Responses of the Lung to Toxic Injury

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Analysis of toxic lung damage may focus on the offending agent and define patterns of bioactivation and interactions with the target tissues. It may also focus on a study of the biological response. While it was originally thought that cell proliferation, particularly Type II epithelial cell proliferation following lung injury, was a common event, it now has become obvious that on occasion proliferation occurs only late after the initial lung damage. Also Type II cell proliferation can occur in the absence of alveolar Type I cell damage. Delayed reepithelialization of the alveolar surface may lead to pulmonary fibrosis. Toxicological interactions often can be best recognized and defined by the extensive lesions that result from concomitant or sequential exposure to such toxic agents as ozone and acidic aerosols or anticancer drugs and oxygen. A correlation of cell proliferation and tumor development in mouse lung has shown that target cell hyperplasia is not a necessary prerequisite for enhanced tumor development. On the other hand, oxygen-induced proliferation of the neuroendocrine cell population results in the short-term development of neuroendocrine lung cell cancer in hamsters. While it is possible to draw some conclusions from an analysis of the lung response to toxic injury, predictions made from such knowledge are sometimes, but not necessarily always, correct.

Introduction

Airborne and bloodborne chemicals may cause toxic lung damage. It is the goal of experimental toxicology to understand the possible underlying mechanisms. A mechanism may be defined as "the fundamental or physical process involved in or responsible for an action, reaction, or other natural phenomenon (as organic evolution)" (*1*). It is certainly fair to say that we know a great deal about mechanisms which underlie what can be defined as actions in toxic lung damage. The metabolism of many drugs has been elucidated in great detail during the past 10 to 15 years. The roles of bioactivation in toxicity, conversion from proximate to ultimate toxins, and pathways of detoxification and of protection are well known for many chemicals, including carcinogens (*2,3*). And for many of the same agents, information is available on the biochemical changes they produce in lung tissue or in isolated lung cells (*4*).

We know less about mechanisms underlying what we may call reactions in the pathogenesis of toxic lung damage. Quite often we are unable to link a specific and mechanistically defined action to a specific and predictable reaction or tissue response. A recent and excellent analysis of our present knowledge of lung damage caused by chemicals stresses two points (*5*). Apparently similar mechanisms leading to cell damage will not necessarily always produce the same ultimate tissue lesions in the lungs of different species. On the other hand, similar lesions are often the result of initially quite different mechanisms. The second point is that studies on the pulmonary toxicity of drugs or chemicals will eventually be remembered not because of the toxicologic, economic, or social consequences of the chemical, but because of the concepts developed through their study (*5*).

The first point made by Smith and Nemery (*5*) clearly emphasizes that we face considerable gaps in our knowledge when we try to understand mechanisms that determine the response or reaction of the lung to toxic injury. Perhaps we should, for the time being, adopt a somewhat different approach to what constitutes a mechanism. We may define it as an initial event or as a sequence of consecutive events which will produce a similar biological response regardless of how the process was initiated (*6*). This implies that in our attempts to understand toxic lung injury and to develop broad concepts, we should focus as much on the tissue response as we usually focus on the metabolic fate of the offending chemicals. We also might consider that a mechanism need not be exclusively an event that is understood at the chemical, i.e., molecular level. A mechanism may also consist of events that can be described at different levels. What a mechanism eventually should give us is the capability to predict the initiation and the evolution of a process leading to acute or chronic disease. In the following, the history and success of a few attempts to determine mechanisms of lung disease along these lines are reviewed and analyzed critically.

