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Experimental Ablation of Emphysematous Rat Lung with Nd: YAG Laser: Lung Changes Studied by Histopathology and SEM

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Akahane, T., Kurokawa, Y., Yaegashi, H., Satomi, S. and Takahashi, T. *Experimental Ablation of Emphysematous Rat Lung with Nd: YAG Laser: Lung Changes Studied by Histopathology and SEM.* Tohoku J. Exp. Med., 1998, **185** (2), 119-129 —— Laser ablation has been employed as a therapeutic measure for chronic pulmonary emphysema. As yet, however, its effect is not understood on firm pathological basis. We aimed to study, both histopathologically and using Scanning Electric Micrscopy (SEM), the changes produced by irradiation with contact Neodymium-yttrium aluminum garnet laser (Nd: YAG laser) in rat lungs with experimentally induced emphysema. Emphysema was produced in 34 rats by instilling elastase via airways. Eight weeks after the instillation, the emphysematous left lung was irradiated under thoracotomy with contact Nd: YAG laser at a power of 5 watts. The animals were sacrificed in acute as well as chronic phase for histopathological observation of lung and scanning electron microscopy. Laser caused necrotic and inflammatory changes in the subpleural zone of lung. Immediately after irradiation, the alveolar septa were destroyed as visualized by SEM, only leaving the elastic skeleton. In a chronic phase, the necrotic zone was collapsed and replaced with a thick fibrous scar which seemed to serve more or less to keep the organ from being excessively inflated. In this model, irradiation induces subpleural dense scarring, which, by "encasing" an emphysematous lung, is expected to more or less normalize the excessive compliance. ———— laser ablation; histopathology; scanning electron microscopy; experimental emphysema

In 1991, Wakabayashi et al. (1991) reported that in patients with bullous emphysema, they managed to attain a significant improvement in pulmonary function by ablating the lung with CO_2 laser under thoracoscopy. Since then, clinical effectiveness of this treatment has been reported by a number of authors

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(Wakabayashi 1993; Brenner et al. 1994; Eugene et al. 1995; Little et al. 1995). Especially, with the recent wide use of thoracoscopy, this has drawn growing attention. We also performed this treatment in about 40 patients of severe bullous emphysema and reported that there was some improvement (Kaiwa et al. 1995). In our series, we used Nd: YAG laser and staplers, the latter applied to the cutting edge of lung when partial resection of lung was combined. On the other hand, some investigators cast doubts on its efficacy for patients with chronic obstructive pulmonary disease (COPD) (Hazelrigg et al. 1996; MacKenna et al. 1996). Also, despite a number of clinical reports, there have been few studies providing basic knowledge on the pathology of this therapy. It has yet to be established what changes are created in the lung by introducing ablation, and how the changes explain the improvement of lung function, if there is improvement at all.

These circumstances urged us to undertake an experimental study. Laser irradiation was performed on rats in which emphysema had been produced by elastase instillation. The aim was to examine the changes produced in the lung in acute and chronic phases after laser irradiation, with which to contribute to better understanding of its therapeutic mechanism. The lungs were subjected to routine histopathology and scanning electron microscopy.

Materials and Methods

Animals

Used were male Wistar rats weighing 254 to 280 g when recruited to the experiment. They were fed and maintained by trained caretakers. The animals were put on quarantine for 7 days before experiment, during which period their physical states were checked. To investigate the effects of Nd: YAG laser irradiation on elastase-induced emphysema, a total of 57 rats were divided into four groups according to the following design:

Non-emphysema groups

Group 1: control, only endotracheal instillation of sterile saline: 7 rats

Group 2: control, saline, thoracotomy and laser: 16 rats

Emphysema groups

Group 3: elastase instillation alone: 8 rats

Group 4: elastase, thoracotomy and laser: 26 rats.

To evaluate the progression of emphysema, each elastase-instilled rat (Group 3) was sacrificed at the 1 hour, and 1st, 2nd, 3rd, 4th, 6th, 8th and 12th week after instillation. In 13 survived rats (Group 4), each three on 1 hour, Day 3, and Day 7 and two at the 2nd and 4th week were sacrificed after irradiation, to study lung changes produced by laser.

