REVIEWS

Origins of major human infectious diseases

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Many of the major human infectious diseases, including some now confined to humans and absent from animals, are 'new' ones that arose only after the origins of agriculture. Where did they come from? Why are they overwhelmingly of Old World origins? Here we show that answers to these questions are different for tropical and temperate diseases; for instance, in the relative importance of domestic animals and wild primates as sources. We identify five intermediate stages through which a pathogen exclusively infecting animals may become transformed into a pathogen exclusively infecting humans. We propose an initiative to resolve disputed origins of major diseases, and a global early warning system to monitor pathogens infecting individuals exposed to wild animals.

Human hunter/gatherer populations currently suffer, and presumably have suffered for millions of years, from infectious diseases similar or identical to diseases of other wild primate populations. However, the most important infectious diseases of modern food-producing human populations also include diseases that could have emerged only within the past 11,000 years, following the rise of agriculture[1,2]. We infer this because, as discussed below, these diseases can only be sustained in large dense human populations that did not exist anywhere in the world before agriculture. What were the sources of our major infectious diseases, including these 'new' ones? Why do so many animal pathogens, including virulent viruses like Ebola and Marburg, periodically infect human hosts but then fail to establish themselves in human populations?

A tentative earlier formulation[1] noted that major infectious diseases of temperate zones seem to have arisen overwhelmingly in the Old World (Africa, Asia and Europe), often from diseases of Old World domestic animals. Hence one goal of this article is to reappraise that conclusion in the light of studies of the past decade. Another goal is to extend the analysis to origins of tropical diseases[3]. We shall show that they also arose mainly in the Old World, but for different reasons, and mostly not from diseases of domestic animals.

These results provide a framework for addressing unanswered questions about the evolution of human infectious diseases—questions not only of practical importance to physicians, and to all the rest of us as potential victims, but also of intellectual interest to historians and evolutionary biologists. Historians increasingly recognize that infectious diseases have had major effects on the course of history; for example, on the European conquest of Native Americans and Pacific Islanders, the inability of Europeans to conquer the Old World tropics for many centuries, the failure of Napoleon's invasion of Russia, and the failure of the French attempt to complete construction of a Panama Canal[4–6]. Evolutionary biologists realize that infectious diseases, as a leading cause of human morbidity and mortality, have exerted important selective forces on our genomes[2,7].

We begin by defining five stages in the evolutionary transformation of an animal pathogen into a specialized pathogen of humans, and by considering why so many pathogens fail to make the transition from one stage to the next. We then assemble a database of 15 temperate and 10 tropical diseases of high evolutionary and/or historical impact, and we compare their characteristics and origins. Our concluding section lays out some unresolved questions and suggests two expanded research priorities. We restrict our discussion to unicellular microbial pathogens. We exclude macroparasites (in the sense of ref. 7), as well as normally benign commensals that cause serious illness only in weakened hosts. The extensive Supplementary Information provides details and references on our 25 diseases, robustness tests of our conclusions, factors affecting transitions between disease stages, and modern practices altering the risk of emergence of new diseases.

Evolutionary stages

Box 1 delineates five intergrading stages (Fig. 1) through which a pathogen exclusively infecting animals (Stage 1) may become transformed into a pathogen exclusively infecting humans (Stage 5). Supplementary Table S1 assigns each of the 25 major diseases discussed (Supplementary Note S1) to one of these five stages.

A large literature discusses the conditions required for a Stage 5 epidemic to persist[2,7]. Briefly, if the disease infects only humans and lacks an animal or environmental reservoir, each infected human introduced into a large population of susceptible individuals must on average give rise during his/her contagious lifespan to an infection in at least one other individual. Persistence depends on factors such as the duration of a host's infectivity; the rate of infection of new hosts; rate of development of host protective immunity; and host population density, size and structure permitting the pathogen's regional persistence despite temporary local extinctions.

Less well understood are two of the critical transitions between stages, discussed in Box 2. One is the transition from Stage 1 to Stage 2, when a pathogen initially confined to animals first infects humans. The other is the transition from Stage 2 to Stages 3 and 4 (see also Supplementary Note S2), when a pathogen of animal origin that is nevertheless transmissible to humans evolves the ability to sustain many cycles of human-to-human transmission, rather than just a few cycles before the outbreak dies out (as seen in modern Ebola outbreaks).

Database and conclusions

Database. Supplementary Table S1 lists 10 characteristics for each of 25 important 'temperate' (15) and 'tropical' (10) diseases (see Supplementary Note S3 for details of this distinction). Our aim was to select well-defined diseases causing the highest mortality and/or morbidity and hence of the highest historical and evolutionary significance (see Supplementary Note S1 for details of our selection criteria). Of the 25 diseases, we selected 17 because they are the ones assessed by ref. 8 as imposing the heaviest world burdens today

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Box 1 | Five stages leading to endemic human diseases

We delineate five stages in the transformation of an animal pathogen into a specialized pathogen of humans (Fig. 1). There is no inevitable progression of microbes from Stage 1 to Stage 5: at each stage many microbes remain stuck, and the agents of nearly half of the 25 important diseases we selected for analysis (Supplementary Table S1) have not reached Stage 5.

- **Stage 1.** A microbe that is present in animals but that has not been detected in humans under natural conditions (that is, excluding modern technologies that can inadvertently transfer microbes, such as blood transfusion, organ transplants, or hypodermic needles). Examples: most malarial plasmodia, which tend to be specific to one host species or to a closely related group of host species.
- **Stage 2.** A pathogen of animals that, under natural conditions, has been transmitted from animals to humans ('primary infection') but has not been transmitted between humans ('secondary infection'). Examples: anthrax and tularemia bacilli, and Nipah, rabies and West Nile viruses.
- **Stage 3.** Animal pathogens that can undergo only a few cycles of secondary transmission between humans, so that occasional human outbreaks triggered by a primary infection soon die out. Examples: Ebola, Marburg and monkeypox viruses.
- **Stage 4.** A disease that exists in animals, and that has a natural (sylvatic) cycle of infecting humans by primary transmission from the animal host, but that also undergoes long sequences of secondary transmission between humans without the involvement of animal hosts. We arbitrarily divide Stage 4 into three substages distinguished by the relative importance of primary and secondary transmission:
  Stage 4a. Sylvatic cycle much more important than direct human-to-human spread. Examples: Chagas' disease and (more frequent secondary transmission approaching Stage 4b) yellow fever.
  Stage 4b. Both sylvatic and direct transmission are important. Example: dengue fever in forested areas of West Africa and Southeast Asia.
  Stage 4c. The greatest spread is between humans. Examples: influenza A, cholera, typhus and West African sleeping sickness.
- **Stage 5.** A pathogen exclusive to humans. Examples: the agents causing *falciparum* malaria, measles, mumps, rubella, smallpox and syphilis. In principle, these pathogens could have become confined to humans in either of two ways: an ancestral pathogen already present in the common ancestor of chimpanzees and humans could have co-speciated long ago, when the chimpanzee and human lineages diverged around five million years ago; or else an animal pathogen could have colonized humans more recently and evolved into a specialized human pathogen. Co-speciation accounts well for the distribution of simian foamy viruses of non-human primates, which are lacking and presumably lost in humans: each virus is restricted to one primate species, but related viruses occur in related primate species[19]. While both interpretations are still debated for *falciparum* malaria, the latter interpretation of recent origins is widely preferred for most other human Stage 5 diseases of Supplementary Table S1.

(they have the highest disability-adjusted life years (DALY) scores). Of the 17 diseases, 8 are temperate (hepatitis B, influenza A, measles, pertussis, rotavirus A, syphilis, tetanus and tuberculosis), and 9 are tropical (acquired immune deficiency syndrome (AIDS), Chagas' disease, cholera, dengue haemorrhagic fever, East and West African sleeping sicknesses, *falciparum* and *vivax* malarias, and visceral leishmaniasis). We selected eight others (temperate diphtheria, mumps, plague, rubella, smallpox, typhoid and typhus, plus tropical yellow fever) because they imposed heavy burdens in the past, although modern medicine and public health have either eradicated them (smallpox) or reduced their burden. Except for AIDS, dengue fever, and cholera, which have spread and attained global impact in modern times, most of these 25 diseases have been important for more than two centuries.

Are our conclusions robust to variations in these selection criteria? For about a dozen diseases with the highest modern or historical burdens (for example, AIDS, malaria, plague, smallpox), there can be little doubt that they must be included, but one could debate some of the next choices. Hence we drew up three alternative sets of diseases sharing a first list of 16 indisputable major diseases but differing in the next choices, and we performed all 10 analyses described below on all three sets. It turned out that, with one minor exception, the three sets yielded qualitatively the same conclusions for all 10 analyses, although differing in their levels of statistical significance (see Supplementary Note S4). Thus, our conclusions do seem to be robust.

Temperate/tropical differences. Comparisons of these temperate and tropical diseases yield the following conclusions:

- A higher proportion of the diseases is transmitted by insect vectors in the tropics (8/10) than in the temperate zones (2/15) ($P < 0.005$, χ^2-test, degrees of freedom, d.f. = 1). This difference may be partly related to the seasonal cessations or declines of temperate insect activity.
- A higher proportion ($P = 0.009$) of the diseases conveys long-lasting immunity (11/15) in the temperate zones than in the tropics (2/10).
- Animal reservoirs are more frequent ($P < 0.005$) in the tropics (8/10) than in the temperate zones (3/15). The difference is in the reverse direction ($P = 0.1$, NS, not significant) for environmental reservoirs (1/10 versus 6/15), but those environmental reservoirs that do exist are generally not of major significance except for soil bearing tetanus spores.
- Most of the temperate diseases (12/15) are acute rather than slow, chronic, or latent: the patient either dies or recovers within one to several weeks. Fewer ($P = 0.01$) of the tropical diseases are acute: 3/10 last for one or two weeks, 3/10 last for weeks to months or years, and 4/10 last for many months to decades.
- A somewhat higher proportion of the diseases ($P = 0.08$, NS) belongs to Stage 5 (strictly confined to humans) in the temperate zones (10/15 or 11/15) than in the tropics (3/10). The paucity of Stage 2 and Stage 3 diseases (a total of only 5 such diseases) on our list of 25 major human diseases is noteworthy, because some Stage 2 and Stage 3 pathogens (such as anthrax and Ebola) are notoriously virulent, and because theoretical reasons are often advanced (but also denied) as to why Stage 5 microbes with long histories of adaptation to humans should tend to evolve low morbidity and mortality and not cause major diseases. We discuss explanations for this outcome in Supplementary Note S5.

