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Environmentally Induced Changes in Immunological Function: Acute and Chronic Effects of Inhalation of Tobacco Smoke and Other Atmospheric Contaminants in Man and Experimental Animals

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INTRODUCTION

Significant progress has been made in understanding the mechanism by which acute exposure to relatively high levels of atmospheric contaminants affect health, but the more subtle problems associated with chronic low level exposure have proven considerably more difficult to assess. Although it is generally accepted that prolonged exposure to cigarette smoke and urban-industrial air pollutants is associated with increased prevalence of a variety of acute and chronic respiratory diseases, the mechanisms involved have not been clearly defined.

In this review, attention is drawn to the long-term effects of atmospheric contaminants in general (and cigarette smoke in particular) on immunological control mechanisms that are accepted as playing a vital role in the maintenance of health. The review argues that a hostile environment within the respiratory tract created by inhalation of air contaminants compromises local immunological function in the short term, and ultimately depresses systemic immunological function. The changes observed in both man and experimental animals exposed for long periods to air contaminants in many respects parallel those associated with normal aging and may represent an acceleration of the process of senescence.

The precise mechanism(s) by which air contaminants affect immunological function remains speculative, but the relative resistance of specified-pathogen-free animals to these agents infers a central role for the hosts' normal bacterial flora in the process.

SMOKING, AIR POLLUTION AND IMMUNE FUNCTION-ANIMAL MODELS

In Vitro Effects on Leukocytes

Tobacco smoke (TS) or aqueous extracts thereof have been shown to exert a number of effects in vitro on macrophages from experimental animals. A variety of gases and particulates exert similar effects.

The most readily demonstrable phenomenon is decreased cell viability after exposure. At relatively low concentrations, TS, O_3, SO_2, and NO_2 rapidly kill alveolar and peritoneal macrophages in vitro (40, 43, 73, 114), whereas such agents as CO_2, CO, CH_4, CH_3Cl, acrolein, and acetaldehyde are ineffective at comparable concentrations (114). Lymphocytes may be even more susceptible than macrophages to the lethal effects of these agents (40). When sublethal exposure regimes are employed, a number of metabolic and functional changes occur in macrophages. Phagocytosis is depressed by NO_2 and TS (34, 107), O_3 and TS inhibit the activity

of a number of macrophage enzymes (52, 79), protein synthesis is depressed by TS (117), and oxidative metabolism is depressed by Cd (68).

Stimulatory effects have also been noted. While it depresses phagocytosis and intracellular killing, NO_2 increases the metabolic activity of macrophages (107); similar effects have been observed with TS (43, 57). Results from a number of independent workers suggest that the balance between stimulation and inhibition of macrophage activity is determined by dosage, with stimulation occurring at low exposure levels and inhibition occurring at higher concentrations (44, 56, 118); the most potent stimulation occurs after prolonged exposures to low levels of irritant (44).

Aqueous extracts of TS have also been shown to transiently inhibit the activity of plaque-forming cells (PFC) in vitro, but they exert no direct effect on immunoglobulin G (IgG) or IgM antibody (103).

In Vivo Effects on Leukocytes

Exposure of experimental animals to a variety of air contaminants produces marked effects on the number and activity of leukocytes in the lung. The immediate effects of exposure to TS (43, 45, 86, 88), O_3 (12, 20, 46), Pb (5), and probably NO_2 (31) are to decrease the viability of the pulmonary alveolar macrophage (PAM) population. Longer exposure periods usually result in an increased population of the PAMs in the lung (45, 86, 89), accompanied in some species by increased numbers of polymorphonuclear leukocytes (27, 89). An exception to this rule is Pb exposure (or exhaust fumes containing lead sesquioxide); long-term exposure to these agents depletes the PAM population in the lung (4, 5). Both short- and long-term exposure to irritants decreases pulmonary bactericidal capacity; this has been noted with many agents including TS (85, 87, 98), coal dust (85), NO_2 (1, 31), and O_3 (33, 50, 51). Effects on leukocyte populations distal to the respiratory system have also been noted in animals exposed for long periods to TS. Mice exposed for 6 months to TS from cigarettes which produced high levels of HbCO exhibited leucopenia, whereas leukocyte counts in those exposed to a milder variety remained within normal limits (42).