Production of emphysema

In creating emphysema in animal, we employed the most widely used tech-

nique: Intratracheal instillation in rats of porcine pancreatic elastase (PPE) (Karlinsky and Snider 1978; Busch et al. 1984). It produces changes sufficiently resembling the pan-acinar emphysema of the humans, a non-bullous lesion classified by Wakabayashi (1995a) as Type 3 emphysema. PPE (Elastin Products Inc., Owensville, MO, USA), which was used in this study had a specific activity of 75 IU/mg. Each animal in the elastase groups (Groups 3 and 4) was given 50 IU/body PPE which was dissolved in 0.5 ml warm sterile physiologic saline and immediately instilled intratracheally. Instillation was carried out through a tracheal tube under ether anesthesia, with the animals put on a supine position. The animals inhaled the elastase-containing solution within two or three breathing and then were shaken gently. To produce emphysema severe enough and equally distributed over the whole lungs, elastase instillation was repeated two times a week until a total doses of 200 IU was given. Treatment with elastase or with saline caused no death of the animals.

Thoracotomy and laser irradiation

The animals in Groups 2 and 4, whether elastase or saline solution was given, were submitted to thoracotomy and laser irradiation 8 weeks after the last instillation. The operation was performed under general anesthesia with intraperitoneal administration of 30 to 40 mg/kg bw sodium pentobarbital. In addition, methylprednisolone sodium succinate (20 mg/kg bw) was given i.p. shortly before operation. The animals were intubated and connected to a respirator.

Under anesthesia, left antero-lateral thoracotomy was performed through the 5th intercostal space, with the rat fixed at right lateral position. Using contact Nd: YAG laser, the left lung was irradiated at a power of 5 watts. The laser beam, when applied to the pleural surface, focussed as a spot of about 2×2 mm. The beam was moved over the whole pleura so that the cauterizing spot might stay at a site for 0.5 to 1 second, giving an energy of 0.62 to 1.2 J/cm^2. After the operation, they were extubated on awakening, and kept warm.

Histopathology

Animals were sacrificed according to the experimental design. Immediately after intravenous injection of KCl, the lungs were fixed in situ by instilling intratracheally 10% buffered formalin according to Weibel and Vidone (1961), and the whole body was kept in the same fixative. Twenty-four hours later, the catheter was removed and the trachea was tied up. Then the lungs, together with the heart, were removed from the thorax as a single block and again fixed for another 24 hours in 10% buffered formalin. After fixation was completed, the lungs were kept in absolute alcohol for 3 to 4 days so that they might be hardened enough to escape deformation at slicing. Then the lungs were cut into frontal slices of 3 mm thickness and were embedded in ordinary paraffin. Two sections were cut from each block at 3 μm, one stained with hematoxylin-eosin and the

other, with elastica-Goldner stain.

Scanning electron microscopy (SEM) was performed on a small number of animals. The lungs of the animals were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde dissolved in 8% sucrose in 0.1 M cacodylate buffer (SCB, pH 7.4). The fixative was slowly infused via the trachea at a pressure of 25 cmH_2O. Then the lungs were excised and re-fixed overnight by immersing in 25% and 50% dimetylsulfoxide in 0.1 M SCB. Then the specimens were subjected to freeze fracturing with a freeze cracking device (Eiko Engineering TF-2, Tokyo). Fractured specimens were processed according to the fixation protocol, first in 1% osmium tetraoxide, second in 1% tannic acid, and third, in 1% osmium tetraoxide in 0.1 M SCB. They were dehydrated through a graded series of ethanol and dried by critical point drying method with liquid CO_2. Finally they were metal coated with gold and observed using a scanning electron microscope (S-2250N, HITACHI, Tokyo).

