



## Viral Disease Transmitted by Laser-Generated Plume (Aerosol)



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**Objective:** To evaluate the possibility of disease transmission through liberated plume from virally infected tissue that is exposed to the carbon dioxide laser.

**Design:** Bovine papillomavirus-induced cutaneous fibropapillomas were exposed to the carbon dioxide laser. Laser settings were within the range of clinically used settings. The laser plume (aerosol) was suctioned and collected and then reinoculated onto the skin of calves.

**Setting:** University laboratory research center.

**Main Outcome Measures:** Laser plume viral content and postinoculation tumor growth were analyzed and documented.

**Results:** Collected laser plume contained papillomavirus DNA in all tested laser settings. The viral DNA was most likely encapsulated. Tumors developed at laser plume-inoculated sites for all laser parameter settings. Histological and biochemical analyses revealed that these tumors were infected with the same virus type as present in the laser plume.

**Conclusions:** Laser plume has been shown, for the first time to our knowledge, to actually transmit disease. Strict care must be maintained by the laser practitioner to minimize potential health risks, especially when treating viral-induced lesions or patients with viral disease.

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**T**HERE HAS been an increasing awareness of the potential health risk of laser-generated plume (aerosol). Many laser systems, on impact with targeted tissue, produce a plume of smoke containing debris and vapor, which is released into the surrounding area. Concerns involving aerosolized carbonized material, viable tumor cell dispersion, and infection transmission have been evaluated.

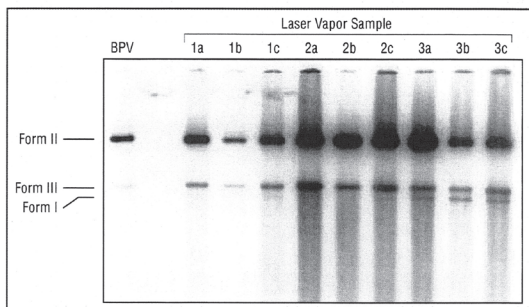
The carbon dioxide (CO<sub>2</sub>) laser is used by various medical specialties to vaporize, ablate, or cut tissue. This instrument emits light energy in the lower infrared range (10 600 nm), which is effectively absorbed by water. Because of the relatively high water content of tissue, the laser energy is readily converted to heat. Copious amounts of plume are generated, which necessitates constant suction away from the procedural area through a filter system.

Early studies that evaluated the laser plume for aerosolized infectious material were prematurely reassuring. Besides occasional bacterial spores recovered from experimentally inoculated tissue,

there was no other discovered aerosolized infectious material.<sup>1-4</sup> In 1988, intact bovine papillomavirus (BPV) and human papillomavirus (HPV) DNA specimens were recovered from the plume of CO<sub>2</sub> laser-treated human and bovine lesions.<sup>5</sup> Several subsequent investigations<sup>6-8</sup> have confirmed these results with the papillomavirus, while viable bacteriophages have been found in the CO<sub>2</sub> laser plume using an agar model.<sup>9</sup> Although a simian immunodeficiency virus model failed to recover any virus from the laser plume,<sup>10</sup> positive results were obtained with an in vitro study<sup>11</sup> of the human immunodeficiency virus. Also, clinical surveys of laser users have revealed increased user infections with HPV; however, direct lesional contact may be the source of infectivity.<sup>12-14</sup>

Intact papillomavirus DNA is a potential infectious agent.<sup>15-17</sup> Although in vitro methods have detected liberated infective virus in the collected plume, in the papillomavirus<sup>6</sup> and the human immunodeficiency virus<sup>11</sup> models using the CO<sub>2</sub> laser, and in an in vitro study<sup>18</sup> of erbium (ER):YAG laser-generated aerosol,

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**Figure 1.** Collected laser plume samples contain bovine papillomavirus (BPV) DNA. DNA specimens extracted from plume samples were electrophoresed in parallel with BPV-2 virion DNA, subjected to Southern blotting, and probed with phosphorus 32-labeled BPV-1 DNA. Numbers 1 through 3 correspond to the 3 different sets of laser parameters described in the "Materials and Methods" section; they are presented in triplicate (a-c).

the induction of actual infection by the laser plume, to our knowledge, has not been documented. Reproducing the infection, with identification of the causative agent, would confirm the potential of laser aerosol in transmitting disease.

