Comparative morphology of *Lacazia loboii* (syn. *Loboa loboii*) in dolphins and humans

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*Lacazia loboii* (syn. *Loboa loboii*), the etiological agent of lobomycosis, was compared in human and dolphin tissue using light and electron microscopy, and computer-assisted morphometrics. The histological features of the lesions were similar; however, preliminary electron microscopy data indicates that cell wall destruction may vary in the two hosts. Calcofluor stained tissue sections of human and dolphin tissue were examined with UV light microscopy and the images digitized. Measurements of area, minimum and maximum diameters, and perimeter were made. Student’s *t*-test (*p = 0.01*) revealed that *L. loboii* cells infecting dolphin tissue were significantly smaller than those infecting human tissue. This study represents the first comparative analysis of the morphology of the etiological agent of this disease in its two known natural hosts. The data indicate that the organism may not be identical in the two hosts.

**Keywords** bottlenose dolphin, comparative morphometrics, human, *Loboa loboii*

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**Introduction**

Lobomycosis, or Lobo’s disease, was first described in humans by Jorge Lobo in 1931 [1]. Humans were considered the only known natural host until 1971 when Migaki *et al.* [2] reported a chronic, cutaneous–subcutaneous granulomatous disease in a bottlenose dolphin with gross and microscopic characteristics of Lobo’s disease. Grossly, the lesions in both hosts are described as crusty, verrucous and ulcerated [2]. Some of the histological diagnostic features of the disease include granulomas consisting of Periodic Acid Schiff (PAS)- and Gomori’s methenamine silver (GMS)-positive foamy histiocytes and multinucleated giant cells. Yeast-like fungal cells are PAS- and GMS-positive and consist of thick-walled round cells having characteristic tubular connections between the cells.

*Lacazia loboii*, previously known as *Loboa loboii* [3], is difficult to study because it has never been cultured *in vitro*. Its phylogeny remains undetermined, although we recently amplified the 5S rDNA from *L. loboii* in dolphin tissue, placing the organism within the Kingdom Fungi [4].

Reported similarities of the disease in its two natural hosts are numerous; however, as far as we are aware, the organism infecting both hosts has not actually been simultaneously compared. Instead, when comparisons have been made, investigators have described either dolphin or human disease and relied upon published reports for the other data. Therefore, it is unclear whether the lesions in the two host species result from a single pathogen, or whether different, related pathogens produce strikingly similar lesions in very dissimilar hosts. In this study we compared features of human and dolphin lobomycosis evident on light and electron microscopy and through concurrent morphometric analysis of *L. loboii* in the tissue of both hosts.
Materials and methods

Paraffin blocks were made available from four bottlenose dolphins (Tursiops truncatus); two from the Texas coast [4,5], one from Sea World of Texas and one from the Brazilian coast [6], and surgical specimens from four humans (one from Columbia and three from Brazil). Blocks were sectioned at 5 μm, deparaffinized, rehydrated and stained with Calcofluor white (Cellulofluor; Polysciences, Inc., Warrington, PA, USA) and either Evans Blue or hematoxylin counterstain, hematoxylin and eosin, PAS or GMS. Hematoxylin and eosin, PAS and GMS sections were permanently coverslipped and viewed with light microscopy. Calcofluor stained slides were dehydrated and wet-mount coverslipped using 10% glycerin in 1× phosphate-buffered saline, pH 7.4.

Electron microscopy

Pieces of lesional tissue were cut from paraffin blocks of a stranded dolphin and a human from Columbia. The tissue was deparaffinized and post-fixed in 1% osmium tetroxide. They were then embedded in PolyBed 812 resin (Polysciences, Inc.) thin sections cut and stained with uranyl acetate and lead citrate, and examined and photographed under a Phillips CM-100 electron microscope (FEI Company, Hillsboro, OR, USA).

Morphometrics

Calcofluor, which preferentially binds to β-hexosamines [7] and is used as a cotton whiteners which fluoresces on exposure to UV light, was used to stain L. loboi cell walls for morphometric comparisons. Calcofluor was chosen because we suspected there would be less potential for cell wall distortion compared to using other routine stains for demonstrating fungi in histological sections, such as PAS or GMS.

