Infection during infancy and long incubation period of leprosy suggested in the case of a chimpanzee used for medical research

Running title: 30-year incubation period of leprosy

Koichi Suzuki, Toshifumi Udono, Michiko Fujisawa, Kazunari Tanigawa, Gen’ichi Idani, and Norihisa Ishii

Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan; Chimpanzee Sanctuary Uto, Sanwa Kagaku Kenkyusho, Kumamoto, Japan; and Department of Welfare and Longevity Research, Wildlife Research Center, Kyoto University, Kyoto, Japan

* Corresponding author: Mailing address: Dr. Koichi Suzuki, Leprosy Research Center, National Institute of Infectious Diseases, 4-2-1 Aoba-cho, Higashimurayama-shi, Tokyo 189-0002, Japan. Phone: 81-42-391-8211. Fax: +81-42-394-9092. E-mail: koichis@nih.go.jp.
ABSTRACT

The length of incubation period of leprosy following *Mycobacterium leprae* infection has never been proven owing to the lack of a method to demonstrate the asymptomatic infection. We report a rare case of leprosy in a chimpanzee, in which a 30-year incubation period was strongly suggested by 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 studies on hepatitis B for 6 years and in studies on hepatitis C for another 4 years before she was retired to live out her life in a sanctuary. Chimpanzees were kept according to the guidelines of the Chimpanzee Sanctuary Uto, Wildlife Research Center, Kyoto University, Japan. They are treated as human patients and all physical examinations were carried out solely for the purpose of diagnosis and treatment, which was approved by the sanctuary. Despite her experimental history, she is seronegative for HBsAg, HBsAb, and HBcAb, as well as for the presence of HCV RNA. Results of other laboratory tests were all normal.

In January 2009, at around age 31 (average life span of a chimpanzee is 40-50 years and rarely exceeds 50 years), swellings and nodules were noted on her face (FIG. 1A) which had not been observed in the past (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 globi formation (FIG. 1C and 1D, respectively). A skin TB test was negative. Histologic examination of the skin lesion showed granulomatous accumulation of foamy histiocytes in the upper dermis that contained numerous acid-fast bacilli (FIG. 1E and 1F, respectively), which were positive for PGL-I immunostaining (PGL-I is an antigen specific to *M. leprae*). Polymerase chain reaction (PCR) amplification of DNA purified from skin biopsy was positive for *Mycobacterium leprae* (*M. leprae*)-specific Hsp-70 (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 rifampicin, according to the protocol of the World Health Organization (WHO). These drugs were administered by mixing with bananas or in vegetable juice. Within 2 months of treatment, the skin lesions were 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 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 determined 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 endemic area (7). However, there have been some arguments for the specificity of PGL-I for leprosy, as well as for 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). Analysis of archived serum samples from Haruna was negative for anti-PGL-I antibody until October 25, 2007 (ca. 1 year before her skin lesions were observed), became positive on May 13, 2009, after the appearance of skin lesions, but returned to negative on October 8, 2009, approximately five 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.
Leprosy has afflicted humans for millennia and is caused by chronic infection with *M. leprae*. It is believed that the disease 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 is only apparent after a long incubation period (3). Although it is thought that *M. leprae* parasitizes histiocytes in the dermis and Schwann cells of the peripheral nerves (15), there is no available serologic or biologic method to demonstrate subclinical infection during latency. Therefore, it has not yet been possible to demonstrate the length of incubation following infection.

*M. leprae* infection in this chimpanzee is 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 *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 bacterial load in the patient, but may not be a marker for subclinical *M. leprae* infection.

To date, only three cases of leprosy in chimpanzees have been reported in the literature. All three were born in Africa and brought to the US at a young age (4-6). One male chimpanzee captured in Sierra Leone developed leprosy sometime 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 2-3 month period when the chimpanzees were housed in outdoor cages while awaiting shipment after capture (4).

Nonetheless, leprosy is clearly a common disease among humans and apes. Even after worldwide efforts to reduce the disease burden of leprosy as spearheaded by WHO (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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FIGURE LEGENDS

FIG 1. (A) Facial leproma-like lesions of the chimpanzee, Haruna (May 13, 2009). (B) The same individual without lesions 1 year earlier (May 16, 2008). (C) Ziehl-Neelsen staining of a nasal swab specimen showing globi-filled acid-fast bacilli ($\times 1000$). (D) A skin smear from a left forearm nodule also showing multiple acid-fast bacilli ($\times 1000$). Tissue staining was performed as previously described (18). (E) Hematoxylin and eosin staining of a skin biopsy from a right forearm nodule showing accumulation of foamy histiocytes in the upper dermis ($\times 400$). (F) Fite staining demonstrating numerous acid-fast bacilli within the histiocytes ($\times 400$). (G) PCR analysis demonstrating $M. leprae$ Hsp-70 DNA. Tissue DNA was prepared using QIAamp DNA Micro kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer’s protocol. Polymerase-chain reaction (PCR) was performed as 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 of 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.

FIG 2. Sequence analysis of the three reported SNPs of $M. leprae$ DNA. PCR was performed using described primer sets (13) and the PCR products were sequenced using an ABI PRISM 310 Genetic Analyzer and GeneScan Collection software (Applied Biosystems).

FIG 3. Changes in serum anti-PGL-I antibody titer before and after disease onset. Arrow indicates the approximate date when skin lesions appeared. Shaded area indicates negative range.
Serum anti-PGL-I antibody titer was measured using a gelatin particle agglutination test kit, SERODIA-Leprae® (Fujirebio, Tokyo, Japan), according to the manufacturer’s instructions. Antibody titers >$2^5$ were judged as positive.