To determine whether laser-generated plume from infected tissue can reproduce disease, the bovine fibropapilloma, a BPV-induced lesion, was used. Various CO<sub>2</sub> laser settings were evaluated, and laser plume at each laser setting was collected and inoculated into animals. Typical BPV lesions containing BPV developed for all laser settings. These viral tumors confirm the ability of the laser plume to produce infection.

## MATERIALS AND METHODS

Bovine cutaneous fibropapillomas, produced from the inoculation of BPV-1, were surgically excised from the cattle and promptly frozen and stored at -65°C. All study of animal subjects received prior institutional review board approval. Fibropapillomas positive for BPV by the peroxidase-antiperoxidase technique<sup>19-21</sup> were exposed to various CO<sub>2</sub> laser exposures in triplicate. All of the fibropapillomas were at room temperature during the laser exposures. Laser settings included the following: (1) 12 W and a 2-mm circular spot size delivered in a continuous fashion (power density, 380 W/cm<sup>2</sup>; and spatially averaged energy fluence, 400 J/cm<sup>2</sup>); (2) 4 W, a 2-mm spot size, and continuous exposure (power density, 130 W/cm<sup>2</sup>; and spatially averaged energy fluence, 130 J/cm<sup>2</sup>); and (3) 8 W, a 0.2-mm spot size, and a pulse duration of 0.1 second (power density, 25 400 W/cm<sup>2</sup>; and spatially averaged energy fluence, 2540 J/cm<sup>2</sup>).

A bubble chamber containing phosphate-buffered saline solution (pH, 7.4) was placed within a vacuum suction line (500 mm Hg) used to collect the laser-generated plume. The suction tip was placed approximately 2 cm from the tumor. Extreme care was taken not to have the suction tip directly contact the papilloma.<sup>22</sup> The collected material was initially evaluated for BPV content and later inoculated in duplicate on the scarified skin of 3 calves. Control BPV inoculates were also placed on the skin of these animals. Growth at inoculation sites was excised after 106 days and analyzed histologically and biochemically for viral content and typing.

DNA extraction from the collected plume material in phosphate-buffered saline and from each tumor was performed. Deoxyribonuclease sensitivity experiments used pooled vapor material mixed with purified pGEM5 plasmid DNA (Promega Corp,

Madison, Wis). The sample was then divided into 3 equal volumes and digested with 1.0 µg, 0.1 µg, or no deoxyribonuclease (Worthington Biochemical Corp, Lakewood, NJ). Purified DNA samples were subjected to gel electrophoresis, transferred to membranes<sup>23</sup> (Duralon; Stratagene, La Jolla, Calif), and UV cross-linked (Stratagene). A purified BPV-2 virion DNA sample was used as a positive control. Bovine papillomaviruses 1 and 2 have homologous DNA, and both types are causative agents of bovine fibropapillomas. Hybridizations were performed with a BPV-1-cloned DNA probe labeled by the random primer method<sup>24</sup> (Boehringer-Mannheim, Mannheim, Germany), as previously described.<sup>20</sup> Signals were detected by autoradiography.

## RESULTS

All of the laser plume samples for the 3 studied laser parameters contained substantial amounts of BPV DNA, as revealed by hybridization of the DNA extracts (**Figure 1**). Although form II (circular DNA) was the most prominent in all samples, forms III (linear DNA) and I (supercoiled DNA) were present. The positions of these DNA forms corresponded exactly to those obtained with DNA from control BPV virions (Figure 1). This direct correlation is evidence that the plume-collected viral DNA was intact.

