

## Cigarette smoking and autoimmune disease: what can we learn from epidemiology?

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## ENVIRONMENTAL EXPOSURE

# Cigarette smoking and autoimmune disease: what can we learn from epidemiology?

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**Key words:** cigarette; smoking; autoimmune disease; rheumatoid arthritis; systemic lupus erythematosus; multiple sclerosis; Graves'; hyperthyroidism; primary biliary cirrhosis; epidemiology; risk factor; gene-environment; interaction

## Introduction

There is ample evidence that environmental exposures are important in the development of autoimmune diseases. The concordance rates for autoimmune diseases in monozygotic twins are well below 100%, pointing to the influence of environmental factors interacting with genetics in determining disease susceptibility. Among the environmental exposures that have been the best studied, crystalline silica from agricultural and other occupational sources,<sup>1–4</sup> Epstein–Barr virus<sup>5–8</sup> and cigarette smoking have been associated with the development of multiple autoimmune diseases. Cigarette smoking has been causally linked to the development of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), Graves' hyperthyroidism, and primary biliary cirrhosis (PBC)<sup>9–16</sup>, among others.

## Cigarette smoke components and systemic effects

Cigarette smoke contains hundreds of potentially toxic components, including tars, nicotine, carbon monoxide and polycyclic aromatic hydrocarbons among others. As an impure mixture, cigarette smoke has multiple known and unknown actions in the human body (Table 1). Two phases of cigarette smoke exist: a tar or particulate phase and a gaseous phase, both of which contain extremely high concentrations of free radicals and cigarette smoke activates endogenous sources of free radicals as well.<sup>17</sup> These toxins and free radicals can interact with DNA,<sup>18</sup> and could cause genetic mutations and gene activation responsible for the development of autoimmune disease. In addition, cigarette smoke has been shown to increase the expression of Fas (CD95) on B and CD4 T lymphocyte cell surfaces.<sup>19</sup> Increasing the sensitivity of these cells to apoptotic signals could add to the burden of apoptotic material to be cleared by an inefficient clearance mechanism in patients at risk for autoimmunity.

The pro-inflammatory effects of cigarette smoke have been well studied in relation to the risk of

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**Table 1** Biologic effects of cigarette smoke

Mechanism		Reference
Tissue damage and increased apoptosis	<ul style="list-style-type: none"> <li>• Contains free radicals</li> <li>• Generates endogenous free radicals and lipid peroxidation</li> <li>• Increases expression of Fas on lymphocytes causing increased sensitivity to apoptotic signals</li> <li>• Leads to release of matrix metalloproteinases</li> </ul>	17, 18, 27 129 19
Inflammatory	<ul style="list-style-type: none"> <li>• Elevates serum fibrinogen</li> <li>• Increases peripheral leukocyte counts</li> <li>• Increases neutrophil chemotaxis and recruitment of PMNs, monocytes and macrophages</li> <li>• Increases levels of CRP, IL-6, serum ICAM-1, and E-selectin</li> </ul>	24, 25 26, 27 21–23 28, 29, 130
Immunosuppressant	<ul style="list-style-type: none"> <li>• Induces abnormalities in T cells and suppresses T cell activation</li> <li>• Reduces NK cell activity</li> <li>• Decreases serum levels of IgG and IgM</li> </ul>	30–32, 34 32, 34, 130, 131 32–34
Anti-estrogenic	<ul style="list-style-type: none"> <li>• Enhances formation of inactive 2-hydroxy estrogens</li> </ul>	35–38

cardiovascular disease and emphysema.<sup>20</sup> Cigarette smoke affects the influx and activation of neutrophils, macrophages and monocytes and increases the release of tissue-damaging matrix metalloproteinases.<sup>21–23</sup> Both current and past smokers have higher fibrinogen levels than non-smokers, and the increase correlates with the number of cigarettes smoked per day.<sup>24,25</sup> Cigarette smoke elevates peripheral blood leukocyte counts<sup>26,27</sup> and is associated with increases in C-reactive protein and IL-6,<sup>28,29</sup> important markers of inflammation in autoimmune diseases. Abnormalities in T-cell function,<sup>30,31</sup> reduction in NK cells<sup>32</sup> and impairment of both humoral and cell-mediated immunity<sup>32–34</sup> have been observed in smokers.