Resistance to Challenge In Vivo

Exposure of experimental animals to a variety of air contaminants elicits changes in host resistance that are clearly time dependent. Acute exposure of experimental animals to TS lowers resistance to bacterial infection (95); similar effects have been noted with NO_2 (21, 39) and O_3 (11, 12). Nonhuman primates exhibit similar susceptibility (21, 39). Short-term exposure to NO_2 has been shown to reduce viral-induced resistance in alveolar macrophages, probably by inhibiting interferon production (106).

The effects of long-term exposure are more complex. Mice exposed to NO_2 for moderate periods exhibited markedly increased resistance to aerosol challenge with influenza virus (58). Squirrel monkeys exposed to NO_2 for several months did not differ from controls in susceptibility to influenza virus infection (23). These latter results contrast sharply with those of other workers employing short-term exposure of monkeys to NO_2; resistance to aerosol challenge with *Klebsiella pneumoniae* was markedly diminished (21, 39). It is possible that different immunological mechanisms may be of central importance in resistance to influenza versus *Klebsiella* in nonhuman primates, and the differing susceptibilities of these immune mechanisms to NO_2 exposure may explain the divergent results obtained (e.g., see reference 1).

The results of studies on resistance to challenge with viable tumor cells in mice exposed for different periods to TS are of interest in this regard. It has been observed that mice exposed for 2 to 3 months to TS exhibit enhanced resistance to tumor cell challenge (10). However, as exposure time increases, resistance progressively decreases, eventually falling below control levels (10, 42, 101). The divergent results obtained above with squirrel monkeys exposed for short or long periods to NO_2 may be explicable in similar terms.

Continuing exposure to air contaminants therefore appears to elicit changes in host resistance that occur in three phases: acute depression (hours to days), followed by stimulation (weeks to months), eventually culminating in severe depression. The absolute time periods involved would be dose and species dependent. Evidence in support of this thesis is presented below.

Humoral and Cellular Immune Responses

The short- and long-term effects of exposure of air contaminants have been studied in detail in two murine models, and the results of both are in close accord.

SO_2 and carbon exposure. Mice were exposed for up to 192 days to SO_2 (at 2.0 ppm) and to carbon (at 1,211 μg/mg per m^3). PFC responses were measured in spleens and regional

lymph nodes of the respiratory system after aerosol and systemic challenge with antigens. Serum antibody and the responsiveness of lymph node and spleen cells to mitogens were also examined (67, 120–122). Acute exposure depresses the spleen PFC and serum antibody responses. The depression is greatest when the antigen is administered as an aerosol (rather than by systemic inoculation), indicating that the irritants exert their strongest effects close to the point of entry. As exposure time increased, enhanced responsiveness was observed first in the regional lymph nodes and later in the spleen. Prolonged exposure ultimately resulted in a severe depression in both local and systemic antibody responses. Systemic cellular immune reactivity (as measured by antigen-induced lymphocyte transformation) also exhibited transient stimulation following moderate periods of exposure; mitogen-induced transformation of B-lymphocytes from the regional lymph nodes of these mice also exhibited stimulation after moderate exposure, but systemic effects on B-lymphocyte responses (as indicated by assays employing spleen cells) were not found.

Fresh TS exposure. Humoral and cellular immune function have been examined in different strains of mice exposed daily to their bodyweight equivalent of fresh smoke from 20 to 30 cigarettes. These studies employed a machine designed to mimic the human smoking habit as closely as possible in laboratory animals, with particular care being taken to avoid changes in the chemical characteristics of the smoke before inhalation (19). Mice were sampled after varying exposure periods up to 42 weeks.

