METHODS FOR EVALUATING THE TOXICOLOGICAL EFFECTS OF GASEOUS AND PARTICULATE CONTAMINANTS ON PULMONARY MICROBIAL DEFENSE SYSTEMS

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INTRODUCTION

Methods to evaluate the toxicity of airborne chemicals (gases and particulates) are of the utmost importance to society. Increasing numbers of potentially toxic contaminants are an invariable consequence of otherwise beneficial industrial processes. Accordingly, toxicological information is essential to assess the need for control measures to protect occupational workers and the public from contaminant-induced health injury. Ideally, these studies should be performed in man using epidemiological or volunteer methods of investigation. Unfortunately, the expense and complexity of epidemiological investigations limit these techniques to the study of selected substances that are either widely disseminated or already suspected of causing human illness. In designing volunteer studies, ethical as well as practical considerations often preclude appropriate toxicological appraisal. Because of these investigational limitations, much reliance must be placed on experiments conducted with animal or cell systems.

Traditionally, nonhuman studies have used physiological, pathological, and biochemical measurements to assess toxicity. Recognition of the association between exposure to noxious gases [ozone (1, 2), nitrogen dioxide (3–5), sulfur dioxide (1, 6)] or airborne particulates [sulfates (6), silica (7), asbestos (8)] and the development of respiratory infection has resulted in the use of microbiological and immunological parameters to evaluate toxicity. Normally, the lungs are protected from bacterial

and viral infection by the integrated activity of the mucociliary, phagocytic, and immune systems. Methods are available to assess components of these defense systems, the systems themselves, and their function as a unit in preventing pulmonary bacterial and viral invasion. The results of these experiments in which baseline measurements of one or more defense parameters are compared with measurements made following exposure to test compounds have provided valuable information concerning the toxicity of airborne contaminants (9–12). This article reviews the methods used to assess different microbial defense parameters, their sensitivity in detecting abnormality, the importance of the abnormality as regards susceptibility to infection, and the extent to which the method has been applied in toxicology.

TRANSPORT SYSTEMS OF THE LUNG

The mechanical removal of bacteria from the lung is a primary means of pulmonary protection against bacterial infection (13, 14). Three mechanisms of varying capabilities participate in this process: the mucociliary system, which removes bacteria from the tracheobronchial tree; the alveolar system, which is important for the removal of particulates but may also transport bacteria from alveoli; and cough reflexes, which expel secretions and bacteria from all pulmonary regions (14). The available evidence indicates that of the three, mucociliary transport is the most important. The mucociliary system extends from the nares to the terminal bronchioles (13). Ninety percent of particles that deposit on ciliated epithelium are transported out of the lungs within hours (15, 16). In contrast, the alveolar system requires days to remove intra-alveolar materials (17, 18). Because of the rapidity of bacterial proliferation, such slow rates are unlikely to prevent bacterial infection. The significance of cough mechanisms for maintaining pulmonary sterility is unknown. Cough is of undoubted importance when the lung is already diseased. Whether cough also compensates for ineffective mucociliary removal in the normal lung has not been determined. Depression of the cough reflex may ultimately be shown to be a factor in reducing pulmonary resistance to infection. However, until such data appear, the significance of alterations in cough frequency and intensity as regards infection cannot be evaluated. In accordance with these considerations, this section is confined to a discussion of the methods used to assess different components of the mucociliary transport system and the effect of airborne toxicants on these components.

Tracheobronchial Architecture

Architecturally, the nasopharynx and tracheobronchial tree may be considered as a series of bifurcating tubes of ever diminishing caliber. This configuration allows certain aerodynamic generalizations regarding sites of deposition of inhaled bacteria. Because many factors in addition to anatomic considerations interact to determine particle movement (inertia, velocity, diffusion, gravity, respiratory frequency, tidal volume), these generalizations apply only to the majority of particles of a given size. Bacteria are 0.5 to 2.0 μm in diameter and usually traverse the bronchi suspended in air by Brownian movement to settle in alveolar regions.

Agglomerations of bacteria that form larger particles, 2.0 to 10.0 μm in diameter, are influenced more by gravity than by Brownian movement. These bacterial particles often settle within the tracheobronchial tree (14). Bacterial particles greater than 10 μm in diameter tend to sediment almost immediately in the nasal or pharyngeal cavity. Because the longest diameter of most bacteria is 1.0 to 2.0 μm, single or paired bacteria are likely to reach the alveoli where they are relatively safe from mechanical removal; small groups of bacteria tend to settle on tracheobronchial surfaces where they are amenable to rapid removal, and bacterial clumps are usually too large to enter the lung.

