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Animal Models and the Development of an HIV Vaccine

Ray Greek*
Americans for Medical Advancement, Refugio Rd, Goleta, USA

Abstract

The failure to find a vaccine against HIV/AIDS has been attributed to numerous factors including the diversity and mutability of the virus and the lack of a good animal model. I readily acknowledge that HIV presents unique challenges and that these have been problematic for vaccine development. However, the search for, and reliance on, animal models is part of the problem and not a means to a solution. I outline why one evolved complex system should not be expected to be predictive for another, especially one that has a different evolutionary trajectory, regardless of extensive similarities. I also discuss general concepts of scientific methodology, specifically what the concept *predict* means and why animal models fail to qualify as predictive systems for drug and disease response.

Keywords: AIDS; Animal model; Biological complexity; Evolution; HIV; Predictive models; Vaccines

Immunodeficiency Viruses in Humans and Animals

Human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS), and the search for an HIV vaccine provide an excellent example of why small differences between two otherwise very similar complex systems negate the ability to extrapolate the results of perturbations such as drugs and disease from one complex living system to another. HIV and simian immunodeficiency virus (SIV), the virus used to approximate HIV infections in nonhuman primates (NHPs), which is the animal model most frequently used in vaccine research, share many features, as do NHPs and humans. When studying complex systems, however, the relatively infrequent dissimilarities can be more important than the numerous similarities. In terms of vaccine development, some of the dissimilarities are not subtle, for example, SIV and HIV are different viruses and the NHPs used to model HIV/AIDS are a different species from the humans that will be administered the vaccine.

Consequently, although a large number of vaccines are effective in NHPs, the vaccines against HIV that were developed and tested on chimpanzees and monkeys, have all ultimately failed in humans [1]. Since the first HIV vaccine trial in 1987, fifty vaccines aimed at prevention and thirty aimed at therapy had been tested by the US National Institute of Allergy and Infectious Diseases (NIAID) by 2007 [2]. Reasons listed for why a vaccine has not been forthcoming despite decades and billions of dollars in research funding include: 1. HIV is able to mutate very easily; 2. HIV has many groups and subtypes; and 3. HIV not only evades the body's immune system but also attacks it. Some would add that there are no animal models that are predictive for human response to interventions such as vaccines or that respond as humans do, mechanistically, to HIV [1,3]. I will elaborate on this lack of an animal model and attempt to place it in a greater context.

Current vaccine testing on NHPs has not only failed to predict human response, but has resulted in vaccines that ultimately proved harmful to humans [4-7]. This is obviously a suboptimal record of accomplishment. Gamble and Matthews have noted the importance of this, stating: "Until a model can be derived that will allow for observation of each stage of infection, progression of disease, and response of the immune system in a way that is comparable to this process in humans, we will not be able to logically predict which vaccine candidates should be moved forward to clinical trials" [1]. I will argue that even if a model could be developed that reproduced those human manifestations and mechanisms; it would be inadequate for predictive purposes.

HIV and SIV are presumably descendants of a common ancestor virus and have many similarities [8]. SIV jumped the species barrier from NHPs to humans, at least eleven times. SIV_{cpz} crossed from chimpanzees (*Pan troglodytes troglodytes*) to humans resulting in HIV-1 groups M, N, O, and P [9,10]. SIV_{smm} from sooty mangabeys (*Cercocebus torquatus atys*) crossed and resulted in HIV-2 groups A-H [11,12]. Each group has various subtypes or clades. Among mammals, intra- and inter-species differences have been revealed that are likely responsible for differences in susceptibilities and response [13,14]. HIV has only nine genes but since it lacks the usual viral repair mechanisms, mutations are common. This makes it interesting from an evolutionary point of view and a problem from a treatment and prevention perspective.

