from $6.2 \pm 0.8\%$ in the normotrophic type II cells to $15.8 \pm 2.1\%$ in the hypertrophic cells, compared to $1.5 \pm 0.01\%$ in type II cells isolated from saline-treated control animals.

We conclude that the hypertrophic alveolar type II cells isolated after silica-induced lung injury are progressing through the cell cycle and maintain a commitment to DNA synthesis in primary culture.

**Epithelial Injury is a Critical Factor in the Development of Pulmonary Fibrosis Following Multiple Episodes of Inflammation**

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Many studies have suggested that injury to the alveolar epithelium, the resulting epithelial repair, and pulmonary fibrosis are interrelated processes. To examine this relationship, reactive oxygen species-mediated lung injury in the hamster induced with intratracheally instilled mixtures of glucose, glucose oxidase, and lactoperoxidase (GO) was allowed to repair for 1 wk; animals were then reinnjured with GO at weekly intervals for a total of 3 wk.

Control hamsters received heat-denatured GO. On d 1 and d 6 after each treatment, hamsters from both groups were killed. On d 1 after the third treatment, plasma levels of thromboxane B$_2$ (TXB$_2$) from GO-treated hamsters were significantly elevated. On d 6 after the second and third exposures, bronchoalveolar lavage (BAL) fluid from GO-exposed hamsters contained significantly more total cells, polymorphonuclear cells (PMNs), and macrophages, whereas the total PMN in BAL fluid from control hamsters decreased after each exposure. $\beta$-Glucuronidase and lact dehydrogenase activity and protein levels in BAL fluid were significantly increased in GO-exposed animals immediately after each dose but returned to control levels before the next dose. Total collagen levels and prolyl hydroxylase activity also increased significantly 6 d after the second and third exposures to GO. On d 6 after each treatment, morphometric analysis showed a greater volume of parenchymal lesion in GO-exposed hamsters. Lesions were characterized by thickened alveolar septa, alveolitis, and type II cell hyperplasia. Large fibrotic foci were observed after the second and third exposures.

To test the hypothesis that multiple pulmonary inflammatory responses alone (without substantial epithelial injury) would not result in fibrosis, hamsters were treated with human recombinant C5a instilled intratracheally; at the same time, control animals were given saline. PMNs increased by 3-, 33-, and 34-fold (compared with the corresponding controls) 24 h after the first, second, and third doses but were not different from levels in control animals 6 d after each treatment. Hydroxyproline levels in C5a-exposed hamsters were not different from levels in controls.

Protein levels were significantly increased 24 h after the second and third doses and 6 d after the third dose, compared with levels in controls. Histologic examination showed occasional foci of PMNs in alveolar spaces 24 h after each dose; these foci were less frequent by 6 d. Pulmonary inflammation resulting from repeated influx of PMNs in response to multiple instillations of C5a did not cause sufficient injury to result in pulmonary fibrosis. These studies suggest that repeated epithelial injury and repair are critical factors in the development of pulmonary fibrosis.

**Modulation of Cellular Repair Response Patterns**

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The repair response to acute lung injury induced by hyperoxia exposure varies among species. Specifically, rats are extremely sensitive to oxygen toxicity and show a high proliferative response to hyperoxia injury. This proliferative response is dominated by endothelial cells. In contrast, mice and marmosets, species that tolerate longer periods of oxygen exposure, show a much lower proliferative response that is dominated by type II pneumocytes. A unique response of the rat is the development of tolerance to the toxicity of 100% oxygen by preexposure to 85% oxygen. We sought to determine whether preexposure to 85% oxygen for 5 d alters the cellular milieu of the rat lung such that the repair response to 100% oxygen becomes more similar to that seen in other species.

**MATERIALS AND METHODS**

Sixty-day-old pathogen-free Sprague-Dawley rats weighing 200-250 g were assigned to 1 of 3 treatment groups. Animals in group 1 were exposed to 100% oxygen for 48 h. Animals in group 2 were exposed to 85% oxygen for 5 d, followed by exposure to 100% oxygen for an additional 48 h. Immediately after exposure of groups 1 and 2, osmotic pumps containing $H$-thymidine were implanted subcutaneously. The rats were given a total of 5 mCi over the weeklong labeling period, then killed. Animals in group 3 were exposed to 85% oxygen for 5 d, then injected intraperitoneally with 1 µCi/g of $H$-thymidine, returned to 85% oxygen for the next hour, then killed. For all groups, control animals exposed to room air were similarly labeled with $H$-thymidine.

Tissue from the lower lobes was prepared as autoradiographic histologic slides as previously described. At least 1,000 cells, excluding intravascular leukocytes, within a grid at an oil immersion magnification were counted in random fields of alveolar parenchyma. Cells with 4 or more grains over their nuclei were considered to be labeled. The labeling index was defined as the number of cells labeled per total cells counted, multiplied by 100. The labeled cells were also differentiated into the major cell types seen in peripheral lung parenchyma; endothelial, interstitial, and alveolar epithelial pneumocytes, or macrophages. Statistical analysis of the data were performed utilizing SAS.

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