

# Infection during Infancy and Long Incubation Period of Leprosy Suggested in a Case of a Chimpanzee Used for Medical Research<sup>∇</sup>

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**The length of the incubation period of leprosy following *Mycobacterium leprae* infection has never been conclusively determined, owing to the lack of a method to demonstrate the presence of an asymptomatic infection. We report a rare case of leprosy in a chimpanzee in which a 30-year incubation period was strongly suggested by single nucleotide polymorphism (SNP) analysis.**

## CASE REPORT

A female chimpanzee (*Pan troglodytes*) named Haruna was caught in Sierra Leone, West Africa, and brought to Japan in 1980 when she was around 2 years old. She was used in hepatitis B studies for 6 years and in hepatitis C studies for another 4 years before she was retired to live out her life in a sanctuary. She and her fellow chimpanzees were kept according to the guidelines of Chimpanzee Sanctuary Uto, Wildlife Research Center, Kyoto University, Japan. Resident chimpanzees are treated with the same level of care as human patients in hospitals, and all physical examinations were carried out solely for the purpose of diagnosis and treatment, as approved by the sanctuary. Despite her experimental history, she is seronegative for HBsAg, HBs antibody (HBsAb), and HBcAb, as well as for the presence of hepatitis C virus (HCV) RNA. Results of other laboratory tests were all normal.

In January 2009, at around age 31 (the average life span of a chimpanzee is 40 to 50 years and rarely exceeds 50 years), swellings and nodules (Fig. 1A) that had not been observed in the past were noted on her face (Fig. 1B). A thorough examination under anesthesia revealed multiple nodular lesions around the eyes, lips, abdomen, forearms, and crus. Nasal swabs and skin smears from a forearm nodule revealed a large number of acid-fast bacilli, with formation of globi (Fig. 1C and 1D, respectively). Results of a skin tuberculosis (TB) test were negative. Histologic examination of the skin lesion showed a granulomatous accumulation of foamy histiocytes in the upper dermis that contained numerous acid-fast bacilli (Fig. 1E and F) that were positive for PGL-I immunostaining (PGL-I is an antigen specific to *Mycobacterium leprae*). PCR amplification of DNA purified from a skin biopsy sample was positive for *M. leprae*-specific Hsp70 (Fig. 1G), and the DNA sequence of 16S rRNA completely matched the reported *M. leprae* sequence (2).

At this point, a diagnosis of lepromatous leprosy was made and the patient was treated with multidrug therapy (MDT), including diaphenylsulfone, clofazimine, and rifampin, according to the protocol of the World Health Organization (WHO). These drugs were administered after being mixed with bananas or vegetable juice. Within 2 months of treatment, the skin lesions had significantly improved, and within 5 months, nasal swabs tested negative for acid-fast bacilli.

To determine the possible origin of *M. leprae* detected in this case, we analyzed single nucleotide polymorphisms (SNPs) for three reported loci in the *M. leprae* genome (13). By using PCR amplification followed by direct sequencing, positions 14,676, 1,642,875, and 2,935,685 of *M. leprae* DNA were identified as “T,” “T,” and “C,” respectively (Fig. 2). This genotype, which has been identified only in West Africa, was classified as SNP type 4; it is thought to have been introduced to parts of the Caribbean and South America, probably via the slave trade, but has not been identified in Japan or other Asian countries (13, 14).

Increased levels of serum anti-PGL-I antibody have been used for the diagnosis of lepromatous leprosy (1). High levels of serum anti-PGL-I antibody have also been reported in healthy household contacts in an area where the disease is endemic (7). However, there have been some arguments about the specificity of PGL-I for leprosy as well as about the clinical relevance of measuring the level of anti-PGL-I antibody for the diagnosis of leprosy, particularly for the detection of subclinical *M. leprae* infection (9). The results of analysis of archived serum samples from Haruna were negative for anti-PGL-I antibody until 25 October 2007 (ca. 1 year before her skin lesions were observed) and became positive on 13 May 2009, after the appearance of skin lesions, but returned to negative on 8 October 2009, approximately 5 months after MDT was administered (Fig. 3). Eight other chimpanzees imported to Japan in the same year and five others living in the same cage with Haruna for several years were all negative for serum anti-PGL-I antibody.

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Leprosy has afflicted humans for millennia and is caused by chronic infection with *M. leprae*. It is believed that the disease