NHPs are the species most closely related to humans and the various NHPs share many genes and characteristics with humans. Thus, NHPs have been the model of choice for the study of HIV/AIDS. Chimpanzees were the original model of choice despite a number of relevant characteristics that demonstrate the important differences between chimpanzees and humans. For example, chimpanzees do not develop cirrhosis following infection with hepatitis B or C, or suffer from rheumatoid arthritis, bronchial asthma, type I diabetes, malaria, or Alzheimer's disease. Another difference proved to be that infection with HIV-1 does not progress to AIDS in chimpanzees [8,15,16] (or any other animal species except humans) [17-20]. Various differences between chimpanzees and humans have been offered in explanation of this. Chimpanzees have a higher body temperature [21-26]. HIV does not reproduce well in chimpanzees [27,28] and the chimpanzee immune system mounts little antibody-mediated or cell-mediated responses to HIV-l [28]. Chimpanzees do not develop any of the characteristic symptoms of AIDS, such as opportunistic infections or malignancies [29-31] and they develop only transient lymph node swelling in response to infection [29,30]. Moreover, chimpanzees do

***Corresponding author:** Ray Greek, MD, Americans for Medical Advancement, 2251 Refugio Rd, Goleta, CA 93117, USA, Tel: 805-6856812; E-mail: DrRayGreek@aol.com

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not experience degeneration in lymphoid follicles [21-26,32]. CD4+ to CD8+ lymphocyte ratios differ in humans and chimpanzees, CD4 decline is observed only in humans [27,28] and CD4+ T helper cells continue to regenerate in chimpanzees [21-26,32]. Chimpanzees do not manifest HIV infection of brain tissue or macrophages, and HIV has not been found in chimpanzee cerebral spinal fluid or saliva [19,33]. Their monocytes and macrophages also resist infection with HIV isolates [21-26,32].

Monkeys were the second choice of model system, although differences also exist between monkeys and humans and among various species of monkeys. For example, the rhesus macaque (RM) has failed to predict human response to medications for asthma [34-36]. Infection with SIV progresses to AIDS in RMs infected with SIV_{sm} but not in chimpanzees or sooty mangabeys (SM), or African green monkeys (AG) infected with SIV_{agm}. Macaques infected with a hybrid of SIV and HIV (SHIV) progress to AIDS but the time to progression is significantly different from that observed in humans infected with HIV. The pathology revealed in SIV- or SHIV-infected monkeys does not duplicate human HIV. In addition, the replicative capacity and other parameters differ from HIV [37,38]. SIV and HIV are different viruses [39] and although HIV is more closely related to SIV_{cpz}, SIV_{sm} and SIV_{mac} are used in most studies [40]. SIV and HIV envelope proteins are different [39] and SIV has the gene *vpx* while HIV-1 has *vpu* [41]. The envelope of SIV is unaffected by antibodies that neutralize HIV and vice versa [39]. Cytotoxic T lymphocytes (CTLs) against HIV do not react against SIV-infected cells and vice versa [39]. SIV_{mac} infection occurs via the CD4 receptor and primarily the CCR5 co-receptor whereas HIV uses CD4 and the co-receptors CXCR4, CCR5, and DC-SIGN [21,39]. These co-receptors are expressed differently in various tissues and thus may affect disease course [40,42]. In approximately 50% of humans, HIV begins by using the CCR5 coreceptor but switches to the CXCR4 coreceptor [39]. SIV_{mac} is only approximately 50% homologous with HIV in terms of nucleotide sequences [43]. SIV_{mac} does not transmit from mother to child [21] and SIV infection is more aggressive and advances more rapidly in macaques [44]. The main route of transmission for SIV_{mac} is nonsexual [21] and SIV results in "rapid and selective depletion of memory CD4 cells in the gut-associated lymphoid tissue (GALT)" [3].

More differences exist. For example, macaques exhibit a different neurological response to different strains SIV and SHIV of with no one model reproducing the human response. Different subspecies of macaques also demonstrate variability in their susceptibility to the central nervous system (CNS) effects of the viruses [45]. RMs differs from humans in their gut flora, which might impact on aspects of mucosal immunity [21,46]. Considerable inter- and intra-species variation among NHPs has been found in the nucleotide sequence of the CD4 receptor gene [47,48]. RMs has twenty-two MHC class 1 loci active genes or haplotypes compared to six for humans. Humans have an HLA-C while RMs does not. The controller haplotypes in RMs is Mamu-B08 and Mamu-B03 (>50%), and Mamu-B17 (>20%). Humans make use of HLA-B57 and HLA-B27 (<2%). RMs have more Mamu-DRB genes in their MHC class II loci than humans [21].