Results

Emphysematous changes induced by elastase instillation

In the elastase-instilled rats (Groups 3 and 4), severe and diffuse pulmonary

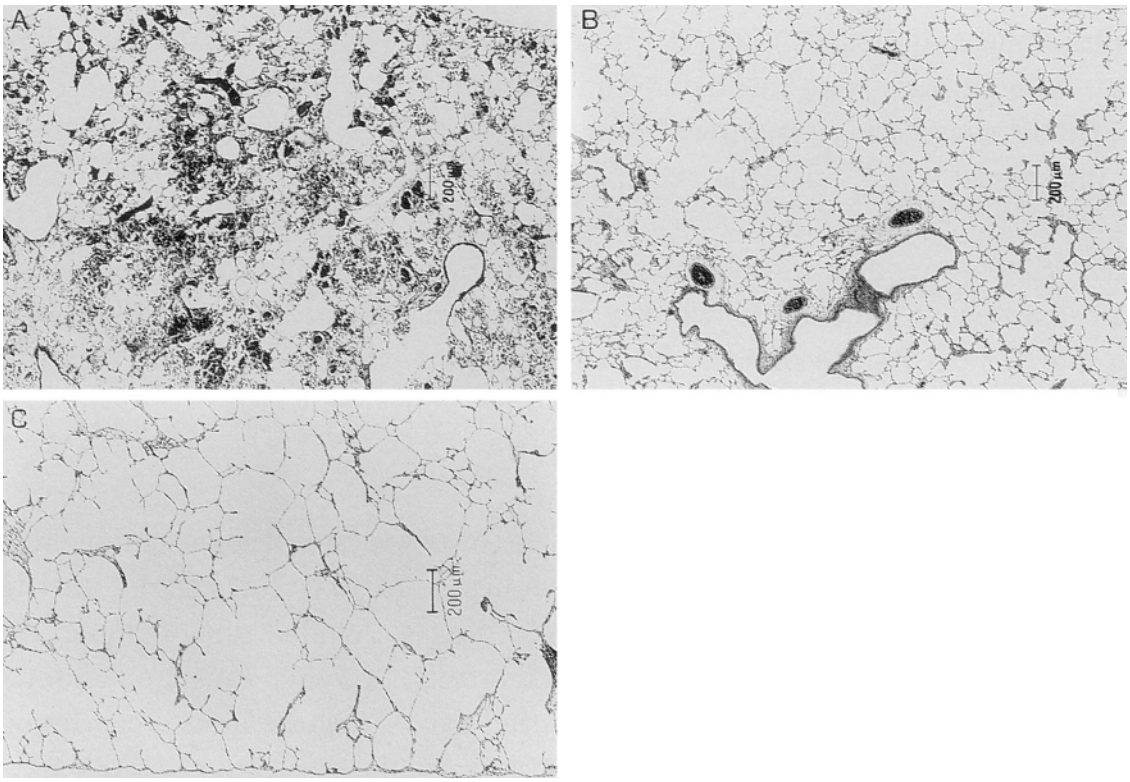

Fig. 1. Microscopic appearance of the lung instilled with PPE (hematoxylin-eosin stain). A: One hour after treatment; severe and diffuse pulmonary bleeding is obvious. B: Seven days after treatment, with apparent dilatation of air spaces. Focal infiltration of mononuclear cells remaining sporadically in the wall of bronchi. C: Eight weeks after treatment, pan-acinar emphysematous changes were progressive.

bleeding was found to emerge, already one hour after the treatment, in almost all areas of lung. At this stage, dilatation of air spaces was not apparent microscopically. By Day 7, blood disappeared from the alveoli, while focal infiltration of mononuclear cells remained sporadically in the wall of bronchi, without stenosing the airways (Figs. 1A and B). Thereafter, the lung proved to become gradually emphysematous. The basic changes that occurred were a marked sparseness of alveolar septa with consequent widening of air spaces, a state quite comparable to the panlobular or distal acinar type emphysema of the humans (Fig. 1C). However, no air spaces larger than 2 mm were confirmed to emerge. Bullae, in the strict sense of the word, were not detected even in the subpleural zone.

Changes of emphysematous lungs after Nd: YAG laser irradiation

Immediately after irradiation, the site of lung where Nd: YAG laser was applied became macroscopically swollen, hyperemic and crumpled. The superficial zone of lung was edematous, stiff, and much less flexible than normal.

Microscopically, the most remarkable finding in this acute phase was necrosis of subpleural alveolar tissue in the irradiated area: It was shown extending over a thickness of 745 ± 18 μm from the pleural surface. Here the constituent cells of the alveolar septa were mostly destroyed, only leaving small number of pyknotic nuclei (Fig. 2). There was severe edema in the air spaces surrounding the necrotic septa that were strikingly hyperemic. The overlying visceral pleura was thickened with edema, swelling of collagenous matrices and superficial deposition of proteinaceous exudate. Fig. 3 is a scanning electron micrograph of the necrotic zone taken at this stage. It demonstrates the whole alveolar septa destroyed, with