As a consequence of these factors, experiments in which radioisotopically labeled bacterial aerosols are used to study the effect of an airborne toxicant on pulmonary defense systems are a poor means of evaluating mucociliary transport; the finely divided aerosol deposits the majority of bacteria within the alveoli (19). Calculation of mucociliary transport by measuring rates of pulmonary removal of the radioisotope for 4- or 5-hr experimental periods invariably shows unaltered mucociliary function in test animals when compared with controls (11, 19–21).

An additional point of importance pertains to differences in respiratory anatomy among rodents, canines, and primates. For example, the respiratory anatomy of rats differs from that of primates in not having interlobular septae, having fewer generations of airways, utilizing distal bronchioles for respiration rather than alveoli, and in pulmonary vasculature (22). There are also important differences among primates and rabbits, mice, pigs, and dogs (23). These distinctions in anatomy may explain why identical exposures to an airborne toxin may cause diverse mucociliary responses and make interpretation that is relevant to disease in man exceedingly difficult.

Tracheobronchial Mucosa, Mucus, and Mucociliary Movement

The celullar components of the mucociliary system are goblet, clara, ciliated epithelial cells, and apocrine glands (13, 24). The goblet and clara cells and the apocrine glands continuously produce large amounts of a sticky, viscous mucus (0.1–0.3 ml/kg body weight per 24 hr in man) (25), which serves as the backbone of the mucociliary system. This complex fluid has a surface layer with gel-like properties and an underlying layer that behaves like a sol (13). Bacteria that come into contact with mucus are trapped by its adhesiveness. Because of these physicochemical properties, mucus and attached bacteria move as a single layer upward against gravity. Ciliated epithelial cells provide the force for this purposeful movement. The cilia that line the free surface of these cells beat synchronously to propel the overlying mucus at rates of 10 to 20 mm/min (25).

Mechanisms of Injury

Although the mucociliary system is continuously exposed to the atmosphere, environmental injury is uncommon in the absence of technology. A few natural perturbations (extremely dry or cold air, dust, allergens, viruses) can inhibit mucociliary function by hardening the mucus layer, constricting bronchioles, or destroying mucosal cells (26–29). The numerous man-made insults (cigarette smoke, industrial

particulates, gaseous pollutants) are considerably more important as causes of mucociliary dysfunction. Exposure to these substances damages cilia, ciliary cells, and mucus-producing cells (24, 30). Such insults result in dyssynchronous ciliary movements, the production of excessive quantities of mucus, and retarded rates of bacterial transport. Because of the relationship of bacterial transport to infection, the result is an increased susceptibility to pulmonary infection.

Measurements of Mucociliary Function

MUCUS Methods exist for determining the amount of mucus produced, its physicochemical characteristics, and its biochemical consistency. Mucus formation can be measured by collecting respiratory secretions from an anesthetized animal via an intratracheal or endotracheal cannula (31–33). The quantity of secretions collected over defined periods allows calculation of hourly or daily rates of formation. The rate in normal animals is then compared with corresponding rates for animals that have received test treatments. It is also possible for the animal receiving the treatment to be its own control. Important sources of error exist in these techniques. The need to anesthetize and to intubate the animals introduces artifacts of mucus formation (anesthesia) and extraneous substances (intubation). A more physiological method for obtaining respiratory secretions has been described by Wardell and associates (34). A 5 to 6 cm segment of the cervical trachea of a dog is separated and formed in situ into a subcutaneous pouch. The isolated system functions for months in a normal fashion. Milliliter quantities of tracheal mucus can be collected at intervals throughout this period. Studies with this experimental model have shown that exposure to sulfur dioxide interferes with mucus production (35).