Over 1400 proteins that interact with HIV-1 in humans are recorded in the HIV-1 Human Protein Interaction Database [49]. Bozek and Lengauer [14] analyzed 1439 of these genes in humans, chimpanzees, rhesus macaques, and orangutans in order to discover genes and proteins under positive selection pressure. They determined that ~10% of the genes were under positive selection pressure. Many of these genes coded for proteins in the cell membrane or were involved in the innate immune response. These are significant findings especially in light of the interspecies differences in response to HIV. The results also forecast difficulty in inter-species extrapolation in terms of vaccine efficacy and safety.

Kim et al. [50] analyzed peripheral blood mononuclear cells (PBMC) from SMs, humans, chimpanzees, AGs, and RMs for the apoptotic factor TRAIL and found that the levels were higher in species susceptible to AIDS (humans and RMs). They also found that in "human and RM myeloid immature dendritic cells and macrophages, the virus-induced expression of TRAIL and other interferon-inducible genes, which did not occur in the same cells from chimpanzee, SM, and AGM, were Tat-dependent."

Stansell and Desrosiers [51] discovered that the carbohydrates that compose the glycoprotein spikes on HIV-1, thus allowing it to attach to receptors on the cell membrane, differ significantly from those on SIV in the SM.

In addition to the above differences, numerous other differences between HIV, SIV, and SHIV and the various strains and clades have been described [1,3,8,21,27-32,39,40,43,44,52-59]. These differences can be accounted for based the evolutionary trajectories of the viruses including the host-virus interactions. Further complicating matters, humans manifest intra-species differences in response to viruses and vaccines [60-83]. Klein et al. [69] evaluated gene response between men and women to the yellow fever vaccine. They analyzed microarray data and found that 660 genes in women, but only 67 genes in men, were differentially expressed after vaccination. They also "established that most of the reported TLR [Toll-like receptor]-associated genes that activate the interferon pathway are upregulated to a greater extent in women than in men during the first 10 days after vaccination" [69]. Men and women also differ in the pathogenesis of viral diseases [84-86]. A greater inflammatory and cellular immune response occurs in women. Because of this intra-species variation, Poland et al. have proposed the field of *vaccinomics* [71]. The above is similar to the differences between men and women [87-95] and among ethnicities [96-103] observed in response to other drugs and diseases. Monozygotic twins also differ in vaccine response [64,82,104-106] as well as in disease susceptibility [107-111]. Genetic variation influencing vaccine response has also been observed in animals [112,113].

There is a genetic basis to at least some of the above observations. Polymorphism occurs in immune response genes three times more frequently than other gene families. Kimman et al. [115] state: "Comparing sequences of genes common to rodents and humans for example revealed that proteins involved in host defense have diverged both within and between species three times as quickly as other proteins [114]. Vaccines against measles and hepatitis B, for example, fail in 2-20% of cases and genetic variability influences response to the HBV vaccine [116,117]. Genetic variation in infants has likewise been described in the case of vaccines for polio, pertussis, and tetanus [118] as well as with other vaccines [64,82,119,120]. If intra-species variation caused by genetic differences is troublesome, we should expect interspecies variation to be profound and interspecies prediction perhaps impossible.

Despite the importance of the above, I believe that focusing on these differences and on superficial similarities or dissimilarities leads scientists to ignore the real problem of using animals as causal analogical (or predictive) models [121] of human disease in general and as models for development of an HIV vaccine in particular. The real problem lies deeper and is far more important.

Animal Models

Animal use in science and research can be broken down into essentially nine categories (table 1) [122].

Current use of animals in HIV vaccine research is consistent with categories 1 and 2 in table 1. Both of these practices implicitly or explicitly make the claim that the animal models in use will reproduce the human response and therefore can predict the outcome. This is a very different claim from the use of animal models in categories 5, 7, and 9, which are relevant for basic research. Basic research makes no claim of applicability [123-131]. There are important distinctions between using animals as predictive models and using them for scientific purposes as described by categories 3-9. For example, the Varicella-Zoster Virus (VZV) vaccine was developed without an animal model but did use cells from animals as part of the growth media, which would be an example of category 4 in table 1 [132]. The basis for using NHPs to develop and test vaccines is that humans will respond in the same way as NHPs; and that causally relevant disanalogies do not exist. This leads us to the definition, or more precisely the explanation, of the terms *predict or predictive* as used in science in general and biological science in particular.

