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Generation of Infectious Retrovirus Aerosol Through Medical Laser Irradiation

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A novel model system was used to investigate the spread of infectious particles and live cells through the application of lasers commonly used in clinical medicine. Supernatants from a cell line producing recombinant retroviruses carrying a marker gene (*neoR*) were exposed to Er:YAG-laser beams. Aerosols were collected from various sites and distances from the point of laser impact and were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) for *neoR*. In addition, a susceptible indicator cell line was used to investigate the presence of infectious virions in collected aerosols. To test the possibility of dissemination of viable cells, a cell line was laser irradiated, and the generated aerosols were analyzed for the presence of viable cells. The viral marker gene *neoR* could be detected in 16% (distance: 5.0–6.3 cm) to 59% (0.5–1.6 cm) of wells adjacent to the point of laser impact. The presence of infectious viruses in laser vapors conferring G418 resistance could be detected in 3% (distance 5.0–6.3 cm) to 20% (distance: 0.5–1.6 cm) of wells containing susceptible cells, and subsequent PCR analysis of isolated resistant clones revealed the presence of *neoR*-RNA and -DNA. Viable cells were detected in 40% (distance 0.7–3.6 cm) to 3% (distance 10.7–11.8 cm) of wells adjacent to the point of laser impact. These results demonstrate that laser vapors can contain infectious viruses, viral genes, or viable cells and may promote the spread of infections or tumor cell dissemination. Lasers Surg. Med. 22:37–41, 1998. © 1998 Wiley-Liss, Inc.

Key words: medical laser application; biosafety; laser aerosol; retroviral vectors

INTRODUCTION

Lasers have many applications in medicine. Although laser beams may come in contact with infectious or neoplastic tissues, the risk of infections or inadvertant dissemination of neoplastic cells through laser plume or vapors is thought to be neglegible. Consistent with this notion, neither human papilloma virus DNA [1] nor infectious HIV-1 [2] have been demonstrated in vapors re-

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sulting from laser treatment of laryngeal papillomatosis and concentrated HIV-infected tissue culture pellets, respectively.

However, several reports suggest that laser-derived vapors may contain infectious particles or viral DNA. First, human and bovine papilloma virus DNA (HPV, BPV) has been detected in laser vapors from warts treated with carbon dioxide lasers [3], and laser vapor-derived bovine papilloma virus has been shown to contain infectious particles [4]. Second, a case of laryngeal papillomatosis (HPV type 6 and 11) has been described in a surgeon who treated patients with anogenital warts using a Nd:YAG laser [5]. Finally, viable bacteriophage λ has been demonstrated in vapors after exposure of virus containing agar to a carbon dioxide laser [6]. This all suggests that laser vapors generated during medical procedures may contain infectious materials as well as viable tumor cells [7] and, therefore, could pose a significant risk to health care providers.

The aim of this study was to determine if laser vapors contain infectious particles or viable cells and, therefore, could promote viral infections or tumor cell dissemination. We developed a model system that permits the detection and quantitation of infectious viral particles, viral mRNA, and viable cells in laser vapors in relation to the distance from the point of laser impact.

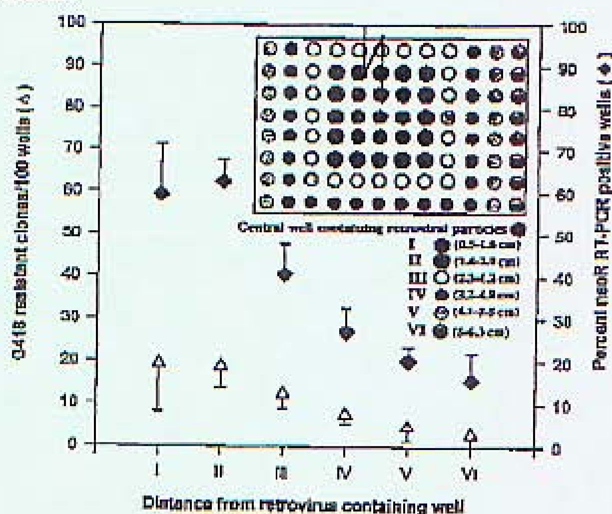

Fig. 1. **Insert:** A 96-well plate and the arrangement of group I to VI wells is shown. X-axis: Group of wells as indicated by the insert. Y-axis: Solid diamonds show the percentage of neoR RT-PCR–positive wells (I to VI) after laser irradiation of the central well. Results of infection assays with airborne retrovirus are presented as G418-resistant NIH3T3 clones per 100 wells (triangles).

MATERIALS AND METHODS

To produce recombinant amphotropic retroviruses PA317 packaging cells [8] were transfected with the pZIPneoSVx plasmid [9], and *neoR*-expressing clones were selected in medium containing 1,000 μg/ml Geneticin (G418). Supernatants from individual clones were analyzed for their ability to induce G418 resistance in NIH3T3 cells, and one clone producing 5×10^5 cfu/ml was selected for all subsequent experiments. Freshly collected supernatants were cleared of cellular debris and analyzed by reverse transcription-polymerase chain reaction (RT-PCR) [10] and DNA-PCR using *env* specific primers which recognize the amphotropic envelope (*env*) coding sequence [11].

