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Discussion

**Dr. Mason:** Will you comment briefly about the specificity of the kind of biochemical changes?

**Dr. Tierney:** We might view this as a change in cell type, from type 1 to type 2 cells, for instance, or almost anything that would increase the G6PD activity which may be used in the repair process. It could also conceivably protect it against oxidant stress. So I don’t think this is very specific for oxygen toxicity.

**Dr. Stephens:** As you know, we have been interested in tolerance development in relationship to NO and ozone and I think the primary lesion is perhaps of particular interest. I was wondering if you could comment on what the primary injury is in oxygen toxicity?

**Dr. Tierney:** I’m speaking to a morphologist whom I respect greatly and I’m very hesitant to state what the primary injury is in oxygen toxicity. There are injuries to the epithelial surfaces within the airways as well as the epithelial surface within the alveoli and I think I have to rely on the reports in the literature. I believe you have progressed beyond that, especially in your studies using other oxidants, NO and O₃. I could visualize G6PD being a mechanism which might protect against oxidants. I don’t know that superoxide dismutase would. I don’t know that superoxide is produced when ozone, or NOₓ are used. I suspect that the G6PD changes will be there.

**Dr. Burri:** In O₃ toxicity, the damage of the endothelial cells seems to be primary and the replacement of type 1 cells by type 2 cells seems to follow.

**Dr. Langdon:** Is there any evidence that at high oxygen concentrations one gets release of lysosomal enzymes, perhaps as a primary injurious event?

**Dr. Tierney:** I think a number of people have thought along those lines.

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**Impairment of Adaptive Tolerance to Oxygen Toxicity by Systemic Immunosuppression**

**Richard Parad, Geneva Simmons, Neil Feldman, M.D., and Gary Huber, M.D.**

Oxygen administered at high concentrations has an essential role in the clinical management of respiratory failure. At increased tensions, continuous exposure to oxygen is accompanied by toxic manifestations that eventually restrict or limit its use. We have previously shown that several of the life-threatening alterations which occur in oxygen toxicity, including pulmonary edema and hyaline membrane formation, can be markedly reduced by the induction of adaptive tolerance. This adaptive tolerance to oxygen toxicity is defined as an attenuation or protection against the otherwise acute lethal effects, pulmonary edema and hemorrhage of oxygen toxicity. One way to induce this adaptive tolerance is to repeatedly expose the host to high concentrations of oxygen intermittently rather than continuously. Adaptive tolerance also occurs in a similar manner with many other environmental oxidants, such as ozone and oxides of nitrogen and there may be a common underlying mechanism in the tolerance produced against these different oxidants. The precise mechanism of the tolerance response, however, is not known. Several investigators have implied that immune reactions might be potentially important in the development of adaptive tolerance to a variety of oxidants. In order to study this, we attempted to determine whether adaptive tolerance to oxygen toxicity could be blocked by systemic immunosuppression.

**Methods**

Approximately 150 male Charles River strain mice, weighing 20-25 grams each, were injected with an initial dose of 0.5 ml of rabbit anti-mouse lymphocytic serum (RAMLS), as prepared by Monaco and coworkers. This injection was followed the next day and then at weekly pulsings thereafter with doses of 0.25 ml RAMLS per mouse for a total of four weeks. Confirmation of immunosuppression in the RAMLS-injected animals was achieved by performing heterologous skin grafts on representative animals. Prolongation of skin graft survival to over 27 days in the RAMLS-treated animals, as compared to 12 days in controls, indicated good immunosuppression. To expose the animals to 100 percent oxygen, the mice were placed in a chamber through which the gas was delivered at a rate of one chamber exchange of oxygen per minute. Humidity and temperature were maintained within normal limits and food and water were provided for ad lib consumption.

