

## Molecular Investigation of the Aum Shinrikyo Anthrax Release in Kameido, Japan

PAUL KEIM,<sup>1\*</sup> KIMOTHY L. SMITH,<sup>1</sup> CHRISTINE KEYS,<sup>1</sup> HIROSHI TAKAHASHI,<sup>2</sup>  
TAKESHI KURATA,<sup>3</sup> AND ARNOLD KAUFMANN<sup>4</sup>

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011-5640<sup>1</sup>; Infectious Disease Surveillance Center<sup>2</sup> and Office of Directors,<sup>3</sup> National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo, 162-8640 Japan; and Stone Mountain, Georgia<sup>4</sup>

Received 5 July 2001/Returned for modification 8 September 2001/Accepted 5 October 2001

**In 1993, the Aum Shinrikyo cult aerosolized *Bacillus anthracis* spores over Kameido, Japan. Spore samples were obtained from the release site, cultured, and characterized by molecular genetic typing. The isolates were consistent with strain Sterne 34F2, which is used in Japan for animal prophylaxis against anthrax.**

Strain identification in *Bacillus anthracis* has been problematic due to a lack of distinguishing features, both phenotypic and molecular (1). With the identification of variable-number tandem repeats (VNTRs), identification of strains (unique genotypes) by multiple-locus VNTR analysis (MLVA) is now possible, and worldwide clone-based diversity patterns have been demonstrated (2). The VNTR loci are hypervariable and have multiple allelic states that provide high discrimination capacity for differentiating among strains and for identifying evolutionary relationships. There are about six major worldwide clonal lineages and nearly 100 unique types now known.

We recently applied this typing system to a forensic study of a *B. anthracis* strain associated with the Aum Shinrikyo cult's activities. Our objective was to determine whether the strain was similar to any other previously known types from our studies of *B. anthracis* worldwide diversity (2). This information could potentially identify the origin of the strain used in the bioterrorism attack and provide insights into how or why the attack was carried out.

In June 1993, the Aum Shinrikyo cult sprayed a liquid suspension of *B. anthracis* from their headquarters building in Kameido, near Tokyo, Japan (3; H. Takahashi, A. Kaufmann, K. L. Smith, P. Keim, and K. Taniguchi, Program 4th Int. Anthrax Conf., p. 27, 2001). While this aerosolization went largely unnoticed, the cult's later (1995) sarin gas attack of a Tokyo subway attracted worldwide attention. It was only with testimony of cult members and a retrospective investigation (3; Takahashi et al., 4th Int. Anthrax Conf.) that the 1993 incident was recognized as an anthrax release. The cult had developed and constructed a delivery system that involved the pumping of a liquid bacterial suspension up eight floors of their headquarters building to an aerosol dispersal device on the roof. During the aerosol dispersal, health authorities received numerous public complaints concerning odors emanating from the building. Upon investigation, they observed and collected a fluid

from the outside of the building. An archived (stored at 4°C) portion of this fluid was analyzed in this study.

We examined the fluid collected from the Aum Shinrikyo headquarters for bacterial content. Microscopically, malachite green and safranin staining revealed stained spores, a large amount of debris, and other bacterial cells. Aliquots of the fluid were cultured by spreading on sheep blood agar plates and incubated at 37°C under ambient CO<sub>2</sub> concentrations. Approximately 4 × 10<sup>4</sup> CFU per ml were observed, though most of the colonies grew only weakly. This weak growth was inconsistent with normal *B. anthracis* characteristics under these conditions, and we did not investigate these poorly growing bacteria further. About 10% of the colonies were typical of *B. anthracis* and had robust nonhemolytic "gray ground glass" colonies, ca. 4 × 10<sup>3</sup> CFU per ml. Forty-eight of these colonies were purified by single-colony streaking and subjected to MLVA. All 48 isolated colonies were determined to be *B. anthracis* and to have identical MLVA genotypes. The DNA isolation and MLVA genotyping were performed as previously described (2), and results were then compared to an electronic database of worldwide *B. anthracis* isolates. The eight VNTR marker analyses resulted in specific PCR amplicon sizes that had been previously observed only in *B. anthracis*. All 48 isolates had the following genotype: *vrrA*, 313 bp; *vrkB*<sub>1</sub>, 229 bp; *vrkB*<sub>2</sub>, 162 bp; *vrnC*<sub>1</sub>, 583 bp; *vrnC*<sub>2</sub>, 532 bp; CG3, 158 bp; pXO1-aat, 129 bp; pXO2-at, no amplification. The lack of PCR amplification at the pXO2 markers is consistent with strains that are missing the pXO2 plasmid entirely.

The MLVA genotype and the lack of amplification from the pXO2-at marker were consistent with results obtained with the Sterne vaccine strain. The Sterne strain is a member of the A3.b diversity cluster, and only four naturally occurring *B. anthracis* strains had the same seven-marker genotype (2). No other natural isolate that is also missing the pXO2 plasmid marker with this genotype has been observed. Given the commercial availability of the Sterne 34F2 vaccine strain in Japan for veterinary purposes, it is reasonable to conclude that the *B. anthracis* strain collected from the Aum Shinrikyo headquarters building is indeed Sterne. There have been reports that the cult had obtained a veterinary vaccine strain of anthrax (3). Our MLVA results substantiate this and lend credence to the

\* Corresponding author. Mailing address: Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640. Phone: (928) 523-1078. Fax: (928) 523-0639. E-mail: Paul.Keim@NAU.EDU.

scenario that the cult was using this strain in their activities and possibly in bioterrorism attacks. It would seem to be a relatively easy undertaking for the cult to obtain a vial of the animal vaccine and cultivate the Sterne strain. It is clear from our analysis that the *B. anthracis* dispersal by the Aum Shinrikyo had little possibility for endangering human life.

This work was supported by funding from the CBNP-NN20 program at the U.S. Department of Energy, the National Institutes of Health (RO1-GM60795), and the Cowden Endowment in Microbiology.

#### REFERENCES

1. **Brachman, P. S., and A. F. Kaufmann.** 1988. Anthrax, p. 95–107. *In* A. S. Evans and P. S. Brachman (ed.), *Bacterial infections of humans: epidemiology and control*, 3rd ed. Plenum Medical Book Company, New York, N.Y.
2. **Keim, P., L. B. Price, A. M. Klevytska, K. L. Smith, J. M. Schupp, R. Okinaka, P. Jackson, and M. E. Hugh-Jones.** 2000. Multiple-locus VNTR analysis (MLVA) reveals genetic relationships within *Bacillus anthracis*. *J. Bacteriol.* **182**:2928–2936.
3. **Smithson, A. E.** 2000. Rethinking the lessons of Tokyo, p. 71–111. *In* A. E. Smithson and L.-E. Levy (ed.), *Ataxia: the chemical and biological terrorism threat and the US response*. Stimson Center report no. 35. Stimson Center, Washington, D.C.