Short-term exposure to extremely high levels of smoke in a cruder smoking apparatus was shown to exert an immediate depressive effect on systemic antibody responses (22). However, long-term exposure to lower (more "physiological") levels of TS (employing the machine described above; 19) produced changes in humoral immune responsiveness analogous to those observed with SO_2 and carbon. In general terms, systemic spleen and regional lymph node responsiveness (particularly following intratracheal antigenic challenge) showed biphasic changes, with moderate exposure periods producing marked enhancement and prolonged exposure producing severe depression (42, 99, 102, 103, 105). Humoral responses following challenge with subunit influenza vaccine exhibited similar biphasic changes (64). It should also be noted the PFC responses of lymphocytes in the lung itself exhibited depression immediately following the commencement of exposure; enhanced responsiveness was never observed in this cell population (102).

Antigen-specific and gross cellular immune reactivity in TS-exposed animals were also monitored during exposure. The phytohemagglutinin (PHA) reactivity of lymphocytes from the regional lymph nodes, the spleen, and the peripheral blood again were increased by moderate exposure and severely depressed by long-term exposure (100). Identical results were also obtained when tumor-antigen-specific T-lymphocyte reactivity was assessed in exposed animals following inoculation with viable tumor cells (10, 41). The time-course of these changes could also be altered by varying the TS dosage. At a time when mice that had been exposed to TS from standard high tar-high nicotine cigarettes exhibited depressed humoral responses to a T-lymphocyte-dependent antigen, syngenic animals exposed to a milder type (low tar-low nicotine) were still in the stimulatory phase (42). A notable observation has been the constancy of B-lymphocyte function in the face of continued TS exposure. At 41 weeks of exposure, antibody responses to a T-lymphocyte-dependent antigen approximated 25% of control levels; in contrast, responsiveness to a B-cell antigen (polyvinyl-pyrollidone) remained normal (105).

The effect of long-term exposure to air contaminants on immune function in nonhuman primates has been reported in one study only. In these experiments, squirrel monkeys were exposed for 413 days to 1.0 μl of NO_2 per liter. The animals were repeatedly challenged with aerosols of monkey-adapted influenza virus throughout the exposure period, and serum neutralizing antibody formation was monitored. Exposed monkeys exhibited significantly enhanced antibody production, even after 16 months of continuous exposure (23).

Synergism

The possibility that simultaneous exposure of experimental animals to low levels of two or more air contaminants may result in synergistic effects in relation to damage of immune processes has been tested in a number of laboratories.

The simultaneous exposure of guinea pigs to NO_2 and carbon results in a considerably greater infiltrate of PAMs and greater damage to lung tissue than exposure to either agent alone (6). Guinea pigs exposed to MNO_2 particles and SO_2 exhibited a greater depression in lung clearance rates (particles and live bacteria) than was observed in animals exposed to either of the two agents singly (92); comparable

results were obtained when the inflammatory response in the lungs of these animals was examined (91).

Depressed thymus-dependent antibody production following respiratory tract challenge has been described in mice exposed to mixtures of air contaminants. Synergistic effects have been observed employing SO_2 and carbon (120) and TS in combination with mixtures of gases (74).

Conclusions and Predictions for Man

The exposure of experimental animals to a wide variety of chemically unrelated agents elicits a distinct pattern of change in immunological function. The dosages required to elicit these effects are low (especially when exposure time is high), and it would appear that altered immune function may be one of the most sensitive biological indexes of the toxicity of atmospheric contaminants. It is also obvious that many air-borne agents have the potential for both immunosuppression and immunostimulation, depending on the dose regime employed.

Based on the data above, the following may be proposed as possible sequelae of human exposure to polluted atmospheres: (i) an expanded population of PAMs, exhibiting heightened metabolic activity but deficient in a number of functional aspects, particularly those associated with bacterial clearance; (ii) depressed immune reactivity at sites associated with the respiratory tract and, in particular, within the lung itself; (iii) enhanced activity of immune functions dependent upon T-lymphocytes at sites distal to the respiratory system, except in cases of long-term exposure—the scanty data available from exposure of nonhuman primates suggest that the period of enhanced activity (during continuous exposure) in man may stretch over several years; (iv) long-term exposure to atmospheres containing mixtures of contaminants, even when the concentration of all the individual components are within acceptable "safe" limits, may severely impair immune function.

