Complex Relationships of Smoking, HLA–DRB1 Genes, and Serologic Profiles in Patients With Early Rheumatoid Arthritis: Update From a Swedish Population-Based Case–Control Study

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Objective. Smoking is associated with an increased risk of rheumatoid arthritis (RA) in subsets of patients defined according to the presence or absence of anti–citrullinated protein antibodies (ACPAs) and rheumatoid factors (RFs). Moreover, an interaction between smoking and the HLA–DRB1 shared epitope (SE) has been demonstrated to be a risk factor for seropositive RA. The aim of this study was to investigate the interplay between smoking and the HLA–DRB1 SE with regard to risk of RA in different patient subsets based on ACPA and RF status.

Methods. Incident cases of RA (3,645 cases, 5,883 matched controls) were divided into 4 subgroups based on the presence or absence of RF and anti–cyclic citrullinated peptide 2 (anti-CCP2) antibodies. The influence of smoking on the risk of disease was determined in each RA subgroup, using logistic regression models with calculation of odds ratios and 95% confidence intervals (95% CIs). The potential interaction between smoking and HLA–DRB1 SE genes was evaluated by calculating the attributable proportion due to interaction (AP).

Results. In the RF+/anti-CCP2+ subset of RA patients, both smoking and the presence of the HLA–DRB1 SE conferred independent disease risks, and there was a strong interaction between the 2 risk factors (AP 0.4, 95% CI 0.3, 0.5). In the RF−/anti-CCP2+ patient subset, the HLA–DRB1 SE conferred an increased risk of RA, whereas the independent influence of smoking was limited. However, there was a significant interaction between the HLA–DRB1 SE and smoking (AP 0.2, 95% CI 0.02, 0.5). In the RF+/anti-CCP2− patient subset, there was an increased risk of disease among smokers, which was only marginally affected by the presence of the HLA–DRB1 SE, and no interaction between the 2 factors was observed (AP 0.002, 95% CI −0.3, 0.3). In the RF−/anti-CCP2− patient subset, neither smoking nor the presence of the HLA–DRB1 SE conferred an increased risk of RA.

Conclusion. These findings demonstrate different effects of smoking and HLA–DRB1 in the 4 serologically defined RA subsets.

INTRODUCTION

Rheumatoid arthritis (RA) is an immune-mediated inflammatory disease resulting from the complex interaction between genetic constitution and environmental triggers. The most important genetic risk factor for RA defined to date is the shared epitope (SE) of HLA–DRB1 (1–3), and smoking has been identified as the most important environmental factor in the development of RA (4–6). The effects of these 2 risk factors, the HLA–DRB1 SE and smoking, and the interaction between them have been shown to be confined to the subset of RA patients whose disease is defined by the presence of anti–citrullinated protein antibodies (ACPAs) and/or rheumatoid factors (RFs), and a hypothesis regarding the etiology of this subset has been formulated based on the interaction between the HLA–DRB1 SE and smoking, as well as between the HLA–DRB1 SE and other airway exposures (7,8). However, the potential roles of RF and ACPAs in the pathogenesis of different subsets of RA have not yet been fully elucidated. We used an updated version of the Swedish population-based case–control study Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study supported by the Swedish Medical Research Council, the Swedish Society for Medical Research, the Swedish Council for Health, Working Life and Welfare, the 80-Year Foundation of King Gustaf V, the Swedish Rheumatism Foundation, Stockholm County Council, AFA, and IMU-supported BeTheCure projects.

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to investigate the interplay between smoking and the HLA-DRB1 SE with regard to risk of RA in different serologically defined patient subsets grouped according to ACPA and RF status.