Most (10/15) of the temperate diseases, but none of the tropical diseases ($P < 0.005$), are so-called 'crowd epidemic diseases' (asterisked in Supplementary Table S1), defined as ones occurring locally as a brief epidemic and capable of persisting regionally only in large human populations. This difference is an immediate consequence of the differences enumerated in the preceding five paragraphs. If a disease is acute, efficiently transmitted, and quickly leaves its victim either dead or else recovering and immune to re-infection, the epidemic soon exhausts the local pool of susceptible potential victims. If in addition the disease is confined to humans and lacks significant animal and environmental reservoirs, depletion of the local pool of potential victims in a small, sparse human population results in local termination of the epidemic. If, however, the human population is large and dense, the disease can persist by spreading to infect people in adjacent areas, and then returning to the original area in a later year, when births and growth have regenerated a new crop of previously unexposed non-immune potential victims. Empirical epidemiological studies of disease persistence or disappearance in isolated human populations of various sizes have yielded estimates of the population required to sustain a crowd disease: at least several hundred thousand people in the cases of measles, rubella and pertussis[2,7]. But human populations of that size did not exist anywhere in the

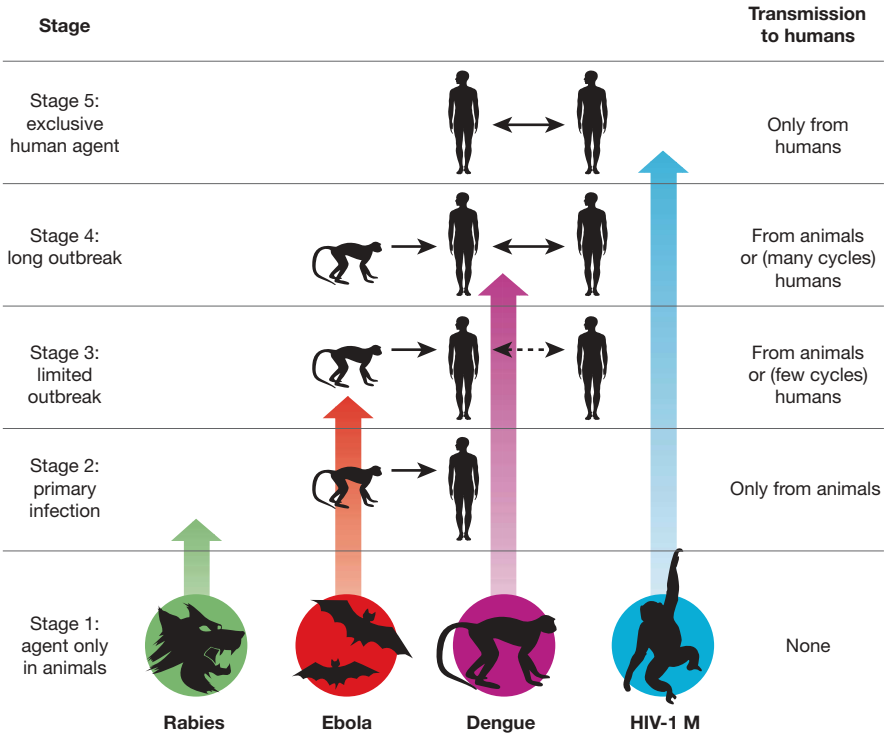

Figure 1 | Illustration of the five stages through which pathogens of animals evolve to cause diseases confined to humans. (See Box 1 for details.) The four agents depicted have reached different stages in the process, ranging from rabies (still acquired only from animals) to HIV-1 (now acquired only from humans).

world until the steep rise in human numbers that began around 11,000 years ago with the development of agriculture[1,9]. Hence the crowd epidemic diseases of the temperate zones must have evolved since then.

Of course, this does not mean that human hunter/gatherer communities lacked infectious diseases. Instead, like the sparse populations of our primate relatives, they suffered from infectious diseases with characteristics permitting them to persist in small populations, unlike crowd epidemic diseases. Those characteristics include: occurrence in animal reservoirs as well as in humans (such as yellow fever); incomplete and/or non-lasting immunity, enabling recovered patients to remain in the pool of potential victims (such as malaria); and a slow or chronic course, enabling individual patients to continue to infect new victims over years, rather than for just a week or two (such as Chagas' disease).

Pathogen origins. (See details for each disease in Supplementary Note S10). Current information suggests that 8 of the 15 temperate diseases probably or possibly reached humans from domestic animals (diphtheria, influenza A, measles, mumps, pertussis, rotavirus, smallpox, tuberculosis); three more probably reached us from apes (hepatitis B) or rodents (plague, typhus); and the other four (rubella, syphilis, tetanus, typhoid) came from still-unknown sources (see Supplementary Note S6). Thus, the rise of agriculture starting 11,000 years ago played multiple roles in the evolution of animal pathogens into human pathogens[1,4,10]. Those roles included both generation of the large human populations necessary for the evolution and persistence of human crowd diseases, and generation of large populations of domestic animals, with which farmers came into much closer and more frequent contact than hunter/gatherers had with wild animals. Moreover, as illustrated by influenza A, these domestic animal herds served as efficient conduits for pathogen transfers from wild animals to humans, and in the process may have evolved specialized crowd diseases of their own.

It is interesting that fewer tropical than temperate pathogens originated from domestic animals: not more than three of the ten tropical diseases of Supplementary Table S1, and possibly none (see Supplementary Note S7). Why do temperate and tropical human diseases differ so markedly in their animal origins? Many (4/10) tropical diseases (AIDS, dengue fever, *vivax* malaria, yellow fever) but only 1/15 temperate diseases (hepatitis B) have wild non-human primate origins ($P = 0.04$). This is because although non-human primates are the animals most closely related to humans and hence pose the weakest species barriers to pathogen transfer, the vast majority of primate species is tropical rather than temperate. Conversely, few tropical but many temperate diseases arose from domestic animals, and this is because domestic animals live mainly in the temperate zones, and their concentration there was formerly even more lop-sided (see Supplementary Note S8).

A final noteworthy point about animal-derived human pathogens is that virtually all arose from pathogens of other warm-blooded vertebrates, primarily mammals plus in two cases (influenza A and ultimately *falciparum* malaria) birds. This comes as no surprise, considering the species barrier to pathogen transfer posed by phylogenetic distance (Box 2). An expression of this barrier is that primates constitute only 0.5% of all vertebrate species but have contributed about 20% of our major human diseases. Expressed in another way, the number of major human diseases contributed, divided by the number of animal species in the taxonomic group contributing those diseases, is approximately 0.2 for apes, 0.017 for non-human primates other than apes, 0.003 for mammals other than primates, 0.00006 for vertebrates other than mammals, and either 0 or else 0.000003 (if cholera really came from aquatic invertebrates) for animals other than vertebrates (see Supplementary Note S9).

Geographic origins. To an overwhelming degree, the 25 major human pathogens analysed here originated in the Old World. That proved to be of great historical importance, because it facilitated the European conquest of the New World (the Americas). Far more Native Americans resisting European colonists died of newly introduced Old World diseases than of sword and bullet wounds. Those invisible agents of New World conquest were Old World microbes to which Europeans had both some acquired immunity based on individual exposure and some genetic resistance based on population

Box 2 | Transitions between stages

Transition from Stage 1 to Stage 2. Most animal pathogens are not transmitted to humans, that is, they do not even pass from Stage 1 to Stage 2. This problem of cross-species infection has been discussed previously[20–23]. Briefly, the probability-per-unit-time (p) of infection of an individual of a new (that is, new recipient) host species increases with the abundance of the existing (that is, existing donor) host, with the fraction of the existing host population infected, with the frequency of 'encounters' (opportunities for transmission, including indirect 'encounters' via vectors) between an individual of the existing host and of the new host, and with the probability of transmission per encounter. p decreases with increasing phylogenetic distance between the existing host and new host. p also varies among microbes (for example, trypanosomes and flaviviruses infect a wide taxonomic range of hosts, while plasmodia and simian foamy viruses infect only a narrow range), and this variation is related to a microbe's characteristics, such as its ability to generate genetic variability, or its ability to overcome host molecular barriers of potential new hosts (such as humoral and cellular defenses or lack of cell membrane receptors essential for microbe entry into host cells).

These considerations illuminate different reasons why a given animal host species may or may not become a source of many infections in humans. For instance, despite chimpanzees' very low abundance and infrequent encounters with humans, they have donated to us numerous zoonoses (diseases that still mainly afflict animals) and one or two established human diseases (AIDS and possibly hepatitis B) because of their close phylogenetic relationship to humans. Despite their large phylogenetic distance from humans, many of our zoonoses and probably two of our established diseases (plague and typhus) have been acquired from rodents, because of their high abundance and frequent encounters with humans in dwellings. Similarly, about half of our established temperate diseases have been acquired from domestic livestock, because of high local abundance and very frequent contact. Conversely, elephants and bats are not known to have donated directly to us any established diseases and rarely donate zoonoses, because they are heavily penalized on two or three counts: large phylogenetic distance, infrequent encounters with humans, and (in the case of elephants) low abundance. One might object that Nipah, severe acute respiratory syndrome (SARS) and rabies viruses do infect humans from bats, but these apparent exceptions actually support our conclusion. While bats may indeed be the primary reservoir for Nipah and SARS, human infections by these viruses are acquired mainly from intermediate animal hosts that frequently encounter humans (respectively, domestic pigs, and wild animals sold for food). The rare cases of rabies transmission directly to humans from bats arise because rabies changes a bat's behaviour so that it does encounter and bite humans, which a healthy bat (other than a vampire bat) would never do.

Transition from Stage 2 to Stage 3 or 4. Although some Stage 2 and 3 pathogens, such as the anthrax and Marburg agents, are virulent and feared, they claim few victims at present. Yet if they made the transition to Stage 4 or 5, their global impact would be devastating. Why do animal pathogens that have survived the initial jump across species lines into a human host (Stages 1 to 2) usually reach a dead end there, and not evolve past Stages 3 and 4 into major diseases confined to humans (Stage 5)? Barriers between Stages 2 and 3 (consider the rabies virus) include differences between human and animal behaviour affecting transmission (for example, animals often bite humans but humans rarely bite other humans); a pathogen's need to evolve adaptations to the new human host and possibly also to a new vector; and obstacles to a pathogen's spread between human tissues (for example, BSE is restricted to the central nervous system and lymphoid tissue). Barriers between Stages 3 and 4 (consider Ebola virus) include those related to human population size and to transmission efficiency between humans. The emergence of novel pathogens is now being facilitated by modern developments exposing more potential human victims and/or making transmission between humans more efficient than before[24–27]. These developments include blood transfusion (hepatitis C), the commercial bushmeat trade (retroviruses), industrial food production (bovine spongiform encephalitis, BSE), international travel (cholera), intravenous drug use (HIV), vaccine production (simian virus 40, SV40), and susceptible pools of elderly, antibiotic-treated, immunosuppressed patients (see Supplementary Note S2 for details).

exposure over time, but to which previously unexposed Native American populations had no immunity or resistance[1,4–6]. In contrast, no comparably devastating diseases awaited Europeans in the New World, which proved to be a relatively healthy environment for Europeans until yellow fever and malaria of Old World origins arrived[11].

Why was pathogen exchange between Old and New Worlds so unequal? Of the 25 major human diseases analysed, Chagas' disease is the only one that clearly originated in the New World. For two others, syphilis and tuberculosis, the debate is unresolved: it remains uncertain in which hemisphere syphilis originated, and whether tuberculosis originated independently in both hemispheres or was brought to the Americas by Europeans. Nothing is known about the geographic origins of rotavirus, rubella, tetanus and typhus. For all of the other 18 major pathogens, Old World origins are certain or probable.

Our preceding discussion of the animal origins of human pathogens may help explain this asymmetry. More temperate diseases arose in the Old World than New World because far more animals that could furnish ancestral pathogens were domesticated in the Old World. Of the world's 14 major species of domestic mammalian livestock, 13, including the five most abundant species with which we come into closest contact (cow, sheep, goat, pig and horse), originated in the Old World[1]. The sole livestock species domesticated in the New World was the llama, but it is not known to have infected us with any pathogens[1,2]—perhaps because its traditional geographic range was confined to the Andes, it was not milked or ridden or hitched to ploughs, and it was not cuddled or kept indoors (as are some calves, lambs and piglets). Among the reasons why far more tropical diseases (nine versus one) arose in the Old World than the New World are that the genetic distance between humans and New World monkeys is almost double that between humans and Old World monkeys, and is many times that between humans and Old World apes; and that much more evolutionary time was available for transfers from animals to humans in the Old World (about 5 million years) than in the New World (about 14,000 years).

Outlook and future research directions

Many research directions on infectious disease origins merit more effort. We conclude by calling attention to two such directions: clarifying the origins of existing major diseases, and surveillance for early detection of new potentially major diseases.

Origins of established diseases. This review illustrates big gaps in our understanding of the origins of even the established major infectious diseases. Almost all the studies that we have reviewed were based on specimens collected opportunistically from domestic animals and a few easily sampled wild animal species, rather than on systematic surveys for particular classes of agents over the spectrum of domestic and wild animals. A case in point is our ignorance even about smallpox virus, the virus that has had perhaps the greatest impact on human history in the past 4,000 years. Despite some knowledge of poxviruses infecting our domestic mammals, we know little about poxvirus diversity among African rodents, from which those poxviruses of domestic mammals are thought to have evolved. We do not even know whether 'camelpox', the closest known relative of smallpox virus, is truly confined to camels as its name implies or is instead a rodent virus with a broad host range. There could be still-unknown poxviruses more similar to smallpox virus in yet unstudied animal reservoirs, and those unknown poxviruses could be important not only as disease threats but also as reagents for drug and vaccine development.