The rheologic or flow properties of mucus can be measured with various types of viscometers (36–39). Considerable technical expertise is required to perform these delicate measurements. Mucus is subject to shear degradation, and only the gentlest of handling will prevent mechanical breakdown. Furthermore, the test stresses must be similar in magnitude to normal intrapulmonary forces, if the results are to be biologically applicable. When properly performed, viscoelastic measurements provide useful information, for increases in viscosity or elasticity are associated with reductions in mucociliary function (40). These kinds of tests have been used to compare the effectiveness of different mucolytic agents in reducing the viscosity of sputum (37, 41). More recently, investigations in which mucus was obtained from a canine pouch demonstrated that exposure to sulfur dioxide changed the viscosity (35). Future studies with other noxious gases or with particulate inhalants may reveal similar effects.

The concentrations of various constituents of mucus (acid polysaccharides, neutral mucopolysaccharides, sialic acid, and sulfonated compounds) can be measured biochemically (42) and histochemically (43, 44). At present the value of the biochemical measurements is uncertain because relationships between chemical concentrations and mucociliary function have not be established (45). Reid has

determined by histochemical testing the percentage of bronchial cells in organ culture explants that secrete glycoproteins at 4 hr after exposure to cholinergic or anticholinergic drugs. The secretory index (percentage of cells secreting at 4 hr) increased in the presence of acetylcholine and decreased with atropine (44). These methods can be used to test the effect of noxious gases and possibly also particulate toxicants on intracellular production of mucus constituents; abnormality would be correlated with hypersecretion.

CILIARY ACTIVITY Much more is known about ciliary activity than about mucus. Ciliastasis has been used as a bioassay for the detection and assessment of hazardous agents for years (9, 46–49). These bioassays are performed in vitro by removing a section of trachea, visually measuring changes in ciliary rate, and then comparing either changes in rate or in time to cessation of ciliary movement in control and test specimens. Numerous technical variables are inherent in the methodology. Observer error occurs in judging ciliary rate or ciliary cessation. Ciliary rates differ among animals of the same species and even in the same animal at different periods. Lastly, difficulties may occur in reproducing treatment procedures. Because of these variables, statistical evaluations using blind protocols and randomized testing are a necessary part of these experiments (50). Nevertheless, these techniques have yielded valuable information concerning the adverse effects of cigarette smoke (47, 51), chemicals (51, 52), and gaseous and particulate air pollutants (9, 26, 53) on ciliary function. Such results have improved our understanding of the pathogenesis of the toxicant-induced decrease in respiratory resistance to infection and also in some instances (cigarette smoke) allowed testing of protective devices (51).

Errors in visualization can be minimized by using objective methods for measuring ciliary rates. Ciliary activity can be continuously photographed (54, 55) or the rate of flickering can be counted photoelectrically (56, 57) or stroboscopically (58–61). Photography appears to be the most reliable of these methods. Very accurate measurements of ciliary rates can be made by means of high speed photography. Preliminary observations with photoelectric methods indicate that this method will also allow precise determination. Stroboscopy has been successful in measuring slow frequencies of ciliary activity in nonmammalian systems (57). However, attempts to use this technique to record the more rapid rates of ciliary movement in mammals have not had the same success (55). Methods are also available for observing in vivo the rate of ciliary movement (52). A microscope with a vertical light is attached to the trachea via tracheostomy. This system allows measurement of ciliary activity while the animal is exposed to noxious gases or particulates.

Nonvisualization techniques have been used to assess the effects of drugs (62, 63), cigarette smoke (64, 65), and air pollutants on cilia (65, 66). These methods have value in demonstrating beneficial as well as toxic effects of various agents. Increases in ciliary activity have been observed following treatment with several adrenergic drugs, thereby documenting the potential utility of these agents (62, 63). In contrast, exposure to realistic concentrations of cigarette smoke produced zones of ciliary

inactivity similar to those observed in bronchitis (64, 65). Preliminary experiments suggest that these techniques can be used to construct dose-response curves (63).

MUCOCILIARY TRANSPORT Mucociliary transport rates are determined by visually monitoring the rate of movement of particles placed within an excised tracheobronchial system (66), or an incised and externalized in situ system (67–71). More nearly physiological measurements may be obtained by externally monitoring the movement of inhaled radioactive particles in intact animals and humans (72–76). The visual methods that require less expertise and costly equipment have been used in most studies. In these experiments the progress of carbon particles, india ink, or graphite has been measured microscopically within murine, feline, or canine tracheas (67–71). Since all particles do not move at the same rate, arbitrary endpoints such as the fastest rate of transport are recorded. Although the evidence is sometimes inconclusive, particle transport appears to be accelerated by epinephrine (77), inhibitied by numerous chemicals (acrolein, formaldehyde, acetone (51), by cigarette smoke (50), and by various noxious gases (SO_2, NO_2, NH_3) (9).