**PATIENTS AND METHODS**

**Study design and study subjects.** The present study investigated data from the ongoing EIRA project, which is a population-based case–control study comprising subjects ages 18–70 years in the middle and southern parts of Sweden. All hospital-based and most privately run rheumatology units in the study area participated in recruiting incident RA cases to the study. All patients identified as an incident case fulfilled the American College of Rheumatology 1987 classification criteria for RA (9). During the study period (November 1996 to September 2014), completed questionnaires were obtained from 3,724 RA cases and 5,935 matched healthy controls. Subjects who could not provide detailed information on smoking habits were excluded, as were patients whose ACPA or RF status was not available. A flow chart depicting the distribution of subjects is presented in Supplementary Figure 1 (available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40852/abstract). For each case recruited between November 1996 and October 2005, 1 control subject was randomly selected from the national population register, matched by age in 5-year age strata, by sex, and by residential area (EIRA I). For each case recruited between October 2005 and September 2014, 2 control subjects were selected using the same matching criteria (EIRA II). The response proportion was 92% for the cases and 75% for the controls. All aspects of the study were approved by the ethics committee of the Karolinska Institutet.

**Anti–cyclic citrullinated peptide 2 (anti-CCP2) and RF analyses.** Cases were categorized into either anti-CCP2 positive or anti-CCP2 negative based on the results of an Immunoscan-RA Mark2 enzyme-linked immunosorbent assay (anti-CCP2 test). An antibody level exceeding 25 AU/ml was regarded as a positive result. RF positivity or RF negativity was determined locally by the unit entering the case into the study.

**Data collection and definition of smoking status.** Information regarding lifestyle factors and different environmental exposures was collected using a standardized questionnaire. Detailed information on smoking was obtained by asking each subject about current and previous smoking habits, including duration of smoking, average number of cigarettes smoked per day, and type of cigarettes. For each case, the time of the initial appearance of RA symptoms was used as an estimate of the date of disease onset, and the year in which this occurred was defined as the index year. The corresponding controls were given the same index year. Information regarding smoking was considered prior to or during the index year in the cases and during the same period of time in the corresponding controls. Subjects who had smoked during the index year were defined as current smokers, those who had stopped smoking prior to the index year were defined as past smokers, and those who had never smoked before or during the index year were defined as never smokers.

**Genotyping.** Blood samples were available from participants who answered the questionnaire between November 1996 and May 2012. HLA-DRB1 genotypes were obtained using a previously described method (10). Data on genotypes were available for 3,355 cases (63%) and 2,840 controls (48%). The HLA-DRB1*01, HLA-DRB1*04, and HLA-DRB1*10 alleles were classified as the SE alleles.

**Statistical analysis.** Using logistic regression analyses with calculation of odds ratios (ORs) and 95% confidence intervals (95% CIs), the risk of occurrence of each RA serologic subset in patients with different smoking habits was compared with that in never smokers. The occurrence of RA among those who had started and stopped smoking in different life periods was compared with that among never smokers. A trend test for a dose-response relationship regarding cumulative dose of smoking and risk of each subset of RA was performed using a continuous

| Table 1. Rate of anti-CCP2 positivity among RA cases categorized by RF status, number of SE alleles, and smoking status* |
|---------------------------------------------------------------|
| No. of RA cases | Anti-CCP2 positive, no. (%) |
|-----------------|-------------------------------|
| RF-negative      |                               |
| 0 alleles       |                               |
| Never smoker    | 179                           |
| Ever smoker     | 254                           |
| 1 allele        |                               |
| Never smoker    | 209                           |
| Ever smoker     | 317                           |
| 2 alleles       |                               |
| Never smoker    | 73                            |
| Ever smoker     | 111                           |
| RF-positive     |                               |
| 0 alleles       |                               |
| Never smoker    | 128                           |
| Ever smoker     | 296                           |
| 1 allele        |                               |
| Never smoker    | 313                           |
| Ever smoker     | 808                           |
| 2 alleles       |                               |
| Never smoker    | 192                           |
| Ever smoker     | 496                           |
| Anti-CCP2 = anti–cyclic citrullinated peptide 2; RA = rheumatoid arthritis; RF = rheumatoid factor; SE = shared epitope.
variable for cumulative dose of smoking (expressed in pack-years) in a logistic regression model.

In addition, we investigated the interaction between smoking and SE genes with regard to each RA subset. The potential interaction was analyzed using departure from additivity of effects as the criterion for interaction and was evaluated by calculating the attributable proportion due to interaction (with 95% CIs) (11, 12).