Calcofluor sections were visualized and photographed under UV light with a Zeiss Axioplan microscope (Carl Zeiss, Inc., Thornwood, NY, USA). The fungus in tissue was digitized using a Nikon Scanner (model LS-1000; Nikon, Melville, NY, USA). Images were converted to grayscale for measurement. Digitized images were analyzed with the University of Texas Health Science Center at San Antonio (UTHSCSA, Texas, USA) Image Tool Program and the plug-in 'Segment Tool' which allows tracing. Image Tool is available from the internet by anonymous FTP from 'http://www.mxrad6.uthscsa.edu'. L. loboi cells in each image were traced and measurements calibrated to real-time using the photo bar as the reference. Tracings were repeated three times with the average being used as the measurement for each cell. All but one sample had enough cells to measure a minimum of 100 L. loboi cells per lesion. One dolphin had a very shallow biopsy which resulted in only 19 detectable cells. Data collected included area, perimeter and major and minor axis lengths. Mean and standard deviation were calculated for dolphin and human fungi for area, perimeter, and major and minor axis lengths. A one-tailed Student's t-test was used to determine if significant differences existed between measurements of L. loboi from dolphins and humans.

Results

Light microscopy

The lesions in both hosts were composed of thickened epidermis overlaying subepidermal non-caseating granulomas which formed around round refractile organisms. The granulomas caused tissue destruction, particularly at the epidermal/dermal junction, which contained many multinucleate giant cells. The organisms, which stained intensely with GMS and PAS, occurred singly, in pairs and in short chains, occasionally connected by short tubular connections (Fig. 1B). They were found in multinucleate histiocytes and free in the interstitium of the lesions. Foamy histiocytes stained PAS- and GMS-positive. In side-by-side comparison, the organism infecting dolphin tissue appeared smaller than that in human tissue.

Electron microscopy

In paraffin-embedded samples, L. loboi cell cytoplasm and nuclei were not well preserved in most cells but the cell walls retained their ultrastructure. Under electron microscopy, some of the cell walls appeared normal and intact with some organisms being connected by a narrow, tubular portion of the cell wall. A highly electron dense outer rim lined a relatively homogenous electron dense cell wall. Connections between cells had a distinct, slightly less electron dense line (Fig. 1C).

In addition to intact cells, two patterns of cell wall destruction (Fig. 2) were revealed. In the dolphin tissue, L. loboi appeared to be broken down by the peeling of external layers of the cell wall, analogous to skinning an onion. The fragments were engulfed by histiocytes. In the human tissue, the cell wall was degraded in a somewhat different fashion, appearing to be degraded by host enzymes perforating the cell wall, resulting in holes through the entire cell wall.

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Fig. 1 Comparisons of size of *L. loboi* in dolphin (left panels) and human (right panels). (A) Range of area for *L. loboi* cells plotted for each tissue section. Mean for each sample is indicated by the horizontal bar. (B) GMS stain of dolphin and human tissue infected with *L. loboi*. Note the foamy histiocytes in the dolphin (arrowheads) and the difference in size of the *L. loboi* cells (arrows) between the two hosts. (magnification × 400). (C) Normal ultrastructure of *L. loboi* with intact cell wall in dolphin and human. Arrows in dolphin indicate tubular connections between cells (magnification × 10400).

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Morphometric analysis

The four measurements (in micrometers), area, major axis, minor axis and perimeter, were significantly smaller ($\alpha = 0.05$) in dolphin than in human lesions. Tissue sections were measured from four humans and four dolphins. The mean ± SD for area was 34.3 ± 12.3 in dolphins compared to 75.8 ± 21.3 in humans. Area data including range are plotted in Figure 1A. The diameter, or major axis, differed 7.2 ± 1.4 (with a range of 4.1–13) for dolphins to 10.5 ± 1.5 (range 6.95–15.9) for humans. The organisms in human tissues were twice the area and 30% longer in the major axis than those in dolphin tissue. The minor axis measured 5.96 ± 1.12 (range 3.49–8.67) in dolphins and 9.11 ± 1.36 (range 5.93–14.52) in humans. Perimeter was 25.85 ± 5.34 (range 14.1–41.19) in dolphins and 38.64 ± 5.31 (range 25.65–61.65) in humans.