Theories in science influence what should even be considered as possible predictive modalities and we need to understand what a theory is before we consider prediction. In science, theory does not mean a hypothesis or idea, nor does it refer to a mathematical conclusion that has been proven. Theory means a scientific position that has been extensively studied and is supported by a vast amount of evidence as well as adhering to consilience; the position supports and explains observations and facts in other fields of science [133,134]. Theories of science include the Theory of Evolution, the Germ Theory of Disease, and Chaos and Complexity Theory. The Germ Theory, for example, would lead one to believe that even testing the predictive capacity of devices that purport to measure *chi*—the Chinese life force or energy—would be unproductive.

Individuals vary from one another and because of this, statistics are used in various ways to evaluate a biological phenomenon and arrive at certain conclusions. One method used to determine the predictive value of a practice, process, technique, or test is to compare the reality, or gold standard, to the answer being obtained indirectly through the process or test under scrutiny. This can be accomplished using the 2X2 binomial classification table and calculations shown in table 2. Physicians are familiar with this table as it is used to calculate values for practices they encounter, for example the positive predictive value (PPV) of a biomarker or practice or intervention, or the negative predictive value (NPV) of a blood test. It is important to note that PPVs and NPVs must be very high to be useful in endeavors that have little tolerance for error, as is the case in the practice of medicine. For example, if a blood test for cancer of the gall bladder has a PPV of 0.7 and a NPV of 0.5, it would not be useful, as these predictive values are totally inadequate in medicine.

1.	Animals are used as predictive models of humans for research into such diseases as cancer and AIDS.
2.	Animals are used as predictive models of humans for testing drugs or other chemicals.
3.	Animals are used as “spare parts”, such as when a person receives an aortic valve from a pig.
4.	Animals are used as bioreactors or factories, such as for the production of insulin or monoclonal antibodies, or to maintain the supply of a virus.
5.	Animals and animal tissues are used to study basic physiological principles.
6.	Animals are used in education to educate and train medical students and to teach basic principles of anatomy in high school biology classes.
7.	Animals are used as a modality for ideas or as a heuristic device, which is a component of basic science research.
8.	Animals are used in research designed to benefit other animals of the same species or breed.
9.	Animals are used in research in order to gain knowledge for knowledge sake.

Table 1: Categories of animal use in science and research [122].

		Gold Standard	
		GS+	GS-
Test	T+	TP	FP
	T-	FN	TN
T+ = Test positive T- = Test negative T = True F = False P = Positive N = Negative GS+ = Gold standard positive GS- = Gold standard negative			
Sensitivity = TP/TP+FN			
Specificity = TN/FP+TN			
Positive Predictive Value = TP/TP+FP			
Negative Predictive Value = TN/FN+TN			

Table 2: Binomial classification method for comparing a modality, practice, or test with a gold standard and for calculating sensitivity, specificity, positive predictive value, and negative predictive value.

The values regarding the use of animals as predictive models for the study of disease and drug development have been calculated in this fashion and found to be far below those needed in medical science [135-144]. These values are more similar to what would be expected from chance events or a coin toss than from a scientifically viable modality. However, when NHPs are used to predict human outcomes for vaccines against HIV, one does not need to perform the above calculations. The number of successful vaccines has been zero while the number of attempts and/or successes in NHPs approximates one hundred. Even if a vaccine were discovered tomorrow that resulted in immunity in both macaques and humans, one would still be forced to conclude that the PPV for the NHP model is approximately 0.01. (The NPV is unknown, as negatives in NHPs are not tested on humans thus raising the question of lost vaccines). Therefore, the monkey model for HIV vaccine development is not a predictive modality. This leads to the question: “Why is it being used?”