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Cell Proliferation As a Common Lung Response to Toxic Injury

A review of some of the literature available 15 to 20 years ago showed that in many instances toxic lung injury appeared to follow a somewhat monotonous and, more importantly, predictable pattern (*7*). The cells apparently most vulnerable to many toxic agents in the lung are the Type I alveolar epithelial cells, cells which cover about 95% of the alveolar surface. Their peculiar shape and localization within the alveolar walls precludes further division (*8*). Once damaged, they cannot be replaced by a division of adjacent undamaged sister cells. To repair defects in the alveolar epithelium, the lung has to rely on a second cell type, the Type II alveolar epithelial cells. These cells are often called the stem cells of the alveolar epithelium. They begin to divide and will eventually assume both the shape and the functional role of the Type I epithelial cells. Transition of Type II into Type I cells has been documented indirectly with autoradiography (*9*) and directly with morphologic methods (*10*).

Although other cells of the alveolar wall such as interstitial cells and capillary endothelial cells undergo division following toxic lung injury, the main emphasis in the analysis of cell proliferation following lung damage was usually placed on the changes occurring in the epithelial cell population. Type II cell division as a response to injury of Type I cells remains a key element in our appraisal on how damaged lung repairs structure and function. For example, it is believed that agents that produce only Type I cell damage usually leave only mild or moderate permanent lung lesions. Examples might be the antioxidant butylated hydroxytoluene (BHT) and many of the common air pollutants. On the other hand, chemicals that have the capability to damage and destroy both Type I and Type II cells are much more harmful and often lead to fatal lung lesions. Examples might be the herbicide paraquat and the antineoplastic drug bleomycin. Maintaining a healthy population of Type II alveolar cells seems to be a prerequisite for the lung to defend itself successfully against chemical injury (*11*).

In 1978 a small but carefully performed study established a direct relationship between the extent of damage to the alveolar Type I epithelium, which was measured by quantitative morphometry, and the subsequent proliferation of the Type II cell population, which was measured by the incorporation of radiolabeled thymidine into cell nuclei (*12*). This particular piece of work turned out to be rather influential. Once the concept was established and accepted that Type II cell proliferation was proportional to the initial lung injury, toxic lung damage was quite often detected and analyzed by the simple expedient of measuring the incorporation of radiolabeled thymidine into total pulmonary DNA (*13,14*). The biochemical procedure has the advantage of being quantitative, allowing researchers to study many more tissue samples in a shorter time than would be possible with morphometric or autoradiographic methods. It was, of course, well recognized that a measurement of thymidine incorporation into total DNA could by no means provide accurate data on Type II cell proliferation; rather, it would reflect overall DNA synthesis in the entire lung cell population. Since autoradiographic studies did suggest that the prominent cell population proliferating after lung injury was indeed the Type II cell population, representing up to 70% of all dividing lung cells (*15*), the assumption was not entirely unreasonable that a biochemical technique would directly relate to reparative cell proliferation and, therefore, to initial lung damage.

During the same time, several morphologic studies reported an observation which seemed at first puzzling and contrary to the established knowledge about finding increased cell proliferation in the lungs of animals exposed to various dusts and fumes without seeing apparent lung lesions to explain the phenomenon. Such observations were made in animals exposed to diesel engine emissions, fly ash, and metal oxide dusts believed to be devoid of any biological action (*16–18*). It was noted in these experiments that there was usually a large influx of inflammatory cells into the lung.

In subsequent experiments it was shown that, indeed, migration of inflammatory cells across the blood-air barrier was sufficient to trigger a proliferative response in the alveolar epithelium (*19*). Mice were made leukopenic by internal radiation with ^{89}Sr and challenged with an intratracheal instillation of biologically inert carbon particles. As expected, the proliferative response of the lungs of leukopenic animals to carbon—which in itself did not produce any lung damage—was considerably less than it was in the lungs of normal animals.

The hypothesis put forward was that inflammatory cells passing between epithelial cells in the lung triggered a round of cell division either by breaking contact inhibition or by releasing specific growth factors. The results of this experiment no longer allow us to equate Type II cell proliferation with Type I cell damage under all circumstances.