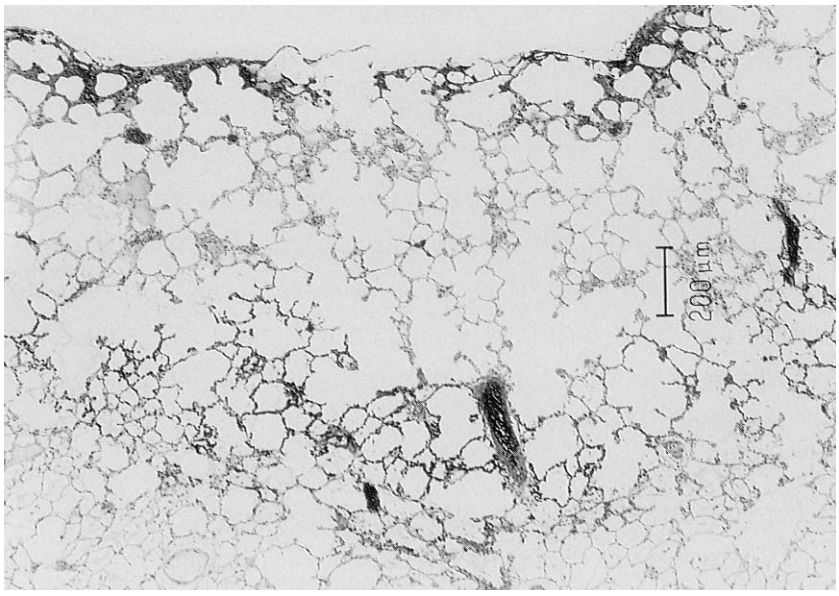

Fig. 2. Microscopic appearance of Group 4 emphysema, on 1 hour after laser irradiation. The constituent cells of the alveolar septa were mostly destroyed, only leaving small number of pyknotic nuclei (hematoxylin-eosin stain).

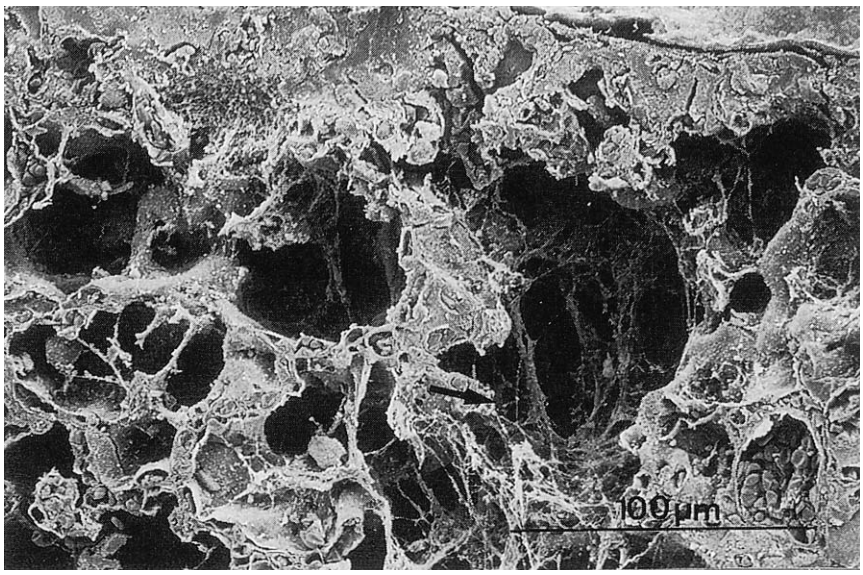

Fig. 3. Scanning electric micrograph of the same lung as shown in Fig. 2. The almost all alveolar septa are destroyed, with web-like grid of fibrin (arrow).

web-like grid of fibrin, stuck with amorphous exudate and debris.

On Day 3, the necrotic zone was shown demarcated from the viable part of lung lying below. The demarcation was given by a thin layer of granulation tissue organizing the ruins, that developed so as to interpose between, and divide the two layers. Thus, a three-tiered stratification was clearly visible: from the pleura downward, Zone 1 (necrosis, including the pleura), Zone 2 (organization) and Zone 3 (viable tissue) (Fig. 4A). At this stage, Zone 1 was 696 ± 24 μm thick and comprised alveolar septa that were in a state of typical coagulation necrosis (Fig. 4B). Zone 2 presented as a membranous granulation tissue 409 ± 12 μm thick (Figs. 4C and D). It consisted of collapsed alveoli, with marked edema and hyperemia, and interspersed with inflammatory cells, but there were no other features than those found in usual, non-specific inflammatory response.