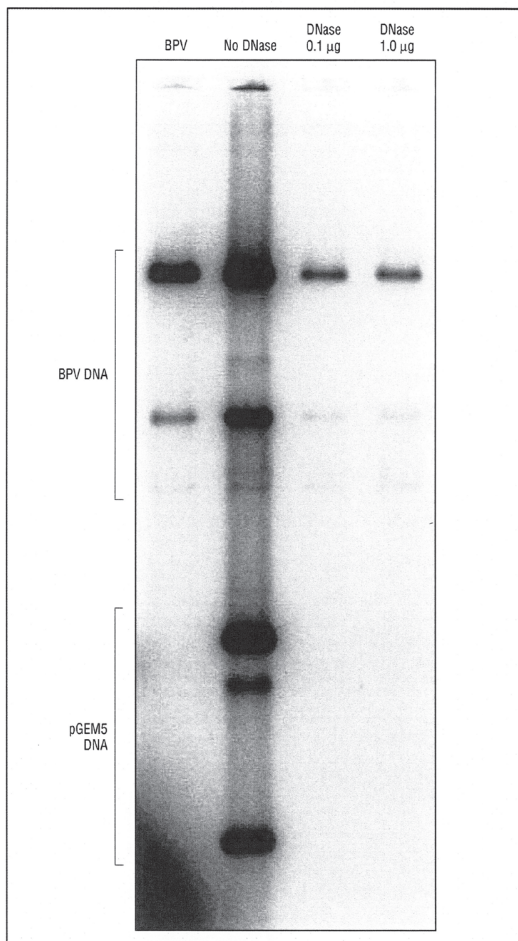
No signal for the naked plasmid DNA was observed in the laser plume samples treated with deoxyribonuclease, whereas a portion of the BPV DNA in the sample survived enzyme treatment with concentrations of 0.1 and 1.0 µg (**Figure 2**). Because the plasmid DNA is present in the same mixture, it can be concluded that the enzyme activity is not inhibited by components of the laser plume sample. Thus, the selective protection of the collected BPV DNA suggests that part of the viral DNA in the laser plume is still encapsulated.

Of 3 calves, 2 developed marked lesions in sites of control BPV concentrate inoculum (**Figure 3**). The third calf had only minimal growth. A varying degree of papillomavirus infection susceptibility occurs in this animal model.<sup>25</sup>

Lesions developed at laser plume inoculation sites for all 3 laser parameters. Of the 2 calves with a strong BPV control response, one developed lesions with material collected using each of the 3 parameters (**Figure 4**) and the other developed lesions with material collected from the laser plume, which corresponded to the highest power density and energy fluence. The calf that had a minor response to the control BPV inoculum did not develop other lesions.

The results of a histological evaluation of the excised laser plume-induced lesions were typical of BPV fibropapillomas. The epidermis revealed hyperkeratosis, acanthosis, and papillomatous changes. Foci of large vacuolated cells appeared in the upper epidermal layers. Sections stained positive for BPV capsid antigen in all lesions.

DNA extracts from each of the 3 induced tumors also contained high levels of BPV DNA (**Figure 5**), confirming that the lesions arose by BPV infection. The more rapid migration of form II DNA, relative to the control DNA in extracts from 2 of the tumors, is presumably due to the presence of large amounts of



**Figure 2.** Viral DNA in collected laser plume samples is resistant to deoxyribonuclease (DNase) I digestion. Laser plume material containing purified pGEM5 plasmid DNA was digested with varying concentrations of deoxyribonuclease (DNase) I, as described in the "Materials and Methods" section. Southern blot hybridization was accomplished with a probe consisting of bovine papillomavirus (BPV) and plasmid vector sequences.