Equally basic questions arise for other major pathogens. While *falciparum* malaria, an infection imposing one of the heaviest global burdens today, seems to have originated from a bird parasite whose descendants include both the *Plasmodium falciparum* infecting humans and the *P. reichenowii* infecting chimpanzees, malaria researchers still debate whether the bird parasite was introduced to

both humans and chimpanzees[12] a few thousand years ago in association with human agriculture, or instead more than five million years ago before the split of humans and chimpanzees from each other[13]. Although resolving this debate will not help us eradicate malaria, it is fascinating in its own right and could contribute to our broader understanding of disease emergence. In the case of rubella, a human crowd disease that must have emerged only in the past 11,000 years and for which some close relative may thus still exist among animals, no even remotely related virus is known; one or more may be lurking undiscovered somewhere. Does the recent identification of porcine rubulavirus and the Mapuera virus in bats as the closest known relatives of mumps virus mean that pigs infected humans, or that human mumps infected pigs, or that bats independently infected both humans and pigs? Is human tuberculosis descended from a ruminant mycobacterium that recently infected humans from domestic animals (a formerly prevalent view), or from an ancient human mycobacterium that has come to infect domestic and wild ruminants (a currently popular view)?

To fill these and other yawning gaps in our understanding of disease origins, we propose an 'origins initiative' aimed at identifying the origins of a dozen of the most important human infectious diseases: for example, AIDS, cholera, dengue fever, *falciparum* malaria, hepatitis B, influenza A, measles, plague, rotavirus, smallpox, tuberculosis and typhoid. Although more is already known about the origins of some of these agents (AIDS, influenza A and measles) than about others (rotavirus, smallpox and tuberculosis), more comprehensive screening is still likely to yield significant new information about even the most studied agents, as illustrated by the recent demonstration that gorillas rather than chimpanzees were probably the donor species for the O-group of human immunodeficiency virus (HIV)-1[14]. The proposed effort would involve systematic sampling and phylogeographic analysis of related pathogens in diverse animal species: not just pigs and other species chosen for their ready availability, but a wider range of wild and domestic species whose direct contact (for example, as bushmeat) or indirect contact (for example, vector-mediated) with humans could plausibly have led to human infections. In addition to the historical and evolutionary significance of knowledge gained through such an origins initiative, it could yield other benefits such as: identifying the closest relatives of human pathogens; a better understanding of how diseases have emerged; new laboratory models for studying public health threats; and perhaps clues that could aid in predictions of future disease threats.

A global early warning system. Most major human infectious diseases have animal origins, and we continue to be bombarded by novel animal pathogens. Yet there is no ongoing systematic global effort to monitor for pathogens emerging from animals to humans. Such an effort could help us to describe the diversity of microbial agents to which our species is exposed; to characterize animal pathogens that might threaten us in the future; and perhaps to detect and control a local human emergence before it has a chance to spread globally.

In our view, monitoring should focus on people with high levels of exposure to wild animals, such as hunters, butchers of wild game, wildlife veterinarians, workers in the wildlife trade, and zoo workers. Such people regularly become infected with animal viruses, and their infections can be monitored over time and traced to other people in contact with them. One of us (N.D.W.) has been working in Cameroon to monitor microbes in people who hunt wild game, in other people in their community, and in their animal prey[15]. The study is now expanding to other continents and to monitor domestic animals (such as dogs) that live in close proximity to humans but are exposed to wild animals through hunting and scavenging. Monitoring of people, animals, and animal die-offs[16] will serve as an early warning system for disease emergence, while also providing a unique archive of pathogens infecting humans and the animals to which we are exposed. Specimens from such highly exposed human populations could be screened specifically for agents known to be present in the animals they hunt (for example, retroviruses among hunters of non-human primates), as well as generically using broad screening tools such as viral microarrays[17] and random amplification polymerase chain reaction (PCR)[18]. Such monitoring efforts also provide potentially invaluable repositories, which would be available for study after future outbreaks in order to reconstruct an outbreak's origin, and as a source of relevant reagents.

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Origins of HIV and the Evolution of Resistance to AIDS

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Origins of HIV and the Evolution of Resistance to AIDS

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The cross-species transmission of lentiviruses from African primates to humans has selected viral adaptations which have subsequently facilitated human-to-human transmission. HIV adapts not only by positive selection through mutation but also by recombination of segments of its genome in individuals who become multiply infected. Naturally infected nonhuman primates are relatively resistant to AIDS-like disease despite high plasma viral loads and sustained viral evolution. Further understanding of host resistance factors and the mechanisms of disease in natural primate hosts may provide insight into unexplored therapeutic avenues for the prevention of AIDS.

Human immunodeficiency viruses HIV-1 and HIV-2, the causes of AIDS, were introduced to humans during the 20th century and as such are relatively new pathogens. In Africa, many species of indigenous nonhuman primates are naturally infected with related lentiviruses, yet curiously, AIDS is not observed in these hosts. Molecular phylogeny studies reveal that HIV-1 evolved from a strain of simian immunodeficiency virus, SIVcpz, within a particular subspecies of the chimpanzee (*Pan troglodytes troglodytes*) on at least three separate occasions (*1*). HIV-2 originated in SIVsm of sooty mangabeys (*Cercocebus atys*), and its even more numerous cross-species transmission events have yielded HIV-2 groups A to H (*2*, *3*). The relatively few successful transfers, in contrast to the estimated >35 different species of African nonhuman primates that harbor lentivirus infections, indicate that humans must have been physically exposed to SIV from other primate species, such as African green monkeys. However, these SIV strains have not been able to establish themselves sufficiently to adapt and be readily transmitted between humans. Thus, it is important to understand the specific properties required for successful cross-species transmission and subsequent adaptation necessary for efficient spread within the new host population. Notably, among the three SIVcpz ancestors of HIV-1 that have successfully crossed to humans, only one has given rise to the global AIDS pandemic: HIV-1 group M with subtypes A to K. Here, we survey genetically determined barriers to primate lentivirus transmission and disease and how this has influenced the evolution of disease and disease resistance in humans.

Origins and Missing Links

A new study of SIVcpz not only confirms that HIV-1 arose from a particular subspecies of chimpanzee, *P. t. troglodytes*, but also suggests that HIV-1 groups M and N arose from geographically distinct chimpanzee populations in Cameroon. Keele *et al*. (*1*) combined painstaking field work collecting feces and urine from wild chimpanzee troupes with equally meticulous phylogenetic studies of individual animals and the SIV genotypes that some of them carry. These data have enabled a more precise origin of HIV-1 M and N to be determined. The origin of group O remains to be identified, but given the location of human cases, cross-species transmission may have occurred in neighboring Gabon.

Although HIV-1 has clearly come from SIVcpz, only some of the extant chimpanzee populations harbor SIVcpz. SIVcpz itself appears to be a recombinant virus derived from lentiviruses of the red capped mangabey (SIVrcm) and one or more of the greater spot-nosed monkey (SIVgsn) lineage or a closely related species (*4*). Independent data reveal that chimpanzees can readily become infected with a second, distantly related lentivirus (*5*), suggesting that recombination of monkey lentiviruses occurred within infected chimpanzees, giving rise to a common ancestor of today's variants of SIVcpz, which were subsequently transmitted to humans (Fig. 1A).

It is tempting to speculate that the chimeric origin of SIVcpz occurred in chimpanzees before subspeciation of *P. t. troglodytes* and *P. t. schweinfurthii*. However, this proposed scenario raises several questions: Why is SIVcpz not more widely distributed in all four of the proposed chimpanzee subspecies? Why is it so focal in the two subspecies in which it is currently found? These issues raise further questions regarding the chimpanzee's anthropology, its natural history, the modes of transmission of SIVcpz among chimpanzees, and the reasons that it is not a severe pathogen (*5*). These questions lead to other hypotheses that speculate about the intermediate hosts that might have given rise to SIVcpz and ultimately to HIV-1 (Fig. 1, B and C).

Diversity

Although the interspersal of SIVcpz and SIVsm in the molecular phylogeny of HIV-1 and HIV-2, respectively, reveals successful cross-species transmission events, there are a surprisingly limited number of documented cases, and direct evidence of a simian-to-human transmission is still missing. This suggests that, in contrast to a fulminant zoonotic (a pathogen regularly transmitted from animals to humans), a complex series of events (for instance, adaptations and acquisition of viral regulatory genes such as *vpu*, *vif*, *nef*, and *tat* and structural genes *gag* and *env*) was required for these SIVs to infect a human and to sustain infection at levels sufficient to become transmissible within the local human population. Closer examination of HIV-1 and HIV-2 groups and subgroups reveals differences in variants and genetic groups and rates of transmission in different populations even after infection is well established. This complex picture is beginning to merge with our understanding of the dynamics of evolving lentiviral variants that infect the natural nonhuman primate hosts. For instance, within the eight HIV-2 groups, A and B are endemic, whereas the others represent single infected persons clustering closely to SIVsm strains (*2*, *6*). These observations reinforce the notion that important adaptations have been necessary for the virus to acquire the ability to be efficiently transmitted.

Since its emergence, HIV-1 group M has diverged into numerous clades or subtypes (A to K) as well as circulating recombinant forms (CRFs) (*7*). There appears to have been an early "starburst" of HIV-1 variants leading to the different subtypes. CRFs have segments of the genome derived from more than one subtype, and two of these—CRF01_AE in Southeast Asia and CRF02_AG in West Africa—have relatively recently emerged as fast-spreading epidemic strains. Currently, subtype C and subtype A + CRF02_AG account for approximately 75% of the 14,000 estimated new infections that occur daily worldwide.

Regarding HIV in the Americas, subtype B was the first to appear in the United States and the Caribbean, heralding the epidemic when AIDS was first recognized in 1981. Subtype B remains the most prevalent (>80%) throughout the Americas, followed by undetermined CRFs (9%), F (8%), and C (1.5%) (*7*). There is a particularly high degree of genetic diversity of HIV-1 in Cuba, unparalleled in the Americas and similar to Central Africa (*8*), perhaps be-

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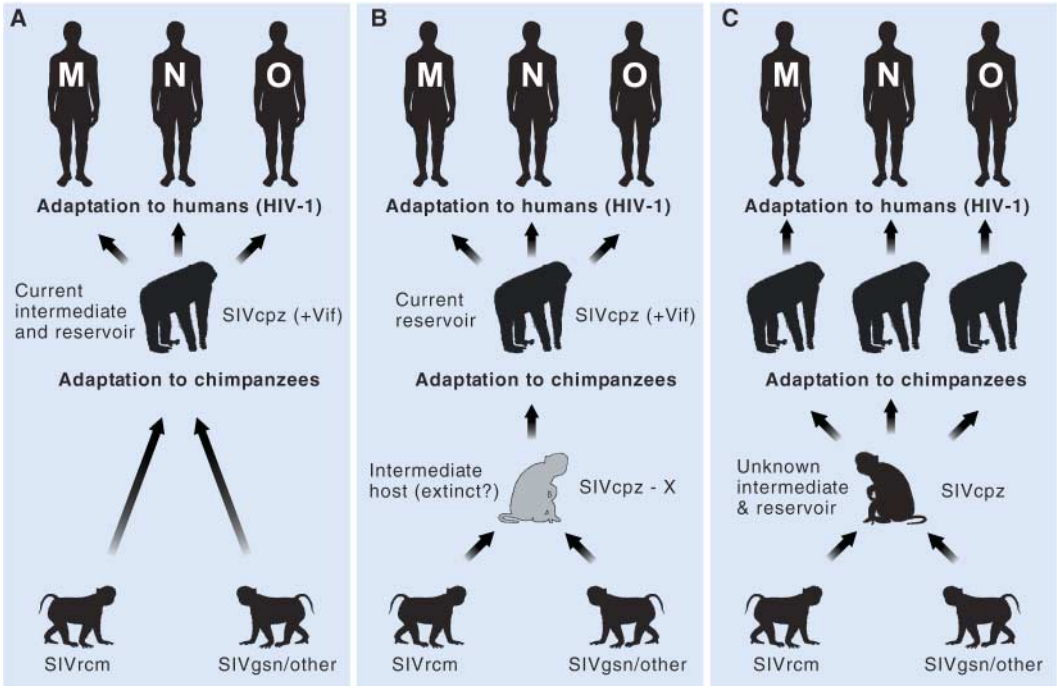

Fig. 1. Possible cross-species transmission events giving rise to SIVcpz as a recombinant of different monkey-derived SIVs. Three different scenarios are considered. (**A**) *P. t. troglodytes* as the intermediate host. Recombination of two or more monkey-derived SIVs [likely SIVs from red capped mangabeys (rcm), and the greater spot-nosed (gsn) or related SIVs, and possibly a third lineage]. Recombination requires coinfection of an individual with one or more SIVs. Chimpanzees have not been found to be infected by these viruses. (**B**) Unidentified intermediate host. The SIVcpz recombinant develops and is maintained in a primate host that has yet to be identified, giving rise to the ancestor of the SIVcpz/HIV-1 lineage. *P. t. troglodytes* functions as a reservoir for human infection. (**C**) An intermediate host that has yet to be identified, which is the current reservoir of introductions of SIVcpz into current communities of *P. t. troglodytes* and *P. t. schweinfurthii*, as a potential source of limited foci of diverse SIVcpz variants.

cause Cuban troops served there for the United Nations. Less than 50% of Cuban infections are subtype B, and sequences of all subtypes are represented either as subtypes or in CRFs. The incidence of subtype C appears to be increasing rapidly in Brazil, just as it has in Africa and in East Asia.

