resistance early in infection influences host health during the infection. It is entirely likely that the increased resistance mediated by *E. faecium* is simply delaying the death of the worms rather than promoting tolerance. This notion is further supported by the fact that worms without *E. faecium* show increased *S*. Typhimurium invasion beyond the intestinal lumen, which is indicative of a resistance defect. In the mouse, Rangan *et al.* [1] found no difference in the levels of *S*. Typhimurium being shed in the feces of *E. faecium* and control animals. As fecal levels are informative only regarding the amount of pathogen being transmitted, and not infecting, in future studies, it will be important to determine the infecting levels of *S*. Typhiumurium in target tissues of the mouse, including liver, spleen, intestinal tract, Peyer's patches, and mesenteric lymph nodes to determine if there is a similar resistance phenotype in mice conferred by *E. faecium*. It is crucial to measure pathogen burdens in the relevant target tissues and use the analyses described here when trying to determine whether a particular condition is influencing resistance or tolerance defenses because, without them, we are vulnerable to biasing our conclusions.

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Spotlight

'Patient 0' and the Origin of HIV/AIDS in America

Zhen Gong,[1] Xiaoyu Xu,[1] and Guan-Zhu Han[1,*]

The origin of the HIV/AIDS epidemic in North America remains contentious. A recent study uses phylogenetic and historical approaches to investigate the early history of HIV-1 group M subtype B in North America and shows that 'Patient 0' is not the source of the North American HIV/AIDS epidemic.

AIDS was first recognized in the United States (US) in 1981. The origin and subsequent spread of HIV-1 group M subtype B in North America remains contentious [1]. Molecular archeology, that is, recovering 'fossil' sequences from archival samples, has succeeded in revealing the emergence of HIV-1 group M at the beginning of the 20th century [2]. Thus it might provide a direct approach to investigate the early history of the HIV/AIDS epidemic in North America. However, recovering viral RNA from archival samples represents a major technical challenge, given that the samples contain low-titer viral copies, and the viral RNA was highly degraded due to long-term storage. In a recent study [3], Worobey *et al.* developed the RNA 'jackhammering' approach, which greatly increases the capability to recover viral RNA from archival specimens. Essentially a multiplex PCR, this approach amplifies many short, overlapping fragments with panels of primer pairs simultaneously. This approach has important applications in the field of molecular archeology, such as assembling genome-scale sequences of archival viruses. Indeed, with this approach, Worobey *et al.* recovered eight coding-complete HIV-1 genomes isolated from patients in New York City and San Francisco during 1978–1979 [2]. Until this report, the only available HIV genome of the pre-1980s is the one of HIV-1 strain Z321B, which was isolated from a Zairian woman with AIDS-like symptoms in 1976 [4]. Analyses of these novel HIV-1 genomes provide a snapshot of the origin and early history of HIV-1 in North America.

Although the early sequences assembled by Worobey *et al.* [3] represent nearly all of the oldest subtype B viral genomes, they are not phylogenetically basal to the other subtype B isolates. Instead, these sequences, together with other subtype B sequences from the US and elsewhere around the world, fall within the diversity of subtype B strains of Caribbean origin. This phylogenetic pattern strongly supports the hypothesis that the subtype B viruses spread from the Caribbean to the US [5]. Consistent with a previous study based on partial genome sequences [5], the jumping from Caribbean countries to the US was estimated to take place around 1971 (95% confidence interval: 1969–1973). This means that HIV-1 had been cryptically circulating for at least 10 years in the US before the initial recognition of AIDS. Phylogeographic analyses demonstrate that the ancestor of the US subtype B epidemic might circulate in New York City. However, this conclusion is based on early sequences from a limited number of the US states. More early genomes from other locations

in the US are needed to fully understand the early spread of HIV-1 in North America.

One of the most significant features of the work of Worobey *et al.* [3] lies in recovering the genome of the so-called 'Patient 0'. Worobey *et al.* present a detailed historical analysis of how the legendary status of 'Patient 0' was formed [3]. Let's make a long story short. According to the study of Worobey *et al.* [3], Patient 0 was initially numbered as Case 057, when the report reached the Centers for Disease Control and Prevention (CDC) in the US. An epidemiologic study that started with patients from California identified a cluster of homosexual men with AIDS-like symptoms, which was historically important in suggesting the sexual transmission of AIDS [6]. When Patient 0 was placed near the center of this cluster, he was renamed as 'Patient O', meaning a patient residing 'Out[side]-of-California'. Unfortunately, 'Patient O' was misinterpreted as 'Patient 0' when the CDC investigators rearranged the cluster cases by the disease onset dates. Although the identity of Patient 0 has never been disclosed by the CDC, the California-based journalist Randy Shilts revealed it (Gaétan Dugas, a highly sexually active Canadian airline steward) in his popular book *And the Band Played On* [7]. In this book, Dugas was claimed to deliberately spread the disease and was described as 'the Quebeçois version of Typhoid Mary' [7,8]. Subsequent media coverage of the book insinuated that Patient 0 was the source of the HIV/AIDS epidemic in North America. Although this idea has drawn criticism and clarification [8], it is still enjoying considerable attention by the public. The HIV-1 genome recovered from Patient 0 should directly test this idea. If it is true, the genome would be the earliest branching isolate among the US subtype B infections. However, Worobey *et al.* found that the viral sequence of Patient 0 was nested within the diversity of other US subtype B strains, which shows clear-cut evidence that Patient 0 was not the index case but is 'just one of many thousands infected prior to the recognition of HIV/AIDS' [3]. This finding should finally lay to rest the theory of Patient 0 as the source of the North American epidemic.

Although Worobey *et al.* succeeded in answering when and where the North American epidemic emerged, the question remains as to how the subtype B viruses entered North America. There are several plausible scenarios: (i) Caribbean immigrants brought the virus into North America; (ii) American individuals were infected by the virus when visiting the Caribbean, possibly via sex tourism; and (iii) contaminated commercial blood products imported from the Caribbean led to American infections [5,9]. To disentangle these possibilities, further virus archeological investigations of relevant samples should be performed.

The > 2500 serum samples which Worobey *et al.* serologically screened were collected for studying hepatitis B virus in the first place [3]. Worobey *et al.*'s work would not be possible without these archival samples. Archival samples play crucial roles in studying the emergence and evolutionary history of not only HIV-1 but also many other infectious agents, such as the 'Spanish flu' influenza virus [10]. Moreover, they are of potential importance in tracking the origin, diversification, and evolution of newly emerging diseases that circulate but have been neglected, such as Zika virus. Unfortunately, many archival samples are going to dwindle as their collectors fade away and thus will never be discovered. Therefore, the scientific community should conceive of ways to preserve archival samples.

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Spotlight

Spray-Induced Gene Silencing: a Powerful Innovative Strategy for Crop Protection

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Plant pathogens cause serious crop losses worldwide. Recent new studies demonstrate that spraying double-stranded RNAs (dsRNAs) and small RNAs (sRNAs) that target essential pathogen genes on plant surfaces confer efficient crop protection. This so-called spray-induced gene silencing (SIGS) strategy of disease