For irradiation experiments, 250 μL of retrovirus supernatant was irradiated in a single well of a 96-well plate (Fig. 1) with a pulsed ER:YAG-laser. The Er:YAG laser is a mid-IR laser with a wavelength of 2.94 μm. The strong absorption by tissue water in this wavelength region is responsible for increasing medical use of this laser type, both for soft and hard tissue ablation. Parameters used for irradiation were pulse energy 60 mJ, spot diameter 1.2 mm, pulses 250 μs, repetition frequency 7 s^{-1}, 500 pulses corresponding to a total irradiation time of 70 s.

The laser beam was directed perpendicularly to the center of the irradiated well (Fig. 1). Surrounding wells contained 100 μL of GuSCN lysis buffer [10] for detection of viral genes by RT-PCR or, alternatively, NIH3T3 cells (approximately 60% confluent) for detection of infectious particles conferring G418 resistance. RT-PCR was performed as described [10] using neoR primers: 5′-GAACAAGATGGATTGCACG-3′ and 5′-TATTCGGCAAGCAGGCATCG-3′. To detect infectious particles, NIH3T3 cells in medium [8] containing 6 μg/ml polybrene were co-cultivated with collected laser vapors for 6 hours and thereafter grown in the absence of polybrene. Three days post-infection, NIH3T3 cells were selected for *neoR* expression by adding G418 to the medium (400 μg/ml from day 3 to day 6, 1,000 μg/ml after day 7) and maintained for 14 to 20 days.

For all experiments, wells adjacent to the central well containing laser-treated retrovirus supernatant were designated as group I (distance from central well was 0.5–1.6 cm; eight wells), group II (distance 1.4–2.9 cm; 16 wells), group III (distance 2.3–4.2 cm; 24 wells), group IV (distance

3.2–4.8 cm; 23 wells), group V (4.1–5.5 cm; 16 wells), or group VI (5–6.3 cm; eight wells).

To analyze laser vapors for the presence of viable cells, PA317 cells were grown to confluency in 35-mm dishes in semisolid medium containing 3% agar and exposed to laser beams (5.3 J/cm^2, 70 sec). Culture dishes were kept in an upright position and placed on an automatic support that provided slow bidirectional movements during the irradiation. For collection of viable cells in laser vapors, 96-well plates with culture medium were positioned beneath (vertical distance between point of laser impact on irradiated cells and medium in 96-well plates was 4 cm) the 35-mm dishes that were exposed to the laser beam (row 12 consisting of eight wells represented the farthest distance to the irradiated cells). The 96-well dishes were then incubated for up to 3 weeks and examined for the presence of growing cell clones.

RESULTS

Laser Vapors Contain Infectious Viral Genes

Tissue culture wells containing viral supernatants were irradiated with a pulsed Er-YAG laser for 70 seconds and then assayed for the presence of viral marker genes as well as infectious viral particles. First, we analyzed wells that were positioned around a laser-treated well (group I to VI; Fig. 1, insert) containing retrovirus supernatant for the presence of *neoR* mRNA by RT-PCR. Figure 1 shows the means and standard deviations of four experiments. In 16% (distance VI = 5–6.3 cm) to 59% (distance I = 0.5–1.6 cm) of adjacent wells viral marker gene sequences (*neoR*) that originated from the laser-treated retrovirus supernatants could be demonstrated (Fig. 1, solid diamonds). The corresponding RT-PCR products of *neoR* for wells IV–VI are shown in Figure 2, lanes 4–6 (upper panel).

Laser Vapors Contain Infectious Viruses

If infectious viruses are present in vapors from laser-treated retroviral supernatants, expression of viral genes should be readily detectable in susceptible cells that were exposed to these vapors. We assayed for the presence of viable viruses in laser vapors by exposing wild-type NIH3T3 cells to laser vapors as described above.

These cells normally do not survive G418 concentrations in excess of 0.5 mg/ml. After successful infection with a retrovirus containing the neoR gene G418 concentrations of 1.0 mg/ml are

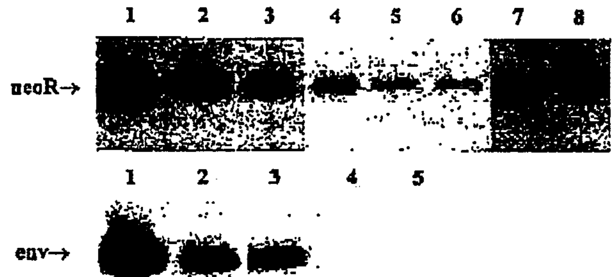

Fig. 2. Autoradiographs of RT-PCR and DNA-PCR products. **Upper panel:** Five microliters (lane 1), 5 µL of a 1:25 dilution (lane 2), and 5 µL of a 1:250 dilution (lane 3) from the retrovirus producer cell line PA317 was processed for RT-PCR as described (10). **Lanes 4–6** represent RT-PCR products derived from airborne retrovirus collected from group IV, V, or VI wells, respectively. DNA-PCR (lane 7) and RT-PCR (lane 8) products derived from G418-resistant clones generated through infection of NIH3T3 cells by airborne retrovirus. **Lower panel:** The RNA from one PA317 producer cell (lane 1) and a 1:10 dilution of RNA (lane 2) was processed for RT-PCR using env-specific primers (11). **Lane 3** shows the DNA-PCR env product from one PA317 cell. Five microliters of producer cell line supernatant was processed for RT-PCR (lane 4) or DNA-PCR (lane 5). Note the absence of env PCR products in lanes 4 and 5, demonstrating absence of retroviral DNA and RNA sequences in the supernatant.

