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Review article

Immune and inflammatory function in cigarette smokers

Tobacco smoking is associated with increased prevalence of various diseases, both in the respiratory tract and in distal organs. The possibility that tobacco smoke induced changes in immune and inflammatory processes may play a part in the aetiology and pathogenesis of many of these diseases was first recognised in the mid 1960s. The ensuing decade witnessed a steadily increasing research effort centred on toxicological aspects of chronic inhalation of tobacco smoke in relation to immune and inflammatory cell function in experimental animals. This work (reviewed by Holt and Keast[1]) defined a discrete pattern of change in animals during the course of tobacco smoke exposure, with the following salient features: (1) systemically, biphasic fluctuations were observed in primary T cell dependent humoral and cellular immune responses, initial enhancement during the first few months of exposure eventually giving way to suppression in chronically exposed animals; (2) T cell independent immune responses (for example, to bacterial polysaccharide antigens) and secondary immune responses dependent on T memory cells remained normal in these animals; (3) an immediate and profound suppression of T cell function was noted in the respiratory tract, together with (4) the steady development of an expanded and functionally altered population of alveolar macrophages.

Recognition of the possibility that similar effects occur in man has provided the impetus for various direct studies on aspects of immune and inflammatory cell functions in human smokers. Furthermore, it has become accepted practice to segregate the smokers within "control" populations, particularly in studies using bronchoalveolar lavage. The available data from these human studies are reviewed below in the light of the information available from earlier animal experiments.

Studies on peripheral blood

CELL POPULATIONS

The phenomenon of *smokers' leucocytosis*, characterised by increases of the order of 30% in the major classes of leucocytes in the peripheral blood, was first recognised in large scale surveys.[2 3] Subsequent studies have confirmed these observations, and have provided a range of information on the function of individual cell types.

Polymorphonuclear leucocyte populations in the circulation of smokers are increased by up to 44%,[4] but appear normal with respect to chemotactic, microbicidal and secretory activities.[5 6] *Monocytes* display similar increases[4 7] and appear normal in conventional chemotaxis and phagocytosis assays, but may be partially defective in their capacity to kill intracellular *Candida*.[7]

The number of circulating *basophils* has been reported by one laboratory to drop significantly as an acute response to tobacco smoking,[8] and counts in smokers after a 12 hour abstinence are claimed to be higher than in non-smokers.[9] These same authors reported that smoking directly triggers basophil degranulation.[10]

Natural killer cell activity has been examined in two recent studies,[11 12] both of which reported depressed levels of activity in smokers.

In studies of the cell types present in the peripheral blood of smokers *T lymphocytes* have attracted most attention. There is general agreement in recent published reports that the total number of T lymphocytes is increased in smokers.[13-16] Analysis of T lymphocyte subsets in smokers by flow cytometry indicates increases in the OKT3$^+$ (total T cells) and OKT4$^+$ (T helper) populations in light and moderate smokers,[12 17] followed by a relative decrease in OKT4$^+$ (and thus an increase in the OKT8$^+$ T suppressor cytotoxic subset) in heavy smokers.[17 18] In two studies on women smoking (at rates unspecified) was associated with increases of up to 30% in circulating OKT4$^+$ T cells, and smaller increases in the OKT3$^+$ and OKT11$^+$ subsets.[15 16] The difference in the OKT4$^+$ subset between smoking and non-smoking women was also noted during pregnancy, in particular during the third trimester.[16]

The functional capacity of smokers' circulating T cells also appears altered, although the nature of the changes is controversial. While some studies have failed to detect differences between smokers' and non-smokers' T cells in in vitro assays of polyclonal T

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cell activation,[12 19 20] others report depressed responses,[21] associated with either the sex (lower in men[22]) or the age of the smokers.[23] It has also been reported that T cells from smokers under the age of 40 show enhanced in vitro responses,[24] analogous to those observed in animals,[1] which indicates that cross sectional studies on T cells from smokers require careful stratification of the study population for detection of the full spectrum of smoking induced functional changes. It is also obvious that a wider range of T cell functions require assessment, as in vitro responses to specific antigen has been examined in only one study,[22] as has T suppressor activity (which was depressed[12]). T helper function per se has not been studied.