In the past, the sensitivity of mucociliary components (mucus, mucus transport, ciliary movement) to noxious inhalants was determined in an isolated fashion without concomitant measurement of overall mucociliary function. Recently, an in vivo feline model has been designed that allows the simultaneous measurement of mucus load and rheology, tracheal transport velocity, and ciliary beat frequency (71). Future studies with this model should allow assessment of the effect of noxious inhalants on each of these parameters, and on overall mucociliary function. Such data should be extremely helpful in evaluating the significance of threshold impairments to individual mucociliary components.

The intrapulmonary movement of radioactive particles (Au^{198}-Fe_2O_3, Cr^{51}-Fe_2O_3, $Mn^{54}O_2$) can be determined by externally monitoring radioactivity (72–76). A source of uniformly sized γ emitting particles and an Anger camera, or similar measuring device, is required. The human subject or the experimental animal inhales the radioactive particles from an aerosol generator. Sequential measurements of particle location are made with γ-ray–detecting cameras. Because the particles are distributed throughout the lungs, alveolar as well as tracheal clearance rates can be determined from the radioisotopic scans. These techniques have a number of potential advantages for studying airborne toxicants. The methods are physiological, nonhuman primates can be studied, the animal serves as its own control, and clearance rates are measured precisely and sequentially over prolonged periods.

The effects of inspired sulfur dioxide and carbon dust have been studied on mucociliary clearance rates using technetium-99, labeled albumin, and teflon particles. Sulfur dioxide at a concentration of 5.0 ppm had, at most, a minor effect on clearance (76), whereas carbon dust caused very rapid clearance of the radioactive spheres (78, 79). These techniques have also been used to study the deposition and clearance of 2 μm particles by individuals with a history of smoking as compared to nonsmokers (72). In the smokers, tracheobronchial clearance was delayed for periods of one to four hours.

ALVEOLAR MACROPHAGE SYSTEM

The distal alveolar regions of the lung are protected against bacterial infection by the alveolar macrophage system (14, 80). These ubiquitous pulmonary phagocytes are dispersed throughout the alveoli in a manner that allows them to intercept invading bacteria within minutes after their entry into the lung (81). The bactericidal armamentarium of the macrophage inactivates and degrades the ingested bacteria in the ensuing 2 to 4 hr (82–84). The efficiency of this phagocytic system maintains the sterility of the alveolar region under normal conditions despite the constant introduction of microorganisms (14, 85).

The extraordinary ability of the macrophage to seek out, ingest, and inactivate invading bacteria results from the integration of a number of complex biophysical reactions (86). Phagocytes are attracted to bacteria by chemotactic factors that are elaborated by the bacteria themselves, or are formed from the interaction of bacteria and host tissues (86). Simultaneously, serum opsonins attach to the bacterial cell surface rendering the microorganism susceptible to phagocyte ingestion. This opsonizing process is extremely important, because phagocytes that are surrounded by equally palatable particles will selectively ingest ones that have been opsonized (87). Once ingested, bacteria are internally isolated with phagosomes (88); microbicidal enzymes that were in inactive states (secondary lysosomes) are activated and then fuse with the phagosome, to form the phagolysosome. This process allows the delivery of highly active enzymes to the operational site without subjecting the cell's cytoplasm to potentially injurious effects (86, 88). Although the exact role of each of the many enzymes and toxic substances that participate in intracellular bacterial inactivation is not known, the available evidence suggests that lysozyme, catalase, hydrogen peroxide, and malonyldialdehyde, a catabolite of lipid peroxide with antibacterial activity, are among the more important bactericidal substances (86, 88, 89).

The complexity of the above sequence of phagocytic events provides numerous potential sites for an environmental toxin to interfere with phagocytic function. The toxin can impair chemotaxis by destroying chemotactic substances or by reducing phagocytic mobility secondary to the formation of edema. The toxin can inhibit the ingestive process by damaging the phagocytic membrane. Lastly, the toxin can damage the cell itself or the enzymatic systems involved in bacterial inactivation and degradation.