A second concept derived from analysis of epithelial cell proliferation following lung injury deals with the development of fibrotic lung disease. In 1976 it was suggested that in oxygen-damaged mouse lungs an undisturbed reepithelialization of the air-blood barrier following the initial injury was necessary in order to obtain proper healing (*20*). If too much toxic damage to the alveolar Type II cell population delayed or even prevented the process, fibrosis developed.

The original model was recently refined (*21*). Mice were exposed to 95% oxygen first. After different lengths of exposure, lung damage was assessed with morphologic and biochemical techniques. At the same time, lung explants were prepared for further maintenance and study in culture. The cultures made it possible to follow precisely the development or resolution of the oxygen-induced lesions. In lungs that had been damaged *in vivo*

for 4 days and showed comparatively mild lesions, the injury remained limited mostly to capillary endothelial cells. Recovery after removal from hyperoxia was good, and interstitial cells did not show excessive proliferative activity.

In more seriously damaged lungs (6 days in oxygen) there was marked toxicity to the epithelial cells, preventing them from recovery. Under these circumstances, the interstitial cell population started to proliferate and to produce substantially more collagen. Since the lung explants contained only very few alveolar macrophages or polymorphonuclear cells, it was concluded that these cells did little to influence the developing lesions. Severe injury to and delayed repair of the alveolar epithelium may thus give the underlying fibroblast population a chance to overgrow and to produce abnormal amounts of collagen, leading to the development of diffuse interstitial pulmonary fibrosis.

A similar conclusion on the critical role that reepithelization plays in a damaged lung was reached with a different experimental approach. Mouse lung is readily damaged by the antioxidant BHT (*15,21*). Detailed cell kinetic analysis showed that following initial damage (widespread necrosis in the Type I epithelium and, to a lesser extent, in the capillary endothelial cells), there would follow, 2 to 4 days later, a burst of Type II cell proliferation. Only later interstitial cells and capillary endothelial cells also proliferated. When mice with lungs damaged by BHT were exposed to an atmosphere of 100% oxygen, it was found that oxygen inhibited pulmonary cell division only 2 or 4 days after BHT, but not later (*22*). The conclusion drawn was that dividing epithelial cells would be more sensitive to the cytotoxic action of oxygen than would dividing interstitial cells. It was speculated that interference with epithelial cell regeneration might favor the development of fibrotic changes in a damaged lung.

Evidence to support this hypothesis was found in several experiments (*23,24*). When animals with BHT-damaged lungs were exposed to hyperoxia during the phase of epithelial cell proliferation, fibrosis developed. In the most severely altered septa, lining cells were often absent, and interstitial connective tissue fibers were exposed directly to the air spaces (*25*). The fibrotic changes persisted up to 1 year and included not only excess collagen accumulation, but also changes in the ratio of Type I to Type III collagen (*26*). The morphologic changes seen in the lungs of mice treated with BHT and oxygen were quite similar to the lesions observed in human lungs afflicted with idiopathic pulmonary fibrosis (*25*). On the other hand, when oxygen exposure was delayed until epithelial recovery was well under way or complete, no fibrosis developed (*23*).

Analysis of patterns of cell proliferation in the lung helped to examine another important question: Is there evidence that different species would respond differently to identical amounts of the same toxic agent? Species differences in the response of lung to foreign chemicals are well known. For example, hamsters seem to be much more resistant to inhaled carcinogens and other toxic agents than are most other species (*27,28*), and BHT damages mouse lung only, but not the lungs of other species (*14*). It is, of course, possible that many such differences may be explained by the ways foreign chemicals are handled. Differences in uptake by target cells and tissues, in metabolism, detoxification mechanisms, and elimination all might account for variations in the biological response. Possibly there is also an inherent difference between different species in the response of the lung tissue to toxic materials. The fact that rats are the only species which can be made tolerant to 100% oxygen by preexposure to 85% oxygen appears to support such a possibility (*29*).

