concerns surrounding the use of chimpanzees in biomedical research, there is a scientific concern that is just as critical: does medical research and testing involving chimpanzees contribute to human health and medical progress? This question is paramount: if it *doesn't*, then we shouldn't be doing it, whether it is morally acceptable or not.

Two of the main ways captive chimpanzees have been used are (i) in research, to *model* human diseases as 'surrogate humans' to help us learn about out the basis of those diseases; and (ii) in tests for new drugs and chemicals to assess their efficacy (drugs) and potential toxicity (chemicals) before humans encounter them. But is the biological similarity between humans and chimpanzees *enough* for chimpanzees to be used in these ways? Or do the small differences matter?

To illustrate how small differences combine and manifest, it is useful to look at the two most significant uses of chimpanzees: hepatitis C (HCV) and human immune deficiency virus (HIV) research.

Hepatitis C

HCV affects up to 200 million people worldwide, and is a leading cause of liver failure and transplants. Viral infection often proceeds to liver cirrhosis and even liver cancer, and is directly responsible for 40% of chronic liver disease in the US.

Chimpanzees have been used as a model of HCV infection since it was first discovered in 1989. So we can ask: were chimpanzees *essential* in the discovery and isolation of HCV? Have they made a positive contribution to our knowledge of the virus and to our understanding of the progress of clinical symptoms in humans in this time? If so, has this positive contribution *absolutely relied* on the use of chimpanzees, providing information that could not have been obtained in another way, perhaps more quickly, easily and reliably?

<u>Similarities and Differences</u>

There *are*, as one would expect, some similarities in HCV infection in chimpanzees and humans – we can infect chimpanzees with HCV for example, use them to grow the virus so we can harvest it from their blood, and the target organ – the liver – responds in a similar way by producing elevated levels of specific liver enzymes.

However, there are critical differences, meaning the course of viral infection and the host's responses to it are very different. There is a much lower rate of chronic infection in chimpanzees (30-50% of cases versus 75% in humans)

due to higher viral clearance; there is a lack of liver fibrosis and cirrhosis in chimpanzees, which both occur regularly in humans; hepatocellular carcinoma (liver cancer) is frequent in humans but extremely rare in chimpanzees, and there exists a lack of mother-to-infant virus transmission in chimpanzees. Differences in immune response between humans and chimpanzees exist (antibodies are raised to different antigens), which could explain why chimpanzees appear not to be protected from re-challenge with HCV following apparent recovery, and why HCV persists in infected humans despite significant immune responses.

Reflection on years of research in this field has prompted some experts to promote an alternative way forward: for example,

> Human liver transplantation represents the only available model system to study HCV, as a suitable animal model (the endangered chimpanzee model has significant limitations) and tissue culture systems for its propagation do not exist.
> - Hugo R. Rosen, MD. *Semin Liver Dis* 20(4):465-480, 2000. Hepatitis B and C in the Liver Transplant Recipient.

Since this 2000 quote, tissue culture systems for the propagation of the virus *have* been developed, which will undoubtedly accelerate tangible translational research (1).

<u>Better contributors to progress and promising current research</u>

The real basis of progress lies in molecular biological techniques. Although the sequencing of the nucleic acid of the virus did use virus isolated from chimpanzees, this is of no relevance – a human source of HCV could easily have been used. Methods of identifying and quantifying HCV infection have all been *in vitro* ('test tube'). Human-based research (epidemiology, clinical studies, biopsies of liver tissue etc) led to discovery of HCV (formerly known only as non-A non-B hepatitis), and characterized different courses of the disease in different human individuals.

Currently, studies of intravenous drug users are underway to prospectively determine immune responses early in acute human HCV infection. Because 15% of infected people clear acute HCV infection, comparative human studies can be done to determine the genetic/molecular biological basis of this. If successful, this could lead to a vaccine.

Production of engineered, lab-grown 3-D human liver tissue is now possible, enabling *in vitro* research to be done in a human context and providing an in expensive and efficient source of HCV virus, eliminating the need for chimpanzee 'reservoirs,' as well as a good model to study human-specific pathology and potential antiviral treatments.