Chemotaxis

Rates of chemotaxis can be measured in vitro by removing pulmonary macrophages and then testing them in Boyden-type chambers (90–92). Highly purified macrophages are obtained by lavage techniques (93). The cells are placed in the upper compartment of the chamber and allowed to migrate through a micropore filter toward an attracting medium. After a period of incubation, the number of macrophages resting on the lower membrane is counted microscopically. Although the results are affected by many technical factors, e.g. adhesiveness of cells to the filter membrane, tortuosity and size of pore channels in the membrane, and detachment

of cells into the underlying medium, the tests are sufficiently standardized to assess differences in chemotactic function (94, 95). Technical improvements such as the use of a second filter that is impermeable to cells (96) or measurement of the cell front within the filter (97) should allow better quantitation of chemotactic rates.

The few toxicological studies that have been performed with pulmonary phagocytes indicate that macrophages from otherwise healthy cigarette smokers do not have chemotactic impairments (92), and that extracts of cotton capable of causing byssinosis also do not impair chemotaxis by pulmonary macrophages (98). A wide variety of drugs have been tested with regard to their effect on the movement of polymorphonuclear leukocytes. Glucosteroids (99, 100), chloroquine (99), and colchicine (101) have been shown to suppress chemotaxis in vitro. Future tests of the effects of exposure to airborne gaseous and particulate contaminants on chemotaxis of alveolar macrophages should result in useful toxicological information. These experiments can be performed either by exposing the intact animal to the toxicant and then removing the macrophages, or by exposing macrophages that have already been removed from presumably normal animals.

Phagocytic Ingestion

A number of in vitro tests have been advised to measure rates of phagocytic ingestion (102–109). Macrophage monolayers can be prepared and tested with microbial or particulate suspensions (102–107). Alternatively, bacteria can be injected intratracheally into animals, alveolar macrophages lavaged, and the number of intracellular bacteria counted (108, 109). Different test organisms *Staphylococcus aureus* (102, 103), *S. epidermidis* (102), *Pseudomonas auriginosa* (104, 105), *Aspergillus fumigatus* (106), as well as inert particles (106, 107) have been used in these tests. Time-related visual measurements of the number of ingested microorganisms (102, 103) or particulates (106, 107), or in some instances in which radiolabeled bacteria were studied, measurements of intracellular radioactivity (104, 105) allow assessment of the ingestion rate. Visual measurements of intracellular microorganisms or particulates are sometimes complicated by an inability to differentiate intracellular from extracellular cell-associated bacteria or particulates. This technical problem can be overcome by using *S. aureus,* because this bacteria is uniquely susceptible to lysostaphin, a muralytic enzyme that does not enter phagocytes. The addition of lysostaphin to a culture dish containing macrophages and staphylococcus results in rapid lysis of extracellularly located staphylococci enabling precise determination of the number of intracellular bacteria.

The literature contains few studies of the effect of airborne toxicants on the ingestive function of alveolar macrophages (108–111). The rate of ingestion of intratracheally injected streptococci by rabbit macrophages is reduced following exposure to ozone or nitrogen dioxide (108, 109). Similar reductions in the rate of uptake of radiophosphorus labeled *S. epidermidis* were observed in cell systems in which macrophages were exposed in vitro to ozone (110). The effect of other airborne toxicants on bacterial ingestion by alveolar macrophages has received much less attention (111). Because of the importance of this parameter it is anticipated that measurement of the effects of airborne agents on ingestion rates of

alveolar macrophages will be evaluated more thoroughly in future toxicologic experiments.

Bactericidal Activity of Macrophages

The techniques applied in vitro to determining bacterial ingestion rates can also be used to measure rates of intracellular bacterial killing (bactericidal activity). Instead of laborious microscopic scrutiny of phagocytes to count the number of ingested bacteria, the cell suspensions or monolayers are lysed, and the numbers of viable bacteria are determined by pour-plate techniques (103, 105, 106). Because the test results vary for different bacteria to macrophage ratios (112), fixed ratios must be present when comparing treatment and control groups or tests from different days. Much variation also exists in the ability of macrophages to kill different microorganisms (113, 114). These considerations, as well as differences due to technical factors in performing bactericidal assays, make interpretation of results exceedingly difficult.