SMOKING AND IMMUNE FUNCTION IN MAN

In Vitro Effects on Leukocytes

In comparison to the exhaustive studies carried out with leukocytes from experimental animals, very little work has been done with human cells. Two groups have examined the acute effects of TS (or extracts) on lymphocyte transformation in vitro. At levels comparable to those encountered in the circulation of human smokers, nicotine produces a slight (but significant) depression of PHA-stimulated deoxyribonucleic acid synthesis in human peripheral blood lymphocytes (70). Identical results were obtained with TS (particularly the gas phase); however in the absence of PHA, lymphocytes-deoxyribonucleic acid synthesis was enhanced (18).

In Vivo Effects on Leukocytes

The structure, function, and metabolic activity of PAMs collected from smokers and nonsmokers by endobronchial lavage have been investigated by a number of groups.

Lavage fluids from smokers consistently yield more PAMs than those from nonsmokers (36, 80, 81). The PAMs of smokers exhibit elevated metabolic rates (36), increased adherence (60), increased activity of lysozomal hydrolases (61), markedly elevated rates of random migration (111, 113), and numerous ultrastructural anomolies (36, 60, 80, 81). PAMs from smokers do not manifest depressed capacity to phagocytose heat-killed or viable bacteria (13, 36); however, rates of intracellular killing have not been examined. It is significant in this regard that murine macrophages exposed to appropriate dosages of TS in vitro exhibit essentially normal phagocytic rates, concomitant with depressed rates of intracellular killing (W. R. Thomas, P. G. Holt, and D. Keast, unpublished data).

Systemic effects on leukocyte populations in smokers have also been reported. Peripheral blood leukocytes from smokers exhibit impaired chemotactic responsiveness (72). Smokers also invariably exhibit leukocytosis (14, 30, 49, 94), characterized by increased numbers of all the major classes of peripheral leukocytes (14). Some of these surveys have been carried out on samples of several thousand subjects and are therefore of considerable significance.

Serology

Smokers exhibit a number of nonspecific serological abnormalities compared to nonsmokers. For example, sera of smokers contain significant levels of C-reactive protein (CRP), an abnormal serum protein associated with inflammatory disease processes (37). They also contain elevated levels of SF, an abnormal serofloccculant for the ethyl ester of choladienic acid associated with a variety of diseased states (37). Titers of natural antibodies are depressed in smokers' sera relative to nonsmokers' sera (26), together with depressed levels of circulating IgG (at least in heavy smokers; see reference 108). Titers of *Haemophilus influenzae* agglutinins are elevated in smokers (63).

Immunological Function

The available data on immune function in human smokers in many respects parallel those from the animal models. Humoral immune function has been investigated in two large studies involving influenza vaccination (n = 289) and sensitization against HL-A antigens (n = 2,499). In the former trial, the smokers in the population studied (young males) exhibited increased susceptibility to illness during influenza outbreaks (25). Prior to immunization with influenza vaccine, the smokers exhibited significantly lower titers of hemagglutination inhibition (HI) antibody than did the nonsmokers (24). Immediately following vaccination of both groups, the smoker's HI antibody "deficit" was abolished, which led the authors to suggest a slightly hyperactive antibody response in the smokers. However, HI titers in the smokers had fallen behind their nonsmoking counterparts within a few weeks, and by one year postvaccination, the smokers exhibited markedly depressed levels of circulating HI antibody (24). Similar results were recently obtained in these laboratories (65) in a study of 1,000 subjects.

In a larger trial, sera from 2,499 pregnant women were tested for the presence of lymphocytotoxic antibodies against a 48-donor panel. The smokers exhibited a significantly lower incidence of lymphocytotoxins than did the nonsmokers, and the divergence between these groups increased with the number of deliveries (75). Urinary tract infection and febrile and nonfebrile virus infections during pregnancy were observed significantly more often in the smokers in this trial (75).

