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What Does Detection of *Mycobacterium ulcerans* DNA in the Margin of an Excised Buruli Ulcer Lesion Tell Us?▽

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We determined by real-time PCR the distribution of *Mycobacterium ulcerans* DNA in the excised lesion of a Buruli ulcer patient. A new lesion developed adjacent to the site of excision in the patient. The excised margin around the primary lesion contained a small amount of mycobacterial DNA in the area where the secondary lesion developed. These results suggest that a relatively small number of infiltrating mycobacteria can lead to the development of a recurrence.

CASE REPORT

A 23-year-old man from the Ga District of Ghana presented at the Amasaman Health Centre in November 2002 with an ulcerated plaque (23 by 15 cm) on the right forearm (Fig. 1a). An ulcer (3 by 2 cm) was observed within the plaque. Laboratory analyses (PCR, culture, and histopathology) confirmed the clinical diagnosis of Buruli ulcer (BU). After the clinical diagnosis of BU, the plaque lesion was surgically removed en bloc on 18 November 2002 with a margin of 2 to 3 cm of healthy-looking tissue (Fig. 1b). For the analysis of the spread of *Mycobacterium ulcerans* in the lesion, samples were immediately collected along the long axis of the excised tissue (Fig. 1b), and individual specimens of about 100 mg each were stored in liquid nitrogen until they were analyzed. The risk of cross contamination was minimized by taking the samples starting with the macroscopically healthy looking margins and working toward the center of the lesion, with a separate disposable scalpel used to cut each piece. Twenty-two specimens adjacent to each other were obtained from this strip of tissue. Two extra samples (samples 23 and 24 in Fig. 1b) were taken from the margin in a region where, on the basis of the macroscopic appearance of the tissue, the surgeon had decided to extend the excision beyond the originally decided margins (Fig. 1b). Fifty nanograms of DNA/sample was used for *M. ulcerans* real-time PCR quantification, as described previously (11). This analysis revealed a focal distribution of *M. ulcerans*, with the highest mycobacterial burden near the center of the ulcer (sample 14 in Fig. 2). While the *M. ulcerans* content in this sample was >100-fold higher than that in any other sample, smaller peaks were also observed at the edges of the ulcer (samples 8 and 11). No significant amounts (<50 genome copies/ml) of *M. ulcerans* DNA were found in some of the samples from the healthy-appearing margins of the excised tissue (samples 1, 2, and 24), while mycobacterial spreading had extended to others (samples 22 and 23).

The patient underwent skin grafting of the postoperative wound 50 days later, on 6 January 2003. The wound healed completely with a sound scar, and he was discharged in good condition on 17 February 2003. On 15 March 2003, 1 month after discharge—nearly 4 months after the first excision—he presented to the Amasaman Health Centre with a new BU lesion located adjacent to the site where the excised margin had contained a significant amount of *M. ulcerans* DNA (sample 23). Administrative delays prevented excision of the new lesion until 8 April 2003, 3 weeks later. Since the repeated excision, successful skin grafting on 20 May 2003 (Fig. 1c), and final discharge on 21 July 2003, no further lesion has developed up to the time of the last follow-up observation in March 2006.

M. ulcerans disease, commonly called BU, is a progressive necrotizing infection of the skin and the subcutaneous tissue (2). The mode of transmission of BU is not entirely clear, but once *M. ulcerans* is introduced into the dermis or subcutaneous tissue, it proliferates and produces a toxin, known as mycolactone (6). This polyketide toxin has cytopathic activity (7) and causes necrosis of the dermis, panniculus, and fascia, usually leading to relatively painless manifestations like subcutaneous nodules, ulcers, edema, plaques, and ulcers. A focal distribution of mycobacteria, with tissue destruction extending into areas with low mycobacterial burdens, is a common feature of BU lesions. Additional peaks of mycobacterial DNA mark sites where satellite lesions in the vicinity of the primary focus are developing (10). *M. ulcerans* may also spread, presumably by lymphatic and hematogenous pathways, to distant locations, where metastatic skin and occasionally bone lesions arise (9). Until recently, the only definitive treatment of BU was the surgical removal of the infected tissue (4), although it does not always ensure the complete removal of the bacilli (3). Frequent delays between the first appearance of lesions and admission to a health care facility often result in the spread of the disease, necessitating extended surgical interventions and long periods of hospitalization. Recurrence rates in hospital-treated BU patients between 6.1% (5) and 47% (13) have been reported. Amofah et al. found a local recurrence rate of 16% at the same site within a year of follow-up (1).