The ability to now grow HCV in culture is immensely promising. Historically this has been a giant leap forward in several areas of virus and vaccine research (for example polio), and should be such a leap in HCV too:

> The development of *in vitro* systems greatly facilitated progress in this field, enabling scientists to make better quantitative estimates of the amount of virus in a sample, to determine the target cells of a particular virus, and to see whether the virus produced a cytopathic effect… Although techniques for tissue culture were a development of the 1930s, the techniques have been continually refined up to the present. A big advance came in the 1950s when John Enders grew the poliovirus in cell culture. The 1950s and 1960s became one of the greatest periods of medical virology because of these cell culture advances.
> - Dr Robert Gallo, Gallo *Virus Hunters* Basic Books, Harper Collins, 1991.

It is important to also look at the use of chimpanzees in hepatitis *B* virus (HBV) because it is often cited as a reason to continue with chimpanzee research into HCV because of its impact and contribution to HBV vaccines. Notably, reviewing the history of hepatitis the physician Paul Beeson concluded:

> Progress in the understanding and management of human disease must begin, and end, with studies of man...Hepatitis, although an almost 'pure' example of progress by the study of man, is by no means unusual…
> - Beeson, PB. The growth of knowledge about a disease: hepatitis. *American Journal of Medicine* 1979:67;366-370.

HBV was differentiated from hepatitis A virus (HAV) on the basis of human clinical studies. In early vaccine development, chimpanzees *were* involved – but they were used only as bioreactors to 'grow' the virus, not to understand the course of the human disease; this would not be possible as chimpanzee HBV infections are asymptomatic. We can therefore conclude that this vaccine wouldn't have arisen *as it did* if it had not been for chimpanzees – but not that it wouldn't have been developed at all. The perfection of cell culture methods has historically been a watershed in vaccine development, facilitating the process (as with polio), and there is no reason to believe that a robust cell culture system could not have been developed to study HBV during those years of work on chimpanzees. In fact, HBV vaccines are now made using genetically engineered yeast.

Human Immunodeficiency Virus (HIV)

Proponents of animal experimentation claim that those who advocate an end to it are threatening to impede medical progress – that without the use of animals science will be losing its most powerful tool in the search for treatments and cures for all manner of human diseases. These same claims have been made throughout history, and we are now in a position to analyze some of the older ones. For example, Robert Yerkes, considered a pioneer of chimpanzee research, wrote in 1943:

> …I am wholly convinced by my own experience and as well as by that of others that the various medical sciences and

> medical practice have vastly more to gain than has yet been achieved, or than any considerable number of medical experts imagine, from the persistent and ingenious use of the monkeys and anthropoid apes in experimental inquiry.
> - Yerkes, RM. *Chimpanzees*. Yale University Press. 1943. p 290.

As these claims were overly speculative and without an empirical basis then, so too are the promises of near-term benefits and breakthroughs using chimpanzee models made now. This view is supported by quotes from scientists who were (or even still are) involved in non-human primate (NHP) research:

> The chimpanzee model doesn't get a lot of support in the scientific community.
> -Steven Bende, research coordinator at the National Institute of Allergy and Infectious Disease, *The Scientist* August 1999.

> I just don't see much coming out of the chimp work that has convinced us that that is a particularly useful model…I can't tell you what it is that those studies have given us that has really made a difference in the way we approach people with this disease.
> -Thomas Insel, former Director of the Yerkes Regional Primate Center, *The Scientist* August 1999.

> The *Handbook of Laboratory Animal Science*, in 1994 called primate models of AIDS "unsuccessful."

> Defending the usefulness of the chimpanzee as a model for HIV research has not only become a difficult task, but also a controversial one…Following viral inoculation of chimpanzees…responses do tend to differ from those observed in HIV-positive humans. In fact, chimpanzees rarely develop full-blown AIDS. [In fact, around 1 in 200 do, though with significant caveats]
> -Nath et al. (2000) The chimpanzee and other non-human primate models in HIV-1 vaccine research. *Trends in Microbiology* 8(9), 426-431.