To test the hypothesis directly, one would require an agent that is handled by all species the same way and can be applied in strictly comparable doses. Oxygen, of course, is such an agent; it is presumably handled the same way by all mammals. As far as comparable doses are concerned, it could be argued that there can hardly be a more comparable dose than to expose animals for identical lengths of time to 100% oxygen.

We exposed mice, rats, hamsters, and marmosets for 48 hr to an atmosphere of 100% oxygen (*30*). After exposure, a minipump filled with radiolabeled thymidine was implanted IP or under the skin of the rodent's back. One week later the animals were killed and autoradiographs of their lungs were prepared. Analysis of patterns of cell proliferation was used to examine how the lungs dealt with recovery from oxygen toxicity.

We did find substantial differences between the four species, both quantitatively and qualitatively. The greatest lung damage occurred in rats and the least in hamsters and marmosets. In rats, the predominant cell type to proliferate following lung injury was the capillary endothelial cells. In mice and marmosets recovery was characterized by an epithelial proliferative pattern. When mice appearing to be less sensitive to oxygen than rats were exposed for longer than 48 hr to oxygen, an increase in overall cell proliferation was observed, but there was no change in the pattern of cell proliferation. Again, the predominant response was epithelial.

The experiment clearly highlights inherent species differences in what most likely is an identical and comparable insult to the lung. It also raises the question to what extent studies with rat lungs are representative of oxygen toxicity in man. The recovery patterns found in mice and marmosets appeared to resemble much more what we know of oxygen toxicity in human lungs than did findings made in rats (*31*).

Toxicologic Interactions in the Pathogenesis of Lung Injury

Exposure of the lung to two or more chemicals may occur in heavily polluted atmospheres or perhaps in critical care medicine. On occasion, this results in lung damage that is more serious than might be expected from our knowledge of the actions of single chemicals (*32*).

The occurrence of interactions is often difficult to predict. Qualitative and quantitative analysis of the responses of the lung is practically the only way to recognize toxicological interactions.

It has been known for some time that exposure of paraquat-treated rats to high concentrations of oxygen in the inspired air is much more lethal than that expected from exposing rats to paraquat alone or to oxygen alone (*33*). The question might be asked whether paraquat enhances oxygen toxicity or whether oxygen enhances paraquat toxicity. The answer to this question came from a detailed morphologic analysis of the lung damage produced by the combined two agents (*34*). The cell type most severely damaged was the Type II alveolar cells. Paraquat is one of the compounds that causes permanent damage to the Type II cell population. Oxygen thus appeared to enhance paraquat toxicity. If paraquat had enhanced oxygen toxicity in rat lung, then one might have expected to find damage due to a paraquat-oxygen interaction predominantly in the capillary endothelial cells—the primary cells of oxygen toxicity in rat lung. This example shows how a study of the biological response may, indeed, give us some mechanistic clues. At the same time it remains puzzling why a biochemical measurement indicative of acute paraquat toxicity failed to pick up an interaction between the herbicide and oxygen. NADPH depletion may be a key element in pulmonary paraquat toxicity (*35*). Yet, in the lungs of rats treated with paraquat, the effects of inhaled oxygen on the reducing status of the NAPD/NADPH system remained the same as in animals kept in air (*36*).