To induce adaptive tolerance, animals were alternately exposed in this chamber to 12 hours of ambient air and to 12 hours of continuous 100 percent oxygen. This pre-treatment was carried out for five sequential days of intermittent

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mice were then exposed to continuous 100 percent oxygen to induce oxygen toxicity. Representative oxygen-exposed animals and appropriate controls were evaluated after 0, 24, 48, 72 and 96 hours of exposure. At sacrifice, the left upper lobe of the lung was removed for an evaluation of intrapulmonary fluid content. The left lower lobe was removed and processed for histologic examination.

RESULTS

Shown in Figure 1 is the effect of exposure to oxygen on intrapulmonary fluid in the groups studied over the 96 hours of exposure. The RAMLS-immunosuppressed animals, the non-RAMLS air controls and the RAMLS-immunosuppressed mice with intermittent oxygen exposure maintained a relatively constant or normal intrapulmonary fluid content. The non-tolerant RAMLS-immunosuppressed and non-RAMLS or untreated animals in continuous exposure to oxygen increased their fluid content at approximately the same rate, as is characteristic of the edemagenic response of oxygen toxicity. The most important difference here, however, can be seen in the curve representing the RAMLS-immunosuppressed mice that were first exposed to intermittent and then to continuous oxygen. These animals accumulated intrapulmonary fluid at a greater rate than any of the other oxygen-toxic animals and had a 100 percent mortality rate beyond 72 hours of exposure.

Histologic examination of lungs from oxygen-tolerant mice revealed a proliferation of cells at the blood-air barrier, particularly involving type 2 epithelial cells. There also was a general hypercellularity in the pulmonary interstitium and a prominence of alveolar capillaries in the lungs of these animals. Typical morphologic changes in the lungs of oxygen-toxic mice included atelectasis, pulmonary hemorrhage, alveolar and interstitial pulmonary edema and alveolar hyaline membrane formation. Histologic examination of the lungs of animals injected with RAMLS for four weeks, exposed to intermittent 100 percent oxygen for five days and then 100 percent oxygen for 72 hours revealed clear-cut morphologic manifestations of pulmonary oxygen toxicity.

FIGURE 1

EFFECT OF EXPOSURE TO OXYGEN ON INTRAPULMONARY FLUID

![Graph showing effect of exposure to oxygen on intrapulmonary fluid.]

DISCUSSION

The toxic manifestations that develop in animals following exposure to high tensions of oxygen can be prevented in animals that develop adaptive oxidant tolerance through preconditioning with equivalent exposures of intermittent oxygen before continuous exposure. Nonimmunosuppressed animals exposed intermittently 12 hours at a time over a period of days to 100 percent oxygen and to air develop tolerance to the toxic pulmonary morphologic responses of oxygen toxicity. In our studies, non-tolerant immunosuppressed and nonimmunosuppressed animals were equally susceptible to the toxic manifestations of continuous exposure to 100 percent oxygen. Immunosuppressed animals, however, were not only less able to develop oxidant tolerance when exposed intermittently to 100 percent oxygen, but were more susceptible than non-immunosuppressed mice to oxygen toxicity. Apparently, immunosuppression has blocked some response, presumably an immune response in nature, which is an intrinsic part of the tolerance mechanism. By raising the questions of immunologically mediated responses in the development of oxygen tolerance, we do not mean to imply that oxygen toxicity or oxygen tolerance are solely immunologic phenomena. An immune response, however, may at least in part explain the observed tolerance which develops during repeated exposures to high concentrations of oxygen.

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Discussion

Dr. Daniele: There seems to be a preferential attack on cellular rather than humoral immunity. Did you measure antibody levels in these mice?

Dr. Parad: The skin graft survival showed immunosuppression. Antibody levels weren't measured. As far as the cellular response is concerned, I am not really sure what immune response is being blocked by the immunosuppression.

Dr. Petty: We have seen proliferative and fibrotic lesions consistent with oxygen toxicity in our longterm home oxygen patients. It's a small number of patients and I don't know what it means, but these individuals have apparently failed to develop tolerance.