If a model is being used as heuristic, as in category 7 of table 1, then it should not be judged based on its ability, or lack thereof to predict human response. Nevertheless, some scientists still tout the monkey model as a predictive modality and state or imply that the results from studies with NHP models translate directly to humans [145-151]. This practice is not confined to AIDS research [152]. Furthermore, it is widely believed that animal models yield results that have a one to one correspondence to the human situation [123]. Science journals are also complicit in this stance [153], as are the media [154,155]. In contrast, some scientists and journalists have commented that NHP models are not predictive for human responses. An editorial in *Nature Biotechnology* stated:

The best large animal model for HIV, for example, is simian

immunodeficiency virus infection of macaques (chimpanzees injected with HIV fail to develop a human-like disease). To recognize the limitations of such models, look no further than the recent high-profile failure of Merck's HIV trivalent V520 vaccine—which *monkey studies had predicted* would be protective [156]. (Emphasis added).

Connor and Green writing in *The Independent* in 2008 state:

One of the major conclusions to emerge from the failed clinical trial of the most promising prototype vaccine, manufactured by the drug company Merck, was that an important animal model used for more than a decade, testing HIV vaccines on monkeys before they are used on humans, does not in fact work [157].

Connor and Green quote Anthony Fauci, then-director of the US National Institute of Allergy and Infectious Diseases (NIAID) as saying: "We've learnt a few important things [from the clinical trial]. We've learnt that one of the animal models, the SHIV model, really doesn't predict very well at all." Yet the SHIV model is still being touted in grant applications and media interviews as being predictive.

The failure of the NHP model to predict human response has been acknowledged by others [158-162]. Historically, monkeys have failed to reproduce human responses in drug and disease response including in HIV-related matters. For example, zidovudine was ineffective in preventing SIV infection in monkeys but has performed well in preventing mother to infant transmission [163-165]. Actinomycin-D, one of the first of the chemotherapy drugs, has very different effects in monkeys than humans [166]. Cancer research using NHPs has been an unqualified failure [167]. The vaccine for Alzheimer's disease, AN1792, was tested on monkeys but was withdrawn from development after the discovery that fifteen patients, out of 360, had developed severe brain inflammation [168-170]. Drugs known to damage the human fetus are found to be safe in 70% of cases when tried on primates [171]. Despite this history, there are conflicting opinions regarding the role of monkey models. Überla states:

Since only a limited number of vaccines can be tested for efficacy in phase 3 studies in humans, a filter is needed allowing selection of the most promising ones. Although differences between HIV infection in humans and simian immunodeficiency virus infection in nonhuman primates (NHP) might limit the predictive value of these models, comparative efficacy studies in NHPs could facilitate ranking of vaccine candidates [172].

This statement is confusing since it gives the impression that Überla is stating mutually exclusive concepts, namely, that monkey models are not predictive but that science should continue to use them as a screening tool—a practice that is based on the assumption of predictive ability. I believe Überla's statement accurately reflects the current situation in HIV vaccine research. For example, Vödrös and Fenyö [40] state: "Animal models cannot determine whether a vaccine will be effective against HIV-1 in human, however, challenge experiments to the macaque models can potentially broaden our knowledge on safety and efficacy of the candidate vaccines [173]." The following from Shedlock et al. is more realistic: "In light of the STEP trial, the data from rhesus macaque challenge models should not be used as a gatekeeper for Phase I clinical trials . . ." [21]. In addition to failing to predict inefficacy, an oft-overlooked problem with using animal models as predictive modality is the fact that efficacious vaccines may have been lost because of such practices. Such has probably occurred with anti-neoplastic drugs [174].

Analyzing past vaccine successes and failures can help us make intelligent decisions moving forward. The pursuit for an HIV vaccine has been compared to the search for a vaccine for polio. NHPs were successfully used in polio vaccine research as reservoirs for the virus and to type the virus, but they were not successful as models of the disease [175,176]. The vaccine against polio virus typifies the successful use of animals in category 4 in table 1, as bioreactors, and the failure of animal models in categories 1 and 2, as predictive models for disease and drug response. Of more concern, however, is the fact that NHP models of polio misled researchers in various ways. For example, despite evidence from humans that the poliovirus entered through the gut, researchers pursued the nasal portal and neurotropism hypothesis based on results from NHPs [21,175,177]. Shedlock et al. state:

However, at the time it was unknown that the rhesus macaque, unlike the cynomolgus macaque (*Macacafascicularis*), is one of the rare monkeys in which poliovirus does not replicate in the digestive tract and subsequently does not cause an orally acquired infection. Unfortunately, Flexner's conclusions that vaccines may be impossible to develop owing to the absence of a blood replication stage for poliovirus and that vaccine candidates should be grown only in neural cell lines, ideas that were widely embraced by the poliovirus research field, delayed the development of an effective poliovirus vaccine by as many as 40 years. Thus, this interpretation from the rhesus macaque model system shows that scientific assumptions of the importance of a particular primate infection model, based on the manifestation of similar disease symptoms and in the absence of known human correlates, may be ultimately misleading [21].