Additionally, cigarette smoking has anti-estrogenic effects through the formation of inactive 2-hydroxy catechol estrogens.<sup>35–38</sup> Women who are smokers undergo menopause earlier than do non-smokers.<sup>39</sup> Estrogens can affect the Th1/Th2 immune balance and estrogens have either pro- or anti-inflammatory actions depending on their concentration and the estrogen/androgen balance.<sup>40,41</sup> Exogenous estrogens can suppress collagen-induced arthritis in mice,<sup>42</sup> as well as decrease susceptibility to its development.<sup>43</sup>

### Autoimmune diseases associated with cigarette smoking: the epidemiologic evidence

The association with cigarette smoke has been best established for five important autoimmune diseases: rheumatoid arthritis, systemic lupus erythematosus, Graves' disease, multiple sclerosis and primary biliary cirrhosis. This list is by no means exhaustive and other autoimmune diseases such as ulcerative colitis do not have similar associations with smoking. Our goal in reviewing this group of autoimmune diseases together

is to draw insights and parallels between the related diseases and their relationships to smoking that may point to cigarette smoke-induced mechanisms of autoimmunity.

#### *Rheumatoid arthritis*

An unexpected 2.4 times increased risk of RA among women smokers was first reported by Vessey and colleagues in 1987, in their investigations of oral contraceptive use and the risk of RA in the Oxford Family Planning Association Contraceptive Study.<sup>44</sup> Since then, 11 case-control and four cohort studies have confirmed the increased risk of RA with cigarette smoking (Table 2).<sup>9–11,44–56</sup> Cigarette smoking is now the most conclusively established environmental risk factor for seropositive RA. Epidemiologic studies have revealed that the risk is higher in men (OR range 1.9–4.4 in six case-control studies<sup>45,48,49,52,53,55</sup>) compared to women (OR range 0.6–2.5 in eight case-control studies<sup>44,46–51,53</sup> and three cohort studies<sup>9,11,56</sup>). Among RA patients with first degree relatives with RA, the age at onset is younger in smokers than non-smokers.<sup>57</sup> Both smoking intensity (number of cigarettes smoked per day) and duration are powerful predictors of RA risk, but, as shown in an analysis in the Women's Health Study, smoking duration may be the more important of the two.<sup>9</sup> The risk of developing RA is elevated in both current and former smokers and, in fact, remains elevated for up to 20 years after smoking cessation.<sup>11,56</sup>

Cigarette smoking may be associated with increased RA severity as well, including rheumatoid nodule formation,<sup>58,59</sup> increased joint destruction,<sup>58–60</sup> increased pulmonary disease,<sup>58,61</sup> and decreased functional abilities.<sup>60</sup> A gene–environment interaction may be responsible for the increased severity of RA in smokers. Glutathione-S-transferase (GST) enzymes

are involved in hepatic detoxification of cigarette smoke. Mattey and colleagues have shown that women with RA who had a null polymorphism in the GSTM1 gene (associated with absence of GST enzyme activity) and smoked had much higher levels of radiographic damage, decreased functional outcomes, and higher RF levels than women with RA with either but not both of these factors.<sup>62</sup> Another potential explanation for the increased severity of RA among smokers is a change in the ratio of tumor necrosis factor (TNF)- $\alpha$  to soluble TNF-receptor that may cause increased TNF- $\alpha$  activity.<sup>63</sup>