On Day 7, the necrotic alveoli in Zone 1 was found completely collapsed, no longer allowing to discriminate the individual septa it contained. By this time, the necrotic Zone 1 was merged not only with the pleura but with the demarcation tissue of Zone 2, forming a common fibrosing zone, 200 to 400 μm thick (Fig. 5). It should be born in mind that this zone is not merely presenting a thickened pleura; it contains a sizable amount of alveolar tissue folded into a solid tissue. There were vessels and sporadically mononuclear cells, but the inflammatory process already seemed subsiding.

At the end of the second week, the superficial collapse zone had shrunk and contracted, presenting as a sheet of well developed scar, 144 ± 16 μm thick. In the deep zone of lung, one could find little if any signs of improved emphysema. Although there were places in the lung where the density of alveolar septa seemed to have been elevated more or less, this was likely to be the result of restriction of lung "encased" and distorted by the contracting scar. At the end of the fourth

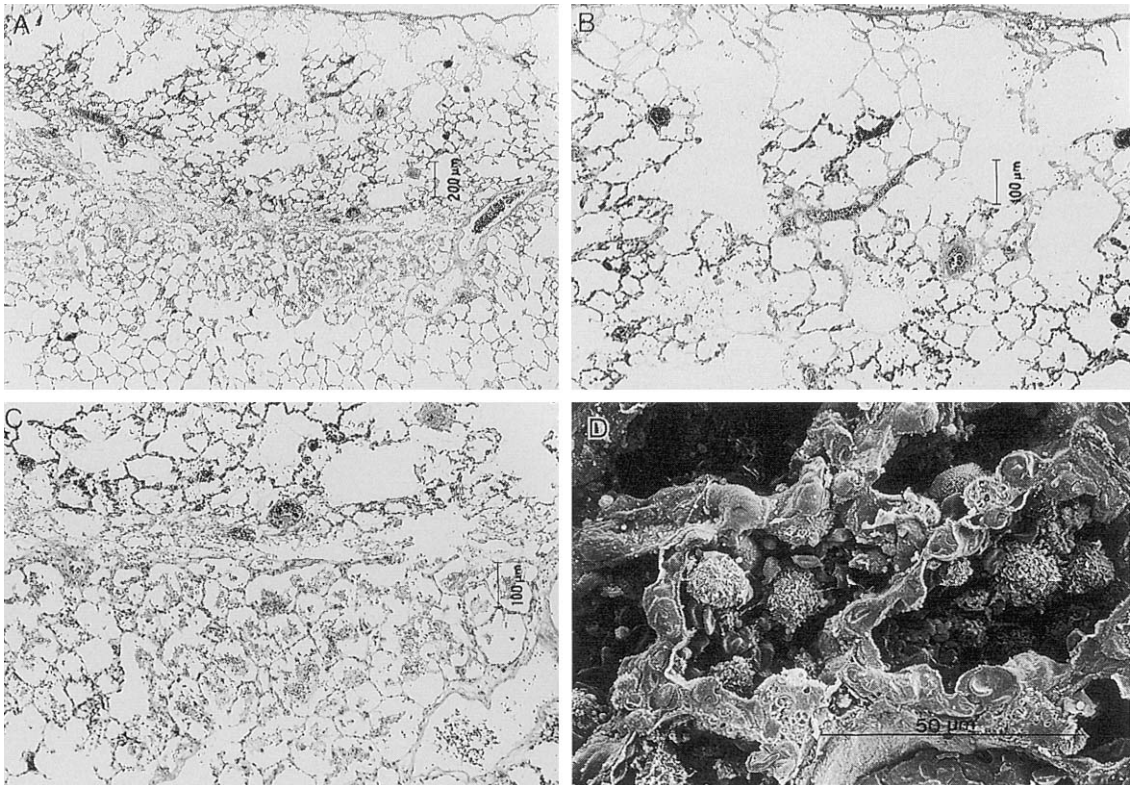

Fig. 4. Microscopic appearance of the lung on Day 3. A: A three-tiered stratification is clear (Zone 1: necrosis, including the pleura, Zone 2: organization, Zone 3: viable tissue) (hematoxylin-eosin). B: High magnification of A. Zone 1 is shown comprising alveolar septa in a state of typical coagulation necrosis (hematoxylin-eosin). C: Zone 2 presenting as a membranous granulation tissue (hematoxylin-eosin). D: Scanning electric micrograph of the same lung as shown in Fig. 4C.