Cellular immune function in smokers has also been investigated by a number of workers. Warr and colleagues have examined the in vitro responsiveness of PAMs lavaged from the lungs of smokers and nonsmokers. PAMs from the smokers failed to respond to migration inhibitory factor (MIF), whereas those from nonsmokers responded similarly to normal peripheral blood leukocytes (111, 112). Conversely, the PAMs of smokers exhibited greater chemotactic responsiveness than did those of controls (113).

Suggestions arising from experiments with smoking mice (47), together with the description of depressed cellular immune reactivity in lymphocytes from marihuana smokers (69), prompted a number of groups to examine T-lymphocyte function in peripheral blood samples from cigarette smokers. In two small studies, lymphocytes from smokers and nonsmokers were tested in vitro for PHA responsiveness, reactivity in the mixed lymphocyte culture, and frequency of E-rosette formation; no differences were found between the groups (97, 116). However, these conclusions were not borne out by the results from studies involving much larger sample sizes where the smoking populations were adequately stratified by age and cigarette consumption. Silverman and colleagues recently surveyed a population of approximately 300 smokers and nonsmokers. PHA reactivity was significantly elevated in smokers less than 40 years of age, or in those with less than a 20 pack-year history of cigarette consumption. T-cell levels in the peripheral blood of these smokers were concomitantly elevated (94). PHA reactivity in older smokers was within normal limits, whereas T-cell counts remained elevated. However, only 19 subjects were tested in this older age bracket, and the authors acknowledged that the data from this subgroup may have been influenced by sampling errors (94). Vos-Brat and Rumke examined PHA reactivity in a much larger sample of older heavy smokers (mean age of 50 years, history of heavy smoking in excess of 20 years, n = 75); PHA responses were significantly lower than those of age-matched, nonsmoking controls (108).

SPECULATIONS ON MECHANISMS

The sequelae of long-term exposure to cigarette smoke in man and experimental animals exhibit a number of significant parallels. In all species examined, changes have been observed in both cellular and humoral immune function in exposed individuals. The degree and direction of these changes appear dependent upon a number of factors, notably duration of exposure and the site of the lymphoid compartment sampled. Consequently, the results of individual studies of the effects of exposure to cigarette smoke on immunological function often appear conflicting.

Examination of the breadth of data obtained from animal models clarifies the situation to a large degree. It would appear that continued exposure produces a biphasic pattern of change in T-lymphocyte-dependent immunological function, with moderate exposure enhancing and prolonged exposure ultimately depressing reactivity. How quickly these changes occur is related to how close the affected tissue is to the point of entry of the irritant: the most rapidly observable changes occur in cell populations within the lung, followed by lymphocytes within the lymph nodes draining the respiratory system, and finally in the spleen. The apparent anomaly of depressed immunological reactivity within the lung lumen concomitant

with enhanced systemic immune function is therefore explicable on a dose-time basis. As described above, high levels of contaminants exert an immediate toxic (and hence suppressive) effect on lymphoid cells, whereas low levels, particularly during prolonged exposure, are stimulatory. Consequently, within the lumen of the lung, immunological function declines rapidly under the influence of direct exposure to inhaled irritants, whereas the regional lymph nodes and later the spleen exhibit enhanced reactivity in response to lower levels of toxicity (see reference 102). The down-turn in immunological reactivity within the regional lymph nodes may be due to the steady accumulation of inhaled contaminants. However, the ultimate immune depression observed distal to the respiratory tract (the spleen) is less likely to be due to direct toxicity but instead may be a consequence of the prolonged hyperreactivity of the thymus-dependent elements of the immune system, both within the thymus itself and at other lymphoid sites seeded by the thymus.

The data obtained from human smokers, while scanty in comparison to those from animal studies, are nevertheless consistent with the latter. The remarkable similarities between the long-term effects on experimental animals of cigarette smoke and a variety of other agents which may be loosely termed "urban air pollutants" further suggest that the latter may also affect immunological function in man. It is of interest in this regard that a recent study suggested a stimulatory effect of polluted air on immunological mechanisms in children (109); it may be extrapolated that continued exposure to such a polluted environment would ultimately produce depressed immunological reactivity in this group in adulthood. Such a situation would supply a plausible mechanism for the well-known gradation in prevalence rates of respiratory disease(s) between rural (nonpolluted) and urban populations.