Cigarette smoking is most closely associated with seropositive RA: both rheumatoid factor (RF) and anti-cyclic citrullinated protein (anti-CCP) antibody seropositivity. This may be in part because seronegative RA is a more challenging diagnosis, and more likely to include misclassified reactive, psoriatic, viral and crystalline arthritides. Work from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) cohort study in Sweden has helped to elucidate an interesting gene-environment interaction that exists between cigarette smoking and the *HLA-DRRB1* 'shared epitope' (SE). A group of *HLA-DRB1* alleles strongly associated with susceptibility to RA share a region of sequence similarity or 'shared epitope' at amino acid positions 70-74 in the third hypervariable region of the *HLA-DRB1* molecule.<sup>49,54,64</sup> They have found that current smokers carrying two copies of the SE are at a 16-fold increased risk of developing RF+ RA (95%CI 7.2, 34.2)<sup>49</sup> and at a 21-fold increased risk of developing anti-CCP + RA (95%CI 11.0, 40.2).<sup>54</sup> Although cigarette smoke is known to induce RF in

healthy subjects,<sup>65,66</sup> in analyses of RF+/anti-CCP- and RF-/anti-CCP+ subjects with RA, the EIRA group has shown that the major association of cigarette smoking is with anti-CCP+ RA, and they have proposed a new and elegant model for the pathogenesis of anti-CCP + RA.<sup>54</sup> Employing immunocytochemical staining of citrullinated peptides in bronchoalveolar lavage cells from smokers, non-smokers, and subjects with other types of pulmonary inflammatory diseases, they demonstrated that smoking and pulmonary inflammation both lead to increased numbers of citrullinated peptides in the lungs.<sup>54</sup> They have proposed that cigarette smoking promotes citrullination (the conversion of an arginine to a citrulline residue in certain peptides) and that the subsequent generation of antibodies to citrullinated proteins (anti-CCP antibodies) occurs preferentially in individuals carrying the SE genotypes.<sup>54</sup> As anti-CCP antibodies occur years before the onset of RA<sup>67-69</sup> and citrullination of peptides appears to render them more prone to bind to HLA class II molecules with the SE,<sup>70</sup> the described interactions between smoking and the SE may give rise to RA in at least some individuals.

### Systemic lupus erythematosus

To date, three case-control studies have reported significantly increased odds ratios for the development of SLE in smokers,<sup>71-73</sup> while six other studies have not found a clear association (Table 2).<sup>74-78</sup> In several of these studies, which were performed in a

**Table 2** Epidemiologic studies of the association between cigarette smoking and selected autoimmune diseases

Autoimmune disease	No. of studies revealing significantly elevated risk/no. of case-control studies	No. of studies revealing significantly elevated risk/no. of cohort studies	Range of observed OR (RR) of developing disease
Rheumatoid arthritis	11/12 <sup>2,10,44-47,49,51-55</sup>	4/4 <sup>9,11,50,56</sup>	0.6-3.4 * higher risk in men * higher risk for RF+ and anti-CCP+ RA * risk related to smoking duration, intensity, and declines slowly after cessation * smoking associated with more severe disease course
Systemic lupus erythematosus	3/8 <sup>71-78</sup>	0/2 <sup>79,80</sup>	0.5-6.7 * risk mainly related to current smoking * anti-dsDNA antibodies related to current smoking * cohort studies may be underpowered to detect elevated risk
Multiple sclerosis	1/3 <sup>93-95</sup>	2/2 <sup>96,97</sup>	1.6-1.9 * risk increases with increasing smoking intensity * smoking associated with worsening course of MS
Graves' disease	8/8 <sup>12,107,110,132-136</sup>	1/1 <sup>109</sup>	1.3-8.2 * higher risk among current than past smokers * risk related to smoking intensity * smoking also risk for Graves' ophthalmopathy * risk may be higher in women than men
Primary biliary cirrhosis	2/2 <sup>16,118</sup>	0	1.6-3.5

wide variety of geographic locations and employed a range of hospital and community-based controls, current smoking was more strongly related to the development of SLE than was past smoking. Only one of these studies showed a dose–response relationship between number of pack years of smoking and the risk of SLE.<sup>72</sup> Three other studies were unable to find a dose effect,<sup>71,73,75</sup> and the remainder did not investigate a dose effect.