Other types of chemical interactions are less well understood, although the biological response has been extensively investigated. The acute pulmonary toxicity of certain anticancer drugs such as bleomycin or cyclophosphamide is enhanced by exposing animals to hyperoxia (*37*). It is possible, although not proven, that similar events may occur in man. Originally it was thought that the mechanism responsible for this particular interaction was similar to the one postulated for the interaction between BHT and oxygen: that oxygen would interfere with reepithelialization following the initial injury. It is no longer certain whether this is indeed the case with oxygen-mediated amplification of bleomycin or cyclophosphamide-induced lung damage. Oxygen is quite effective in potentiating lung damage if given early after the anticancer drugs (*37*). As short an exposure time of 12 hr in 80% oxygen will produce 100% mortality within 72 to 96 hr in hamsters treated with a dose of 0.5 units of bleomycin intratracheally (*38*). Epithelial cell proliferation, on the other hand, is delayed until 10 to 14 days after cyclophosphamide or bleomycin administration (*39*). It would thus appear that potentiation of lung damage occurs because of a direct interaction between the anticancer drug and oxygen, rather than because oxygen interferes with proper repair of the lesion, although interference with early repair processes cannot be excluded. A general influx and proliferation of alveolar macrophages might be an additional factor (*38*). Possibly oxygen also amplifies the generation of hydroxyl radicals, an event thought to be critical in bleomycin pulmonary toxicity. On the other hand, two observations are somewhat difficult to reconcile with simple amplification of lung injury. One is that in hamsters given a single intratracheal instillation of bleomycin and then exposed for 24 hr to oxygen, pulmonary edema develops only some 72 hr later (*38*). The reason for this delayed response is unclear. The second phenomenon is the continuing development of lung damage (*40*). Treatment of rats with a combination of bleomycin and oxygen produces a lung lesion that has all the hallmarks of progressive interstitial pulmonary fibrosis.

A somewhat similar observation was made in a study that examined the long-term effects of a single administration of cyclophosphamide (*41*). It was found that total lung collagen content increased progressively over a 1-year period. The exact mechanisms responsible for this progressing lesion are not understood. The two observations do point out the need for a better understanding of the interrelationships between acute lesions and their eventual transformation into chronic events. Of particular interest is the observation that chronic-progressive lesions may result from a single episode of exposure to one or several drugs; why the disease process, once initiated, seems to sustain itself over a period of weeks or months is obviously of considerable interest.

Examination of the biological response has also been critically important in analyzing the consequences of exposure to more than one air pollutant. It is obvious that in any heavily polluted atmosphere, many noxious agents are present. Most experimental research on acute and chronic health effects of air pollutants deals with exposure to one compound at a time (*42*). While this may be justifiable, it should nevertheless be recognized that a combined exposure to several pollutants may, on occasion, cause more damage than might be expected from a study of the effects of single compounds. Several years ago it was found that exposure to SO_2 and several particulate aerosols would produce an exacerbated airway hypersensitivity, compared to that expected from the known effects of the individual agents alone (*43*). More recently it has been shown that a concomitant exposure to the common air pollutant ozone and to acidic aerosols, particularly H_2SO_4, will greatly enhance the rate of collagen synthesis in the lung (*44,45*). Of particular importance was the observation that such interactions did occur at levels of acidic aerosol and ozone that are close or identical to conditions encountered in the real world. It must be noted that, at present, air quality standards for air pollutants are set and regulated based on data pertaining to individual chemicals only. The question must be asked whether this approach will be sufficient to deal with the possibility of interactions.

Again, it must be pointed out that only the study of the biological response, the apparent synthesis rate of pulmonary collagen, provided evidence that potentially

important interactions between ozone and an acidic aerosol could be of practical importance. As far as mechanisms are concerned, there is only speculation. A reasonable assumption is that inhalation of an acidic aerosol alters the pH within the tracheobronchial tree and perhaps at the centroacinar target sites for ozone. A change in the microenvironment might allow for longer half-lives of active molecular species involved in oxidant damage, thus creating the potential for an exacerbation of lesions.