The same research led scientists to believe there was only one strain of the virus whereas there are actually three. The nasal portal notion also led to interventions such as the application of picric acid to the nasal mucosa, which resulted in loss of olfaction in some patients. Human-based research was responsible for major breakthroughs in the quest for a vaccine and human-based research did not mislead researchers. For example, poliovirus had been found in the gut by 1912 [175]. A vaccine for polio was developed based on the *in vitro* work of Ender, Weller, and Robbins.

Another source for direction is the vaccine against varicella zoster virus (VZV). VZV is similar to HIV in that humans are the only species adversely affected by the virus. The varicella vaccine was developed without an animal model, but the researchers did use animals as heuristic devices as outlined in category 7 in table 1 and for cell cultures (category 4). Eventually the vaccine was tested for efficacy and safety in human clinical trials without going through animal trials [132,178]. An animal model of VZV, using NHPs and simian varicella virus (SVV), was subsequently developed but it did not contribute the development or testing of the VZV vaccine. SVV and SIV share characteristics with the human disease [179].

Modeling Evolved Living Complex Systems

Medical research should be based on, and conform to, knowledge from all pertinent fields of science, a characteristic of science called consilience. I believe medical researchers working on a vaccine against HIV have violated this critical principle. As stated earlier, the real problem with using animals in general, and NHPs in particular, as predictive models for HIV vaccine research lies deeper than dissimilarities in CD4 receptors and virus homology; although these differences are important. I will now elaborate on this concept.

All members of the Kingdom Animalia are examples of living adaptive complex systems and each has a unique evolutionary

trajectory [152,180,181]. Very small variations between two otherwise very similar complex systems can result in opposite outcomes to the same perturbation and evolution uses many such small variations to make new species. Herein lays the problem for using animal models to predict human outcomes for perturbations such as drugs and diseases. Evolution has proceeded by altering genes, molecules, and processes while simultaneously conserving some of the general features of the organisms [182,183]. Moreover, the same outcome can be achieved by very different processes. For example, the human eye and the eye of cephalopods appear, to the first approximation, identical. But these two eyes are examples of *convergent evolution* hence the wiring and even the anatomical features are very different [183]. This has implications for what can be learned about the human eye from studying the cephalopod eye. While the cephalopod eye can certainly be used as a heuristic, it is unlikely to have a high PPV for human response to drugs and other interventions for the eye. However, not every important difference among species is secondary to convergent evolution. The eye of the rabbit, another mammal, responded very differently than the eye of humans to early attempts to correct myopia resulting in loss of vision in some patients [184-190]. I will now describe the characteristics of a complex system and note how evolution affects these properties and what this implies for inter-species extrapolation.

Reductionism has taken us far in our understanding of living systems but there is a point at which a living system must be analyzed as a whole. There are some characteristics of a complex living system that cannot be discovered by examining its constituents, regardless of how thorough such an examination might be [191]. Complex systems are composed of many components and some of these are simple systems—systems that can be completely described by the sum of their parts and that are subject to linear cause and effect relationships. However, for complex systems, the whole is greater than the sum of its parts. One reason for this is that complex systems demonstrate emergent phenomena—properties that only become apparent when the system is studied as a whole [192]. Reductionism cannot be used to discover emergent properties. For example, the emergent property of ice that allows it to float on water cannot be predicted based on complete knowledge of the properties of the atoms hydrogen and oxygen or the analysis of a single molecule of H_2O. Likewise, the fact that isomers have different chemical properties cannot be predicted by reductionism. Relevant to our discussion, gene regulation can be considered an emergent property and different species have evolved, at least in part, by changes in gene regulation. Also relevant to this discussion, the specificity of an antibody and the immunogenicity of an antigen are emergent properties [193,194].