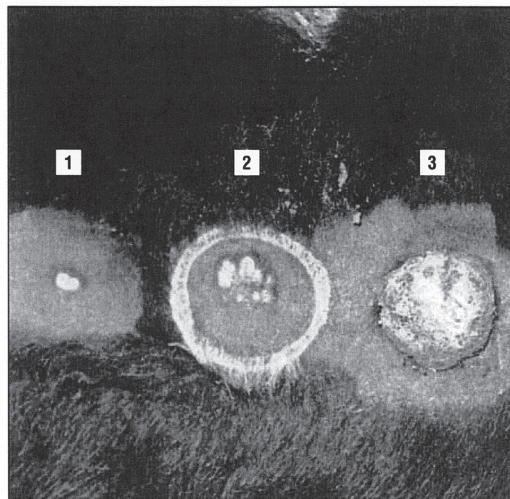
cellular DNA in those samples. Additional bands above form II DNA are likely to represent multimeric forms of BPV. Digestion of a laser plume-induced tumor sample, and a laser plume sample with the restriction enzyme *Bam*HI, demonstrated only 1 band, thus distinguishing the BPV as type 1 (BPV-2 has 2 *Bam*HI restriction sites).<sup>26,27</sup> This correspondence of viral types in the laser plume, and in induced tumors, provides additional evidence for a causative role in lesion induction by the laser plume.

**COMMENT**

The CO<sub>2</sub> laser is an effective instrument in many medical specialties. Water within the tissue absorbs the laser energy, converting it to heat that produces the desired effect and a thick plume of vapor and debris that must be removed by suction. Analysis of the plume has revealed vari-



**Figure 3.** Bovine fibropapillomas occurred at the site of inoculation of control bovine papillomavirus tumor extract, which was not treated with the laser (full size).

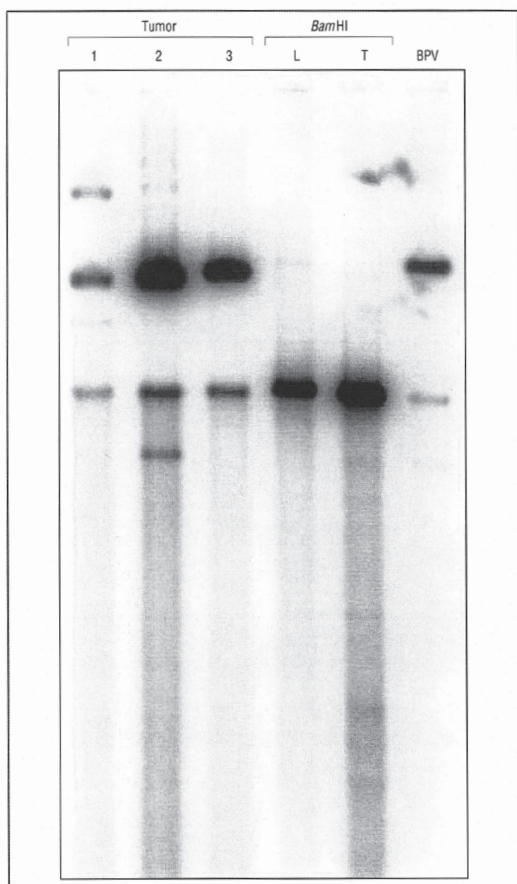


**Figure 4.** Bovine fibropapillomas occurred at the sites of inoculation of collected laser plume. Numbers 1 through 3 correspond to the 3 different sets of laser parameters described in the "Materials and Methods" section. The white ring is a freeze brand, used as a marker for identification of inoculation sites.

ous components besides the water vapor,<sup>28</sup> which can be irritating to the eyes and respiratory tract and are known animal or human mutagens and carcinogens. Laser smoke condensates, from control animal tissue in modified Ames tests, have produced mutagenicity.<sup>29</sup>

The size of the mainly homogeneous particulate matter present in the plume debris can easily spread into the surrounding environment and reach the entire respiratory system, producing, in study animals, pathologic changes.<sup>30-33</sup> Cellular elements are also recovered with the CO<sub>2</sub> laser, most cells are carbonized or distorted, and intact cells have not been viable after placement into tissue culture.<sup>34-36</sup> However, with other laser systems, viable cells have been found.<sup>37</sup>

Since an earlier study<sup>5</sup> of animal and HPV lesions treated with the CO<sub>2</sub> laser revealed intact viral DNA in



**Figure 5.** Laser plume-induced bovine tumors and the laser plume contain bovine papillomavirus (BPV) type 1 DNA. Laser plume-induced tumor extracts (1-3), and a tumor extract (T) and laser plume sample (L) digested with *Bam*HI, were analyzed by Southern blot hybridization. Numbers 1 through 3 correspond to the 3 different sets of laser parameters described in the "Materials and Methods" section.