Because alterations in the rate of bacterial killing relate directly to impairments in pulmonary resistance to infection, many studies of the effect of airborne toxicants on bactericidal function have been performed (104, 110, 115, 116). Ozone (110), nitrogen dioxide (115), cigarette smoke (116), high oxygen tensions (124), and nitrate ion (115) have been tested in phagocytic systems to determine their toxicity. With the exception of oxygen, exposure to concentrations of these agents that were much above ambient caused impairments in intracellular bacterial killing rates.

In Vivo Measurements of Pulmonary Antibacterial Activity

Methods for the evaluation in vivo of the intact pulmonary antibacterial system of rodents (mice, rats, guinea pigs) have been developed (117–120). Briefly, rodents are infected with aerosols of test bacteria. The animals are sacrificed immediately after infection and 4 hr thereafter. The lungs are excised to determine the numbers of viable bacteria by pour-plate techniques. The rate of bacterial inactivation can be determined by comparing the numbers of viable bacteria at each time period. If the bacteria are radiolabeled, precise measurements of pulmonary bacterial inactivation can be made because each animal serves as its own control (118). Histologic determination of the intra- or extracellular locations of the intrapulmonary bacteria at the two time periods reveals the rate of bacterial ingestion by pulmonary macrophages (81). The effects of exposure to ozone (11, 81, 121, 122), nitrogen dioxide (10, 21), sulfur dioxide (19, 123), cigarette smoke (124), automobile exhaust (12), silica (125), and high oxygen tensions (122, 126) have been studied by these experimental methods. According to the data obtained, exposure to above ambient levels of ozone, nitrogen dioxide, and automobile exhaust, but not sulfur dioxide, depresses bactericidal function. Tests with ozone also show that the depression in bactericidal function is due to severe impairments of intrapulmonary phagocytic killing and lesser impairments of bacterial ingestion by the alveolar macrophage (83). In addition to determining threshold levels of toxicity, these methods have also allowed the formulation of dose-response curves (11, 21), and testing of mixtures of airborne toxicants (20). Qualitatively, the results of these studies have compared favorably with other indices of respiratory disease caused by these agents (4, 127).

DEFENSE AGAINST VIRAL INFECTION

A protective function against viral infection has been proposed for the mucociliary transport system, the alveolar macrophage, the interferon system, and humoral and cellular immune mechanisms. The antiviral role of these putative host defenses has been difficult to define, partly because of a lack of the necessary ablative experiments. Studies of the effects of inhaled toxins on resistance to viral infection with concomitant monitoring of specific host defenses have begun to provide not only information on the action of these toxicants, but also information on the physiologic role of the defense mechanisms themselves.

Effect of Pollutants on Viral Respiratory Infection

Epidemiologic data have shown an association between respiratory symptoms indicative of viral infections and increased levels of air pollution (128, 129). Cigarette smoke has been shown to increase the mortality of murine influenza (124). Chronic exposure of squirrel monkeys to nitrogen dioxide renders influenza virus infection fatal, in contrast to the symptomatic, but nonfatal, infection in unexposed animals (130, 131). Mice exposed to 20 ppm of sulfur dioxide for 7 days after infection with influenza virus developed more pneumonia than control animals, although no effect was observed on the growth of influenza virus (132). Conflicting evidence on the effects of oxidants on the pathogenesis of viral infection may result from such factors as the species of animal, the concentration of the toxicant, and the intermittent or continuous nature of the exposure (133, 134). Other effects of toxic gases on the establishment of viral respiratory infection include the increased decay of infectivity of aerosolized Venezuelan equine encephalomyelitis virus in the presence of NO_2 (135) and the increased deposition of vesicular stomatitis virus in the nasal cavity of mice exposed to ozone (136). Studies illustrating detrimental effects of inhaled toxicants on respiratory tract viral infections provide models, which by further study may yield information concerning the involved host defense mechanisms.

Mucociliary Transport

The site of deposition of inhaled particles containing infectious virus may in part determine clinical manifestations. Tracheobronchitis can result from aerosolized rhinovirus type 15; however topical application to the nasal mucosa is a more efficient route for establishing infection, which then spreads down the respiratory tract. In contrast, influenza infection begins in the bronchiolar epithelium with virus swept upward by cilia to cause bronchitis and systemic disease (137, 138).