Complex systems are dependent upon initial conditions. Small differences between two otherwise identical systems can be acted upon by the same perturbation but yield dramatically different outcomes. Moreover, these small differences can cause other changes in the system over time, which leads to even more differences between the two systems. This is what has happened with evolution. Species that share a common ancestor species, for example chimpanzees and humans, have undergone very small changes over time and thus are separate species. These species are composed of different genes and individuals within the species of different alleles. These differences in genes, proteins, gene-gene interactions and protein-gene interactions can result in different outcomes to the same perturbation. Differently regulated genes and gene networks similarly lead to vastly different outcomes to perturbations. The expression of genes varies considerably among species and even among individuals and these results in correspondingly divergent outcomes. Very small differences in the genetic makeup of monozygotic twins, perhaps secondary to epigenetics—an example of the complex system interacting with its environment—can translate to one twin suffering from a disease like multiple sclerosis while the other does not [107-111].

Initial conditions differ because evolution has used changes in genes, different proteins, different regulatory mechanisms and changes in the same regulatory mechanisms, different background and modifier genes, and mutations such as copy number variants and single nucleotide polymorphisms to build new species. Lorenz rounded off a number from six to three significant digits and this resulted in opposite outcomes for the two weather simulations. This is the mathematical equivalent of monozygotic twins experiencing opposite outcomes to the same perturbation. Even if we only considered the above-mentioned gene-based differences between NHPs and humans, those differences in initial conditions would be so great that one should not expect NHP models to predict human responses in vaccine development.

Robustness and redundancy are also characteristics of complex systems [195,196]. Robustness, meaning resistance to change, which exists at least in part because of the redundancy of components, for example gene pleiotropy and alternative splicing, which allows a protein to be produced despite the usually active gene not being present. Because the system is robust, a perturbation may cause no noticeable effect. However, because complex systems display the property of nonlinearity, the same small perturbation may wreak havoc on a similar living complex system. An example would be that some strains of rodents can have a gene knocked out with little consequence while a similar strain will not survive [197,198]. The presence of feedback loops also influences response to perturbations.

Different levels of organization exist in a complex system. The components of complex systems can be grouped in modules [199] that occupy these different levels of organization. However, components or modules that are wholes on one level may be parts on another [200]. The modules interact, for example gene networks interact with proteins, but the same interaction may result in different outcomes because of modifier genes, gene regulation, or epigenetic factors. Components and modules are not like pistons that can be interchanged between engines of the same variety. Mayr states that: "Owing to the interaction of the parts, a description of the isolated parts fails to convey the properties of the system as a whole. It is the organization of these parts that controls the entire system" [201]. This is one reason genetically modified animals have been unable to predict human response to drugs and disease [202-208]. An appreciation of the genetic differences among individual human complex systems has resulted in the field and concept of personalized medicine [209-211]. Given the differences among individuals of the same species to perturbations such as drugs and disease, one must question the claim that inter-species extrapolation of outcomes that involve higher levels of organization is justified.

Applying what is known from the Theory of Evolution and Chaos and Complexity Theory, I believe that we have a broad conceptual theory that explains why inter-species extrapolation of outcomes is problematic when such outcomes are not reducible to, or explained by, a level of organization where the system can be described in terms of a simple system.

The Ideal Model

The associate editor of the *British Medical Journal*, Alison Tonks stated in 2007: "When it comes to testing HIV vaccines, only humans will do" [212]. Nobel laureate Sydney Brenner was quoted in *Nature*

as quipping: "We don't have to look for model organisms anymore because we are the model organism" [213]. MacLennan and Amos anticipated this in 1990, declaring: "There is no doubt that the best test species for Man is man. This is based on the fact that it is not possible to directly extrapolate animal data to Man, due to interspecies variation in anatomy, physiology and biochemistry" [214]. Echoing Horrobin [215], Van Regenmortel affirms: "It remains true that human disease is best studied in human subjects" [180]. (Also see [216]).

