Smoking and pulmonary sarcoidosis: effect of cigarette smoking on prevalence, clinical manifestations, alveolitis, and evolution of the disease

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ABSTRACT Patients with pulmonary sarcoidosis are less likely to smoke than persons of a similar age in the general population. This could be because smoking reduces the likelihood of developing sarcoidosis, or alternatively smoking could reduce the severity of the disease process so that smoking patients are underrepresented among patients with clinically overt disease. To evaluate these possibilities 64 patients with sarcoidosis of recent onset were studied at presentation and after a one year follow up period, clinical, functional, radiographic, and bronchoalveolar lavage fluid criteria being used to identify factors that might account for the lower incidence of sarcoidosis in smokers and to determine whether the disease is less severe in smoking patients. Smoking was less common in the patients with sarcoidosis (30%) than in the control subjects (46%). The study did not support the conclusion that sarcoidosis is less severe in smokers, as clinical, radiographic, and functional abnormalities were similar in smokers and non-smokers at initial evaluation and after a one year follow up period. Nevertheless, smoking did influence various indices used to assess disease “activity.” Cigarette smoking was associated with a significant increase in the serum angiotensin converting enzyme activity (SACE), and patients with very high SACE and pulmonary gallium-67 uptake were smokers. Furthermore, more CD8+ (but not CD4+) lymphocytes were recovered by lavage from smoking than from non-smoking patients, giving a lower CD4 :CD8 ratio in smokers. Fewer alveolar macrophages were recovered by lavage from smokers with sarcoidosis than from normal subjects with a similar smoking history. These findings support the possibility that smokers, particularly those with a prominent accumulation of alveolar macrophages in the lower respiratory tract, may be less likely to develop sarcoidosis.

Introduction

Pulmonary sarcoidosis is a granulomatous lung disease in which granuloma formation is preceded by the appearance of an “alveolitis” characterised by increased numbers of T lymphocytes and inflammatory cells. As this immune and inflammatory response develops in situ within the lung, we may reasonably assume that other factors influencing the number and state of activation of immune and inflammatory cells in the lung might alter the initiation or evolution of the alveolitis.

Cigarette smoking is known to produce various alterations in the number, type, and functional activity of lung immune and inflammatory cells. We and others have suggested that the incidence of sarcoidosis is lower among individuals who smoke than among non-smokers, at least in certain subpopulations of patients. Two explanations could account for this apparent difference: smoking might reduce the likelihood of developing sarcoidosis, or alternatively it could influence the evolution of the disease so that the signs and symptoms are less severe and therefore smoking patients are underrepresented in series of patients with clinically overt disease.

To evaluate these two possibilities we have studied a homogeneous group of patients with sarcoidosis of recent onset at presentation and after a one year follow up period using clinical, functional, radiographic, and bronchoalveolar lavage fluid indices, to identify...
**Smoking and pulmonary sarcoidosis**

Factors that might account for a lower prevalence of sarcoidosis among cigarette smokers and to determine whether the clinical course of sarcoidosis is less severe in smokers than in non-smokers.

**Methods**

**STUDY POPULATIONS**

**Patients with sarcoidosis**

All patients with pulmonary sarcoidosis evaluated at Hôpital Avicenne from February 1984 to August 1986 were considered for this study. The diagnosis of pulmonary sarcoidosis was established according to previously described criteria, which included: (1) a compatible clinical picture; (2) presence of non-caseating granulomas in biopsy samples of lung, bronchus, mediastinal nodes, or the site of a Kveim-Siltzbach reaction; (3) absence of mycobacterial or fungal infections; and (4) absence of exposure to agents known to produce granulomatous disease. For the purpose of this study the duration of disease was regarded as the interval between evaluation and the last normal chest radiograph or since the onset of erythema nodosum.

Among the 133 patients identified, 92 were non-smokers and 39 were current smokers; only two patients were former smokers and these individuals were excluded from the study. There was no significant difference in mean (SD) age between the two groups (non-smokers 36 (11), smokers 32 (12) years; p > 0.2), although men were more likely to smoke (non-smokers, 30 men/62 women; smokers, 24 men/15 women; p < 0.01 by \( \chi^2 \) analysis). Mean cigarette consumption by smoking patients was 10 (SD 8) cigarettes a day. Twenty two patients smoked under 10 cigarettes a day, 11 patients 11–20/d, and six patients over 20/d.