All analyses were adjusted for age, sex, residential area, ancestry, and study. Assessment of ancestry was based on whether or not the subject was born in Sweden, and whether or not either of the subject’s parents had immigrated to Sweden. A subject who was born in Sweden and whose parents had not immigrated was classified as Swedish. Adjustments were also made for educational level (university degree or no university degree), exposure to passive smoking (yes or no), alcohol consumption (number of standardized drinks per week at study inclusion), and body mass index at inclusion in the study (<25 kg/m² or >25 kg/m²). However, these factors had only a minor influence on the results and were therefore not retained in the final analyses. All analyses were conducted using SAS software version 9.4 (SAS Institute).

RESULTS

The majority of the patients with incident RA were both RF positive and ACPA positive (57%), whereas 25% were negative for both classes of antibodies. Nine percent of patients were ACPA positive only, and 9% were RF positive only. The characteristics of the cases and controls are presented in Supplementary Table 1 (on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40852/abstract). There were no significant

| Table 2. | Odds of developing rheumatoid arthritis, stratified by serologic subset, according to different categories of smokers compared with never smokers, in total and by cumulative dose of smoking* |
|---|---|---|---|---|
| | Anti-CCP2−, RF− | Anti-CCP2+, RF− | Anti-CCP2−, RF+ | Anti-CCP2+, RF+ |
| No. cases/no. controls† | OR (95% CI) | No. cases/no. controls† | OR (95% CI) | No. cases/no. controls† | OR (95% CI) | No. cases/no. controls† | OR (95% CI) |
| Total | | | | | | | |
| Never smoker | 367/2,655 | 1.0 (reference) | 133/2,655 | 1.0 (reference) | 111/2,655 | 1.0 (reference) | 594/2,655 | 1.0 (reference) |
| Past smoker | 330/1,909 | 1.1 (0.9−1.4) | 109/1,909 | 1.2 (0.98−1.6) | 116/1,909 | 1.3 (1.02−1.8) | 774/1,909 | 1.8 (1.6−2.1) |
| Current smoker | 218/1,319 | 1.1 (0.9−1.3) | 89/1,319 | 1.3 (1.004−1.7) | 109/1,319 | 1.8 (1.3−2.3) | 695/1,319 | 2.4 (2.1−2.7) |
| <10 pack-years | | | | | | | |
| Never smoker | 367/2,655 | 1.0 (reference) | 133/2,655 | 1.0 (reference) | 111/2,655 | 1.0 (reference) | 594/2,655 | 1.0 (reference) |
| Past smoker | 179/1,062 | 1.1 (0.9−1.4) | 58/1,062 | 1.1 (0.8−1.5) | 62/1,062 | 1.2 (0.9−1.7) | 320/1,062 | 1.2 (1.001−1.5) |
| Current smoker | 68/476 | 1.1 (0.8−1.5) | 28/476 | 1.0 (0.7−1.3) | 28/476 | 1.2 (0.8−1.8) | 148/476 | 1.3 (1.1−1.5) |
| 10−20 pack-years | | | | | | | |
| Never smoker | 367/2,655 | 1.0 (reference) | 133/2,655 | 1.0 (reference) | 111/2,655 | 1.0 (reference) | 594/2,655 | 1.0 (reference) |
| Past smoker | 79/495 | 1.0 (0.7−1.2) | 30/495 | 1.3 (0.9−2.0) | 25/495 | 1.2 (0.8−1.9) | 205/495 | 1.9 (1.6−2.3) |
| Current smoker | 52/325 | 1.1 (0.8−1.5) | 26/325 | 1.6 (1.04−2.5) | 37/325 | 2.4 (1.6−3.6) | 201/325 | 2.7 (2.2−3.3) |
| >20 pack-years | | | | | | | |
| Never smoker | 367/2,655 | 1.0 (reference) | 133/2,655 | 1.0 (reference) | 111/2,655 | 1.0 (reference) | 594/2,655 | 1.0 (reference) |
| Past smoker | 72/352 | 1.2 (0.9−1.6) | 21/352 | 1.5 (0.9−2.4) | 29/352 | 1.9 (1.3−2.7) | 249/352 | 3.1 (2.6−3.7) |
| Current smoker | 98/518 | 1.2 (0.9−1.5) | 35/518 | 1.6 (1.05−2.4) | 44/518 | 2.0 (1.2−3.1) | 346/518 | 3.6 (2.9−4.4) |