SERUM COMPONENTS

In vitro experiments indicate that tobacco smoke extracts are capable of directly activating the complement pathway,[25 26] and raised levels of C5, C9, and C1 inhibitor have been reported in the serum of smokers.[27] Other acute phase reactants reported to be increased are C-reactive protein and the serofiocculant compound SF.[28] Autoantibodies, notably antinuclear and rheumatoid factors, are also found to be increased more frequently in smokers than in non-smokers.[29]

Levels of non-specific agglutinins are lower in the serum of smokers than of non-smokers.[30] There is also general agreement that concentrations of the immunoglobulins IgG, IgM, and IgA are reduced by 10–20% in the serum of smokers.[11 23 31-39]

In contrast to these observations, serum concentrations of IgE are higher in smokers than in non-smokers.[35 40-45] Close analysis of these data as a function of amount of smoking indicates that changes in IgE production induced by tobacco smoke are biphasic, light smoking (1–9 cigarettes/day) being associated with a striking rise in IgE levels (mean 189·8 IU/ml) whereas in heavy smokers ($\geqslant$20/day) serum IgE levels were below those of non-smokers.[46 47] Similar biphasic changes, with up to fourfold rises in light smokers, have also be observed in serum IgD concentrations.[39 48]

Lung lining fluids

NON-CELLULAR COMPONENTS

Concentrations of IgG and IgA in saliva from smokers have been reported as low,[49-51] suggesting smoking induced impairment of local antibody production in the upper respiratory tract.

IgA concentrations in smokers' bronchoalveolar lavage fluids have generally been reported as normal,[52-54] or (in one study) increased.[55] IgA—secretory component was assessed to be decreased in smokers' bronchoalveolar lavage fluid, in the single reported study where this compound was measured.[56] IgG concentrations appear normal[53 57] or raised.[52 54 55 58 59] The disparity between IgG:IgA ratios in bronchoalveolar lavage fluids in these reports and those in saliva may reflect the differential effects of tobacco smoking on immunoglobulin production at different levels of the respiratory tract.

Lung lining fluids from smokers are reportedly deficient in functional antiprotease activity.[60-62] This may be partially compensated for by an increase in the absolute levels of α_1 proteinase inhibitor in their serum.[63 64] The recent description of increased macrophage and neutrophil derived elastase activity in bronchoalveolar lavage fluids collected 30 minutes after smoking[65] casts doubt on the capacity of this serum inhibitor pool to control adequately proteases released into the extravascular space of the lungs in response to tobacco smoke inhalation. This question, however, remains controversial, as other authors[66] have claimed that the reported deficiency in protease inhibitors in smokers' bronchoalveolar lavage fluid may be transient at best, and rapidly reversed (after cessation of smoking) by in vivo reducing systems.

Other components that have been reported to be present in increased amounts in smoker's bronchoalveolar lavage fluid include lysozyme,[67] complement components,[54 68] and fibronectin,[69] collectively indicative of ongoing inflammation. In addition, histamine concentrations in sputum are raised in some smokers,[36] which may be a reflection of increased numbers of intraepithelial mast cells in the airway epithelium.[70]

BRONCHOALVEOLAR LAVAGE CELLS

Lavage cell populations from smokers display characteristic changes, which have been reviewed in detail by Hunninghake *et al*,[71] whose major conclusions remain valid. Smokers' bronchoalveolar lavage fluid contains a greatly expanded population of alveolar macrophages, together with increased numbers of neutrophils.[52 72 73] Lymphocyte populations remain numerically constant, although certain aspects of T cell function, notably mitogen induced proliferation, may be reduced.[74 75] The alveolar macrophages population in smokers displays up to a threefold increase in metabolic activity,[72] and comparable increases in various enzymes, particularly in lysosomes.[67] Morphologically, smokers' alveolar macrophages show increased numbers of endoplasmic reticulum, ribosomes, large lysosomes, phagolysosomes, and cytoplasmic inclusions.[72 76-79] Collectively, these features suggest that smokers' alveolar macrophages are "activated" in vivo.