For evaluation of the influence of cigarette smoking on pulmonary function, indices of disease activity, and evolution, three groups of patients were excluded to restrict the study to a homogeneous population. Patients were excluded for the following reasons: (1) Patients who had received corticosteroid treatment before their initial evaluation by us (n = 27, 15 non-smokers); (2) patients with disease of over two years’ duration (n = 20, 14 non-smokers); (3) patients with disease of less than two months’ duration (n = 20); all of the latter patients presented with erythema nodosum and were excluded because patients with erythema nodosum evaluated less than two months after the onset of the disease have clinical and lavage fluid findings that are significantly different from those observed in patients (with or without erythema nodosum) evaluated after longer intervals from the onset of the disease. Furthermore, patients with erythema nodosum are predominantly female (17 women and three men in this series) and therefore less likely to smoke (18 non-smokers and two smokers). The inclusion of such patients would therefore create a bias as differences resulting only from the early evaluation of these patients would be represented disproportionately in the non-smoking group. The age, sex, and smoking habits of the remaining 64 patients were not significantly different from those of the total study population.

**CONTROL SUBJECTS**

For comparison of tobacco consumption between patients with sarcoidosis and the general population, we used results obtained by a survey conducted by the Société Brule Ville Associés and sponsored by the Comité Français d’Education pour la Santé. The survey sample was representative of the population of France in terms of age, sex, and socioeconomic status. For our study we used only those responses given by people aged 25–49 years.

For comparison of the results of bronchoalveolar lavage, 36 normal volunteers served as control subjects (25 men, 11 women). Fifteen were non-smokers (mean age 40 (SD 13) years) and 21 smoked cigarettes (age 35 (12) years). Mean cigarette consumption by smoking control subjects was 28 (18) cigarettes a day. Four subjects smoked less than 10/d, eight 11–20/d, and nine over 20/d.

**BRONCHOALVEOLAR LAVAGE**

All patients with sarcoidosis underwent bronchoalveolar lavage, as part of a diagnostic evaluation. Patients and normal volunteers gave informed consent before the procedure. Lavage was performed as previously described, five aliquots (50 ml each) of sterile saline being used. The total number of cell recovered/ml lavage fluid was determined by counting cells present in an aliquot of the resuspended original lavage fluid. Cytocentrifuge preparations were made from uncentrifuged lavage fluid and stained with May-Grunwald-Giemsa stain, and a differential cell count was performed by examining at least 1000 cells. Lavage fluid was centrifuged (600 g for 10 minutes) and supernatant fluid was stored at −20°C. Cells were resuspended at a concentration of 10⁷ cells/ml in RPMI-1640 medium containing 25 mM Hepes.

The surface phenotype of lymphocytes recovered by lavage was evaluated by indirect immunofluorescence microscopy as previously described, with monoclonal antibodies recognising CD4 (OKT4 plus OKT4A, Ortho Diagnostics, Raritan NJ) and CD8 (OKT8, Ortho) determinants.

Albumin and immunoglobulin G (IgG) in lavage fluid were measured by immunoprecipitation with a laser nephelometer (Behring Diagnostics, Paris, France) without prior concentration of the supernatant.
MEASUREMENT OF SERUM ANGIOTENSIN CONVERTING ENZYME ACTIVITY, IgG, AND ALBUMIN

Serum angiotensin converting enzyme (SACE) activity was assayed with hippuril histidine leucine as substrate; the normal range (40-7 ± 20-4 units) represents the mean concentration with 2 standard deviations in serum from an independent group of 50 normal volunteers. Serum IgG and albumin were measured by laser nephelometry, as described above for bronchoalveolar lavage fluid.

EVALUATION OF FUNCTIONAL INDICES, PULMONARY GALLIUM-67 UPTAKE, AND RADIOGRAPHIC ABNORMALITIES

Vital capacity and FEV1 were measured by spirometry (Godart water sealed spirometer) and functional residual capacity by multiple breath helium dilution. Carbon monoxide transfer factor (TLCO) was measured by the single breath method21 and scaled for age and height. The results were compared with previously published standards.18-19

The pulmonary gallium-67 index was determined by a modification of the method of Line et al.20-21 The upper limit of normal (23 units) represents the mean + 2 SD for tests performed on 11 normal volunteers. Gallium scans were performed on 31 out of 66 patients. The age, sex, and smoking history of patients evaluated by gallium scans were not significantly different from those of the total study population. Chest radiographs were typed according to the criteria of Siltzbach.22

STATISTICAL METHODS

All data are presented as means with standard deviations in parentheses. One way analysis of variance was used to obtain probability values for comparisons of measurements between groups. All significant differences were confirmed by the non-parametric Kruskall-Wallis method. Paired t tests were used for within group comparisons. The χ2 test was used for data on groups of patients.