The existing case-control studies are a heterogeneous group of studies. Definitions of smoking status (never, past and current), questionnaire response rates in cases and controls, the inclusion of potential confounders, and the timing of the study questionnaire in relation to the onset of SLE, varied widely among the studies. Most strikingly, the results of the Ghaussy study,<sup>71</sup> which was performed in New Mexico and used general medical outpatients as controls, are remarkably higher than those of the rest of the studies: an odds ratio (OR) of 6.69 (95%CI 2.59, 17.30) for current smoking and 3.62 (95%CI 1.22, 10.70) for former smoking, whereas the remainder of the studies reported ORs ranging from 0.9 to 2.3 for current smoking and 0.6–1.2 for former smoking.

A meta-analysis statistically combining the effect estimates from the seven case-control studies and two cohort studies<sup>79,80</sup> that have examined cigarette smoking as a risk factor for SLE,<sup>81</sup> revealed a modestly increased risk posed by current smoking (RR 1.5 [95%CI 1.09, 2.08]), but no increased risk associated with past smoking (RR 0.98 [95%CI 0.75, 1.27]). A sensitivity analysis in which the outlying study by Ghaussy *et al.*<sup>71</sup> was excluded, showed that it did have a large influence on the summary effect estimate, and was responsible for much of the statistical heterogeneity observed between studies. Without this study, the OR for current smoking was still elevated at 1.31 (95%CI 1.02, 1.70). These results suggest that current smoking may be an instantaneous hazard for the development of SLE, much as it is for the development of coronary artery disease, and that, with time after the cessation of smoking, the risk of SLE returns to that observed in those who have never smoked.

One biologic mechanism potentially involved in the link between smoking and SLE was provided by a recent case-control study by Freemer and colleagues that reported an association between current smoking and the presence of anti-dsDNA antibodies among 140 smokers and 270 non-smokers with SLE.<sup>82</sup> In current smokers compared to non-smokers, an OR of 4.0 (95%CI 1.6, 10.4) for anti-dsDNA antibody seropositivity was found. There was no increased risk of these antibodies in former smokers compared to non-smokers. It is hypothesized that smoking causes DNA damage and the formation of DNA adducts, thereby producing

anti-dsDNA antibodies.<sup>82</sup> The estimated half-life of these adducts is nine to 13 weeks,<sup>83</sup> possibly explaining the transient nature of smoking's effect on anti-dsDNA production.

This mechanism would also explain the epidemiologic association of active cigarette smoking with increased severity of SLE. Ward and colleagues reported the more rapid development to end stage renal disease in smokers<sup>84</sup> and Ghaussy and colleagues found increased SLE disease severity (higher SLE Disease Activity Index scores) over a six-month period in smokers compared to non-smokers.<sup>85</sup> Smoking is also associated with discoid lupus<sup>86</sup> and, more predictably, with avascular necrosis<sup>87</sup> and thrombotic complications in lupus.<sup>88</sup>

### *Multiple sclerosis*

The etiology of multiple sclerosis is unknown, but it is thought that MS is mediated by autoreactive T-cells directed against components of myelin.<sup>89</sup> MS affects approximately two times more women than men<sup>90</sup> and is associated with HLA class II alleles. MS has been observed to have increased prevalence in Northern latitudes world wide, although there are many exceptions<sup>91</sup> and this may be in part due to vitamin D deficiency.<sup>92</sup> The relationship between cigarette smoking and MS has been investigated in five epidemiologic studies (Table 2). Two studies of cohorts of women followed for contraceptive practices in the 1990s found suggestive but not significantly elevated rates of MS among female smokers of >15 cigarettes a day.<sup>93,94</sup> A case-control study in Canada reported an elevated risk of MS (RR 1.6, 95%CI 1.0, 2.4) among ever smokers compared to never smokers, and even higher risk among those who smoked 20–40 cigarettes a day (RR 1.9, 95%CI 1.2, 3.2).<sup>95</sup> The prospective Nurses' Health Study cohorts confirmed an elevated risk of developing MS among both current smokers (RR 1.6, 95%CI 1.1, 2.1) and past smokers (RR 1.2, 95%CI 0.9, 1.6), compared to never smokers.<sup>96</sup> A cross-sectional study of 22 312 individuals living in one city in Norway in 1997 found 87 cases of MS with an elevated risk of 1.8 (95%CI 1.1, 2.9) in ever smokers compared to never smokers.<sup>97</sup>