Host-Pathogen Evolution

Upon adaptation of the virus to a new host, Darwinian selection would not only apply to the virus and host, but also to the modes of transmission between individuals in the new species, as well as to efficient replication within the infected individual (*9*). The modes of transmission of SIV likely differ from species to species. For example, parenteral transmission from bites and wounds as a consequence of aggression may be the main route of transmission in many nonhuman primates (*5*), whereas the major current mode of HIV transmission among humans is sexual. Nevertheless, parenteral transmission may well have played a more important role early in the emergence of the African epidemic (*10*), and it remains a risk today when nonsterile injecting equipment is used. Thus, efficient HIV transmission across mucosal surfaces may be a strongly selected secondary adaptation by the virus, given that humans tend to inflict minor parenteral injuries on each other less frequently then simians.

Whether genetic properties of the virus determine the rapid spread of HIV-1 subtypes such as C and CRF02_AG is not clear, although relative to other subtypes, subtype C appears to be present at higher load in the vaginas of infected women (*11*). It is not yet apparent whether certain subtypes are more virulent than others for progression to AIDS, although some indications of differences do exist (*12*).

SIVs do not appear to cause AIDS in their natural African hosts (Table 1). Similar to humans, however, several species of Asian macaques (*Macaca* spp.) develop AIDS when infected with a common nonpathogenic lentivirus of African sooty mangabeys (SIVsm became SIVmac). This observation demonstrates the pathogenic potential of such viruses after cross-species transmission from an asymptomatic infected species to a relatively unexposed naïve host species. Furthermore, SIV infection of macaques has provided a powerful experimental model system in which specific host as well as viral factors can be controlled and independently studied (*13*).

During the AIDS pandemic, it has become clear that host genetic differences between individuals as well as between species affect the susceptibility or resistance of disease progression, revealing a clinical spectrum of rapid, intermediate, or slow progression or, more rarely, nonprogression to AIDS within infected populations. A range of distinct genetic host factors, linked to the relative susceptibility or resistance to AIDS, influence disease progression. In addition to those genes that affect innate and adaptive immune responses, recently identified genes block or restrict retroviral infections in primates (including the human primate). These discoveries provide a new basis for detailed study of the evolutionary selection and species specificity of lentiviral pathogens.

Among the most important antiviral innate and adaptive immune responses of the host post-infection are those regulated by specific molecules of the major histocompatibility complex (MHC) (*13*). It is conceivable that in the absence of a vaccine or antiviral drugs, the human population will evolve and ultimately adapt to HIV infection, in much the same way that HIV is evolving and adapting to selective pressures within its host. Indeed, examples of similar host-viral adaptation and coevolution are evident in lentivirus infections of domestic animals. Nevertheless, greater insight into CD4 tropic lentiviruses and acquired resistance to AIDS has come from African nonhuman primates, which are not only reservoirs giving rise to the current human lentivirus epidemic but also possible reservoirs of past and future retroviral plagues.

Host Resistance Factors Influencing HIV Infection and Progression to AIDS

In humans, a spectrum of disease progression has emerged. Within the infected population, there are individuals with increased susceptibility as well as increased resistance to infection, who display rapid or slow progression to AIDS, respectively. Analyses of several large AIDS cohorts have revealed polymorphic variants in loci that affect virus entry and critical processes for the intracellular replication of lentivirions as well as subsequent early innate and especially highly specific adaptive host responses (*14*). To date, there is a growing list of more than 10 genes and more than 14 alleles that have a positive or negative effect on infection and disease progression (Table 2).

Polymorphic loci that limit HIV infection include the well-described *CCR5Δ32* variants

(*15*, *16*). The chemokine ligands for these receptors also influence disease progression: One example is Regulated on Activation Normal T Cell Expressed and Secreted (RANTES) (encoded by *CCL5*), with which elevated circulating levels have been associated with resistance to infections and disease. Moreover, it is the combination of polymorphisms controlling levels of expression of ligands and their specific receptors that exerts the most profound effect on HIV susceptibility and progression to AIDS; for example, gene dosage of *CCL3L1* acts together with *CCR5* promoter variants in human populations (*17*).

After retrovirus entry into target cells, intracellular "restriction factors" provide an additional barrier to viral replication. To date, three distinct antiviral defense mechanisms effective against lentiviruses have been identified: TRIM5α, a tripartite motif (TRIM) family protein (*18*); apolipoprotein B editing catalytic polypeptide (APOBEC3G), a member of the family of cytidine deaminases (*19*); and Lv-2 (*20*). TRIM5α restricts post-entry activities of the retroviral capsids in a dose-dependent manner (*18*, *21*), and the human form of this protein has apparently undergone multiple episodes of positive selection that predate the estimated origin of primate lentiviruses (*22*). The species-specific restriction of retroviruses is due to a specific SPRY domain in this host factor, which appears to have been selected by previous ancestral retroviral epidemics and their descendant endogenous retroviral vestiges. TRIM5α proteins from human and nonhuman primates are able to restrict further species of lentiviruses and gamma-retroviruses, revealing a host-specific effect on recently emerged lentiviruses.

The cytidine deaminase enzymes APOBEC3G and APOBEC3F also represent post-entry restriction factors that act at a later stage of reverse transcription than TRIM5α and are packaged into nascent virions. The APOBEC family in primates consists of nine cytosine deaminases (cystosine and uracil) and two others that possess in vivo editing functions (*19*, *23*). In the absence of the lentivirus accessory gene "*virion infectivity factor*" (*vif*), APOBEC3G becomes incorporated into nascent virions and inhibits HIV activity by causing hypermutations that are incompatible with further replication. At the same time, this represents a potentially risky strategy for the host, given that in some circumstances it might provide an opportunity for viral diversification (*24*). As with the primate TRIM5α family, APOBEC3G activity shows species-specific adaptations (*25*) emphasizing that coevolution of lentiviruses was a prerequisite for adaptation to a new host after cross-species transmission (*26*). Thus, although APOBEC3G clearly possessed an ancient role in defense against RNA viruses, a function that predates estimates of the emergence of today's primate lentiviruses, APOBEC3G appears to remain under strong positive selection by exposure to current RNA viral infections (*27*).

Evolving Host Resistance in the Face of New Lentiviral Pathogens

Failing the establishment of productive infection by the earliest innate defenses, natural killer (NK) cells of the immune system sense and destroy virus-infected cells and modulate the subsequent adaptive immune response. At the same time, the potentially harmful cytotoxic response of NK cells means that they are under tight regulation (*28*), which is centrally controlled by a raft of activating and inhibitory NK receptors and molecules encoded by genes of the MHC. Viruses have a long coevolutionary history with molecules of the immune system and a classical strategy for evading the cytotoxic T cell response of the adaptive immune system is by altering antigen presentation by MHC class I-A, I-B, or I-C molecules (*29*). In turn, the NK response has evolved to sense and detect viral infection by activities such as the down-regulation of class I MHC proteins.

Human lymphoid cells protect themselves from NK lysis by expression of the human MHC proteins human lymphocyte antigen (HLA)–C and HLA-E as well as by HLA-A and HLA-B. HIV-1, however, carries accessory genes, including *nef*, that act to differentially decrease the cell surface expression of HLA-A and HLA-B but not HLA-C or HLA-E (*30*). Such selective down-regulation may not only facilitate escape from cytotoxic T lymphocytes (CTLs) that detect antigens presented in the context of these MHC proteins but also escape from NK surveillance that might be activated by their loss of expression. However, within human MHC diversity, there may be an answer to the deception of NK cells by HIV. Certain alleles of HLA (HLA-Bw4) have been found to act as ligands for the NK inhibitory receptor (KIR) KIR3DSI and correlations with slower rates of progression to AIDS in individuals with the HLA-Bw4 ligand have been made with the corresponding expression of KIR3DSI expression on NK cells (*31*). The strength of this association between increased NK cell killing and HIV progression will have to bear the test of time as well as the test of the epidemic.

In the event that rapidly evolving pathogens such as HIV are able to evade innate defenses, adaptive defenses such as CTLs provide mechanisms for the recognition and lysis of new virus-infected targets within the host. This recognition depends on the highly polymorphic MHC class I molecules to bind and present viral peptides. However, a long-term CTL response will only be successful if the virus does not escape it through mutation. Additionally, it is advantageous to maintain MHC variability for controlling HIV replication and slowing disease progression (*32*), given that a greater number of viral peptides will be recognized if the infected individual is heterozygous for HLA antigens.

More importantly, there are qualitative differences in the ability of individual class I molecules to recognize and present viral peptides from highly conserved regions of the virus. These differences are observed in the spectrum of rapid, intermediate, and slow progressors in the HIV-infected human population (Table 2). Independent cohort studies have demonstrated the effects of specific HLA class I alleles on the rate of progression to AIDS with acceleration conferred by a subset of HLA-B*35 (HLA-B*3502, HLA-B*3503, and HLA-B*3504) specificities (*33*, *34*). Most notably, HLA-B*27 and HLA-B*57 have been associated with long-term survival. Both of these class I molecules restrict CTL responses to HIV by presenting peptides selected from highly conserved regions of Gag. Mutations that allow escape from these CTL-specific responses arise

Table 1. Natural lentivirus infections without immunopathology in African nonhuman primates.

Naturally resistant species and features of resistance
Examples
Chimpanzees (*P. troglodytes*), SIVcpz (HIV-1 in humans)
Sooty mangabeys (*C. atys*), SIVsm (HIV-2 in humans)
African green monkeys (AGMs) (*Chlorocebus* sp.), SIVagm
Common features of asymptomatic lifelong infection
Persistent plasma viremia
Maintenance of peripheral CD4 T cell levels
Sustained lymph node morphology
High mutation rate in vivo
Marginal increase in apoptosis returning to normal range
Transient low-level T cell activation and proliferation, returning to normal range
Less rigorous T cell responses than those in disease-susceptible species
Observed in one of these species, awaiting confirmation in others
High replication of virus in gastrointestinal tract, transient loss of CD4 T cells
CTL responses to conserved viral epitopes
Maintenance of dendritic cell function
Early induction of transforming growth factor–β1 and FoxP3 expression in AGMs with renewal of CD4 and increase in IL-10

only at great cost to viral fitness, reflected in lower viral loads (*13*) and survival benefit.