As a consequence, and following a review of chimpanzee-based AIDS research, the NIH cut its funding drastically.

<u>Similarities, Differences, and Problems Caused by Chimpanzee use in AIDS Research</u>

In humans, HIV-1 infection is characterized by attrition of CD4+ cells, compromising the immune system and resulting in opportunistic infections and malignancies. In chimpanzees, however, viral infection is classed as 'mild' with little or no drop in CD4+ cells and the ensuing symptoms. HIV genetic material IS detectable for several months, but normally disappears.

Because HIV doesn't 'behave' properly in chimpanzees and other NHPs, a hybrid virus has been created between HIV and Simian IV (SIV), and sometimes SIV itself is used. These viruses, while in some ways alike, are only around 40%-60% genetically similar (depending on the strain) and

contain important structural and genetic differences: some of the common genes have different functions, and the viruses use different routes into the cells that they infect. Chimpanzees have higher levels of CD8+ cells (antibody-producing cells), which respond more forcefully to HIV infection than human CD8+ cells.

These differences have manifested in any number of problems for HIV/AIDS research. For example:

- In the 'French blood transfusion scandal,' HIV was thought to be harmless on basis of chimpanzee experiments, and so contaminated blood was allowed to be used in transfusions that infected 8000 people with HIV.
- More than 80 proposed AIDS vaccines, 'proven' safe and effective in animals (often NHPs including chimpanzees), have failed in over 100 human clinical trials. One of the latest was 'AIDSVAX' which, though safe in chimpanzees, failed to protect over 3000 volunteers in clinical trials.

Successes in HIV/AIDS research

Human clinical and epidemiological studies revealed the existence of HIV and how it is transmitted:

AZT (the first effective drug, a reverse-transcriptase inhibitor) was discovered by *in vitro* screening of existing compounds for anti-viral activity.

Protease inhibitors, designed on computer following the elucidation of HIV protease enzyme structure, proceeded to human trials without animal testing due to pressure from patient advocacy groups fearing time delays.
Subsequent *in vitro* experiments have elucidated the viral structure and life cycle, and enabled different classes of drugs to be designed against it such as non-nucleoside reverse-transcriptase inhibitors like Nevirapine.

Dr. David Ho's clinical research led him to conclude that we should treat HIV positive patients early and aggressively with a multiple drug regime. The British Medical Journal stated,

> Ho's remarkable success, derived from clinical observation of patients is a classic example of what can happen when physicians actually try to learn about disease from humans.

Toxicity Testing

It is notoriously difficult to obtain data in this field because most of it is confidential, residing with privately owned drug and animal testing companies. However, it is widely acknowledged that there is no evolutionary basis to toxicity testing, i.e. just because a chimpanzee is more genetically related to a human than a rat, it doesn't mean it will serve as a more reliable model for testing drugs and chemicals for safety.

Some evidence of the relevance of NHPs in general is available from a comparative teratology study done in 2005 (2), which shows that teratological classifications in NHPs correlate with human classifications only around half of the time: this is no better the correlation between humans and mice or dogs, and actually *worse* than that with rats, hamsters, guinea pigs, pigs and sheep.

Other Areas of Chimpanzee research

Multiple Sclerosis (MS)

A current research project is using chimpanzees to test the hypothesis that MS has a viral cause. This hypothesis is based on human epidemiological studies, information from human clinical studies using beta interferon therapy and the documented presence in humans of cells associated with viral immunity in MS lesions (3). Previous attempts to test this hypothesis in animal models have failed (4).

Respiratory Syncytial Virus (RSV)

The crude nature of animal models of RSV is demonstrated by a review published in 1997 (5), which outlines that RSV disease in humans is a multifaceted disease whose clinical manifestations depend upon age, genetic makeup, immunologic status, and concurrent disease within subpopulations. The review suggests that the choice of an animal model with which you work, ranging from primates to cotton rats, mice, calves, guinea pigs, ferrets, and hamsters, ought to be governed by the specific manifestation of disease to be studied.