Discussion

Lobomycosis has long been described in humans and more recently in dolphins. While L. loboi has been experimentally inoculated into tortoises, hamsters and armadillos [8–10], the only known natural hosts remain humans and dolphins. There are several excellent morphological descriptions of the disease; however, these are limited to either dolphin [2] or human disease [11]. In this study, we concurrently examined and compared the organism in both hosts for the first time.

The disease process in both hosts appears to be similar, as previously reported. In both hosts, L. loboi forms characteristic chains of cells that are suggestive of beads-on-a-string owing to their round form and tubular connections between cells. Histiocytes within both hosts' granulomas have a foamy appearance which stains PAS- and GMS-positive resulting from phagocytosis of portions of the fungal cell wall. The L. loboi cells exhibit a relative homogeneity of cell size within a granuloma. A distinction in the organism's size between the two hosts has not previously been reported. It is noteworthy that the organism infecting humans has twice the area of that infecting dolphins.

In contrast to a similar response at the light microscopic level, preliminary data from electron microscopy of single representatives of each host indicate potential differences in host/organism interaction. While some cell walls remained intact in both hosts and had a similar homogeneous appearance, degraded cells appeared different in the two hosts. The human response appeared to be to penetrate the cell wall at many points, thereby fragmenting it. Electron micrographs of L. loboi in humans in previous studies show a similar pattern of porous

Fig. 2 Ultrastructure of L. loboi being degraded in (A) dolphin and (B) human skin (magnification × 14100). The cell wall of the organism is indicated by arrowheads.
cell wall destruction [12,13]. Our preliminary findings are also supported by prior electron microscopic analysis of *L. loboi* in bottlenose dolphins [14,15], neither of which reported radial projections of the cell wall seen in the human tissues. However, the dolphin response of peeling of successive layers of the cell wall, was also not documented and requires further study for confirmation. In both hosts, the subsequent reaction to the cell wall fragments appears to be the same: engulfment and digestion by histiocytes.

Dolphin disease has been reported in the western and eastern Gulf of Mexico [2,4,5,16], the Surinam river [17] and the Brazilian coast [6]. Human disease has been reported from Central America south to Brazil with the highest number of reported cases associated with patients living in the Amazon river basin [18]. Because of the diversity of the measured samples (i.e. humans from Columbia and Brazil and dolphins from western and eastern Gulf of Mexico and Brazil), it is unlikely that our results were biased due to sampling of the disease in restricted geographic locations.

Several factors could explain the morphometric variability observed. First, humans and dolphins occupy distinct environments; the effects of salinity, temperature and aqueous environment may play a role in the organism’s size. Second, there could be an organism response to the host or physiological differences in the hosts that may affect cellular growth of the organism. While core body temperatures are similar in dolphins (36–39°C) and humans, cooler water could lower the skin temperature in the dolphin creating a different growth environment. Third, the size difference may be a result of similar but different organisms producing an analogous disease in the two hosts. Ongoing investigations are examining the third factor through comparisons of phylogenetic relatedness of the organisms infecting the two hosts.

In summary, this study represents the first simultaneous comparison of *L. loboi* in its two known natural hosts. The histological features of the lesions of both human and dolphin infections with lobomycosis are similar, indicating corresponding host responses to the organism. Preliminary electron microscopy identifies a potential variation in destruction of the thick cell wall of *L. loboi* in its two hosts. Finally, the morphology of the organism in the two hosts is different as indicated by morphometric analysis of the *L. loboi* cells in the tissue. Whether or not the organism is actually two organisms producing a similar host response, will have to be determined through phylogenetic analyses.

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References