Many important questions in relation to man remain unanswered. The data from experimental animals indicate that systemic immunostimulation can exist for long periods side-by-side with respiratory tract immunosuppression. Is this the case in man? The latter would presumably be an important contributory factor to the enhanced prevalance rates of acute and chronic respiratory diseases associated in particular with smoking. Are these effects reversible in man as they are in the mouse (104)? Again this could in part explain the time-dependent return to normality with respect to susceptibility to respiratory infections (and lung cancer?) observed in ex-smokers (84).

The most vexing question is one of mechanism. In the smoker, it is not altogether surprising that aerobically respiring PAMs may be deleteriously affected by an environment contaminated with high levels of such agents as carbon monoxide and, consequently, immunological responses to thymus-dependent antigens within the respiratory tract (for which macrophage cooperation is now accepted as obligatory [82]) may be expected to be depressed. However, there is no evidence of marked alterations in systemic phagocytic function in human smokers (72) or in experimental animals (Thomas, Holt, and Keast, unpublished data), and it is thus more likely that the major defect in T-lymphocyte-dependent immunological function resulting from chronic exposure to a polluted environment resides within the T-cell itself.

Any explanation of this phenomenon must account for two major facts. First, immunostimulation in the face of continued exposure may occur for prolonged periods before immunosuppression is observed; second, specified-pathogen-free (SPF) animals differ markedly from conventional animals in their response to air contaminants—their PAM population does not expand in response to continuing exposure in a fashion akin to that of conventional animals (89, 90, 91)—and more significantly, they do not manifest enhanced immunological reactivity following moderate exposure periods as do syngenic conventional animals (8, 58). Furthermore, Laurenzi and colleagues (55) observed that acute exposure of conventional mice to TS depressed lung bactericidal activity, while that of identically treated SPF mice remained normal. When the SPF mice were exposed to enteric bacteria or were inoculated intraperitoneally with bacterial endotoxin, subsequent exposure to TS depressed lung bactericidal capacity in a similar fashion to that observed in the conventional animals. Davis and colleagues (16, 17) have also remarked upon the relative resistance of their SPF rats (exposed to whole cigarette smoke or its vapor phase) in terms of the development of chronic respiratory disease.

These latter observations suggest that normal bacterial flora, or some product thereof, may affect a host's susceptibility to atmospheric contaminants. In terms of the respiratory tract, it is not unlikely that the simultaneous presence of latent infection would exacerbate any pressure on the resources of the local immune system induced by the inhalation of toxic air contaminants, but any resultant effect on systemic immunological reactivity, particularly in the long term, would seem unlikely. However, it has been suggested earlier that endotoxin(s)

liberated by gram-negative commensals from the intestinal tract may, under conditions of stress, deleteriously affect the capacity of a host to carry out immunological functions dependent upon T-lymphocytes (53). Endotoxin has been recognized for many years as a powerful modulator of immune responsiveness; a number of workers have reported T-cell destruction, T-cell stimulation, and B-cell stimulation at different dose levels. Endotoxin thus has the potential for both marked stimulation and inhibition of immune function (3, 7, 28, 29, 53, 54, 66, 71, 76). The long-term effects of exogenous endotoxin on immunological function in mature animals have not been studied in detail; however, if endotoxin administration is initiated in early postnatal life, severe runting and immunosuppression results (53). Experiments currently in progress in these laboratories indicated that dose levels as low as 2.0 μg per week in mature mice result, by the eighteenth week of exposure, in leukocytosis, elevated PHA responsiveness, and depressed antibody production to a T-dependent antigen (Holt and Keast, unpublished data); this pattern parallels that observed in mice after 15 to 20 weeks of continuous exposure to TS (see above).