Despite decades of research, much of it involving NHPs including chimpanzees, a vaccine is still elusive. Only in 2000, a paper (6) warned against relying on data from NHPs, including chimpanzees, when trying to understand the disease's processes and to develop vaccines:

> The testing of vaccines and drugs in more animals will not be helpful if in the end these animals do not closely resemble humans. Even a vaccine that has 100% efficacy in [animal] models… might still be ineffective in humans. Conversely, a proficient vaccine developed in humans might never show benefit in the animal models. Earlier experiences in vaccine development have stressed the need for great caution when beginning human clinical trials. *Immunization with a formalin-inactivated vaccine for respiratory syncytium virus infection induced immune responses in primates but exacerbated disease in children.*

A 2001 study (7) showed that Interleukin-8 (IL-8), a normal pro-inflammatory molecule, correlates with the severity of RSV-induced disease in humans. It showed that the IL-8 gene is variable in nature, and specific types confer a particular susceptibility to RSV infection. The chimpanzee IL-8 gene showed 108 genetic differences with respect to the typical human IL-8 gene over 7.6 kilobases of the IL-8 genetic locus: this is likely to have a significant impact on

the course of RSV infection and its manifestations in the chimpanzee versus the human situation.

Why Aren't Chimpanzees Used in Other Areas of Human Disease Research?

If chimpanzees are such indispensable research tools due to their genetic similarity to human beings, we must ask the question: "Why aren't they used in *more* areas of research?"

It would seem logical that our closest living species would be used to research cures for cancer, heart disease, stroke, Alzheimer's and Parkinson's (among the leading killers of human beings)…yet they are not. Research instead is focused largely on transgenic mice, which are of questionable applicability to humans.

The reason why chimpanzees are not used in these areas of research is the same reason that their current areas of use in biomedical research are replete with failure: they are just too different from human beings: only around 5% different at most, but this difference has significant ramifications. For example, human DNA is made up of around 3 billion 'units' called base-pairs. 5% of 3 billion is 150 million. So, if you were given just 1 cent for each difference between the genes of a typical human and a typical chimpanzee, you'd be $1.5 million better off… it all adds up.

Furthermore, recent research suggests we may be even more different than we first imagined. For example, scientists comparing human and chimpanzee chromosomes have found large 'insertions' and 'deletions' of DNA, which can be thought of as genetic 'hiccoughs.' These can disable entire genes, with particularly profound implications if such disabled genes regulate other genes. (8-10).

Chimpanzees are not used in cancer research any longer…yet it is interesting to look at the claims of a famous cancer researcher in 1976, during a tribute to Robert Yerkes, asserting that chimpanzees would be critical to curing cancer:

> The importance of biomedical research in human cancer is more evident today than ever before, the obvious important role that the subhuman primate plays in this continued research is evident. Research data accumulated using this experimental animal, so close to man, has in the past and will continue in the future to be directly applicable to the human situation and thus, permits vital investigation that for moral and ethical reasons could have never been considered using human volunteers.
> - Seigler, HF. Immunology and melanoma. In *Progress in Ape Research*. GH Bourne (ed). Academic Press Inc. New York. 1977. (227-230).

The reason chimpanzees are no longer used in cancer research is quite simple: it didn't work. We are now beginning to learn *why.* Recent research has analyzed, in chimpanzees, the equivalents of 333 human genes

implicated in cancer. Although both species share these genes (as expected: genes controlling cell growth are often tightly conserved throughout evolution because they perform essential cellular functions) and although there is a high degree of similarity, there exist some small differences that clearly exert major effects in terms of cancer susceptibility. For example, a small difference was found in the p53 gene (whose function is to turn other genes on and off, especially in response to toxic agents), which has long been known to be mutated in tumors. Also, the chimpanzee version of the BRCA1/NBR2 (a tumor-suppressor gene responsible for DNA repair) has a major deletion in its sequence. All in all, 20 of the 333 genes contained some form of difference, which may go some way to explaining why chimpanzees have an extraordinarily low incidence of cancer – especially of the breast, prostate and lung which account for over 20% of human deaths.