* All analyses were adjusted for age, sex, residential area, and ancestry. Anti-CCP2 = anti–cyclic citrullinated peptide 2; RF = rheumatoid factor; OR = odds ratio; 95% CI = 95% confidence interval.
† Values are the number of exposed cases and controls.
differences between the RA subsets with regard to age, sex, or ancestry.

Among the RA patients, ACPA positivity independently correlated with both the HLA–DRB1 SE status and the RF status (Table 1). Smoking habits did not significantly influence these correlations, but there was a significant dose-dependent relationship between smoking and RF positivity irrespective of anti-CCP2 and HLA–DRB1 SE status \( (P < 0.0001) \).

Compared with never smokers, the overall OR for developing RF-/anti-CCP2− RA among ever smokers was 1.1 (95% CI 0.96–1.3). The corresponding ORs for the other subsets of patients who were ever smokers were as follows: for RF-/anti-CCP2+ RA, OR 1.2 (95% CI 0.98–1.6); for RF+/anti-CCP2− RA, OR 1.6 (95% CI 1.2–1.9); for RF+/anti-CCP2+ RA, OR 2.0 (95% CI 1.8–2.2) (Table 2). The association between smoking and risk of RA increased numerically with increasing exposure to smoking (i.e., increasing pack-years of smoking) in all 3 antibody-dependent subsets, but was largest in the subsets positive for RF \( (P \) for trend < 0.0001 in the RF-positive subsets).

The influence of smoking on the risk of developing RF+/anti-CCP2+ RA increased significantly with the number of SE alleles \( (P \) for trend < 0.0001) (Table 3).

The risk of RA conferred by HLA–DRB1 SE seropositivity was mainly observed in anti-CCP2+ RA patients, irrespective of RF status (Table 4). The interaction between smoking and HLA–DRB1 SE genes, measured as the attributable proportion due to interaction, was highest in the subset positive for both RF and anti-CCP2, but a notable interaction was observed also in the RF-/anti-CCP2+ RA subset for the group consisting of individuals who had smoked more than 10 pack-years (Table 4). This interaction was also stronger among HLA–DRB1 SE homozygotes than among HLA–DRB1 SE heterozygotes (Table 5). No significant interaction was observed between smoking and HLA–DRB1 SE genes with regard to the risk of anti-CCP2− RA, regardless of RF status (Table 4).

A summary of the risk of RA conferred by smoking and the presence of the HLA–DR SE in the 4 different serologically defined subsets of RA patients is provided in Figure 1.

### Table 3. Odds of developing rheumatoid arthritis, stratified by serologic subset, according to HLA SE status and different categories of smokers compared with never smokers*