A comprehensive picture of the effector function or functions of smokers' alveolar macrophages has been steadily built up over the last few years. The responsiveness of these cells to chemotactic stimulants is increased,[80–82] which is consistent with their apparently activated state. In contrast, the phagocytic and microbicidal activity of this population may be deficient, although the capacity to detect differences between smokers and non-smokers may depend on the choice of targets and the nature of the assay systems. Thus, while uptake of fungi appears normal,[72 81 83] phagocytosis of opsonised bacteria or inert particles has been variously reported as normal[72 84] and, in more recent studies, as decreased.[85–87]

Complement-receptor mediated phagocytosis appears to be depressed in smokers' alveolar macrophages,[88–90] which is consistent with earlier observations indicating decreased C3b receptors on these cells.[80 85 91]

With respect to Fc receptors, conventional rosette assay systems applied to unfractionated populations of smokers' alveolar macrophages have failed to detect differences between these and cells from non smokers,[80 85 91] which further adds to the confusion surrounding possible smoking induced effects on Fc receptor mediated phagocytosis. More recent studies by Plowman,[92] however, have shown that the subpopulation of smokers' alveolar macrophages that is heavily laden with characteristic particulate intracytoplasmic inclusions displays reduced Fc receptor expression. Additionally, more sophisticated binding studies, using radiolabelled immune complexes,[93] indicate that the Fc receptors of smokers' alveolar macrophages have a lowered affinity for IgG.

Alveolar macrophage effector functions mediated by products of the cyclo-oxygenase pathway appear to be significantly impaired by tobacco smoke, as the production of prostaglandin (PG) E_2, $PGF_{2\alpha}$, leukotriene (LT) B_4 and 5-HETE are considerably reduced (by up to 90%) in smokers' alveolar macrophages, particularly in the stimulated state.[94–96]

In strong contrast, potentially tissue damaging effector functions of alveolar macrophages in which reactive oxygen metabolites (notably hydrogen peroxide and superoxide anion) are released appear much greater in smokers than in non-smokers.[97–102] Of particular note here is the observation of a twofold increase in the IgE-FcR mediated secretory response (measured by release of superoxide anion and the lysosomal enzyme β glucuronidase) in smokers' alveolar macrophages compared with those of non-smokers.[99]

The accessory cell functions of alveolar macrophages in smokers associated with antigen presentation and activation of T lymphocytes have been reported to be defective, and this is associated with reduced IgA expression.[103 104] A later study, however, failed to reproduce these defects in smokers' alveolar macrophages.[75 102] Given the complexities inherent in the role of alveolar macrophages in this context,[105] detailed studies on the presentation of defined antigens and the production of interleukin 1 by defined subpopulations of smokers' alveolar macrophages will be required to resolve this important question. Regardless of the functional capacity of smokers' alveolar macrophages of a cell by cell basis, however, the milieu created by the presence of greatly increased numbers of alveolar macrophages in the alveolar spaces of smokers is very likely to have an extremely inhibitory effect on local T cell activation. This conclusion follows from the findings of several laboratories (reviewed by Holt[105]) indicating that high concentrations of alveolar macrophages directly suppress T cell proliferation.

Immune responses of smokers to antigenic challenge

Natural exposure to fetal histocompatibility antigens during pregnancy commonly leads to sensitisation against these antigens on the part of the maternal immune system, and the occurrence of the resulting lymphocytotoxic antibodies in serum is positively correlated with the number of deliveries. In a survey of lymphocytotoxic antibodies in 2499 serum samples collected at delivery, Nymand[106] noted that 10·4% of non-smokers developed antibodies during the first pregnancy, rising to 31% after the fourth. The corresponding figures in smokers were 7·8% rising to 17·4%, which indicates significantly reduced immune recognition of foreign histocompatibility antigens among the smoking population.

Various evidence suggests reduced immune responses to inhaled antigens in smokers. Morgan and colleagues[107 108] were the first to report a significantly higher prevalence of serum precipitins to *Micropolyspora faeni* in non-smoking than smoking farmers. These findings have been substantiated in a large survey of farmer's lung disease and serum antibodies to antigens associated with farmer's lung among 1444 Canadian farm workers by Gruchow *et al*,[109] who have reported that the prevalence of antibodies to *M faeni* was eight times higher among non-smokers than among smokers, with similar results for *Aspergillus fumigatus*. Similar results have also been obtained with Quebec dairy farmers.[110]

In a series of studies on serum antibodies to pigeon serum antigens in smoking and non-smoking pigeon breeders, the presence of precipitating antibodies was detected respectively in 18·6% versus 50·5%,[111] 4·3% versus 55·4%,[39] and 5% versus 46%.[112] These results are consistent with those of an earlier study, which detected lower mean levels of precipitins to pigeon 7S globulin among the smoker.[113] Similar differences have also been observed between smoking and non-smoking poultry workers with respect to IgG responses to hen serum antigens,[114] and in processing workers in relation to IgG responses to prawn antigens.[115]