The problem of interactions becomes even more complex when we deal with multichemical exposure. It is not unreasonable to assume that exposure to complex mixtures can, on occasion, result in biologically significant toxicological interactions. It is, of course, impossible to study the toxicity of all single ingredients in such complex mixtures as, for example, represented by cigarette smoke or diesel engine exhaust. In order to assess toxicity, the only practical way is to rely on the biological response. This approach is not always rewarding. Although there is certainly overwhelming epidemiological evidence to link cigarette smoking to lung cancer in man, only comparatively few animal inhalation studies have shown a similar correlation (*46*). The production of lung tumors in rats, mice, or hamsters by inhalation of tobacco smoke has proved to be a difficult and often elusive endeavor. As far as diesel engine emissions are concerned, numerous *in vitro* short-term assays for carcinogenicity suggest the presence of potent carcinogens and mutagens associated with diesel exhaust particles. Studies with whole animals have shown that prolonged exposure to diesel engine emissions produces lung tumors in rats (*27,47*). It was pointed out repeatedly that tumor development might be a consequence of particle overload of the lungs with a resulting decrease in pulmonary clearance (*48*). It was stressed that this overload in the end might be responsible for the carcinogenic action of diesel emission particles. This is an interesting hypothesis. The question asked may be: What are, under less stressing and therefore nontumorigenic conditions of exposure, the ultimate metabolic fate, and biological effects of all these compounds that are associated with diesel exhaust particles and which show considerable mutagenic activity in appropriate short-term assays?

Modification of Tumor Development in the Lung

We have earlier made a tentative attempt to broaden the definition of mechanisms. A mechanism might be a sequence of consecutive events that yield a similar biological response, regardless of how the process was initiated. Several experimental investigations on the biological behavior of lung tumors in mice were precisely undertaken because it was thought such a mechanism was known and, therefore, might produce an anticipated development. It is interesting to note that more often than not, the prediction made originally turned out to be correct, although quite often for the wrong reason.

In a comprehensive review on the mechanism of promoters in carcinogenesis, Boutwell in 1974 (*49*) concluded that the capability to produce epithelial cell proliferation was a critical property of mouse skin tumor promoters. [It also was pointed out that not all agents capable of producing skin cell proliferation would be tumor promoters (*49*).] At the same time, practically no evidence was available that showed two-stage carcinogenesis existing in organs other than mouse skin.

Lung tumors in mice are believed to originate from Type II alveolar epithelial cells (*50*), although some of them might also be of Clara cell origin (*51*). The question for the time being is open to discussion (*52*). Ten years ago the following prediction was made. It was known that many tumors in mouse lung were Type II cell tumors, and that tumor-promoting agents were able to produce cell proliferation in mouse skin. When it was found that BHT would stimulate proliferation of Type II cells in mouse lung, it was postulated that this situation might somehow fulfill the criteria put forward above for mechanisms. In other words, BHT should be capable of promoting tumor development in mouse lung (*53*).

In a series of experiments it was found that in strain A or Swiss-Webster mice treated first with a carcinogen such as urethan or methylcholanthrene, the subsequent administration of BHT (by IP injections or given in the food) would greatly increase the number of lung tumors (*54*). BHT was found to have such an effect even if given for the first time 5 months after the carcinogen (*55*). On the other hand, BHT was not effective in increasing tumor yield in mice treated with subcarcinogenic doses of a carcinogen, and it may be questioned whether the BHT-lung tumor system rigorously fulfills all criteria usually applied to the mouse skin tumor system. Nevertheless, under appropriate experimental conditions BHT enhances tumor development in mouse lung and also in several other organs such as mouse liver and colon; rat liver, colon, urinary bladder, forestomach, and thyroid; and in several *in vitro* systems (*56*).

If the underlying mechanism for enhanced tumor development in mouse lung was the induction of repeated Type II cell proliferation in the lung, then two more predictions should follow. Both of these predictions could be and were tested in appropriate experiments. Both predictions also proved to be wrong.

Several agents other than BHT were used to produce alveolar Type II cell proliferation in mouse lung. They all should have enhanced tumor development. Intermittent exposure of mice treated with a carcinogen to 100% oxygen or injections of methylcyclopentadienyl manganese tricarbonyl (MMT) produced Type II cell proliferation, but they had no enhancing effect on tumor development (*57*). Recently it was suggested that glycerol, given at a concentration of 5% in the drinking water, would produce hypertrophy and hyperplasia of the Clara cell population in the lungs of male ddY mice (*58*). Presumably, through this effect glycerol would enhance tumor development in mouse lung (*59*). Unfor-

tunately, the response to glycerol appears to be unique to the ddY mouse. In our own experiments with A/J or Swiss-Webster mice we did not obtain any evidence that glycerol would increase alveolar or bronchiolar cell proliferation or would enhance tumor development in mouse lung and liver or rat liver (*60*).