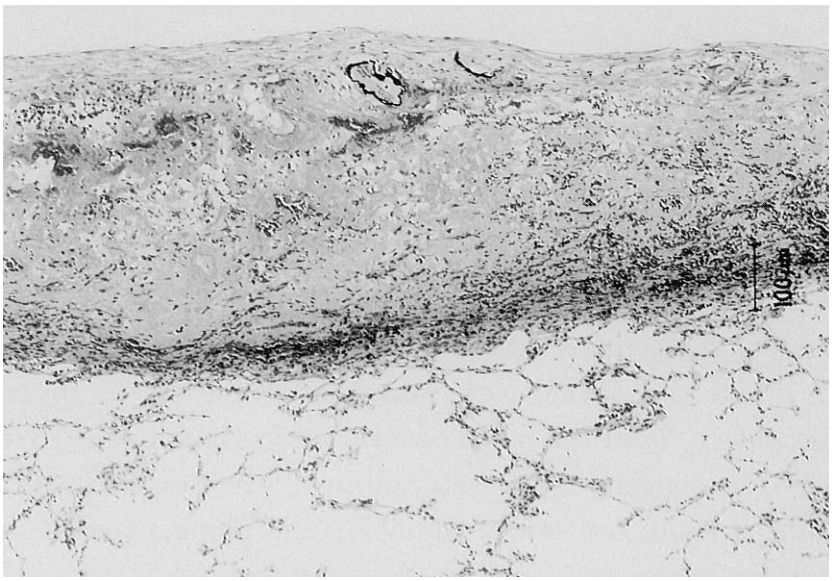

Fig. 5. Microscopic appearance of lung on Day 7. The necrotic alveoli in Zone 1 are completely collapsed, forming a common fibrosing zone joined with the pleura and the granulation tissue (hematoxylin-eosin stain).

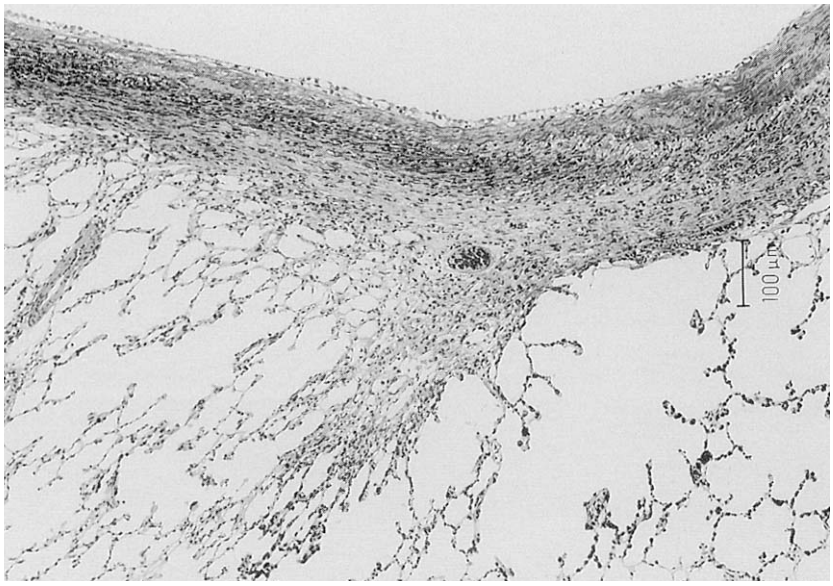

Fig. 6. Microscopic appearance of the lung on 4th week in group 4. The superficial fibrosing zone had shrunk and contracted, presenting as a sheet of well developed scar (hematoxylin-eosin stain).

week, the above findings remained essentially unchanged except that the scar zone became more condensed and firm (Fig. 6).

Changes of non-emphysematous lung after Nd: YAG laser irradiation

Also in the lungs treated only with saline, irradiation produced changes that were essentially the same. On Day 28, we had a dense fibrous scar beneath the pleura, which differed in no respect from that found in Group 4. Around the patches of scar, there were sometimes dilated air spaces beneath the pleura.