Cigarette smoking seems to exacerbate MS, both chronically and acutely. Cigarette smoking also causes a transient worsening of motor functioning on a battery of tests in MS patients, compared to healthy controls.<sup>98</sup> In a retrospective study using data from the General Practice Research Database in Britain, Hernan and colleagues have shown that cigarette smoking increases the risk of transforming a relapsing-remitting clinical course into a secondary progressive course.<sup>99</sup> Nicotine, free radicals, or other substance contained in cigarette

smoke, may cause axonal degeneration or block axonal conduction, especially in axons that are already damaged or demyelinated.

### *Graves' hyperthyroidism*

Graves' disease is one of the most common autoimmune diseases among women, with a prevalence of over 1%.<sup>100</sup> It often occurs in conjunction with autoimmune rheumatic diseases and has increased incidence in the post-partum period. Characterized by hyperthyroidism, goiter, ophthalmopathy and pretibial myxedema, Graves' is mediated by autoantibodies to the thyrotropin (TSH) receptor that stimulate thyroid hormone synthesis and secretion and thyroid growth. Genetics, as for most autoimmune diseases, are clearly important with a concordance rate of 17–35% in monozygotic twins.<sup>101,102</sup> Environmental risk factors for Graves' have been studied in mainly small case-control studies. The onset of Graves' has been associated with stressful life events,<sup>103–105</sup> high iodine intake,<sup>106</sup> and cigarette smoking.<sup>107–109</sup>

In a 2002 meta-analysis of 25 studies of the association between thyroid disease and smoking (only eight of which were limited to Graves' alone), the summary odds ratio for current smoking was 3.30 (95%CI 2.09, 5.22) and it was 1.41 among past smokers (95%CI 0.77, 2.58) (Table 2).<sup>108</sup> In a 2005 study in the Nurses' Health Study II, a prospective cohort of 115 109 women aged 25–42 at entry, 543 incident cases of Graves' were identified between 1989 and 2001. Cigarette smoking was confirmed to be a strong and time-dependent risk factor for the development of Graves'.<sup>109</sup> The relative risk among current smokers was 1.93 (95%CI 1.54, 2.43) and among past smokers it was 1.27 (95%CI 1.03, 1.56). As in RA, the risk was related to smoking intensity and was highest in women who smoked >25 cigarettes a day (RR 2.63, 95%CI 1.71, 4.04). Smoking appears to be an especially strong risk factor for Graves' ophthalmopathy<sup>110–113</sup> and the risk of Graves' declines with smoking cessation.<sup>108</sup> Again, the mechanisms of smoking's effect on Graves' and its ophthalmopathy are unknown. Several effects have been posited, including enhanced generation of reactive oxygen species,<sup>112,114</sup> increased concentrations of soluble adhesion molecules such as s-ICAM-1<sup>111</sup> and increased production of autoantibodies in this autoantibody mediated disease.<sup>115</sup>

### *Primary biliary cirrhosis*

Primary biliary cirrhosis (PBC), characterized by the progressive destruction of intrahepatic biliary ducts, is another autoimmune disease of unclear etiology, associated within individuals and families with other

autoimmune diseases, such as scleroderma, lupus, and autoimmune thyroid disease. Autoantibodies, including antinuclear antibodies and anti-mitochondrial antibodies, are often present at high titer, and the latter react specifically with the components of 2-oxodehydrogenase enzymes in the liver.<sup>116</sup> The concordance rate in first degree family members is high, pointing to an important genetic component of susceptibility.<sup>117</sup> Cigarette smoking has recently been associated with increased risk of incident PBC in two case-control studies (Table 2). In NorthEast England, Howel and colleagues found an elevated risk of developing PBC among smokers (among smokers of 20 or more years, OR 3.5, 95%CI 1.9, 6.3).<sup>16</sup> In a larger, USA-wide case-control study, Gershwin and colleagues assessed risk factors for PBC among over 1000 PBC pts from 23 centers compared to healthy age-, race-, sex- and geography-matched controls. They also reported an increased risk of PBC associated with cigarette smoking (OR for ever smokers of >100 cigarettes 1.6, 95%CI 1.3, 1.9).<sup>118</sup>