It also was not possible to demonstrate unequivocally that diffuse alveolar Type II cell hyperplasia was a necessary prerequisite for enhanced tumor development in mouse lung. Two observations were made: First, in our hands, the minimum number of BHT injections needed to enhance tumor development are four. Yet, it was found that after only two injections of BHT, mice no longer responded with increased cell proliferation; somehow the animals appear to become resistant to further BHT toxicity (*57*). Second, the damaging effects of BHT on lung tissue could be prevented by mixed-function oxidase inhibitors (*14*). As a consequence, cell proliferation was prevented. However, tumor development still was enhanced despite the absence of diffuse cell hyperplasia in the lung. A detailed analysis of the phenomenon showed that, indeed, it is entirely possible to separate enhanced tumor development from diffuse alveolar cell hyperplasia in the lung; the two phenomena are not interdependent (*61*).

Sustained exposure to oxygen is able to elicit a continuing cell proliferation in the Type II cell population of the lung two to four times over basal rate (*62*). Thus, one might expect that such an event would increase tumor development in the lung, should cell proliferation be such a crucial event in carcinogenesis. It could even be speculated that hyperoxia might be doubly effective. Under hyperoxic conditions there could conceivably occur an increased production and flux of reactive oxygen species in the lungs of exposed mice. Reactive oxygen species are believed to be of crucial importance in tumor promotion (*63,64*). However, in several experiments it was shown that chronic hyperoxia, rather than enhancing tumor development, severely curtails tumor formation in mouse lung—even in the continued presence of an active proliferative response in the Type II cell population and an increased activity of pulmonary ornithine decarboxylase, believed to be a hallmark of tumor promotion (*62,65*). It has not been possible to apply some mechanistic considerations developed for mouse skin to the mouse lung tumor system.

Although cell hyperplasia does not appear necessary to enhance the development of lung tumors in animals after exposure to a carcinogen, it might be important to explain the development of spontaneously occurring lung tumors in mice. A good correlation has been found between rates of basal Type II cell proliferation and the apparent sensitivity of various mouse strains to develop spontaneously occurring lung tumors (*66*). And although both oxygen (*62*) or ozone (*67*) inhibit the development of chemically induced tumors in mice pretreated with a carcinogen, both gases increase the average number of tumors and the percentage of tumor-bearing individuals in mice not knowingly exposed to a carcinogenic agent (*62,67,68*). This, of course, could imply both ozone or oxygen as pulmonary carcinogens, at least in mice. There is an alternative explanation that would link Type II cell proliferation to tumor development occurring in sensitive mouse strains and also in mice exposed to ozone or oxygen. An increased number of dividing cells would expand the cell population at risk (the risk currently being unidentified) or place a continually dividing cell population at higher risk to undergo spontaneous transformation.

To explain the decreased number of tumors found in animals pretreated with a carcinogen and then exposed to ozone or to oxygen, another assumption is needed. Oxygen appears to have high cytotoxicity for newly transformed tumor cells in mouse lung, and the gas appears to be capable of preventing individual tumor cells or small clusters of transformed cells from growing into visible tumors (*62*). If this is true, oxygen should prevent tumor development in other species.

To test this hypothesis, rats received a single intratracheal instillation of methylcholanthrene at a dose known to produce, within a few weeks, multiple squamous cell carcinomas in the lung. Exposure of such animals to an atmosphere of 70% or even of 40% oxygen dramatically reduced tumor formation (*65*). The percentage of tumor-bearing rats in the oxygen group was less than half of that found in controls (where practically all animals developed squamous cell carcinomas). The average number of tumors found per lung was 10% of that seen in controls. The original conclusion applied thus to a second experimental system.