In the primary BU lesion described in this report, the highest

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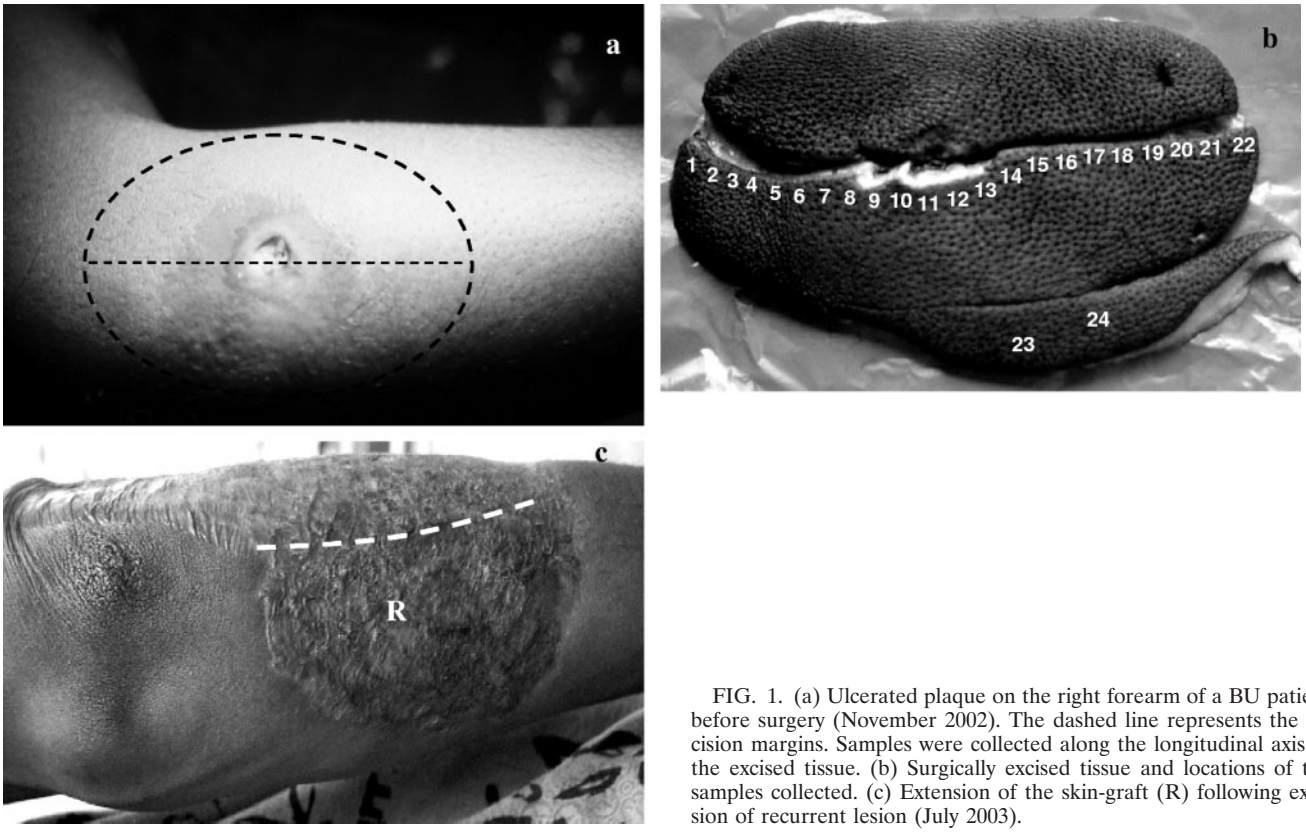

FIG. 1. (a) Ulcerated plaque on the right forearm of a BU patient before surgery (November 2002). The dashed line represents the incision margins. Samples were collected along the longitudinal axis of the excised tissue. (b) Surgically excised tissue and locations of the samples collected. (c) Extension of the skin-graft (R) following excision of recurrent lesion (July 2003).