| HLA SE status | Anti-CCP2−, RF− | Anti-CCP2+, RF− | Anti-CCP2−, RF+ | Anti-CCP2+, RF+ |
|---------------|----------------|----------------|----------------|----------------|
|               | No. cases/no. controls† | OR (95% CI) | No. cases/no. controls† | OR (95% CI) | No. cases/no. controls† | OR (95% CI) | No. cases/no. controls† | OR (95% CI) |
| HLA SE negative |                 |               |                 |               |
| Never smoker  | 161/592 (0.96–1.3) | 1.0 (reference) | 18/592 (0.96–1.3) | 1.0 (reference) | 42/592 (0.96–1.3) | 1.0 (reference) | 86/592 (0.96–1.3) | 1.0 (reference) |
| Past smoker   | 142/501 (0.71–1.3) | 1.0 (0.71–1.3) | 12/501 (0.71–1.3) | 1.0 (0.71–1.3) | 43/501 (1.0–2.3) | 1.0 (0.71–1.3) | 117/501 (1.0–2.3) | 1.0 (0.71–1.3) |
| Current smoker| 89/263 (1.0–2.3) | 1.2 (0.9–1.6) | 11/263 (1.0–2.3) | 1.3 (0.9–1.6) | 48/263 (1.0–2.3) | 2.4 (1.5–3.7) | 88/263 (1.0–2.3) | 2.3 (1.6–3.2) |
| HLA SE heterozygote |     |               |                 |               |
| Never smoker  | 151/521 (0.71–1.3) | 1.0 (0.71–1.3) | 59/521 (0.71–1.3) | 1.0 (0.71–1.3) | 49/521 (1.0–2.3) | 1.0 (0.71–1.3) | 265/521 (1.0–2.3) | 1.0 (0.71–1.3) |
| Past smoker   | 125/464 (0.71–1.3) | 0.9 (0.71–1.3) | 58/464 (0.71–1.3) | 1.2 (0.71–1.3) | 58/464 (1.0–2.3) | 1.6 (0.9–2.0) | 369/464 (1.0–2.3) | 1.6 (1.3–2.0) |
| Current smoker| 95/272 (1.0–2.3) | 1.1 (0.8–1.5) | 46/272 (1.0–2.3) | 1.5 (0.97–2.3) | 49/272 (1.0–2.3) | 1.7 (1.1–2.7) | 336/272 (1.0–2.3) | 2.4 (1.9–2.9) |
| HLA SE homozygote |            |               |                 |               |
| Never smoker  | 35/137 (1.0–2.3) | 1.0 (0.9–2.3) | 38/137 (1.0–2.3) | 1.0 (0.9–2.3) | 15/137 (1.0–2.3) | 1.0 (0.9–2.3) | 177/137 (1.0–2.3) | 1.0 (0.9–2.3) |
| Past smoker   | 35/92 (1.0–2.3) | 1.3 (0.9–2.6) | 29/92 (1.0–2.3) | 1.3 (0.9–2.6) | 11/92 (1.0–2.3) | 1.1 (0.5–2.5) | 238/92 (1.0–2.3) | 2.2 (1.6–3.1) |
| Current smoker| 16/60 (1.0–2.3) | 1.0 (0.6–2.0) | 31/60 (1.0–2.3) | 1.9 (1.0–2.4) | 12/60 (1.0–2.3) | 1.8 (0.8–3.9) | 235/60 (1.0–2.3) | 3.2 (2.2–4.6) |

* All analyses were adjusted for age, sex, residential area, and ancestry. SE = shared epitope; anti-CCP2 = anti-cyclic citrullinated peptide 2; RF = rheumatoid factor; OR = odds ratio; 95% CI = 95% confidence interval.
† Values are the number of exposed cases and controls.
Table 4. Interaction between the HLA SE and smoking in relation to odds of developing rheumatoid arthritis*