Table 1  Pulmonary function tests in patients with sarcoidosis as a function of smoking history

<table>
<thead>
<tr>
<th>Study group</th>
<th>Pulmonary function (mean (SD))</th>
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<tbody>
<tr>
<td></td>
<td>TLC</td>
</tr>
<tr>
<td>NON-SMOKERS (n = 42)</td>
<td></td>
</tr>
<tr>
<td>% of predicted</td>
<td>88 (13)</td>
</tr>
<tr>
<td>No of patients with &gt; 20% decrease from predicted</td>
<td>12/42</td>
</tr>
<tr>
<td>SMOKERS (n = 16)</td>
<td></td>
</tr>
<tr>
<td>% of predicted</td>
<td>88 (17)</td>
</tr>
<tr>
<td>No of patients with &gt; 20% decrease from predicted</td>
<td>7/16</td>
</tr>
</tbody>
</table>

TLC—Total lung capacity; VC—vital capacity; RV—residual volume; FEV1—forced expiratory volume in one second; TLCO—carbon monoxide transfer factor.

Results

TOBACCO CONSUMPTION

The patients with sarcoidosis were less likely to smoke (30%) than people of a similar age in the normal population (46%) (p < 0.05). When men and women with sarcoidosis were considered separately, the percentage of smokers was less in both groups than that observed in the corresponding normal population, although the difference was significant at the p < 0.05 level only for women, (patients v controls: women 19% v 35%; p < 0.01; and men 44% v 57%; 0.1 > p > 0.05). The level of tobacco consumption by smoking patients with sarcoidosis was not significantly different from that of smokers in the general population (p > 0.2), although most patients and controls who were smokers smoked less than 20 cigarettes/day.

RELATION OF CIGARETTE SMOKING TO INDICES OF DISEASE ACTIVITY AND SEVERITY AT INITIAL EVALUATION

Chest radiograph

There was no difference in the proportion of non-smoking and smoking patients with hilar adenopathy alone (type 1: non-smokers 29/45, smokers 12/19; p > 0.2) or pulmonary infiltration with or without hilar lymphadenopathy (types 2 and 3: non-smokers 16/45, smokers 7/19; p > 0.2).

Pulmonary function

The patients with sarcoidosis as a group had a mild reduction in vital capacity, residual volume, and diffusing capacity (table 1). No significant differences were observed between non-smokers and smokers for any index, and the proportion of patients with a greater than 20% decrease compared to predicted values was similar for the two groups (table 1).

Cells recovered by bronchoalveolar lavage

Among the patients with sarcoidosis a significantly greater number of alveolar macrophages was...
recovered by lavage from smoking patients than from non-smoking patients. Among smokers the number of macrophages obtained from patients with sarcoidosis was less than that recovered from control subjects (table 2; p < 0.001 for control v sarcoidosis group).

The reduced number of alveolar macrophages recovered from sarcoid smokers compared with control smokers was not entirely explained by differences in tobacco consumption. When control subjects were subdivided according to cigarette consumption the number of macrophages recovered increased progressively as a function of cigarette consumption (fig 1). In contrast, only those patients with sarcoidosis who smoked over 20 cigarettes a day had more alveolar macrophages than non-smokers with sarcoidosis, and the total number of macrophages recovered from these patients with a heavy smoking history was significantly less than that of controls with a similar smoking history (fig 1; p < 0.001).

The numbers of lymphocytes recovered/ml lavage fluid in the patients with sarcoidosis was not significantly different in non-smokers and smokers (table 2), nor did smoking modify the total number of CD4+ T lymphocytes recovered by lavage (table 3). Smoking, however, was associated with a twofold increase in the number of CD8+ T-lymphocytes recovered (table 3) and as a consequence the CD4 : CD8 ratio was significantly lower among smokers than non-smokers with sarcoidosis.

In the patients with sarcoidosis there were no significant differences between smokers and non-smokers in the numbers of neutrophils, eosinophils, or mast cells recovered by lavage (table 2). The smokers had significantly fewer neutrophils in lavage fluid than the control smokers, but this may reflect the fact that cigarette consumption by the smokers with sarcoidosis was less than that of our control smoking population (p < 0.001).