mycobacterial load was detected at one side of the ulcer, while most of the mycobacteria were obviously washed out from its center, which represented the primary focus. Small amounts of bacterial DNA were also detected in an area of excised healthy-appearing tissue that, during surgery, showed slight changes in texture (sample 23). Within 4 months of the primary excision, a new lesion developed at a distance of about 3 cm from the dorsal margin of the primary lesion (Fig. 1b) and was excised (Fig. 1c). The location of the new lesion strongly suggests that small numbers of remnant mycobacteria were enough to provoke a recurrence. We have recently demonstrated for the first time the genetic diversity of *M. ulcerans* isolates in an African country (8). However, the discriminative power of the newly developed variable number of tandem

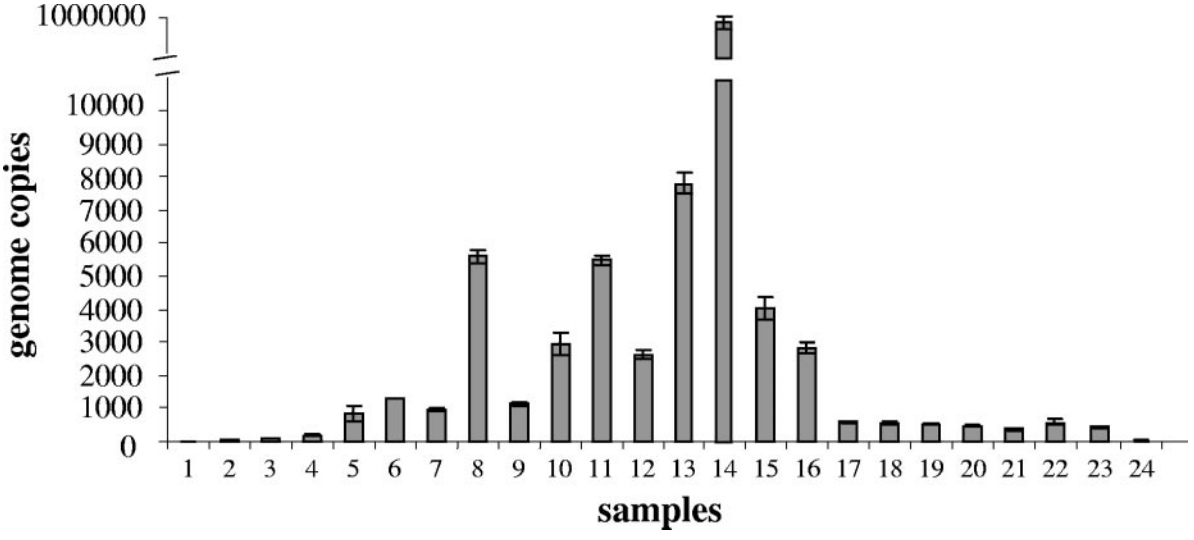

FIG. 2. Real-time PCR quantification of mycobacterial genome copies corresponding to 50 ng of extracted DNA. The threshold level was set to 50 genome copies/ml.

repeats fingerprinting method used was not sufficient to differentiate between reinfection and recurrence in the patient described here, since all 47 recent isolates from the Ga District of Ghana analyzed had the same allele combination (8). However, reinfection in the case described here appears to be very unlikely, considering both the location of the new lesion, which was adjacent to an area where mycobacterial DNA was detected, and the detection of the first signs of texture changes of the excised tissue margin at the time of surgery at this site. Additionally, the recurrences at the same site reported in the literature (5) well mirror the case described here. Although there is good evidence that wide excision reduces the risk of recurrence (13), the mycobacterial threshold levels for the development of a new lesion are far from clear. The genetic background (12) and the immune status of a patient are additional factors which may determine the mycobacterial load that can still be successfully contained. Previous work has shown that mycobacteria can contiguously disseminate and give rise to satellite lesions, even when granulomas provide evidence for the development of cell-mediated immunity (10). It appears that the mycobacteria can spread to some extent locally and diffuse across tissue affected by the disease (10). Satellite lesions therefore do not seem to appear exclusively downstream from the lymphatic flow. As soon as a microcolony containing a critical number of *M. ulcerans* cells has developed by focal bacterial multiplication, a cloud of mycolactone may impair the cellular immune system locally, permitting the development of a new lesion.