## Conclusions

Cigarette smoking is not associated with increased risk of all autoimmune diseases. While RA, SLE, MS, Graves'; and PBC all appear to be associated with cigarette smoking, other autoimmune diseases may not be so. The relationship between smoking and inflammatory bowel disease, for example, is complex. While Crohn's disease is associated with smoking<sup>119–122</sup> and smoking exacerbates the clinical course of the disease,<sup>123–125</sup> smoking is protective against ulcerative colitis.<sup>119,126–128</sup> The pathophysiologic basis for these associations is not known.

There are epidemiologic challenges to studying risk factors for rare diseases. Retrospective case-control studies are prone to recall bias, in which participants who have developed autoimmune disease recall past exposures differently than non-affected control individuals. Case-control studies must also deal with bias that could be introduced by the choice of control subjects that are not representative of the source population. Prospective studies, on the other hand, have the advantage of more accurately measuring cigarette smoke exposure before the onset of disease, but are time-consuming and expensive to perform as many healthy subjects must be followed for many years to collect a sample size of affected individuals suitable for analysis. For example, two large prospective cohort studies, the Nurses' Health Study and the Black Women's Health Study,<sup>79,80</sup> did not observe an association between cigarette smoking and the development of SLE. Thus, the possibility that these cohort studies,

which had 67 and 85 cases of SLE respectively, lacked the power to detect a small effect must be considered. Whether cigarette smoking elevates the risks of diseases such as scleroderma, Sjogren's, and inflammatory myositis is unknown given the difficulty in studying these rare conditions.

Additional methodological challenges to epidemiologic studies are posed by the conglomerate nature of cigarette smoke and the heterogeneous nature of these diseases. The composition of cigarettes over the years has changed and the effects of low-tar and filtered cigarettes on the quantities of inhaled toxins are unknown. Passive exposure to cigarette smoke (second hand smoke) has been linked to a variety of chronic diseases, including asthma, coronary heart disease and cancer, but has not been well studied in relation to autoimmune diseases. Moreover, exposure to second hand smoke may vary considerably by geographic location.

The immunologic and genetic subphenotype of individual autoimmune diseases appears to be important in determining smoking-related risk; smoking elevates the risk of seropositive RA, but not seronegative RA, and even more dramatically the risk of seropositive RA among subjects with the *HLA-DRB1* shared epitope.<sup>49</sup> Similarly, cigarette smoking appears to be related to the risk of anti-dsDNA antibodies within SLE, a very heterogeneous disease. The racial and genetic compositions of the populations examined in the different epidemiologic studies also contribute to imprecise and differing estimates.

The picture of how environmental exposures lead to autoimmune diseases in genetically predisposed individuals is starting to come into focus. Closer scrutiny of the similarities and differences between these autoimmune diseases, in terms of their epidemiology and basic immunology, may help to elucidate some of the mechanisms underlying the pathogenesis of autoimmune conditions. Continued exploration of how cigarette smoking acts as a trigger of autoimmunity is necessary. Animal models and basic research into the biologic effects of the constituents of cigarette smoke, as well as ongoing large cohort studies will advance our understanding of disease mechanisms. Evidence from epidemiologic studies should be put to use on two fronts: 1) the prevention of autoimmune disease: understanding the relationship between smoking and autoimmune disease provides the possibility of preventing it in high risk individuals, if we can prevent or reduce smoking; 2) understanding of the mechanisms of disease pathogenesis. The citrullinated peptide/anti-CCP antibody story coming to light in RA is fascinating, and probably not the only one of its kind. Similar mechanisms may be at work in related autoimmune diseases. Understanding of these mechanisms opens the door to new treatments. The search for new genes, in particular non-HLA genes, which predispose to

autoimmune conditions is ongoing, and the search for gene-environment interactions involving smoking and other environmental exposures should proceed in parallel.

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