Evidence is emerging that HIV-1 is continuing to adapt under pressure from HLA-restricted immune responses in the human population. In a study that examined the relationship between HIV reverse transcriptase sequence polymorphisms and HLA genotypes, virus load was found to be predicted by the degree of HLA-associated selection of viral reverse transcriptase sequence (*35*). In a broader context, these results indicate that HLA alleles in the host population play an important role in shaping patterns of adaptation of viral sequences both within the host and at large.

Recent data have also started to suggest a potential influence that the HIV-1 epidemic may have on descendants of the HIV-infected population. In examining the relative contributions of HLA-A, HLA-B, and HLA-C alleles on restricting effective antiviral CTL, Kiepiela *et al*. (*36*) observed that HLA-B but not HLA-A allele expression influenced the rate of disease progression in that cohort. Thus, certain HLA-B alleles that favor long-term survival with HIV infection, in the absence of treatment, will be positively selected and will continue to evolve more rapidly over time. This coevolution of virus and host would be predicted to continue over generations until a relative equilibrium is reached between host resistance genes and virus infection. This would perhaps be similar to the asymptomatic lentivirus infections currently observed in naturally infected African nonhuman primates.

Disease Resistance in African Nonhuman Primates

Studies of SIVs in their natural hosts have been difficult and limited because of ethical issues and the endangered status of some species. For the most part, SIV natural history studies have been restricted to chimpanzees, sooty mangabeys, and African green monkeys. The chimpanzee is the closest living relative of humans, and two of its subspecies—*P. t. schweinfurthii* in East Africa and *P. t. troglodytes* in Western Central Africa—have certain wild communities with infected individuals (*1*). Although we should be cautious with generalizations, differences in transmission patterns may exist between the naturally infected monkey and ape populations (*5*). The prevalence of naturally occurring SIVsm in sooty mangabeys and SIVagm in African green monkeys appears to be relatively high, between 30 and 60%, increasing with age. However, SIVcpz infection across remaining free-ranging chimpanzee populations appears to be relatively low and regionally focal, restricted to certain troupes or communities in which it may reach levels greater than 20% (*1*, *37*, *38*).

Table 2. Human genes identified that influence HIV infection and disease.

Gene products	Allele(s)	Effect
	Barriers to retroviral infection	
TRIM5α	SPRY species specific	Infection resistance, capsid specific
ABOBEC3G	Polymorphisms	Infection resistance, hypermutation
	Influence on HIV-1 infection	
Coreceptor/ligand		
CCR5	Δ32 homozygous	↓ Infection
CCL2, CCL-7, CCL11 (MCP1, MCP3, eotaxin), H7		↑ Infection
Cytokine		
IL-10	5′A dominant	↓ Infection
	Influence on development of AIDS	
Coreceptor/ligand		
CCR5	Δ32 heterozygous	↓ Disease progression
CCR2	164 dominant	↓ Disease progression
CCL5 (RANTES)	ln1.1c dominant	↑ Disease progression
CCL3L1 (MIP1α)	Copy number	↓ Disease progression
DC-SIGN	Promoter variant	↓ Parenteral infection
Cytokine		
IL-10	5′A dominant	↑ Disease progression
IFN-γ	179T dominant	↑ Disease progression
Innate		
KIR3DS1 (with *HLA-Bw4*)	3DS1 epistatic	↓ Disease progression
Adaptive		
HLA-A, HLA-B, HLA-C	Homozygous	↑ Disease progression
*HLA-B*5802*, *HLA-B*18*	Codominant	↑ Disease progression
*HLA-B*35-Px*	Codominant	↑ Disease progression
*HLA-B*27*	Codominant	↓ Disease progression
*HLA-B*57*, *HLA-B*5801*	Codominant	↓ Disease progression

Few naturally infected chimpanzees have been available for study (*1*), and much of the knowledge of the immune responses to lentivirus in this species has come from animals infected with HIV-1 strains in the late 1980s and 1990s (*39*). In contrast to pathogenic HIV-1 infection of humans or SIVmac infection of rhesus macaques, the hallmarks of lentivirus infection in chimpanzees include the absence of overt CD4 T cell loss, a lack of generalized immune activation, and the preservation of secondary lymphoid structure, specifically with respect to MHC class II antigen presenting cells (APCs) in infected lymph nodes (*39*, *40*). In addition, there is little increase in apoptosis or anergy and no marked loss of interleukin (IL)-2–producing $CD4^+$ T cells after infection (Table 1) (*41*, *42*). These findings further underscore the importance of maintaining intact dendritic cell function and CD4 T cell interaction, which are symptoms of early immune dysfunction in infected AIDS-susceptible species (*40*).

Notably, $CD8^+$ CTLs in chimpanzees recognize highly conserved HIV-1 Gag epitopes, which correspond to almost identical epitopes presented by HLA-B*57 and HLA-B*27 alleles of humans with nonprogression or slow progression to AIDS (*43*). A phylogenetic analysis of MHC class I alleles in chimpanzees as compared with humans reveals an overall reduction of HLA-A, HLA-B, and HLA-C lineages in chimpanzees. Furthermore, comparative analysis of intron 2 sequences strongly supports marked reduction in the MHC class I repertoire, especially in the HLA-B locus (*44*). These data imply that chimpanzees may have experienced a selective sweep, possibly caused by a viral epidemic in the distant past. We could envision such a selective sweep of the modern day human population in the HIV-1 pandemic (in the absence of antiretroviral therapy), with a strong positive selection for HLA-B alleles beneficial for long-term survival (*36*).

It is becoming clearer that infected chimpanzees are relatively resistant to developing AIDS, not because they control virus load better than humans (*45*), but because they avoid the immunopathological events that affect the function of lymphoid tissue in humans and macaques that progress to AIDS. Thus, certain African nonhuman primates, such as chimpanzees, serve as natural lentivirus reservoirs and sustain lentivirus infection without the immunopathology (*40*, *42*) (Table 1). Mature CD4 T cells of chimpanzees are susceptible to SIVcpz or HIV-1 infection and cytopathology, but unlike macaques and humans, chimpanzees retain the renewal capacity to replace and sustain sufficient numbers of immunologically competent CD4 T cells to maintain immunological integrity (*39*).

How Will Humans Evolve in the Era of Medical Intervention?

New generations of more effective antiviral drug combinations are being developed, as are

strategies to reduce virus load and facilitate restoration of CD4+ T cell numbers. The opportunity to convert an HIV-1 viremic patient into an aviremic individual by antiviral chemotherapy is an achievable clinical aim (*46*). Concern remains over the resident proviral population in long-lived lymphocytes and in APCs. Under antiretroviral treatment, aviremic CD4+ T cell tropic primate lentiviruses may also share features with the true "slow" replicating lentiviruses of ruminants. The prototypic lentiviruses of sheep and goats infect and persist in APCs such as dendritic and monotype/macrophage lineages without overt plasma viremia (*47*). Disease development is asymptomatic until late stages and is extremely protracted. Even in the absence of viremia and CD4 T cell loss, symptoms associated with chronic inflammation develop insidiously in diverse tissues resulting in a range of clinical conditions including encephalitis, pneumonia, and arthritis. It is important to consider that after solving the side effects of antiviral therapies such as lipodystrophy, HIV-infected aviremic humans might develop such classical lentivirus symptoms over a longer period of time.

Clearly, prophylactic strategies such as vaccines to prevent infection are the ultimate public health goals. Failing this, there is abundant evidence of previous retroviral epidemics embedded within the human genome. These suggest that there are further undisclosed antiretroviral defenses, which have coevolved and will continue to coevolve in human populations in response to retroviral insurgents.

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AIDS RESEARCH

Reconstructing the Origins of the AIDS Epidemic From Archived HIV Isolates

Five HIV isolates that had been forgotten in freezers for 2 decades are revealing new details about how and when the virus spread from Africa to Haiti and then exploded on the world scene. Evolutionary biologist Michael Worobey of the University of Arizona in Tucson led the new study, which analyzed HIV saved from five Haitian AIDS patients treated in Miami in 1982 and 1983. "It was the next best thing to being able to travel back in time," says Worobey, who obtained the samples through the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.

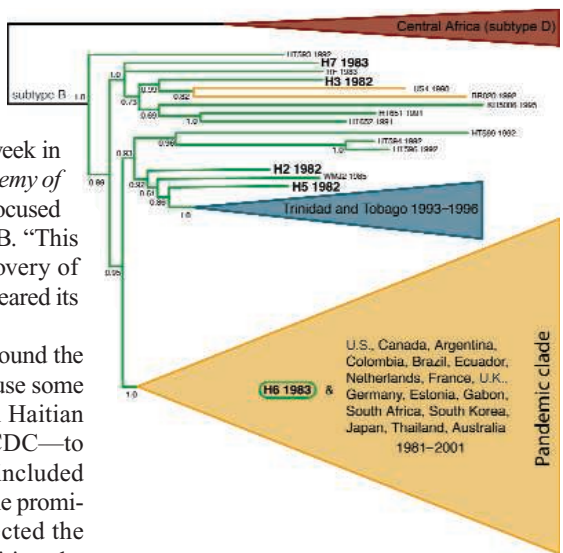
In a paper published online this week in the *Proceedings of the National Academy of Sciences*, Worobey and co-workers focused on what's known as HIV-1 subtype B. "This was the variant that led to the discovery of AIDS and so much of the story that reared its head after 1981," says Worobey.

Much controversy has swirled around the origins of the AIDS epidemic. Because some of the first AIDS cases surfaced in Haitian immigrants to the United States, CDC—to the consternation of many—once included Haitians as a special risk group. Some prominent Haitian researchers have rejected the idea that the virus spread from Haiti to the United States, contending that it likely moved in the other direction.

Molecular analyses of the archival isolates confirmed earlier reports that subtype B traveled from central Africa to Haiti about 1966, entering the United States 3 years later. The researchers' estimated probability that the virus instead traveled from the United States to Haiti—0.00003—is infinitesimal. "The methods are beautiful, and the analysis is elegant," says Bette Korber, an immunologist at Los Alamos National Laboratory in New Mexico, who published similar results in *Science* in 2000 (9 June, p. 1789).

Some are not persuaded. Jean "Bill" Pape, who heads the largest AIDS research program in Haiti, says Worobey and co-workers simply "restate prejudices advanced 2 decades ago." Pape notes that the authors offer no details about the sexual histories of the five Haitian immigrants, who he contends could have been infected by Americans. He also questions whether HIV arrived in 1966, pointing to retrospective studies in Haiti that did not find an AIDS case until 1978.

Other AIDS researchers counter that the Worobey paper offers the clearest picture yet of how the young epidemic matured. "It's a very nice piece of evolutionary sleuthing," says Beatrice Hahn, a virologist at the University of Alabama, Birmingham, and a co-author of the Korber study. One provocative finding, says Hahn, suggests that although several different isolates of subtype B came from Haiti to the United States, only one got a foothold. It had not evolved ways to transmit more readily, says Worobey, and appears to have been "lucky" to have spread among high-risk populations—primarily, gay males in the United States. It then spread to Canada, South America, Europe, Asia, and even back to Africa (see figure).



Descent of HIV. A new analysis of stored blood samples from early AIDS patients shows Haiti (green) as a steppingstone between central Africa and the rest of the world.