This is the first report correlating the development of a new lesion in a BU patient who underwent surgical treatment with the spatial distribution of the mycobacteria within the tissue that was removed. Although the primary lesion had a focal *M. ulcerans* distribution, this case suggests that small numbers of spreading mycobacteria are sufficient to establish a new infection focus at the edge of the previously excised lesion.

Careful clinical and laboratory examination of excised tissue margins around BU lesions may help to assess the risk for local recurrences.

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REFERENCES

1. **Amofah, G., S. Asamoah, and C. Afram-Gyening.** 1998. Effectiveness of excision of pre-ulcerative Buruli lesions in field situations in a rural district in Ghana. Trop. Doct. **28:**81–83.
2. **Asiedu, K., R. Scherpbier, and M. Raviglione.** 2000. Buruli ulcer. Mycobacterium ulcerans infection. World Health Organization, Geneva, Switzerland.
3. **Bretzel, G., V. Siegmund, P. Racz, F. Ngos, W. Thompson, P. Biason, O. Adjei, B. Fleischer, and J. Nitschke.** 2005. Post-surgical assessment of excised tissue from patients with Buruli ulcer disease: progression of infection in macroscopically healthy tissue. Trop. Med. Int. Health **10:**1199–1206.
4. **Buntine, J., and K. Crofts.** 2001. Buruli ulcer. Management of Mycobacterium ulcerans disease. World Health Organization, Geneva, Switzerland.
5. **Debacker, M., J. Aguiar, C. Steunou, C. Zinsou, W. M. Meyers, and F. Portaels.** 2005. Buruli ulcer recurrence, Benin. Emerg. Infect. Dis. **11:**584–589.
6. **George, K. M., D. Chatterjee, G. Gunawardana, D. Welty, J. Hayman, R. Lee, and P. L. Small.** 1999. Mycolactone: a polyketide toxin from Mycobacterium ulcerans required for virulence. Science **283:**854–857.
7. **George, K. M., L. Pascopella, D. M. Welty, and P. L. Small.** 2000. A *Mycobacterium ulcerans* toxin, mycolactone, causes apoptosis in guinea pig ulcers and tissue culture cells. Infect. Immun. **68:**877–883.
8. **Hilty, M., D. Yeboah-Manu, D. Boakye, E. Mensah-Quainoo, S. Rondini, E. Schelling, D. Ofori-Adjei, F. Portaels, J. Zinsstag, and G. Pluschke.** 2006. Genetic diversity in *Mycobacterium ulcerans* isolates from Ghana revealed by a newly identified locus containing a variable number of tandem repeats. J. Bacteriol. **188:**1462–1465.
9. **Pszolla, N., M. R. Sarkar, W. Strecker, P. Kern, L. Kinzl, W. M. Meyers, and F. Portaels.** 2003. Buruli ulcer: a systemic disease. Clin. Infect. Dis. **37:**e78–e82.
10. **Rondini, S., C. Horsfield, C., E. Mensah-Quainoo, T. Junghanss, S. Lucas, and G. Pluschke.** 2006. Contiguous spread of Mycobacterium ulcerans in Buruli ulcer lesions analysed by histopathology and real-time PCR quantification of mycobacterial DNA. J. Pathol. **208:**119–128.
11. **Rondini, S., E. Mensah-Quainoo, H. Troll, T. Bodmer, and G. Pluschke.** 2003. Development and application of real-time PCR assay for quantification of *Mycobacterium ulcerans* DNA. J. Clin. Microbiol. **41:**4231–4237.
12. **Stienstra, Y., T. S. van der Werf, E. Oosterom, I. M. Nolte, W. T. van der Graaf, S. Etuaful, P. L. Raghunathan, E. A. Whitney, E. O. Ampadu, K. Asamoa, E. Y. Klutse, G. J. Te Meerman, J. W. Tappero, D. A. Ashford, and G. van der Steege.** 2006. Susceptibility to Buruli ulcer is associated with the SLC11A1 (NRAMP1) D543N polymorphism. Genes Immun. **7:**185–189.
13. **Teelken, M. A., Y. Stienstra, D. E. Ellen, E. Quarshie, E. Klutse, W. T. van der Graaf, and T. S. van der Werf.** 2003. Buruli ulcer: differences in treatment outcome between two centres in Ghana. Acta Trop. **88:**51–56.