If the conjunctiva of one eye is inoculated with adenovirus type 4, the sequential development of unilateral rhinitis, tonsillitis, and pharyngitis can be observed before infection spreads to the contralateral side (138). Thus, while entrapment in mucus and physical removal by ciliary action are thought to be a primary defense of the respiratory tract, some viral infections may actually spread by this mechanism. Once viral or mycoplasmal infection of the respiratory tract is established, impaired mucociliary clearance of bacteria can be demonstrated (139, 140).

The Alveolar Macrophage

An important antiviral role for the mononuclear phagocyte has been established for infection of the peritoneum, skin, and brain—indeed, the susceptibility of newborn as opposed to weanling mice can be related to the maturation of the virucidal capacity of these cells (141, 142). Valand et al (143) have shown that lung monocytes from rabbits exposed to 25 ppm of nitrogen dioxide for 3 hr failed to produce interferon after infection with parainfluenza virus type 3 and did not develop resistance to challenge with virulent rabbitpox virus. Subsequent studies by Williams et al (144) showed that the failure of interferon production was not due to lack of attachment, penetration, or uncounting of the virus. In fact, more virus was absorbed and penetrated the macrophages from animals exposed to nitrogen dioxide. Suppression of phagocytic activity and virus-induced resistance were observed to be the most sensitive indicators of NO_2 effects on alveolar cells (145).

The establishment of a viral pulmonary infection results in enhanced susceptibility to bacterial superinfections because of the viral-induced defects in bacterial inactivation by macrophages (146, 147).

Protection of the Respiratory Tract by Interferon

A distinction should be made at the outset between a possible physiological role of interferon as a defense mechanism of the respiratory tract and the antiviral effects that have been demonstrated in vivo using high doses of locally applied interferon or interferon inducers. The cells of the respiratory tract must be exposed directly to the drug administered as an aerosol, or given as drops into the nose or trachea, before resistance to viral challenge via the respiratory route can be demonstrated (148, 149). The administration of mouse interferon intranasally or by aerosol has been shown to inhibit virus replication in the lungs of mice challenged 24 hr later with an aerosol of influenza virus (149, 150). Inducers that stimulate the local production of interferon have also been shown to be effective against intranasal challenge with influenza, mouse pneumonia, vesicular stomatitis, and Columbia SK viruses (p. 306, Table 14.2 in reference 151). These inducers have included pyran copolymer (152), statolon (153), poly I/C (152, 154), and other double-stranded RNAs (155–158). In only a few instances, however, have attempts been made to demonstrate the presence of interferon in the respiratory tract following inducer treatment (153, 154).

Human interferon has been detected in respiratory tract secretions in naturally occurring infections with influenza A_2 (159) and parainfluenza (160) viruses as well as in experimental infections with influenza A_2 (161–163) and members of the rhinovirus and Coxsackie A virus groups (164). Locally applied human interferon has been shown to suppress symptoms and decrease the virus titer during experimental rhinovirus 4 infection in a placebo-controlled double-blind trial (165). Interferon inducers have been shown to suppress symptoms (166, 167), prevent illness, and decrease viral titers in experimental rhinovirus infections (167).

In spite of numerous studies demonstrating protection of the respiratory tract by exogenous interferon or inducers, the importance of the interferon system as a physiologic mechanism is difficult to assess. Evidence that interferon plays a physiologic role in resistance to viral infection is circumstantial and is based on its

presence in measurable quantities at the proper time and location to provide beneficial effects to the host. The effect of inhaled toxicants on interferon-mediated resistance has received little study. In order to study the effects of gaseous and particulate pollutants on the interferon system in the respiratory tract, several types of experiments can be envisioned. First, the response to inducers, administered by aerosol, can be determined before and after exposure to toxicants. Two types of measurable response include the level of interferon produced and the degree of protection to virus challenge obtained by the inducer following such exposure. Second, the response to virus challenge after exposure might include observations on the susceptibility to infection, the outcome of infection, and the titer of interferon and virus in respiratory secretions and in the lung.