Anne-Mieke Vandamme, a molecular epidemiologist at the Rega Institute for Medical Research in Leuven, Belgium, and co-author of a 2003 study that arrived at similar conclusions, says the new work underscores a fundamental feature of HIV epidemiology. Most of the early isolates found in Haitians quickly "died out," she notes. "You need an event that boosts the transmission, and the epidemic takes off." In this case, Vandamme says the promiscuity of gay men appears to have boosted the prevalence above a threshold that allowed the virus to thrive. **–JON COHEN**

CREDIT: ADAPTED FROM GILBERT *ET AL.*, *PNAS* (29 OCTOBER 2007)

China Wants More Enviros …

BEIJING—At last month's Communist Party Congress, China's leaders enshrined environmental protection in the country's constitution. Now China's State Environmental Protection Administration (SEPA) has inked a deal to train grassroots conservationists. SEPA's China Environmental Culture Promotion Association and Rare, a conservation group in Arlington, Virginia, will train budding Chinese conservationists in techniques—such as festivals and puppetry—that can stir public interest and pride in biodiversity in order to "translate knowledge into personal, meaningful change," says Brett Jenks, president of Rare. Southwest Forestry University in Kunming City in China will help launch projects at 10 sites next year, most likely in some of China's roughly 2000 nature reserves. **–RICHARD STONE**

… And Heads to the Moon

The first spacecraft launched beyond Earth orbit by a developing nation is on its way to the moon. Chang'e 1, named for the Chinese goddess who flew to the moon, will arrive in lunar orbit 5 November. The 24 October launch drew large crowds near the Sichuan launch center, was broadcast live on national television, and prompted senior Chinese officials to declare plans to share culled science data. The 2300-kg satellite will circle the moon for a year and send back three-dimensional images of the lunar surface and an analysis of moon dust. India and the United States plan to launch moon orbiters next year, and Japan announced this week that it will launch a robotic rover in the next decade. **–ANDREW LAWLER**

Oceans Are Nickel-and-Dimed

HONOLULU—A dozen marine scientists gathered here last week at the behest of the International Seabed Authority to design safeguards against the anticipated damage from the industrial harvesting of potato-sized nodules rich in nickel and copper sitting on a part of the Western Pacific sea floor with great biodiversity. "Practically every individual [organism] is a new species," said Alex D. Rogers of the Zoological Society of London. The scientists inserted a patchwork of nine 400-km-by-400-km protected areas, in between mining claims in an area nearly the size of Australia. Harvesting is expected to start within a decade. If adopted, as expected, the restrictions would be the first such sanctuaries in international waters. **–CHRISTOPHER PALA**

When HIV spread afar

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Since the first cases of AIDS were described in the United States in 1981, the origin of this devastating disease has intrigued scientists and the general public alike. This fascination is reflected in the numerous theories put forth for the emergence of HIV, the most infamous of which involves the alleged use of HIV-contaminated oral polio vaccine in Africa during the late 1950s (1). Thankfully, a steady stream of virological data and phylogenetic analyses now means that the oral polio vaccine theory has rightly been assigned to the back shelves of science fiction (2). The article by Gilbert *et al.* in this issue of PNAS (3) similarly uses an elegant combination of virology and phylogeny to shed light on another key moment in the history of HIV: its spread from an origin in Africa to the Americas.

Haiti: Sink or Source?

From the earliest days of AIDS reporting, it was clear that the Caribbean nation of Haiti was particularly significant in this epidemic. Indeed, HIV/AIDS was initially found to be relatively frequent in persons of Haitian origin, and some Haitian isolates of HIV-1 fell on relatively deep branches in phylogenetic trees, suggesting that the virus took an early foothold in that country. Until now, however, the connection among Africa, Haiti, and industrialized nations like the United States has largely remained the stuff of speculation. By deploying an impressive armory of phylogenetic techniques, Gilbert *et al.* (3) provide the first solid evidence for the role of Haiti in the emergence and evolution of HIV.

Like many RNA viruses and retroviruses, HIV-1 is genetically very diverse, falling into a series of phylogenetically defined clades, or subtypes, that have differing geographic distributions, as well as an ever-expanding set of intersubtype recombinants. The focus of this particular study is HIV-1 subtype B, the form of virus that was first described in U.S. populations in the early 1980s and that still dominates infections in most industrialized nations to the present day. The global spread of subtype B is considered a major event in the history of HIV/AIDS because it marks the point when the virus first entered the large, wealthy, and highly mobile populations of the Western world.

Two factors contribute to the power of the Gilbert *et al.* (3) study: (*i*) the use of sophisticated methods of sequence analysis that are able to account for some of the idiosyncrasies of HIV evolution and (*ii*) the retrieval of gene sequence data from "archival" HIV samples, notably those from patients of Haitian origin who carried the virus in the early 1980s. Although far older samples are available from a number of other viruses (for example, those for human influenza A virus date back to 1918; ref. 4), these HIV viruses are certainly old with respect to the spread of HIV outside of Africa and so provide a unique window into the timescale of viral evolution. With this happy marriage of new sequence data and state-of-the-art bioinformatics, Gilbert *et al.* first show that those subtype B viruses in Haiti have their origins in Africa. They then provide compelling evidence that Haiti has unwittingly acted as the conduit for the spread of HIV to the United States and a wide range of other localities rather than being simply a regional sink.

Of more interest are the attempts of Gilbert *et al.* (3) to put these evolutionary events into an historical time frame. To achieve this result, the authors estimated the time to the most recent common ancestor (TMRCA) of HIV-1 subtype B using a "relaxed" molecular clock (5), which allows the rate of evolutionary change to vary in a lineage-specific manner, a major factor in the evolution of HIV. They estimated that the date for the spread of HIV-1 to Haiti from its ancestry in Africa is between 1962 and 1970 (with a mean of 1966). Importantly, this timescale corresponds well with a period when many Haitians returned to their home country from the Congo, after the latter's independence from Belgium and subsequent political crises. Because the Congo region has been shown to play a pivotal role in the genesis of HIV (6), the correspondence between the travel data and the inferred epidemiological timescale of Gilbert *et al.* provides strong circumstantial evidence that the timescale is broadly correct. This study therefore highlights the role played by socioeconomic factors such as human migration in the history of infectious disease. In addition, the migration of HIV from Haiti to the United States and beyond is dated to the period 1966–1972, perhaps 30–40 years after the virus first established itself in the human population in Africa.

A Slow Fuse for AIDS in the Americas?

Perhaps the most fascinating insight from this exercise in viral archeology is that HIV was spreading in the United States for at least 9 years before its first clinical description. This finding is bound to spark a lively debate because it may seem untenable that such a long period of "cryptic" transmission could be possible in a nation with such an advanced health care system. Some may argue that the now-characteristic symptoms of severe immunodeficiency would have been spotted sooner, even given the time lag between initial infection and the onset of AIDS. Although it is theoretically possible that the high virulence of HIV infection—manifest as AIDS—did not evolve until later in the U.S. epidemic, such that HIV virulence has increased through time, a far more likely explanation for a long history of HIV in the United States before the discovery of "patient zero" is simply that it went undetected or was misdiagnosed. Indeed, this cryptic period is far shorter than an equivalent period in Africa, where the disease may have remained unrecognized for more than half a century. Furthermore, for the "increasing virulence" hypothesis to be true, all of the subtypes of HIV-1 that have independent origins in central-west Africa would have to have evolved the symptoms of AIDS independently, which seems untenable. A slow fuse for the explosion of AIDS in the United States in the early 1980s is also compatible with serological studies that suggest that thousands of individuals may have already been HIV-infected in this country by the late 1970s (7).

Another possibility is that the relaxed molecular clock used by Gilbert *et al.* (3), although a major advance, does not fully capture the nature of HIV evolution. The most likely cause of any clock error is that HIV exhibits rather differ-

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See companion article on page 18566.

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ent evolutionary rates at the intrahost and interhost levels. In particular, there is mounting evidence for an inverse relationship between rates of viral transmission and rates of evolutionary change, with the highest rates observed within individual hosts (8). Consequently, the very rapid spread of HIV through standing networks of gay men and injecting drug users in industrialized nations during the early 1980s may have been characterized by unusually low rates of evolutionary change, which in turn will introduce error into estimates of the TRMCA. This important dynamical relationship has two possible causes: (*i*) that intrahost evolution, in contrast to that occurring among hosts, is dominated by the positive selection of amino acid changes that facilitate immune escape and that elevate rates of evolutionary change over that expected under neutral genetic drift (9), and/or (*ii*) that most of the mutations that occur within hosts are purged at transmission to new hosts because of strong purifying selection in this new environment. For example, a proportion of the HIV genome appears to be "reset" at interhost transmission because of mismatches between mutations that confer escape from host cytotoxic T lymphocyte responses and the HLA type determining the specificity of that response (10). In short, mutations that are advantageous in one individual may be detrimental in another. This continual rewinding may slow the molecular clock in fast epidemics.

The global spread of subtype B is considered a major event in the history of HIV/AIDS.

Although it is possible that the timescale for HIV evolution proposed by Gilbert *et al.* (3) has, to some extent, been adversely affected by changing rates of epidemic spread, the correspondence between the documented movement of individuals from Africa to Haiti and the dates estimated in this paper make it likely that any rate variation is adequately encompassed within the distribution of evolutionary rates estimated under a relaxed molecular clock. While conclusive proof for the cryptic transmission of HIV will obviously require the sampling, sequencing, and phylogenetic analysis of HIV samples from the United States obtained during the 1970s, this paper undoubtedly sets the benchmark for future studies in viral phylogeography.

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The emergence of HIV/AIDS in the Americas and beyond

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HIV-1 group M subtype B was the first HIV discovered and is the predominant variant of AIDS virus in most countries outside of sub-Saharan Africa. However, the circumstances of its origin and emergence remain unresolved. Here we propose a geographic sequence and time line for the origin of subtype B and the emergence of pandemic HIV/AIDS out of Africa. Using HIV-1 gene sequences recovered from archival samples from some of the earliest known Haitian AIDS patients, we find that subtype B likely moved from Africa to Haiti in or around 1966 (1962–1970) and then spread there for some years before successfully dispersing elsewhere. A ''pandemic'' clade, encompassing the vast majority of non-Haitian subtype B infections in the United States and elsewhere around the world, subsequently emerged after a single migration of the virus out of Haiti in or around 1969 (1966–1972). Haiti appears to have the oldest HIV/AIDS epidemic outside sub-Saharan Africa and the most genetically diverse subtype B epidemic, which might present challenges for HIV-1 vaccine design and testing. The emergence of the pandemic variant of subtype B was an important turning point in the history of AIDS, but its spread was likely driven by ecological rather than evolutionary factors. Our results suggest that HIV-1 circulated cryptically in the United States for ≈12 years before the recognition of AIDS in 1981.

evolution | pandemic | phylogeny | archival | Haiti

Viral gene trees can deliver powerful insights into ecological and evolutionary processes (1). Population-level phylogenetic patterns reflect both transmission dynamics and genetic change, which in turn can accumulate because of selection (driven, for example, by host immunity) or drift. In this study, we use a phylogenetic approach and HIV-1 gene sequences recovered from early victims of AIDS to investigate when, where, and how HIV-1 emerged from Africa and spread worldwide. Although it accounts for fewer infections than subtype C, which dominates the HIV-1 epidemics in southern Africa and India and is spreading elsewhere (2), HIV-1 group M subtype B is arguably the most widespread HIV variant. No other subtype or circulating recombinant form predominates in as many countries around the world (3).

Our aim here is to combine phylogenetic, molecular evolutionary, historical, and epidemiological perspectives in an attempt to reconstruct the history of the subtype B pandemic. Such retrospective knowledge can clarify the past but also potentially can be of value for rational vaccine design that takes into account the genetic diversity of the virus (4) and for predicting the future complexity of regional and global HIV-1 genetic diversity. This is a function of how frequently HIV-1 strains disperse to, then successfully colonize, new geographic ranges and host populations, a question we address here.

The idea that Haiti might have played a special role in the unfolding of the AIDS pandemic predates the discovery of HIV. Soon after the initial recognition of AIDS (5), evidence of a high prevalence of the syndrome among Haitian immigrants in the United States (6) helped fuel speculation that Haiti may have been the source of the mysterious newly identified syndrome (7). It has since become clear that the causative agent, HIV-1 group M, actually originated not in Haiti but in central Africa, apparently sometime around 1930 (8, 9).

Nevertheless, the possibility remains that Haiti was the stepping-stone for the emergence of the exceptionally widespread subtype B lineage, and this idea has implications that extend beyond historical interest. Some researchers have noted that Haitian HIV-1 sequences tend to occupy basal positions on the subtype B phylogeny, suggestive of the epidemic originating there (9–11). Others argue vigorously that the Haitian HIV/AIDS epidemic was seeded from the United States, perhaps after Haiti became a popular sex tourism destination in the mid-1970s (12–14). However, these competing hypotheses have never been rigorously tested, despite their importance for understanding the global spread and vaccine-relevant genetic diversity of HIV-1.