tolerated. When NIH3T3 cells were exposed to vapors from laser-treated retroviral supernatants between 3 (VI = farthest distance) and 20 (I = closest distance), G418-resistant indicator cell clones per 100 wells could be detected (Fig. 1, open triangles). Subsequent PCR analysis of isolated resistant clones revealed the presence of *neoR*-RNA (Fig. 2, upper panel, lane 8) and -DNA (Fig. 2, upper panel, lane 7).

Retrovirus supernatants may contain producer cell–derived non-particle–associated viral RNA and/or proviral DNA sequences which could obscure our PCR results. To address this issue, supernatants were analyzed by RT- and DNA-PCR using primers that recognize the producer cell line–derived retroviral *env*-sequence which is not part of the genome of the investigated retroviral particles. Figure 2 (lower panel), lanes 1–3 show *env* products of lysates derived from a single PA317 producer cell as determined by RT-PCR (lanes 1 and 2) or DNA-PCR (lane 3). Supernatants derived from the PA317 producer cell line, however, were negative for *env* products by RT-PCR (lane 4) or DNA-PCR (lane 5). Thus, supernatants contained virus particle–associated retroviral RNA but not cellular RNA and/or DNA sequences.

TABLE 1. Percentage of Wells at the Indicated Distance From the Point of Laser Impact Containing Viable Cells

	Distance from laser impact (cm)	Wells with viable cells (% ± SD)
Row 1	0.7–3.6	40.7 ± 11.7
Row 2	1.8–4.1	40.6 ± 11.9
Row 3	2.2–4.6	37.5 ± 10.2
Row 4	3.5–5.3	28.1 ± 11.9
Row 5	4.4–6.1	15.6 ± 6.2
Row 6	5.3–6.8	12.5 ± 10.2
Row 7	6.2–7.6	9.3 ± 6.2
Row 8	7.1–8.4	6.2 ± 7.2
Row 9	8.0–9.2	3.1 ± 6.2
Row 10	8.9–10.2	0 ± 0
Row 11	9.8–11.0	3.1 ± 6.2
Row 12	10.7–11.8	3.1 ± 6.2

Laser Vapors Contain Viable Cells

To determine if laser vapors can contain viable cells, confluent layers of PA317 cells were irradiated. Ninety-six–well plates that had been positioned around the point of laser impact were assayed for the presence of viable cells. Table 1 shows that between 40% (shortest distance from point of impact) and 3% (longest distance) of wells contained viable cells.

DISCUSSION

Our data suggest that laser irradiation of biological materials can lead to the generation of laser vapors containing viable cells and infectious retroviruses. Our data show that viruses contained in laser vapors remain infectious and remain capable of integrating into the genome of susceptible cells. Aside from viable viruses, laser vapors may also contain partially inactive or incompetent viruses. This is supported by the fact that the *neoR* marker gene is detected with higher frequency than G418-resistant NIH3T3 clones in wells at equal distances from the point of laser impact. A possible explanation is that the direct impact of laser beams may cause fragmentation of some viruses such that they are rendered noninfectious. However, partial viral or oncogene sequences can also pose a significant health hazard for exposed personnel since they may have transforming potential [12]. Similarly, HIV proviral sequences as well as p24 and gag antigens have been detected in susceptible cells after exposure to laser-generated debris [2]. Productive infection of these cells was not detectable, suggesting that only non-infectious viruses were generated in vapors collected at the site of laser impact. Although the occupational risk of HIV transmission appears to be low, laser vapors containing potentially infectious HIV particles pose a serious threat to health care providers. We detected virus-infected cells and viral genes as far as 6.3 cm from the point of laser impact, but laser debris traveling distances of up to 100 cm has been described [13].

The spread of tumor cells has been demonstrated by others [7] and is corroborated by our findings that laser vapors contain viable cells. These findings raise the possibility that lasers used for tumor surgery may contribute to the dissemination of tumor cells and promote local or distant metastasis. Our data suggest that the use of lasers in medicine may constitute a significant biohazard for health care providers. However, continuous-wave lasers with very much lower peak powers than the described Er:YAG-laser may be less biohazardous, which was not investigated in the present study.

By using PCR as described in this report as well as through viability and infectivity assays, more data on an array of medically important viruses and tumors could be obtained. Estimating the risk of medical procedures may aid in establishing safety precautions to protect health care personnel more effectively.

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