|                                | Anti-CCP2−, RF− | Anti-CCP2+, RF− | Anti-CCP2−, RF+ | Anti-CCP2+, RF+ |
|                                | No. cases/no. controls† | OR (95% CI)     | No. cases/no. controls† | OR (95% CI)     | No. cases/no. controls† | OR (95% CI)     | No. cases/no. controls† | OR (95% CI)     |
| Total                          |                  |                  |                  |                  |
| HLA SE negative               |                  |                  |                  |                  |
| Never smoker                  | 161/592          | 1.0 (reference)  | 18/592           | 1.0 (reference)  | 42/592           | 1.0 (reference)  | 86/592           | 1.0 (reference)  |
| Ever smoker                   | 231/764          | 1.1 (0.9, 1.4)   | 23/764           | 1.1 (0.6, 2.0)   | 91/764           | 1.8 (1.2, 2.6)   | 205/764          | 1.9 (1.5, 2.5)   |
| HLA SE positive               |                  |                  |                  |                  |
| Never smoker                  | 184/636          | 1.0 (0.8, 1.3)   | 97/636           | 5.0 (3.0, 8.4)   | 63/636           | 1.4 (0.9, 2.1)   | 441/636          | 4.8 (3.7, 6.1)   |
| Ever smoker                   | 261/848          | 1.1 (0.9, 1.4)   | 160/848          | 6.7 (4.0, 11.1)  | 126/848          | 2.2 (1.5, 3.1)   | 1166/848         | 10.0 (7.8, 12.8) |
| AP†                           | 0.03 (−0.3, 0.3) | 0.2 (0.02, 0.5)  | 0.002 (−0.3, 0.3) | 0.4 (0.3, 0.5)   |
| <10 pack-years of smoking     |                  |                  |                  |                  |
| HLA SE negative               |                  |                  |                  |                  |
| 0 pack-years                  | 161/592          | 1.0 (reference)  | 18/592           | 1.0 (reference)  | 42/592           | 1.0 (reference)  | 86/592           | 1.0 (reference)  |
| <10 pack-years                | 101/340          | 1.1 (0.8, 1.4)   | 10/340           | 1.0 (0.5, 2.0)   | 35/340           | 1.4 (0.8, 2.2)   | 62/340           | 1.2 (0.8, 1.7)   |
| HLA SE positive               |                  |                  |                  |                  |
| 0 pack-years                  | 184/636          | 1.0 (0.8, 1.3)   | 97/636           | 5.0 (3.0, 8.4)   | 63/636           | 1.4 (0.9, 2.1)   | 441/636          | 4.8 (3.7, 6.2)   |
| <10 pack-years                | 119/402          | 1.1 (0.8, 1.4)   | 68/402           | 5.1 (3.0, 8.8)   | 52/402           | 1.7 (1.1, 2.6)   | 374/402          | 6.3 (4.8, 8.3)   |
| AP†                           | −0.06 (−0.5, 0.3) | 0.04 (−0.3, 0.4) | −0.02 (−0.5, 0.5) | 0.2 (0.05, 0.4)  |
| 10–20 pack-years of smoking   |                  |                  |                  |                  |
| HLA SE negative               |                  |                  |                  |                  |
| 0 pack-years                  | 161/592          | 1.0 (reference)  | 18/592           | 1.0 (reference)  | 42/592           | 1.0 (reference)  | 86/592           | 1.0 (reference)  |
| 10–20 pack-years              | 59/198           | 1.1 (0.8, 1.5)   | 6/198            | 1.2 (0.5, 3.0)   | 25/198           | 2.0 (1.2, 3.5)   | 59/198           | 2.3 (1.6, 3.4)   |
| HLA SE positive               |                  |                  |                  |                  |
| 0 pack-years                  | 184/636          | 1.0 (0.8, 1.3)   | 97/636           | 5.0 (3.0, 8.4)   | 63/636           | 1.4 (0.9, 2.1)   | 441/636          | 4.8 (3.7, 6.3)   |
| 10–20 pack-years              | 61/220           | 1.0 (0.7, 1.4)   | 47/220           | 8.5 (4.8, 15.3)  | 33/220           | 2.4 (1.4, 3.9)   | 317/220          | 11.4 (8.5, 15.3) |
| AP†                           | −0.09 (−0.6, 0.4) | 0.4 (0.1, 0.7)   | −0.06 (−0.6, 0.5) | 0.5 (0.3, 0.6)   |
| >20 pack-years of smoking     |                  |                  |                  |                  |
| HLA SE negative               |                  |                  |                  |                  |
| 0 pack-years                  | 161/592          | 1.0 (reference)  | 18/592           | 1.0 (reference)  | 42/592           | 1.0 (reference)  | 86/592           | 1.0 (reference)  |
| >20 pack-years                | 71/226           | 1.2 (0.8, 1.6)   | 7/226            | 1.5 (0.6, 3.6)   | 31/226           | 2.5 (1.5, 4.1)   | 84/226           | 3.3 (2.3, 4.6)   |
| HLA SE positive               |                  |                  |                  |                  |
| 0 pack-years                  | 184/636          | 1.1 (0.8, 1.3)   | 97/636           | 5.0 (3.0, 8.4)   | 63/636           | 1.4 (0.9, 2.1)   | 441/636          | 4.8 (3.7, 6.2)   |
| >20 pack-years                | 81/226           | 1.3 (0.98, 1.8)  | 45/226           | 9.2 (5.1, 16.6)  | 41/226           | 3.1 (2.0, 5.0)   | 475/226          | 18.2 (14, 24)    |
| AP†                           | 0.09 (−0.3, 0.5) | 0.4 (0.1, 0.7)   | 0.09 (−0.4, 0.5) | 0.6 (0.5, 0.7)   |