To test these hypotheses, we recovered complete HIV-1 *env* and partial *gag* gene sequences from archival specimens collected in 1982–1983 from five Haitian AIDS patients, all of whom had recently immigrated to the United States and were among the first recognized AIDS victims (6). Being independent of and much older than the few previously published Haitian HIV-1 full-length *env* strains, these archival sequences offer a unique opportunity for resolving the origin and emergence of subtype B. They provide direct insight into Haitian HIV-1 genetic diversity at an exceptionally early time point and an unbiased sample for testing the *a priori* specified phylogenetic hypotheses addressed here.

Results and Discussion

The Geographical Origin of Subtype B. Under the ''Haiti-first'' model, non-Haitian subtype B strains are expected to be phylogenetically nested within an older and hence more extensive range of Haitian genetic variation, with Haitian lineages branching off closest to the B subtype ancestor. To test whether it is this

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Abbreviations: TMRCA, time of the most recent common ancestor; MCMC, Markov chain Monte Carlo.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. EF159970–EF159974 and EF362773–EF362777).

See Commentary on page 18351.

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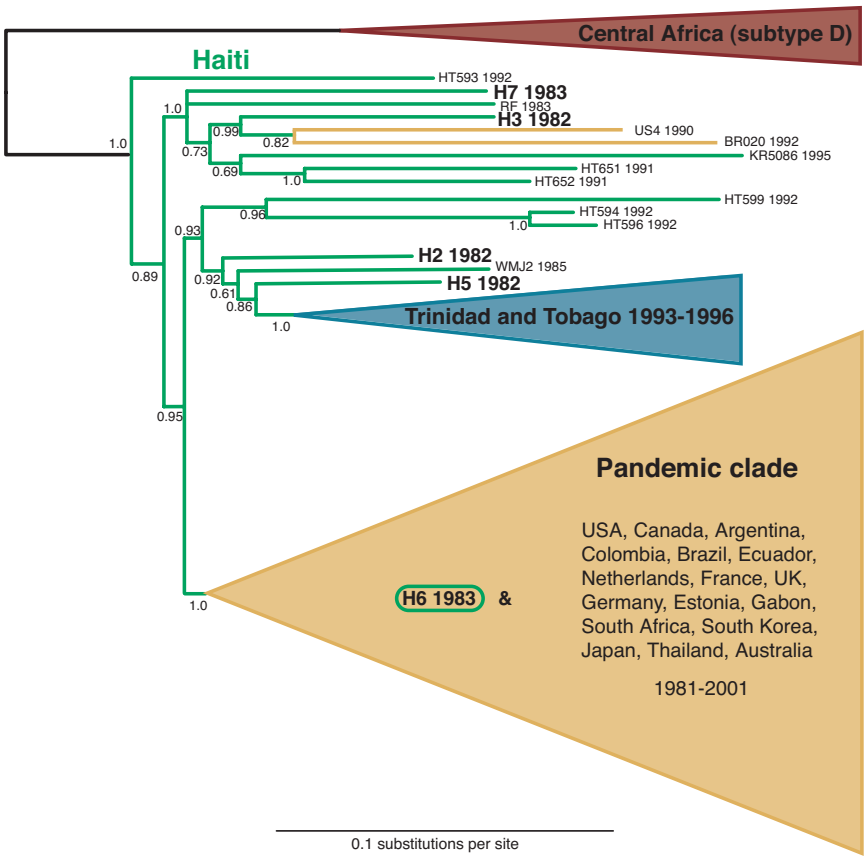

Fig. 1. The abridged majority-rule consensus tree summarizing the results from the MrBayes analysis of complete *env* genes. The branch lengths represent the mean value observed for that branch among the postburnin sampled trees. Posterior probabilities are indicated for each node. As expected under a ''Haiti-first'' model, the non-Haitian subtype B strains are phylogenetically nested within an older and more diverse range of Haitian viral variants. The 11 sequences of the Trinidad and Tobago clade and the 96 sequences of the pandemic clade are schematically represented by the blue and yellow triangles, respectively. Haitian or Haitian-linked sequences are shown in green, with the archival sequences labeled in larger bold text. The unabridged tree is available as SI Fig. 4.

or an alternative pattern that characterizes this HIV-1 subtype [supporting information (SI) Fig. 3], we conducted a detailed Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analysis using an alignment of the Haitian archival sequences plus 117 previously published subtype B *env* sequences from a total of 19 countries. Five African strains of subtype D (the closest relative to subtype B) served as the outgroup.

On the *env* gene phylogeny, the archival sequences occupy basal positions within subtype B (Fig. 1 and SI Fig. 4); there is extremely strong statistical support for a Haitian origin of subtype B, in that the probability of a U.S. or other non-Haitian origin [i.e., the posterior probability of any non-Haitian sequence(s) occupying the most basal position in the B clade] is <0.001. These results indicate, with high statistical significance, that subtype B arrived and began spreading in Haiti before it spread elsewhere and was not originally introduced to Haiti from the United States. In addition, the unequivocal support for the monophyly of subtype B as a whole ($P = 1.0$) supports the previous suggestion of a single (epidemically successful) introduction of this subtype from central Africa to Haiti (10).

Drummond *et al.* (15) found that relaxed-clock models are more accurate and precise at estimating phylogenetic relationships than unrooted methods. In other words, when data have evolved in a somewhat clock-like fashion, incorporating knowledge of the tempo of evolution can make topology estimation more reliable compared with methods that ignore this information. Therefore, we were also interested in what the relaxed-clock results (described below) revealed with respect to where subtype B originated (i.e., the topological relationships among subtype B sequences, regardless of timing/branch lengths). Under the relaxed-clock model, the posterior probability of a U.S. origin was 0.00003, and the probability of a Haitian origin was 0.9979.

The inference that subtype B reached Haiti before spreading to other countries does not depend on a dating analysis. One of the advantages of a Bayesian statistical framework is that it yields direct estimates of the probability of phylogenetic hypotheses, and in this case there is strong evidence to reject a U.S. or other non-Haitian origin of subtype B. This means that even if there is some uncertainty regarding precisely when HIV-1 entered Haiti or the United States (see below), there is little doubt about the sequence of events; the clear-cut topological information implies that the entry to Haiti occurred first. Moreover, our sampling bias in favor of non-Haitian subtype B makes the ''Haiti-first'' inference conservative; the Haitian strains occupy the basal positions within subtype B even though there were many more opportunities for recovering non-Haitian basal strains, if they existed.

The Dispersal of HIV-1 Out of Haiti. We next investigated how many times the older Haitian HIV-1 epidemic has seeded detectable secondary outbreaks elsewhere. We found evidence for only three such events, despite the fact that our data set comprised 109 non-Haitian subtype B strains from the Caribbean, North and South America, Europe, Africa, Asia, and Australia. One instance is confined to Trinidad and Tobago (Fig. 1). All of the HIV-1 sequences from this country form a distinct monophyletic

grouping with the strongest possible support (posterior probability of 1.0). Previous studies have asserted homosexual/bisexual contact with North American foreigners in the late 1970s or early 1980s as the alleged route of introduction (16, 17). Our results indicate this was not the case; the Trinidad and Tobago clade is unequivocally nested among Haitian strains, not North American ones, clear evidence that the predominantly heterosexual epidemic in this country can be explained by a single introduction linked to Haiti.

The next, and most important, secondary epidemic accounts for all but three of the remaining non-Haitian strains, encompassing 96 sequences representing every other country in our data set (Fig. 1). This "pandemic" clade forms another unequivocally monophyletic cluster ($P = 1.0$) nested within the basal Haitian strains. As for the Trinidad and Tobago clade, the most parsimonious explanation for this pattern is that all these subtype B infections from across the world emanated from a single founder event linked to Haiti. This most likely occurred when the ancestral pandemic clade virus crossed from the Haitian community in the United States to the non-Haitian population there.

The only other Haiti-linked outbreak we detected is comparatively insignificant, here comprising a single Brazilian sequence (BR020) and one American one (US4). Additional rare chains of transmission may have emerged from Haiti but remained undetectable in this study. Likewise, pandemic clade viruses may have reentered Haiti but were undetectable. Regardless, it is evident from this large international sample that the subtype B epidemics in most afflicted countries and the bulk of subtype B infections worldwide are caused by viruses belonging only to the pandemic clade.

Three additional nominally non-Haitian strains are noteworthy for falling among the basal Haitian lineages rather than within the three non-Haitian clades (Fig. 1). Each one provides further support for the notion that strains linked to Haiti occupy the deepest branches within subtype B. RF was sampled in 1983 from a Haitian immigrant to the United States. Like our five newly sequenced strains, it represents a Haitian strain that entered the United States via an immigrant host (10). WMJ2 was sampled in 1985 from a perinatally infected infant born to an HIV-positive Haitian immigrant mother (18). KR5086 was recovered in 1995 from a South Korean sailor infected in the Dominican Republic (19), a country directly linked to Haiti in terms of both geography and HIV-1 epidemiology (20). These cases, plus the additional ones considered in our study, show that the virus moved out of Haiti on many separate occasions but did so mostly as dead-end infections that evidently failed to ignite successful epidemics. A key question for future research will be to determine why Haiti-to-U.S. epidemic outbreaks have apparently become established so rarely since the initial introduction in or around 1969, despite presumably frequent movement of the virus because of rising HIV-1 prevalence, continued migration, and the once-thriving sex tourism industry linking Haiti and the United States.

Several additional analyses corroborated these findings. First, we reanalyzed our *env* gene data by replacing the African subtype D outgroup sequences with a variety of alternatives from different subtypes and geographical regions. This had no impact on the basal position of the Haitian strains within subtype B (SI Fig. 5*a*); their ancestral position cannot be dismissed as an artifact of convergent evolution with African subtype D sequences. Second, the *gag* sequences also unambiguously place the Haitian sequences in the most ancestral positions within subtype B ($P = 0.9930$) (SI Fig. 5*b*). We also inspected the nucleotide substitutions that mapped specifically onto the branch leading to the pandemic clade. For *env*, all eight such changes were silent at the amino acid level. For *gag*, only one of the six changes on the relevant branch indicated a change in amino acid between the Haitian strains and the pandemic clade strains. The paucity of amino acid substitutions along this clade-defining branch suggests that the ancestor of the pandemic clade probably possessed no selective advantage over other Haitian strains; its remarkable epidemic success may simply reflect ecological factors rather than evolutionary ones (chance colonization of a new population, as opposed to competitively superior transmission fitness). However, analysis of complete genomes would be necessary to definitively rule out selection.

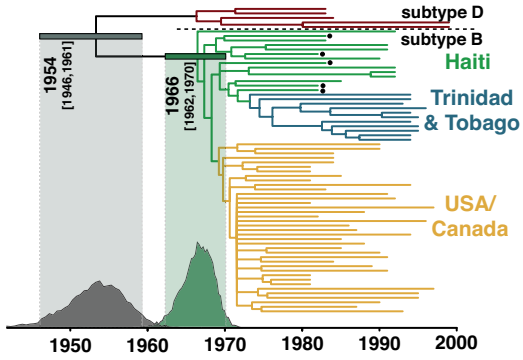

Fig. 2. The consensus tree of the relaxed molecular clock analysis, with the Haitian archival sequences bulleted. The tips of the tree correspond to year of sampling, and the branch lengths reflect the mean of the posterior probability density. The posterior probability density for the TMRCA for subtype B in Haiti is depicted in dark green, and the 95% highest probability density (HPD) is shown by the horizontal bar and light-green shading. The TMRCA means and 95% HPDs for the other key nodes were as follows: subtype B/D ancestor = 1954 (1946–1961); subtype D ancestor = 1966 (1961–1971); Trinidad and Tobago subtype B ancestor = 1973 (1970–1976); and U.S./Canada subtype B ancestor = 1969 (1966–1972). This analysis resolves the position of the archival sequence H6 as basal to the pandemic clade. Under the relaxed molecular clock, $P_{\text{non-Haitian-origin}} = 0.00003$, $P_{\text{simultaneous-origin}} = 0.0021$, and $P_{\text{Haitian-origin}} = 0.9979$.