IMMUNOLOGIC DEFENSES

A detailed presentation of the humoral and cellular immunologic reactions that protect the lung from microbial infection is beyond the scope of this review. Microorganisms that infect the lung stimulate the formation of various protective neutralizing and agglutinating antibodies. The presence of these antibodies may be assayed in respiratory secretions or in blood. Secretions are commonly obtained by pulmonary lavage through a bronchoscope in anesthetized dogs, sheep, and nonhuman primates. Variability in volume and protein concentration of the lavage fluid can be an important source of error (168, 169). Because of this difficulty in specimen collection, whole-lung lavage in terminal experiments is a preferred means for sampling secretions of the lower airways (170–172). Standardized procedures exist for measuring concentrations of neutralizing or agglutinating antibody (173–175). In these procedures the respiratory or serum specimens are serially diluted and reacted against appropriate antigens. An alternative method is to adsorb the antigen on a carrier particle, red blood cells, or latex spheres and to measure antibody-mediated agglutination. Complement fixation or gel immunodiffusion tests are additional methods of measuring antibody (175). The amount of specific antibody in a respiratory or serum sample can be measured with regard to its immunoglobulin class. A fluorescein conjugated anti IgA, IgG, or IgM antibody is added following the reaction of microbial antigen and lavage fluid or serum. If antibodies of the homologous class are bound to the antigen, the particle fluoresces (176).

The few immunologic studies of airborne toxicants indicate that prolonged exposure to carbon or sulfur dioxide can cause a progressive decrease in murine ability to form agglutinating antibodies (177). Cigarette smoke has also been shown to initially enhance and then depress murine antibody response to sheep red blood cell antigens (178). In contrast to these toxicant-induced reductions in antibody formation, continuous exposure for 21 days to nitrogen dioxide did not affect the formation of serum-neutralizing antibody or hemagglutination-inhibiting antibody to influenza virus in nonhuman primates (179). Because of the importance of antibody function in preventing bacterial or viral respiratory infections, further studies of the effect of toxicants on antibody formation are warranted. It is worth emphasizing that appropriate immunological models and test procedures are available for assessing potential toxicity.

It has recently been shown that cellular immune reactions occur locally within the lung (180, 181). These reactions involve the mononuclear population, which in lavage fluid from the normal lung is composed of 78% macrophages and 17% lymphocytes (182). Forty seven percent of the lymphocytes are identifiable as T cells by E rosette formation and 22% as B cells by surface immunoglobulin staining. The B cells are further divided in that 14.5% possess IgM, 9.3% IgG, and only 5% have IgA on their surface. There is in addition a population of cells without markers known as "null cells." Changes in cell number or composition following exposure to toxicants is unknown. Preliminary evidence indicates that in smokers the total number of mononuclear cells is increased and the proportion that are macrophages reaches 95% (182). Cigarette smoke reduces the phytohemagglutinin response of lymphocytes isolated from the peripheral blood, lymph nodes, and spleens of mice (183). The effects of cigarette smoke on pulmonary lymphocytes have not as yet been reported. Since alterations in number and type of lymphocyte can have profound effects on the production of local antibody needed for inactivating invading bacteria or viruses (184), it is likely that these changes will alter resistance to infection.

CONCLUDING REMARKS

In recent years considerable information has accumulated indicating the pathophysiological interrelationship of exposure to noxious atmospheric compounds and enhanced susceptibility to respiratory infection. Recognition of this association has resulted in many studies linking the toxicity of airborne agents to impairments in one or more parameters of pulmonary microbial defense. The development of newer methods of testing microbial resistance allows more precise evaluation of airborne toxicants. Methods are now available for (noninvasive) testing of mucociliary transport, for simultaneously measuring the component parameters of mucociliary function and overall mucociliary transport, and for testing the influence of toxicants on in vivo rates of ingestion and inactivation of bacteria by alveolar macrophages. Additionally, techniques for studying pulmonary susceptibility to viral infection and for studying the effect of toxicants on humoral and cellular immune systems can be used to obtain toxicological information. Although few studies relating exposure to a toxicant on viral susceptibility, or on immune defense have been performed, it can be anticipated that future investigations will provide data regarding these potentially significant interrelationships. Because the above studies can be performed with any inhaled contaminant, the hazard of presently unstudied agents such as pesticides, metallic vapors, and hydrocarbon-containing industrial effluents is assessable by the methods indicated in the test.

One important area for future developmental research concerns the lack of animal models corresponding to human disease states. Present evidence indicates that individuals with underlying illness, especially chronic respiratory disease, are particularly vulnerable to infection following exposure to airborne toxicants. The development and use of animal models that mimic human diseases should significantly enhance our ability to assess the hazard from exposure to man-made airborne contaminants.

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