* All analyses were adjusted for age, sex, residential area, and ancestry. Anti-CCP2 = anti–cyclic citrullinated peptide 2; RF = rheumatoid factor; OR = odds ratio; 95% CI = 95% confidence interval.
† Values are the number of exposed cases and controls.
‡ Attributable proportion (AP) due to interaction between the HLA shared epitope (SE) and smoking.
**DISCUSSION**

The main finding of the present study is that the impact of smoking and HLA-DRB1 genes and their interaction with regard to risk of RA varied between the 4 serologically defined subsets of RA. It has previously been difficult to distinguish the association between the HLA-DRB1 SE and ACPAs from the unique association between smoking and RF, due to the fact that sample sizes have been limited. By using well-defined serologic subsets of RA, we have demonstrated that smoking is indeed a prominent risk factor for RF+/anti-CCP2− RA, whereas the effect of smoking is more limited, but still existing, in RF−/anti-CCP2+ RA. Our results are consistent with those of several previous studies in which it was suggested that smoking may generate RF and ACPAs as well as other autoantibodies in RA (13). The situation with regard to the association with the HLA-DRB1 SE was even more obvious. A clear association with the class II genes was observed for both anti-CCP2–positive RA subsets irrespective of RF status, whereas no association was observed for the RF+/anti-CCP2− RA subset.

Another recent study showed that RF levels are associated with ACPA positivity irrespective of smoking history, and noted that there seemed to be a difference in the importance of the number of SE alleles in determining ACPA positivity between RF-negative and RF-positive RA patients (10). The results of the present study confirmed a correlation between RF and anti-CCP2 positivity in RA patients that was independent of smoking habits. However, the correlation was present regardless of HLA-DRB1 SE status (P < 0.0001).

A question that can now be addressed more distinctly than before is how smoking may be involved in the induction of RF and ACPAs (herein measured as anti-CCP2 antibodies) in individuals with different genetic setups. Our study in the RF+/anti-CCP2– RA subset clearly showed that smoking may induce RF independent of both the HLA-DRB1 SE and presence/induction of ACPAs. This is consistent with findings in previous studies in healthy individuals, which showed that smoking can induce RF production (14). This lack of relationship with the HLA-DRB1 SE status is also compatible with the notion of T cell–independent triggering mechanisms, as demonstrated recently by the findings of a low number of T cell–mediated somatic mutations in single RF-producing B cells from RA patients (15). The situation was substantially different in the RF−/anti-CCP2+ RA group, in whom there was a major and gene dose–dependent effect of HLA-DRB1 and a more limited, but still visible, effect of smoking, particularly in heavy smokers. This finding is compatible with prior reports of high numbers of T cell–dependent somatic mutations in genes coding for anticitrulline-reactive antibodies (15–17).

The situation in the major subset of RA patients who were positive for both RF and anti-CCP2 was also different, with major effects of both smoking and the HLA-DRB1 SE and with a pronounced interaction between the 2 risk factors. Thus, the challenge is to understand the mechanisms that explain why the gene–environment interaction between the HLA-DRB1 SE and smoking is most pronounced in conjunction with the presence of both RF and anti-CCP2 antibodies. As previously described, the data suggest that class II major histocompatibility complex (MHC)–dependent immunity may be triggered at sites in which smoke primarily interacts with the immune system, i.e., in the lungs and related mucosal tissues (18,19). A more precise molecular definition with regard to which structures in the HLA-DRB1 molecule are involved in this interaction has also been provided recently (20).