IgG content in lavage fluid and serum
The patients with sarcoidosis, both non-smokers and
smokers, had higher IgG concentrations in the fluid recovered by bronchoalveolar lavage than the control non-smokers (table 4). As the albumin recovered/ml lavage fluid was similar for control subjects and patients, regardless of their tobacco consumption, the IgG:albumin ratio was higher in patients than in control subjects but similar for non-smoking and smoking patients.

The concentration of IgG in serum was similar for smoking and non-smoking patients with sarcoidosis. When they were considered as a group, the concentration of serum IgG was similar in patients with sarcoidosis and controls. In the sarcoaid group, however, 10 of 30 non-smoking patients and two of 12 smoking patients had clearly increased serum immunoglobulin concentrations (p > 0.2 for smokers vs non-smokers with sarcoidosis by χ² analysis).

Pulmonary gallium uptake
No significant difference in ⁶⁷Ga accumulation was observed between non-smoking and smoking patients (fig 2a). The two patients with very high ⁶⁷Ga indices were both heavy smokers (> 20 cigarettes/day).

Serum angiotensin converting enzyme activity
In the patients with sarcoidosis SACE activity was significantly higher in smokers than non-smokers (SACE: non-smokers 72.4 (32.9) units; smokers 99.2 (66.3) units; p < 0.05), although considerable overlap was observed between the groups (figure 2b).

Individual smokers were also more likely to have increased SACE than non-smokers (SACE > 50 units; non-smokers 36.48; smokers 19.20; p < 0.001). The two patients with very high SACE activity had both smoked 20 or more cigarettes a day. No correlation was observed, however, between the number of alveolar macrophages recovered by lavage from smokers with sarcoidosis and SACE activity in these individuals (p > 0.2).

RELATION OF CIGARETTE SMOKING TO THE EVOLUTION OF SARCOIDOSIS
Treatment with corticosteroids
Information on the clinical course during a follow up period of one year was available for 46 (72%) of the 64 patients with sarcoidosis initially evaluated. There was no significant difference in the number of non-smoking patients (10/33) and smoking patients (5/13) who received corticosteroids (p > 0.2). The primary indication for steroid treatment in both groups was usually the presence of extrapulmonary sarcoidosis or severe systemic symptoms (9/10 non-smokers and 4/5 smokers).
smokers). One patient in each group received corticosteroids solely because of dyspnoea, although a further three of the nine non-smokers and three of the four smokers treated with corticosteroids had dyspnoea on exertion.

Radiographic findings
Most patients had complete or partial resolution of radiographic abnormalities after 12 months of follow up (table 5). The proportion of patients with complete resolution, partial resolution, stability, or deterioration of radiographic abnormalities did not differ between smokers and non-smokers.

Lung function
The non-smoking patients had only slight changes in pulmonary function during the follow up period of 12.2 (4.5) months (table 6), though vital capacity and TLCO showed significant increases. Changes in indices of pulmonary function in smokers did not differ significantly from those observed in non-smokers, even though the average length of follow up was somewhat longer (13.6 (5.6) months). Furthermore, the proportion of patients who showed a greater than 20% decrease in any given index of pulmonary function was similar for non-smokers and smokers (table 6).

Discussion

SMOKING AND THE PREVALENCE OF SARCOIDOSIS
In our study patients with pulmonary sarcoidosis were
less likely to smoke than people of a similar age in the general population. The failure to find a significant difference in cigarette consumption between men with sarcoidosis and men in the general population reflects, at least in part, the small number of patients evaluated. For example, when the results of the present study (131 patients) are combined with those from a previous study by our group (130 different patients), both men and women with sarcoidosis were found to be less likely to smoke than the corresponding groups in the normal population (men: p < 0.02; women: p < 0.001), although the differences for men were not significant for either group when these were considered separately. Only patients with overt pulmonary manifestations, however, were studied and the effect of cigarette smoking, if any, on the incidence of extrathoracic sarcoidosis is unknown.

This study was not a case-control study and potential confounding factors such as socioeconomic status and race were not controlled. Our results, however, are concordant with those of several previous studies suggesting that the incidence of sarcoidosis is higher in non-smokers than smokers. Nevertheless, not all studies have shown clearly that smoking reduces the incidence of sarcoidosis, and further studies will be required to explain the differing results so far presented.