the generated plume, viral DNA has been detected in subsequent studies from bovine fibropapillomas (BPV), plantar warts,<sup>9</sup> and genital verrucae (HPV).<sup>7,8</sup> A bioassay of the plume detected infectious BPV.<sup>6</sup> In a tissue culture study<sup>11</sup> using the CO<sub>2</sub> laser, proviral human immunodeficiency virus DNA was recovered from the suction tubing used to remove the plume. However, no sustained infection occurred in cultured cells inoculated by the laser plume-contaminated tubing.

In the present study, BPV viral DNA was again readily detected in the laser aerosol. In addition, a portion of this DNA was not sensitive to deoxyribonuclease treatment, suggesting that whole virion particles were present in the laser plume. Most important, when the collected plume produced from a wide range of various laser parameters was used as an inoculum, lesions were induced. Based on histopathological and viral typing criteria, the laser plume-induced lesions were identical to the original tumors.

This study addresses the use of the CO<sub>2</sub> laser, either in a continuous mode or pulsed at 100 milliseconds. Lasers used for tissue resurfacing, such as the CO<sub>2</sub> or the Er:YAG laser, are pulsed for short exposures, from 10 to generally less than 1 millisecond. These rapid pulses produce a more explosive response with greater tissue ablation.

A study<sup>38</sup> analyzing CO<sub>2</sub> laser plume captured during resurfacing cases revealed viable bacteria. The filter size used in the study was too porous to capture viruses, and there was no indication of viral infection in these patients. An Er:YAG laser used in resurfacing verrucae vulgaris did not find any HPV DNA in material collected on the laser handpiece.<sup>39</sup> This material may have represented desiccated debris, and perhaps material directly collected in the plume would have revealed HPV DNA. Ziegler et al,<sup>18</sup> using the Er:YAG laser, did demonstrate, through in vitro methods, viable cells, viral genes, and infectious viruses. Therefore, it seems that short-pulsed resurfacing lasers also have the potential of liberating infectious material.

Another type of laser that has a short pulse duration, and is used in ophthalmology for tissue ablation (photorefractive keratectomy), is the excimer laser. At 193 nm, and nanosecond pulse durations, it was able to experimentally disrupt the fairly large (180-200 nm) attenuated varicella-zoster virus, with only fragments present in the laser plume.<sup>40</sup> However, as the researchers themselves comment, it is unknown whether smaller viruses (ie, papillomavirus, hepatitis, and retrovirus) would be undamaged and liberated into the plume. More important, the UV wavelength of the excimer laser immediately disrupts surface cells, allowing for little tissue penetration. The Er:YAG laser at 2940 nm and the CO<sub>2</sub> laser at 10600 nm have greater tissue penetration, producing a deeper ablative effect, potentially expelling much more intact material.

These studies and the findings in the present study increase the concern surrounding the use of aerosol-producing lasers in the treatment of virally induced lesions and virally infected (or potentially infected) patients. With HPV and the human immunodeficiency virus already detected in laser plume, it is possible that other viruses, such as hepatitis, may also be liberated in the plume during laser use. Fortunately, most HPV lesions, particularly those of genital origin, contain fewer particles than those studied in the bovine fibropapilloma model,<sup>41</sup> and the direct inoculation of the laser plume in our study onto the animals may not equal routine clinical exposure. However, there is already a report<sup>42</sup> of a surgeon, who treats anogenital condylomas with the Nd:YAG laser, developing laryngeal papillomatosis containing HPV DNA types 6 and 11.

It is even more relevant, with the proved potential for disease transmission, that safety precautions during laser surgery be strictly maintained. These include limiting the use of aerosol-producing lasers to patients for whom there is a strong therapeutic advantage over other modalities, protection of skin surfaces with gloves and gowns, eye protection, and the use of masks and smoke suction systems that have high flow volume and good filtration.<sup>43</sup>



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