Yet, another model system in which the effects of oxygen on lung tumor development could be examined appeared to be the hamster model that was originally developed by Reznik-Schuller (*69,70*). Here, oxygen had a different effect. While being treated with the carcinogen N-nitroso-diethylamine (DEN), hamsters exposed to 70% oxygen developed, within 8 weeks, multiple lung tumors of neuroendocrine cell origin (*71*). This was the first time that this particular tumor had been produced in a reproducible way in experimental animals.

In man, neuroendocrine lung cancer is commonly subdivided into carcinoid and small cell lung cancer (*72,73*). Carcinoids are generally well-differentiated tumors that rarely metastasize; whereas, small cell lung cancer, which constitutes some fourth of all human lung cancers, is highly malignant and metastasizes early. The tumor usually responds well to initial treatment with chemotherapeutic agents. Resistance then develops rather soon, and the median survival time of patients with diagnosed small cell cancer of the lung is about 12 months. Only 5% to 10% of all patients survive for 3 years or more.

Epidemiological data link this particular tumor with cigarette smoking; the tumor is virtually never found in nonsmokers. Additional contributing risks are exposure to radiation, as in uranium miners, and chronic exposure to asbestos fibers in the air.

How then do we explain the finding that a combination of hyperoxia and DEN would produce neuroendocrine lung cancer in hamsters? Neuroendocrine cells have been identified in the lungs of many species. The cells are comparatively rare in adult lungs, but they occur in great abundance in fetal lungs and immediately after birth (*74,75*). They are believed to play a role in helping the lung to adapt to drastic changes in pulmonary oxygen levels through the production of vasoactive substances as may occur during the transition from intrauterine to extrauterine life. The pulmonary neuroendocrine cells contain vasoactive substances such as serotonin, bombesin, calcitonin or neuron-specific enolase. When the oxygenation of the lung changes (under conditions of hyperoxia or hypoxia) the number of cells also changes, as for example when the lung goes from a comparatively hypoxic environment *in utero* to an environment of physiological oxygen toxicity after birth (*76–80*). In patients suffering from chronic obstructive lung disease and in man living at high altitudes, the number of neuroendocrine cells in the lung is also increased (*81*).

Conditions of oxygen imbalance in the lung may thus alter the proliferative status of pulmonary neuroendocrine cells and explain why, in hamsters exposed to hyperoxia, tumors develop in response to DEN administration. Since the occurrence of neuroendocrine cell tumors in man also appears to be linked to conditions of oxygen imbalance in the lung (chronic obstructive lung disease is found mostly in smokers), the availability of this new animal model of a specific human lung disease should allow researchers to carry out important mechanistic studies in the future.

Conclusions

The study of the lung response to toxic injury eventually should help to predict the development of acute and chronic lung disease from first principles. Unfortunately, first principles are much harder to develop for the biologic response than for the biotransformation and activation of toxic chemicals. On occasion, first principles derived from analyzing the biological response are successful, at least in some systems. One such example might be that inhibition of proper reepithelialization of a damaged alveolar wall will lead to the development of fibrotic changes.

In an analysis of other examples it was found that what was first believed to be a universal mechanism did not necessarily apply to all circumstances. General target cell hyperplasia and the enhancement of tumor development are clearly separable events in mouse lung and need not be linked to each other, as has been postulated for numerous other organs and tissues. There appear to be species differences in the response of lung to toxic injury, as exemplified by the different patterns of cell proliferation observed during recovery in the lungs of mice, rats, hamsters, and marmosets. Even more dramatic are the observations that when rat lungs are exposed to a potent carcinogen, oxygen inhibits tumor development, whereas in hamsters, hyperoxia together with a systemically acting carcinogen results in neuroendocrine lung cancer, a disease previously not induced in the lungs of experimental animals.

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