Both IgG and IgA antibodies in nasal secretions and serum against cotton dust bacterial flora were up to 10 times lower in smoking than in non-smoking cotton mill workers.[116] Similarly, precipitating antibodies to antigens of microorganisms growing in humidified water were examined in serum from workers in a cigar plant and found to be present in 93·3% of non-smokers, compared with 31·7% of smokers[37]; antibodies to microbial antigens normally encountered by other routes (notably *Eschesichia coli* and *Candida*) were detected with equal frequency in the two groups in this study, suggesting that the humoral immune defect in the smokers may be more pronounced in the respiratory tract.[37]

In contrast to results obtained with IgG and IgA, increased allergic sensitisation accompanied by increased IgE antibody production and the manifestation of asthma symptoms has been reported to occur up to five times more frequently in smokers than in non-smokers in several specific industrial settings. The occupational allergens include coffee bean dust,[41 117] ispaghula powder in the pharmaceutical industry,[41] phthalic anhydride in factories using epoxy plastics,[118] prawn antigen in processing plants,[115] and possibly bacterial enzymes in detergent factories.[119 120]

It is noteworthy that smoking does not appear to promote sensitisation to common seasonal aeroallergens such as pollens, as IgE levels in smokers do not display the characteristic spring rises seen in most non-smokers.[40] The fact that increased IgE production in smokers is apparently restricted to occupational exposure suggests that prolonged inhalation of relatively high levels of antigen may be required to elicit sensitisation. It is also possible that concomitant exposure to other irritants in the workplace may be an important factor in this process. Stopping smoking has been reported to worsen the symptoms of asthma in some patients,[121] but in the absence of information on the nature of the triggering factors operating in this group it is difficult to speculate on the nature of the mechanism or mechanisms underlying this phenomenon.

The immune response that follows immunisation with influenza vaccine is also impaired in smokers. While postvaccination titres are similar in smokers and non-smokers during the three months immediately after challenge,[122] they are depressed in smokers after one year,[123 124] suggesting that postvaccination protection against infection may be more short lived in the smokers.

The degree of this apparent defect in smokers has been subjected to detailed analysis in a large clinical trial in which healthy volunteers were immunised in the face of an expected influenza epidemic.[124] The trial compared subunit vaccine administered parenterally with a live attenuated virus vaccine given intranasally in 799 subjects. Postvaccination HI antibody titres were initially similar in smokers and nonsmokers given the killed subunit vaccine, but the subsequent rate of decline in antibody titres (particularly after 30 weeks) was more rapid in the smokers,[124] in agreement with the earlier findings of Finklea *et al.*[123]

Close examination showed that this difference between the two populations could be accounted for entirely by the smokers who had little or no prevaccination antibody, as responses in those with prevaccination titres of 24 or more were indistinguishable from those of the non-smokers. In contrast, a significantly higher proportion of smokers showed seroconversion after receiving the live vaccine (indicating greater susceptibility to the subclinical infection), and in the subgroup with seroconversion postvaccination titres here were similar in smokers and non-smokers.[124] This suggests that the defect in the smokers' immune response was restricted to primary responses to non-replicating antigen, which is compatible with earlier findings in mice exposed to tobacco smoke.[1] Furthermore, it was noted that smokers in the placebo group with pre-epidemic HI titres of 6 or less were considerably more susceptible to epidemic influenza than were comparable nonsmokers, and that seroconversion (that is, to titres of 24 or more) after vaccination conferred almost complete protection on both groups.[124]

Reversibility of tobacco smoke effects

The cessation of tobacco smoke exposure in experimental animals is associated with the restitution of normal immune and inflammatory functions.[1] The changes observed in human smokers also appear reversible. These include the effects of tobacco smoke on serum concentrations of the immunoglobulins IgM, IgG, and IgA,[34 125] IgD,[48] and IgE,[40 43] changes in the number[12 18] and function of both circulating T lymphocytes[12] and natural killer cells[12 125]; and capacity to develop IgG antibodies after antigen inhalation.[39 107 110]

Cell populations present in lavage fluids from exsmokers are numerically and morphologically indistinguishable from those derived from non-smokers, but relevant functional studies have not been reported.

Conclusions

The data reviewed above indicate that there are clear parallels between the effects of chronic tobacco smoke exposure on immune and inflammatory functions in experimental animals[1] and those in man. In both cases, fluctuations (often biphasic on a dose-time basis) occur in systemic activity, which are paralleled by much larger changes in the same indices within the respiratory tract, presumably reflecting relative tobacco smoke exposure levels of the effector cell populations concerned.