The Timing of the Emergence of Subtype B. We used a second Bayesian MCMC method that simultaneously estimates phylogenetic relationships and times of most recent common ancestors (15) to perform a supplementary phylogenetic analysis on a reduced data set. This method uses a "relaxed molecular clock" model, so-called because it relaxes the need to assume a constant rate of molecular evolution across the tree to obtain date estimates from gene sequences. We estimated the time of the most recent common ancestor (TMRCA) of subtype B at 1966 (1962–1970) (Fig. 2), a date that suggests its arrival in Haiti may have occurred with the return of one of the many Haitian professionals who worked in the newly independent Congo in the 1960s (21).

The TMRCA of the U.S. epidemic is estimated to be 1969 (1966–1972) (Fig. 2), suggesting that HIV-1 was circulating cryptically in the United States for ≈12 years before the initial recognition of AIDS in 1981. The evidence of a single origin of the pandemic variant of subtype B allowed us to date the beginning of the actual U.S. HIV-1 epidemic, rather than the ancestor of multiple viruses introduced from Haiti; if multiple introductions from Haiti had occurred, the "U.S. MRCA" would actually correspond to a Haitian virus that predated the initial entry of HIV-1 to the United States (11). Serological evidence of an ≈5% prevalence of HIV-1 by 1978 in men who have sex with men (MSM) populations in both San Francisco (22) and New York City (23) suggests that several thousand individuals in the United States would already have been infected by then. Even assuming the fastest-documented growth rates for HIV-1 (24), this implies that the virus had been spreading in the MSM population for several years before this point, consistent with our

findings here. The virus may well have been spreading slowly for an extensive period, perhaps in the heterosexual population, before entering the highest-risk MSM subpopulation, where it spread explosively enough to finally be noticed. We contend that the phylogenetic estimate, with appropriate confidence intervals, provides more reliable information on the date of the origin of the U.S. epidemic than the available epidemiological data, which cannot resolve this question.

Nevertheless, although our relaxed-clock methods should be reasonably robust to rate variation among lineages and uncertainty in phylogenetic inference, some caution is always warranted when such inferences are made. For example, the relatively sparse sampling of both Haitian and Trinidadian sequences means it is conceivable that more intensive future sampling could recover deeper-branching lineages and push back these TMRCA estimates slightly. This is unlikely to apply to the U.S. TMRCA, on the other hand, because of the already dense sampling of this HIV-1 population. If obtainable, additional archival sequences should help clarify the early spread of subtype B with greater precision.

The three-decade gap between the estimated timing of the HIV-1 M group ancestor (9) and the earliest evidence of HIV/AIDS in Africa (25, 26) seems unexceptional in comparison to the U.S. cryptic period, especially because a good deal of tuberculosis-caused mortality in Africa must have gone unrecognized as AIDS-related, then as now. Taken together with our dating analysis, including the subtype B/D ancestor dated to 1954 (1946–1961) (Fig. 2), the extensive cryptic period in the United States, therefore, provides compelling corroboration of an early 20th century M group ancestor (9).

Conclusion

Our findings imply that Haiti has the oldest-known HIV/AIDS epidemic outside of sub-Saharan Africa, which helps explain the high prevalence of AIDS and HIV-1 among Haitians in the early 1980s. Because of its 40-year history, the HIV-1 epidemic in Haiti exhibits a greater range of viral genetic diversity than the rest of the world's subtype B strains combined, much as the HIV-1 epidemic in the Democratic Republic of the Congo does for group M as a whole (27). This raises the possibility that subtype B strains in Haiti or elsewhere might exhibit distinct or more diverse antigenic properties compared with pandemic clade viruses. Vaccines derived from consensus or other central sequences should perhaps be based on extensive sampling of Haitian HIV-1 if they are intended to cover both Haitian subtype B strains as well as the pandemic clade.

Although it has long been clear that population bottlenecks and founder effects are a feature of the unfolding HIV/AIDS pandemic, the series of bottlenecks that punctuated the global emergence of subtype B is remarkable. The lack of evidence for selection associated with the spread of the pandemic clade of subtype B, moreover, points to the importance of chance events and ecological interactions in driving what was perhaps the most explosive worldwide dispersal of HIV-1.

Our phylogenetic estimates of timing anchor previous epidemiological observations that, on their own, cannot reliably date the origin of regional epidemics. Taken together, these sources of information suggest that HIV-1 was circulating in one of the most medically sophisticated settings in the world for more than a decade before AIDS was recognized.

Methods

The Archival Samples. Peripheral blood mononuclear cell (PBMC) samples were collected in 1982 and 1983 at Jackson Memorial Hospital in Miami, FL, during one of the first investigations establishing that Haitians in Haiti and elsewhere were at risk for AIDS (6). One of the six PBMC samples obtained for this study failed to yield any amplifiable HIV-1 PCR products. As described in Pitchenik *et al.* (6), all of the patients were Haitian immigrants who had entered the United States after 1975 and progressed to AIDS by 1981 and hence were presumably infected with HIV-1 before entering the United States. The position of these sequences on the subtype B phylogeny (distinct from and basal to the dominant U.S. variant of subtype B) is consistent with this sequence of events.

Amplification and Sequencing of Archival HIV-1 DNA. DNA was extracted from 10 μl of peripheral blood mononuclear cells by using QIAamp DNA micro kits (Qiagen, Valencia, CA), following the manufacturer's instructions for extractions from blood. After extraction, DNA was eluted into 100 μl of elution buffer AE and stored frozen at −20°C until required for DNA analyses. DNA was PCR-amplified from the extracts by using a nested PCR approach (28). First-round PCRs were undertaken in 25 μl of final volume reactions, using 0.1 μl of Platinum Taq HiFi enzyme (Invitrogen, Carlsbad, CA)/0.1 μl of 25 mM dNTP mix/2.5 mM (final concentration) $MgSO_4$/10× PCR buffer/1–5 μl of DNA extract. Second-round amplifications were performed on 1 μl of the first-round PCR product by using the same reagent concentrations. Enzyme activation, dissociation, and extension temperatures followed the manufacturer's guidelines, with an extension time of 3 min. Annealing temperatures varied by extract in response to initial amplification success rates.

After amplification, the PCR products were visualized on 0.8% agarose gels stained with ethidium bromide and then purified by using QIAquick spin columns (Qiagen). Purified products were sequenced by using several overlapping primer pairs (28) by the University of Arizona Genomic Analysis and Technology Core Facility with ABI Big Dye 3.1 chemistry (Applied Biosystems, Foster City, CA) on Applied Biosystem 3730xl DNA Analyzers. Each sample was extracted, PCR-amplified, and sequenced twice to ensure that the sequences generated were not modified through low template copy number. We recovered five full-length *env* sequences and five partial (0.7- to 1.2-kb) *gag* sequences.

Sequence Alignments. We used the Los Alamos National Laboratory HIV sequence database (29) to download all full-length published *env* and *gag* gene sequences of subtypes B and D. We then subjected the resulting sequence set to strict quality control measures to remove (*i*) incomplete sequences; (*ii*) sequences not published in a peer-reviewed journal; (*iii*) multiple sequences from the same patient; (*iv*) sequences suspected *a priori* of possibly anomalous evolutionary patterns (from long-term nonprogressors with *nef* deletions, laboratory workers infected accidentally, sequences exhibiting evidence of hypermutation, etc.); (*v*) sequences with midpeptide stop codons, frame-shift mutations, or nonnucleotide characters; or (*vi*) sequences for which there was any uncertainty regarding which subtype they belonged to. To search for such sequences, which might include unidentified intersubtype recombinants, we screened all sequences using the REGA HIV-1 Subtyping Tool (30) and then removed any sequence with bootstrapping support <100% or bootscanning support <1.0 for clustering with subtype B or D.

To ensure that no very-early-diverging subtype B strains were removed by this procedure, we inferred additional phylogenies that included the "cleaned" sequences (data not shown). For *env*, the only such strains that were positioned basal to the pandemic clade were from Trinidad and Tobago, and these clustered with the other sequences from the Trinidad and Tobago clade. Similarly, for *gag*, the only nonpandemic clade sequences that were removed (US4 and RF) were ones that had already been identified as basal in the *env* analysis; all other sequences with bootstrap or bootscan support <100% or 1.0 were from the pandemic clade. Unlike intersubtype recombinants, the possible nonexclusion of intrasubtype recombinants is not expected to

affect inferences regarding the geographical origin and emergence of subtype B because intrasubtype recombination cannot plausibly lead to strains from one locality systematically falling basal to all of the others. Moreover, although unidentified intrasubtype recombination might increase the variance of dating estimates, it is unlikely to systematically bias these dates in one direction or the other in an exponentially growing population (31).

The resulting data sets were codon-aligned and then adjusted by eye in Squint Ver. 1.0 (M. Goode, University of Auckland, Auckland, New Zealand), and regions of ambiguous alignment were removed. We constructed three additional *env* alignments replacing the D subtype outgroup with a subtype C sequence from India, a subtype A sequence from Kenya, or a CRF01 (A/E) sequence from Thailand. All previously published sequences (see the taxon labels in SI Figs. 4 and 5*b*) are available from the Los Alamos National Laboratory HIV sequence database. All alignments are available from the authors upon request.

The Bayesian MCMC Phylogenetic Analysis and Estimation of the Probability of a Haitian or non-Haitian Origin of Subtype B. We used MrBayes, Ver. 3.1 (32), to perform two independent runs of 20 million steps (for *env*). Examination of the MCMC samples with Tracer, Ver. 1.3 [A. Rambaut (University of Edinburgh) and A. J. Drummond (University of Auckland, Auckland, New Zealand); http://beast.bio.ed.ac.uk], indicated adequate mixing of the Markov chain. We discarded the first 2 million steps from each run as burn-in and combined the resulting MCMC samples ($n = 36{,}002$) for subsequent estimation of posteriors. Fewer steps (5 million) were required for convergence and adequate mixing in the *gag* analyses.

We used tree filtering in PAUP, Ver. 4.0b10 (33), to calculate the posterior probabilities of a Haitian, non-Haitian, or effectively simultaneous Haitian/non-Haitian origin of subtype B. Briefly, we removed from the posterior sample any tree with a Haitian sequence(s) in the most basal position. Only 4 of 36,002 *env* trees had a non-Haitian basal sequence ($P_{\text{non-Haitian-origin}} = 0.00011$), and 24 others placed the Haitian and non-Haitian sequences in reciprocally monophyletic clades ($P_{\text{simultaneous-origin}} = 0.00066$), which left 35,974 with a Haitian sequence or group of sequences, in the most-ancestral position(s) within subtype B ($P_{\text{Haitian-origin}} = 0.9992$). A similar approach was followed for the estimation of the probability of a Haitian or non-Haitian origin of subtype B for the *gag* and the relaxed-clock analyses.

For both the *env* and *gag* data sets, we used a parsimony approach as implemented in MacClade, Ver. 4.08 (34), to identify the nucleotide substitutions that mapped onto the branch leading to the pandemic clade of subtype B. We then determined whether these changes were synonymous or nonsynonymous.

The Relaxed Molecular Clock Analysis. To infer the timescale of HIV-1 group M subtype B evolution, we used a Bayesian molecular clock method (15), as implemented in BEAST (http://beast.bio.ed.ac.uk), under an uncorrelated log-normal relaxed molecular clock model with a Bayesian Skyline coalescent tree prior. For this analysis to run in a reasonable time, we considered only subtype B sequences from Haiti, Trinidad and Tobago, and the United States (plus one from Canada), and we removed some pandemic clade sequences from overrepresented years and localities. The maximum *a posteriori* tree from the MrBayes analysis (available upon request) revealed that the sequences were scattered across the entire pandemic clade, consistent with a U.S. entry of the founding virus. We ran 10 independent MCMC analyses, with each run consisting of 100 million steps, and then discarded the first 5 million steps from each run as burn-in and combined the resulting postburn-in MCMC samples for subsequent estimation of posteriors.

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