**THE EFFECT OF CIGARETTE SMOKING ON THE SEVERITY OF SARCOIDOSIS**

Our findings do not support the conclusion that the apparent lower incidence of sarcoidosis observed in smokers resulted from the fact that the disease is less severe in these patients. No significant differences were found between smokers and non-smokers in the comparisons of radiographic and functional indices at initial evaluation, and follow up of the two groups did not disclose differences in clinical symptoms, the proportion of patients requiring corticosteroid treatment, or subsequent changes in the results of functional tests or chest radiographs. Although our study did not show major differences in the evolution of lung function in smoking and non-smoking patients studied over one year, we would emphasise that smoking may influence the long term evolution of the disease. For example, Dutton et al have shown that residual volume is higher in smokers than non-smokers with chronic sarcoidosis, and we have confirmed this finding in a small group of patients with sarcoidosis of more than three years' duration (R Georges, unpublished results).

Nevertheless, cigarette consumption did modify some, though not all, indices used in assessing the “activity” of sarcoidosis. Cigarette smoking was associated with a significant increase in SACE activity in sarcoid patients, and all patients with very high SACE activity and 67Ga accumulation smoked 20 or more cigarettes a day. Gallium uptake but not SACE activity have been reported to be higher in smokers than in non-smokers with sarcoidosis. Both the release of angiotensin converting enzyme and 67Ga uptake are thought to represent, at least in part, alveolar macrophage activity and presumably relate both to the number of alveolar macrophages present and their state of activation. Cigarette smoking alone, which increases the number of alveolar macrophages considerably, results in only a modest increase in 67Ga uptake and no significant increase in SACE activity. Furthermore, we observed no correlation between the number of alveolar macrophages recovered by lavage and these two indices. These findings suggest that the state of activation of alveolar macrophages is the predominant factor determining the level of these indices. Nevertheless, the increased number of alveolar macrophages that results from smoking in combination with appropriate activation signals (present in some patients with sarcoidosis) may explain the very high 67Ga uptake and SACE activity seen in some, but not all, patients with sarcoidosis who smoked.

Smoking was also associated with a twofold increase in CD8+ T lymphocytes recovered by lavage from patients with sarcoidosis. Similarly, twice as many CD8+ T lymphocytes were observed in smoking as in non-smoking controls, as reported previously. As smoking did not affect the recovery of CD4+ T lymphocytes, the CD4 : CD8 ratio was significantly lower in smoking than in non-smoking patients, though it was clearly abnormal in almost all patients who smoked.

In this study, like some but not all previous studies, the number of alveolar macrophages recovered from smoking patients with sarcoidosis was much less than the number recovered from controls, subjects with a similar smoking history. This finding is consistent with the possibility that smokers who develop a large increase in the number of alveolar macrophages in the lower respiratory tract are unlikely to develop pulmonary sarcoidosis, and might explain in part the lower incidence of sarcoidosis in smokers. In this context, the alveolitis of sarcoidosis is dependent on the proliferation of T lymphocytes within the lung parenchyma, and most previous studies have shown that “excessive” numbers of alveolar macrophages inhibit T lymphocyte proliferation.

Cigarette smoking is recognised not only as increasing the number of alveolar macrophages in the lung but also as influencing their functional activity. As changes in the number and activity of alveolar macrophages may be associated with each other, our results do not exclude the possibility that changes in...
functionality of these cells induced by cigarette smoking may be important in reducing the likelihood of developing sarcoidosis. For example, some previous studies\(^3\) indicate that alveolar macrophages from smokers have less accessory cell activity than equal numbers of alveolar macrophages from non-smokers.

Alternatively, the smaller number of alveolar macrophages in smokers with sarcoidosis than in smoking control subjects may be the result of the disease process. For example, sarcoidosis can have an important impact on the phenotype of alveolar macrophages recovered by lavage,\(^4\) and the development of sarcoidosis could alter the distribution of alveolar macrophages within the lung in such a way that fewer cells are recovered by lavage. The fact, however, that just as many alveolar macrophages were recovered from non-smoking patients with sarcoidosis as from non-smoking controls does not support this hypothesis.

In conclusion, our study supports the idea that smoking reduces the incidence of sarcoidosis. Furthermore, smoking modifies the results of various tests that have been used in the evaluation of these patients, and this variable must therefore be taken into consideration in interpreting these results. Finally, our results are consistent with the possibility that the appearance of increased numbers of macrophages in the lower respiratory tract induced by cigarette smoking contributes to the lower incidence of pulmonary sarcoidosis in smokers.

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