Other Crucial Differences between Humans and Chimpanzees

Other comparative genomic research has outlined important genetic differences between humans and chimpanzees, which are likely to have far-reaching consequences regarding the suitability of chimpanzees as 'human surrogates' in research, and also to explain exactly *why* we're so different:

- Comparison of a group of genes coding for 'proteases' (enzymes that are important in a wide variety of cellular processes) also revealed important differences (11). The 559 chimpanzee and 561 human genes showed a high degree of similarity, but a significant number of genes were deleted in their entirety (7 genes), contained small insertions or deletions, or sequence changes that can lead to gene inactivation – many of these changes affected genes of the immune system.
- 169 genes in the cerebral cortex are expressed differently in humans and chimpanzees (12).
- A study examining the expression of around 12,000 genes in the pre-frontal cortex (PFC) of the brain found that 39% of these genes were expressed in the human PFC, but only 34% in the chimpanzee PFC…a difference of 621 genes. Of the genes that were found to be commonly expressed between the 2 species, 20% had a different expression profile:

Attention was then focused on those genes linked to, for example, Alzheimer's disease. Although there was a common presence of some genes such as amyloid precursor and tau, other important genes such as apolipoprotein E and TNF-alpha showed differences. Differences were also noted in gene presence and expression for genes linked to Parkinson's and Huntington's diseases… researchers found that 19 genes were expressed to a much higher level in chimpanzees compared to humans (including a gene called GluR2 that mediates excitatory neurotransmission), and another 33 genes to a much lower level, including 4 genes related to the process of actual gene expression itself…with potentially promiscuous effects. In fact, differences in transcription have been proposed as the main basis of differences between humans and chimpanzees.

Can't We *Use* Knowledge of Human/Chimpanzee Differences for Our Benefit?

It is often claimed that the differences between humans and chimpanzees and in our responses to infectious agents, for example, are pivotal to our efforts to understand and treat human disease. The premise of this argument is that if we can discover exactly *why*, for example, chimpanzees infected with HIV or HCV don't develop the same symptoms of disease as humans, we can home in on areas of those viruses or our immune systems that differ in order to guide the development of drugs and identify susceptible people.

There are two main responses to this argument: first, the study of human/chimpanzee differences does not require the ethically troublesome practice of confining chimpanzees and experimenting upon them, causing them to suffer – instead, tissue samples and cells grown from chimpanzees in sanctuaries can be used following their removal/sampling during surgery and *post mortems*, enabling detailed and comprehensive comparative studies, of which there are many examples. Second, modern technology allows scientists to investigate the differences between *humans* that are resistant to certain diseases and infectious agents, and those that are not – thus avoiding the confounding aspects of the myriad other species differences that exist when one looks outside the human context.

The salient point is simple: studying biological differences *can* be highly informative in medical research, but we can do this in a purely human context. Where there may be an argument to study the differences between humans and chimpanzees, this can be achieved using ethically obtained tissue samples with no need for harmful experiments on captive animals.

In Summary

The salient point is that small, subtle differences in genes can manifest in massive biochemical and physiological differences in the whole organism. For example, the diseases of cystic fibrosis and sickle-cell anemia are caused by single 'point' mistakes in genes, which cause the protein products of those genes to 'mis-fold' and completely ablate their function and/or alter the other thousands of proteins that it must interact with in the cell or tissue where it is situated and/or affect the expression of downstream genes that it turns on or off…. ad infinitum. Genes can have 'global' effects in an organism, and a small change can cause an avalanche of detrimental sequelae.

We now know too much about the differences between species that make animal based research – even in our closest relatives, chimpanzees – confounding and even futile. To persist with it eschewing better human-specific options that technology has provided is a dereliction of duty that will continue to keep the treatments we seek for so many human diseases at arms' length.

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