Discussion

There are several studies comparing the effect of Nd: YAG laser upon lung with that of CO_2 laser. Brenner et al. (1996) undertook this comparison by studying microscopically the changes of normal, non-emphysematous lungs of rabbit, which were subjected to irradiation with Nd: YAG as well as CO_2 laser, both given at an energy level as low as clinically applicable to patients. The changes of lung that occurred were congestion, ischemia, edema, necrosis and fibrosis, which in Nd: YAG laser were found involving only a superficial zone of the organ while in CO_2 laser the range of injuries was much more extensive. This was considered to be suggesting a greater clinical advantage of Nd: YAG laser in view of the difficulties in uniformly radiating the pleural surface with a spotty beam.

Of the changes that ensued, the most remarkable was zonal necrosis of alveolar tissue uniformly distributed beneath the pleura, which subsequently was organized, collapsed and condensed into a sheet of fibrous scar. The organization was confirmed to begin as early as on Day 3. It advances so as to demarcate the

necrotic mass from the underlying viable part of lung, forming an interposing zone of granulation tissue. With the absorption of debris and collapse of destroyed alveoli, it finally leaves a sheet of fibrous tissue. By the end of the second week, the scar came to "encase" the irradiated emphysematous lung as a solid shell. This is a state in which the lung may be restricted more or less, as experienced in patients occupationally exposed to asbestos, in whom the visceral pleura often is hardened by diffuse fibrous thickening. In the deep layer of lung exempted from laser-induced necrosis, no improvement of emphysematous changes seemed to occur. Sometimes the spatial density of alveolar septa seemed elevated to a certain degree, but this could be an apparent-than-real improvement, brought about by the distortion of lung tissue encased and restricted by the dense superficial scar. Thus, what we found was, in terms of histopathology, a non-specific process of injury repair. We think that in the emphysematous lung of humans subjected to laser, the same may be taking place.

Cole and Wolfe (1987) observed in an experiment using canine lung in which they gave an external incision how the process of wound healing is influenced by high energy Nd: YAG laser. Although there are some aspects common to their observation and ours, the aim, and also the situation in which the experiment was done, completely differ. Moreover, the level of radiation energy they employed was much higher (80 W, vs. 5 W of our experiment).

Sawabata et al. (1995) attempted laser irradiation on lung specimens surgically resected from patients with subpleural bullae or bullous emphysema. They paid attention to a "gelatinous" change of the lung surface which they considered to give rise to hardening and strengthening of the pleura, keeping the bullae from rupturing. We suspect that the change might correspond to the edema we confirmed in the necrotic subpleural zone. In any event, so long as using a resected material where the vital response no longer endures, little may be understood on the course of event that follows an irradiation.

If the laser ablation can ever have a therapeutic effect on emphysematous lung, how does it work ? Wakabayashi (1995b) emphasizes that with the laser treatment, an emphysematous lung can restore the "fine parenchymal structure." However, in our recent clinical trial of Nd: YAG laser irradiation on lungs of emphysematous patients, we confirmed by microscopic examination of resected material that changes attributable to laser only involved a superficial zone not deeper than 0.5 mm from the pleural surface (data not shown). In terms of tissue volume this may be a very minute fraction, seeing that the lungs were voluminous, with a total volume of 7000 ml or more. Also in the present experiments, laser-associated changes proved to reach not deeper than 0.7 mm from the surface. Therefore, we supposed the encasement effect and reduction value of lung volume would be minimal in humans since only small portions of the lung surface would be irradiated. Also, with bilateral volume reduction surgery, about 20-40% of each lung volume could be resected with staplers. We supposed that the stapler

method is more useful than laser pneumoplasty in volume reduction therapy. From all these it may be quite improbable that the mere subpleural necrosis and fibrosis can correct the structure in the bulk of the organ even if there is some improvement of lung function. Reduction of lung volume, which has been said to improve the function of emphysematous lungs (Gelb et al. 1996), does not seem to work at this tiny reduction. On the other hand, "restoration of fine parenchymal structure" seems to be consistent with the results of our experiments, only if it implies an elevated density of alveolar septa once thinned out with the advancement of emphysema. Indeed, in our rat model with pre-existing emphysema, laser irradiation seemed to leave somewhat elevated density of septa. However, this is likely to be the result of tissue distortion in a lung restricted by the subpleural dense scarring, not reflecting the restoration of alveolar structure itself. Or one may talk about an "encasement" effect of lung packed in a non-pliable scar. Whether the density of septa is really elevated and to what degree, has to be made clear by morphometric studies. On the other hand, improvement of lung function in terms of reduced compliance awaits to be corroborated by physiological studies. We have already extended our studies into these directions, but the results will be presented in a forthcoming report.

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