The most potentially important of these in relation to disease processes are smoking induced changes in antibody production, particularly in response to foreign antigens that impinge on the respiratory mucosa, and alterations in the effector functions of smokers' alveolar macrophages. The latter involve considerably enhanced secretion by alveolar macrophages of cytotoxic effector molecules (such as superoxide anion) after stimulation, with reduced production of regulator molecules derived from the cyclooxygenase pathway, which normally fine tune the inflammatory response. Given the 5–10 fold numerical increase in smokers' alveolar macrophage populations, their cellular response to erstwhile innocuous inflammatory stimulants in the lower respiratory tract is likely to be exaggerated relative to that of non-smokers, and this constitutes a potential source of chronic tissue damage. This may be offset to a degree by the reduced immune receptor activity of smokers' alveolar macrophages, but the evidence suggests that the reduction affects only a proportion of a greatly expanded population of alveolar macrophages.

The exaggerated secretory response of smokers' alveolar macrophages is probably a direct result of a generalised biochemical "activation" of these cells in vivo by irritants present in tobacco smoke, an adaptive process common to all macrophages, which is associated with enhanced survival capacity in the presence of a wide range of potentially cytotoxic agents.[1]

With respect to overall levels of antibody production, the paradoxical selective pattern of change characteristic of smokers—that is, *decreased* IgG responses and *increased* IgE responses—does not appear explicable simply on the basis of the known tobacco smoke induced increase in the permeability of the respiratory epithelium.[126] These findings may be attributable, however, to tobacco smoke induced suppression of regulatory T lymphocyte function. Identical changes in immune function involving highly selective stimulation of IgE synthesis can readily be induced in experimental animals by administration of low concentrations of various cytotoxic agents, which act preferentially upon subsets of T lymphocytes that are rapidly turning over[127]; in man similar changes are also recognised clinically as relatively common sequelae of drug induced immunosuppression, or hereditary immunodeficiency states, characterised by depressed T cell function.[128–130]

The precise mechanism or mechanisms by which tobacco smoke affects the activity of particular T lymphocyte subsets remain to be elucidated. An intriguing possibility is suggested by recent reports, from my laboratory, on the nature of the T cell population revealed by enzymatic digestion of lung tissue.[131 132] These studies have shown that in the steady state the extravascular compartment of the lung (excluding the alveolar spaces) contains considerably more T cells than are present in the overall peripheral blood pool. The general absence of organised lymphoid structures with germinal centres in the lung parenchyma suggests that these T cells are temporary immigrants, as opposed to members of a lymphoid population derived from the lung. Whether their local extravasation is random or selective has yet to be determined; but the physical changes associated with T cell activation—that is, increased size and surface membrane adhesiveness associated with blast transformation—are likely to be sufficient in themselves to trap these cells temporarily during their passage through the lung vascular bed, and thus set the extravasation process in motion.[133] Preliminary data derived from T cell cloning experiments indicate that passage into the lung tissue also considerablly lowers the capacity of these T cells for future proliferation, which hints at a possible role for this process in general immune regulation. We may speculate that in chronically inflamed lung tissue, such as that of the smoker, this growth regulatory function may be exaggerated. The T cell subsets that would be most susceptible to an increased attrition rate during circulation through the lung would be those with the shortest initial half life—that is, those that rely most on steady proliferation to maintain their relative levels within the T cell population at large. Perhaps coincidentally, such properties have been ascribed to those T cells thought to control overall levels of IgE production.[127]

It is, however, in light smokers that these selective effects on IgE levels are principally seen.[47] They are not observed with the heavy smoking (over 20 cigarettes a day) that is associated with depressed overall levels of IgG production, and especially IgG antibody responses against inhaled antigens. In this particular case depressed immune reactivity may be the result of more generalised toxic effects on T helper cells ($OKT4^+$), which are reduced relative to $OKT8^+$ suppressor cells in heavy smokers (see above). Additionally, as these effects are clearly more evident with immune responses initiated in the lung itself, local smoking induced changes (for example, in antigen presenting cells) may also have a contributory role.

These changes in T cell function may indirectly "protect" against the development of some relatively rare immunologically mediated lung disease such as farmer's lung, pigeon breeder's disease and sarcoidosis,[134] all of which are less prevalent in smokers. As, however, normal T cell function in the lung plays an essential part in the defence against the development of both infectious disease and neoplasia, the benefits of cigarette smoke induced immunosuppression must be considered dubious.

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