There is presently no direct evidence to suggest that prolonged exposure to a polluted atmosphere stimulates the passage of endotoxin from the gut into the circulation, nor is there any evidence that such leakage would escape the highly efficient liver clearance mechanisms. However, Fine's group (15) have reported (in the rabbit) that traumatized tissues liberate a vasoactive substance which produces an endotoxemia of intestinal origin in the original host, or in recipients following passive transfer of serum. The possibility that lung tissue traumatized by prolonged exposure to inhaled air contaminants releases a similar factor is testable.

It is also possible that the host's commensal flora may contribute indirectly to this process. Due to the absence of a gram-negative endotoxin-producing intestinal flora, the immune system in SPFs may operate at a lower level of activity than in conventional animals and, as a consequence, may be able to tolerate a higher degree of stimulation before reaching a state of immunological hyperreactivity. Under identical conditions of exposure, immunological function in SPFs would therefore be expected to remain within normal limits for longer periods than that in syngenic conventional animals.

AGING

The changes observed in immunological mechanisms in man and experimental animals exposed for long periods to polluted atmospheres resemble in many respects those associated with aging, and may therefore represent an acceleration of what is essentially a normal process. A number of observations on human smokers support this. (i) The mean life expectancy of human smokers is significantly below that of nonsmokers; death rates from a multitude of diseases usually associated with old age are raised in smokers (35, 84). (ii) Morbidity, resulting particularly from chronic respiratory diseases normally associated with old age, is again more prevalent among smokers (84). (iii) A variety of autoantibodies (including antinuclear and rheumatoid factors), which are associated with aging (48, 83, 93), are more prevalent in smokers (62). (iv) Nonspecific serological abnormalities (the presence of C-reactive protein and abnormal seroflocculants normally associated with aging) are again more prevalent in smokers (37). (v) Heavy smokers exhibit significant circulating levels of carcino-embryonic antigen (96); comparable levels are not observed in sera from individuals free of neoplastic disease, with the exception of a small proportion of the aged (119). (vi) Titers of agglutinins to exotic antigens, which decline in normal individuals after childhood (83, 110), do so more rapidly in smokers (26). (vii) In vitro PHA responsiveness of circulating T-lymphocytes in smokers over the age of 50 is depressed (108); a decline in PHA responsiveness accompanies aging in normal individuals (77, 115).

These data are also supported by studies in experimental animals. (i) Mice chronically exposed to cigarette smoke exhibit markedly depressed primary immune responses, whereas secondary responses exhibit much smaller changes (99, 105); a similar situation occurs with age (9, 110). (ii) Immune responsiveness to T-lymphocyte-dependent antigens is depressed in long-term smoking mice (99, 102), whereas responsiveness to T-independent antigens remains normal (105); normal aging in mice is associated with a progressive decline in responsiveness to thymic-dependent antigens (59) and a much lesser and later occurring decrease in responsiveness to thymic-independent antigens (32).

The impetus for accelerated aging of the thymus-dependent elements of the immune system in this situation may well be the chronic overstimulation discussed above. As was noted earlier, continuous exposure to a polluted atmosphere is associated with a prolonged period of T-lymphocyte-dependent hyperreactivity. It is conceivable that such prolonged stimulation may hasten the progressive involution of the thymus (as described in reference 38) by strain-

ing the resources of thymus progenitor-cell populations via a process akin to the Hayflick phenomenon.

The precise identification of the stimulus resulting from prolonged exposure to atmospheric contaminants and the elucidation of its mechanism(s) of action must await further investigation. However, the observation by Pollard and colleagues (78) of markedly increased life-spans in germ-free (and hence endotoxin-free) rodents argues strongly for a contribution from normal bacterial flora to the process.

CONCLUSIONS

Evidence from a variety of human studies suggests that increased prevalence rates of infections (and perhaps neoplastic diseases) resulting from chronic exposure of air contaminants are associated with impaired immunological control mechanisms.

Whether such a decline in immunological homeostasis is due directly to toxicity, or indirectly to accelerated aging of susceptible elements of the immune system, is speculative.

These data are supported by a variety of information from animal studies; the latter also suggest that the host's normal bacterial flora may influence susceptibility to environmental stress of this nature.

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