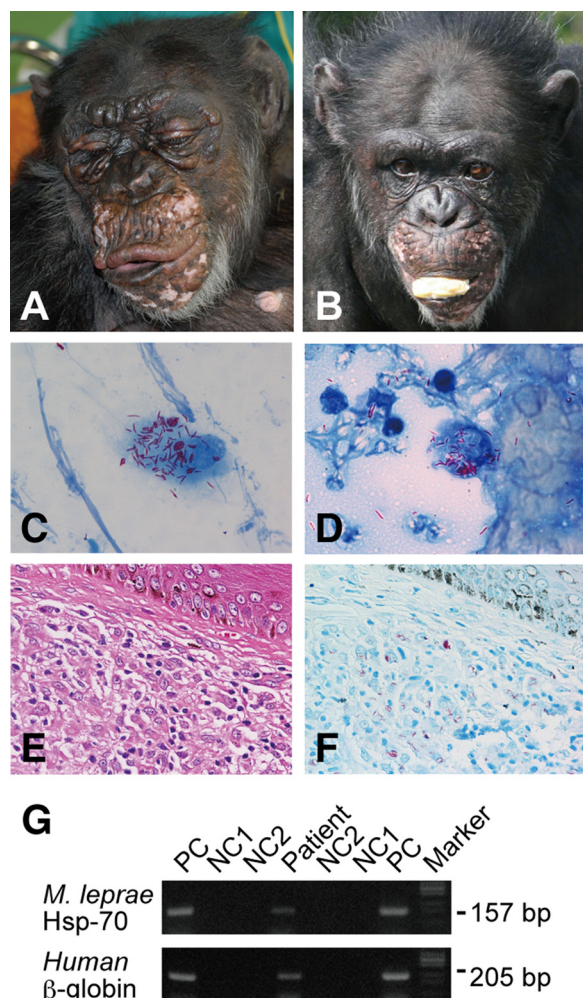


FIG. 1. (A) Facial leproma-like lesions of the chimpanzee Haruna (13 May 2009). (B) The same chimpanzee without lesions 1 year earlier (16 May 2008). (C) Ziehl-Neelsen staining of a nasal swab specimen showing globus-filled acid-fast bacilli ( $\times 1,000$  magnification). (D) A skin smear from a left forearm nodule also showing multiple acid-fast bacilli ( $\times 1,000$  magnification). Tissue staining was performed as previously described (18). (E) Hematoxylin and eosin staining of a skin biopsy sample from a right forearm nodule, showing accumulation of foamy histiocytes in the upper dermis ( $\times 400$  magnification). (F) Fite staining demonstrating numerous acid-fast bacilli within the histiocytes ( $\times 400$  magnification). (G) PCR analysis demonstrating *M. leprae* Hsp70 DNA. Tissue DNA was prepared using a QIAamp DNA Micro kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. PCR was performed as previously described (18) using specific primers (10, 17). PCR products were sequenced using an ABI Prism 310 genetic analyzer and GeneScan Collection software (Applied Biosystems). PC, positive control; NC1, negative control 1 (nuclease-free water was used as a template for PCR); NC2, negative control 2 (nuclease-free water was used instead of skin tissue to purify DNA). Human  $\beta$ -globin served as a positive control for DNA extraction from the skin biopsy sample.

is primarily transmitted by repeated airborne exposure to *M. leprae* through the nasorespiratory passage via close contact with multibacillary leprosy patients during infancy or early childhood and that the clinical disease becomes apparent only after a long incubation period (3). Although it is thought that *M. leprae* parasitizes histiocytes in the dermis and Schwann

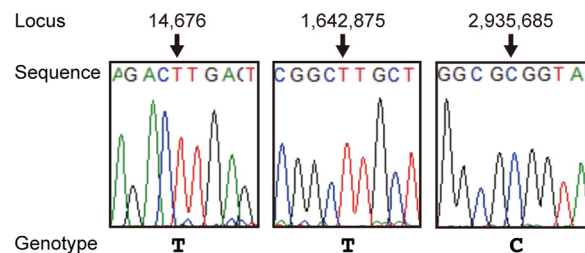


FIG. 2. Sequence analysis of the three reported SNPs of *M. leprae* DNA. PCR was performed using previously described primer sets (13), and the PCR products were sequenced using an ABI Prism 310 genetic analyzer and GeneScan Collection software (Applied Biosystems).

cells of the peripheral nerves (15), there is no available serologic or biologic method to demonstrate the presence of subclinical infection during latency. Therefore, it has not yet been possible to determine the length of incubation following infection.

*M. leprae* infection in this chimpanzee was highly unlikely to have occurred in Japan, particularly given the strict biosafety standards of primate housing facilities in experimental laboratories and the very low prevalence of leprosy in Japan. Therefore, the evidence strongly suggests that Haruna contracted a *M. leprae* infection when she was in West Africa before the age of 2 and that she developed lepromatous leprosy after a 30-year incubation period. Our results also suggest that the levels of serum anti-PGL-I antibody reflect the bacterial load in the patient but may not represent a marker for subclinical *M. leprae* infection.

To date, only three cases of leprosy in chimpanzees have been reported in the literature. All three chimpanzees were born in Africa and brought to the United States at a young age (4–6). One male chimpanzee captured in Sierra Leone developed leprosy some time between 5 and 6 years of age (4). Gormus et al. suggested the possibility that *M. leprae* might be transmitted among chimpanzees in Africa (5). Others have suggested that contact with an infected human had potentially occurred during the 2-to-3-month period when the chimpanzees were housed in outdoor cages while awaiting shipment after capture (4).

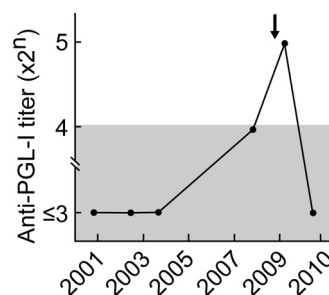


FIG. 3. Changes in the serum anti-PGL-I antibody titer before and after disease onset. The arrow indicates the approximate date when skin lesions appeared. The shaded area indicates the negative range. The serum anti-PGL-I antibody titer was measured using a gelatin particle agglutination test kit, Serodia-Lepre (Fujirebio, Tokyo, Japan), according to the manufacturer's instructions. Antibody titers  $> 2^5$  were judged to represent positive results.

Nonetheless, leprosy is clearly a common disease among humans and apes. Even after the worldwide efforts to reduce the disease burden of leprosy spearheaded by WHO ([http://www.searo.who.int/LinkFiles/GLP\\_SEA-GLP-2009\\_3.pdf](http://www.searo.who.int/LinkFiles/GLP_SEA-GLP-2009_3.pdf)) were successfully completed (8, 16), isolated leprosy cases in chimpanzees or other animals (e.g., armadillos) may still exist in the wild (11, 12, 19) and serve as potential sources of human infection. Therefore, it might be of particular importance to perform a survey of chimpanzee leprosy in West Africa (11).

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