An obvious hypothesis for a triggering scenario would be that RF generated by T cell–independent effects of smoking (14) would enhance class II MHC–dependent T cell activation against

| Table 5. Odds of developing rheumatoid arthritis, stratified by serologic subset, in subjects categorized by the number of HLA SE alleles and smoking status* |

|                | Anti-CCP2+, RF− | Anti-CCP2+, RF+ |
|----------------|-----------------|-----------------|
|                | No. cases/no.   | OR (95% CI)     | AP† |
| HLA SE         | controls†       |                 |     |
| 0 alleles      |                 |                 |     |
| Never smoker   | 18/592          | 1.0 (reference) |     |
| Ever smoker    | 23/764          | 1.1 (0.6–2.0)   |     |
| 1 allele       |                 |                 |     |
| Never smoker   | 59/501          | 4.0 (2.3–6.8)   |     |
| Ever smoker    | 100/697         | 5.3 (3.2–8.8)   | 0.2 (0.02–0.5) |
| 2 alleles      |                 |                 |     |
| Never smoker   | 38/135          | 7.8 (5.4–17.8)  |     |
| Ever smoker    | 60/151          | 14.0 (8.0–24.3) | 0.3 (0.04–0.7) |
|                |                 |                 |     |
|                |                 | 86/592          | 1.0 (reference) |
|                |                 | 205/764         | 1.8 (1.4–2.4)   |
|                |                 | 264/501         | 3.6 (2.7–4.7)   |
|                |                 | 693/697         | 6.9 (5.3–8.8)   |
|                |                 | 473/151         | 21.5 (16.0–28.8) |

* Anti-CCP2 = anti–cyclic citrullinated peptide 2; RF = rheumatoid factor; OR = odds ratio; 95% CI = 95% confidence interval. † Values are the number of exposed cases and controls. ‡ Attributable proportion (AP) due to interaction between the HLA shared epitope (SE) and ever smoking.
citrullinated proteins at mucosal sites where smoke encounters the immune system. Such enhancing effects on antigen presentation from RF and other mechanisms that generate immune complexes are well known (21). This scenario may be further strengthened from the generation of RF due to reactivity of T cells against antigens present in local immune complexes (22). Taken together, these data suggest that the dramatic interaction between the HLA–DRB1 SE and smoking in conferring a risk of RF-positive RA (8) and ACPA-positive RA (6,23) requires the simultaneous presence of both of these antibodies.

Interestingly, the presence of both RF and ACPAs also appears to provide the highest risk for subsequent development of RA in antibody-positive, but still nonarthritic, individuals (24,25). The synergizing effects between ACPAs, RF, and immune complexes have also been described in models of effector phases of joint inflammation in RA (26–28). All of these prior studies addressed in vitro–formed immune complexes, but the first report of an evaluation of ACPA-containing immune complexes obtained in vivo was recently published (29).

Our study was designed as a case–control study with incident RA cases, and information regarding smoking habits and exposure to passive smoking was collected retrospectively. Recall bias was minimized by using incident cases of RA. We took great effort to obtain information on lifestyle factors and environmental exposures from the RA patients in a way that was identical to that used for the controls. Furthermore, the questionnaire contained a wide range of questions regarding many potential environmental risk factors, and no section in the questionnaire was given prime focus.

A potential selection bias may arise when recruiting cases and controls. The proportion of respondents with regard to participation in the EIRA study was 92% for cases and 75% for controls. Since the structure of the Swedish public health care system provides equal access to medical services for all Swedish citizens, it is most likely that almost all cases of RA are referred to public rheumatology units, and it is not likely that the few unidentified cases would cause a substantial bias in our calculations. Selection bias among controls is likely to be modest, since the prevalence of smoking among controls, seen as an indicator of lifestyle, was consistent with that observed in the general population at equivalent ages (30).

In summary, our findings describe how smoking and the HLA–DRB1 SE may play different roles in the pathogenesis of different serologically defined subsets of RA, and that RF and ACPAs appear to act together in both the triggering and the effector phases in the major RF+/ACPA+ subset of RA.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version for publication. Dr. Hedström had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Rönneklid, Klareskog, Alfredsson.

Acquisition of data. Klareskog, Alfredsson.

Analysis and interpretation of data. Hedström.

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