In the final analysis, if one wants to learn about human disease then he must study humans and HIV allows many such opportunities. Approximately 0.5% of all people infected with HIV do not succumb to the virus and approximately 3% are long-term non-progressors (LTNPs) [217]. This is a valuable population for learning more about the pathophysiology of HIV. In 2007, Hütter apparently cured AIDS with a bone marrow transplant that contained the Δ32 mutation in the gene coding for the CCR5 receptor [218,219]. Physicians desperately need a way to insert genes into patients. The antigenic structure of HIV-1 has been described in detail and yet there is no vaccine. The belief that structural information will lead to a vaccine has proven unfounded. It should be recalled that science does not understand the mechanisms of many very successful interventions, drugs, and vaccines currently in use. Van Regenmortel has emphasized the importance of empirical-based vaccine development and has advocated that immunogens should be empirically tested in small clinical trials [220-223]. Van Regenmortel states that the current emphasis on rational drug design:

Denigrates the empirical approach in science and is highly misleading since modern science actually blossomed after the 17th century when empirical observations replaced the earlier reliance on scholastic and rational analysis for studying natural phenomena. The subsequent immense accumulation of scientific discoveries in the experimental sciences did not arise from deductive thinking and purposeful design but from the unpredictable outcomes of controlled experimental observations that mostly followed a trial and error approach [222].

Funding from the US government for AIDS research went from approximately $200 million in 1985 to billions in 2006 [224]. Much of this went to NHP models of HIV. If this money had gone to human-based research the situation today might be different. Moreover, even if scientists develop a vaccine for HIV that tests well on NHPs and proves effective in humans, this will not be because the model was predictive but secondary to sheer brute force of trying many different vaccines. Even with such a success, the positive predictive value for the NHP model would still be dismal. Current medical research standards revolve around the Declaration of Helsinki and Nuremberg Code both of which demand animal testing before an intervention is tried in humans. While that idea was based on the best science of the mid 20th century, science has advanced much since that time.

Conclusion

The human body and immune system are examples of complex systems that vary over time. The immune system you have today is not identical to the one you were born with. Likewise, the human body in general also changes with time. These changes result in the manifestation of diseases that one would not have been susceptible to decades ago as well as in different responses to the same drug. For example, the same patient may require different anesthetic management because of changes resulting from aging or concomitant diseases. Thus, we see intra-individual differences. The next level of consideration would be intra-species, for example, the differences between men and women, or among ethnic groups, or between monozygotic twins in the form of different responses to drugs and disease. Here we see even more variation in response to perturbations such as drugs and disease. In reality, the differences in response to drugs are so profound that otherwise useful drugs are removed from the market because of adverse reactions in a very small percentage of the population. The allowable margin of error in medical science is so small that even intra-species prediction has not been completely successful. Finally, we come to the inter-species level of comparison where we have empirical evidence that interspecies differences are simply too great to expect even species as closely related to humans as NHPs are, to predict human responses to perturbations such as vaccines.

All of the above is explained by the same set of scientific facts. All members of Animalia are living evolved complex systems. This means that the individual in question, regardless of species, is subject to the mechanisms of evolution and the results of the evolutionary process are subject to rules of complex systems. No two living complex systems have the same initial conditions and this alone allows us to predict dramatic differences in outcomes to perturbations. Other characteristics of complex systems such as the whole being greater than the sum of its parts, emergence, nonlinearity, and different levels of organization further jeopardize our ability to make predictions by extrapolating from one complex system to another. Complexity theory and evolutionary theory allow us to state with certainty that while one species may share traits with another, the first species will never be able to fulfill the criteria for being a predictive model for the second at levels of organization where disease and drugs act. I again note that occasional shared responses do not qualify a model as being predictive. Science, as well as serious scholarship, requires that words and concepts be used with utmost precision and not be changed merely to suit particular circumstances. No doubt, many NHP models have shared specific responses with humans to various drugs and diseases. However, medical science requires a positive predictive value so high that inter-species extrapolation simply cannot meet this requirement. NHP models of vaccine development will never be predictive and have a history of being misleading.

Our current use of animal models in HIV/AIDS vaccine development is similar to playing the lottery. Playing the lottery may result in wealth but winning is secondary to luck and is not science. We have relied on what is essentially random chance in developing an HIV/AIDS vaccine. Comparative studies will continue to inform about the differences among species and the evolution of traits, but they will not allow us to predict the effectiveness of a vaccine. It is time to acknowledge that the best science currently available demands a new approach.

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