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A New Model for an Etiology of Rheumatoid Arthritis

Smoking May Trigger HLA–DR (Shared Epitope)–Restricted Immune Reactions to Autoantigens Modified by Citrullination

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Objective. **To investigate whether smoking and HLA–DR shared epitope (SE) genes may interact in triggering immune reactions to citrulline-modified proteins.**

Methods. **In a case–control study involving patients with recent-onset rheumatoid arthritis (RA), we studied interactions between a major environmental risk factor (smoking), major susceptibility genes included in the SE of HLA–DR, and the presence of the most specific autoimmunity known for RA (i.e., antibodies to proteins modified by citrullination). Immunostaining for citrullinated proteins in cells from bronchoalveolar lavage fluid was used to investigate whether smoking is associated with citrullination in the lungs.**

Results. **Previous smoking was dose-dependently associated with occurrence of anticitrulline antibodies in RA patients. The presence of SE genes was a risk factor only for anticitrulline-positive RA, and not for anticitrulline-negative RA. A major gene–environment interaction between smoking and HLA–DR SE genes was evident for anticitrulline-positive RA, but not for anticitrulline-negative RA, and the combination of smoking history and the presence of double copies of HLA–DR SE genes increased the risk for RA 21-fold compared with the risk among nonsmokers carrying no SE genes. Positive immunostaining for citrullinated proteins was recorded in bronchoalveolar lavage cells from smokers but not in those from nonsmokers.**

Conclusion. **We identified an environmental factor, smoking, that in the context of HLA–DR SE genes may trigger RA-specific immune reactions to citrullinated proteins. These data thus suggest an etiology involving a specific genotype, an environmental provocation, and the induction of specific autoimmunity, all restricted to a distinct subset of RA.**

Rheumatoid arthritis (RA) is one of the complex immune-mediated diseases for which an understanding of the etiology is dependent on the definition of environmental triggers that, in a restricted genetic context, may initiate immune reactions having the potential to contribute to disease development. To date, no consistent hypothesis containing all these elements has been formulated for RA or for most other complex immune diseases. However, compelling data have recently been accumulated that provide us with the possibility of formulating such a hypothesis, at least for some cases of

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RA. These background data are as follows: 1) Smoking is the major known environmental risk factor for RA, as is evident from epidemiologic studies (1–5), although little is known about the mechanisms involved. 2) HLA–DR shared epitope (SE) genes comprise the major genetic risk factors for RA (6,7), although again little is known about how these genes restrict development of potentially arthritogenic immune reactions. 3) A dramatic gene–environment interaction between smoking and HLA–DR SE genes as risk factors for RA was described by us, and notably both of these risk factors were seen exclusively in rheumatoid factor (RF)–positive RA (8). However, no good biologic hypothesis that might explain this interaction has been provided. 4) Antibodies to autoantigens modified by citrullination through deimination of arginine to citrulline are present in about two-thirds of all RA patients but are rare (<2%) in healthy individuals and relatively rare in other inflammatory conditions (9–11).

Since antibodies to citrullinated peptides have been demonstrated to precede development of RA by several years (12–15), anticitrulline immunity has increasingly been hypothesized to be causatively involved in the development of RA (16,17). Citrullination of a rat autoantigen (type II collagen) has also been demonstrated to render this antigen more arthritogenic than the unmodified molecule (18). It remains undefined, however, how smoking, HLA–DR SE genes, and immunoreactivity to citrullinated autoantigens may act in concert to contribute to the development of RA.

In order to generate new data that might help us to formulate an etiologic hypothesis on the basis of the abovementioned background data, we performed a series of epidemiologic and experimental studies, initially investigating effects of smoking on anticitrulline immunity. Since these experiments demonstrated that smoking was associated with both citrullination and anticitrulline immunity, we investigated the interaction between smoking and HLA–DR SE genotypes with regard to development of RA. By using a large case–control study involving patients with early RA, we were able to show a dramatic gene–environment interaction between smoking and HLA–DR SE genes in the development of anticitrulline antibody–positive RA. In contrast, no such interaction could be demonstrated in anticitrulline antibody–negative RA. Taken together, these data permitted us to formulate a hypothesis on how smoking may act together with genetic factors and the immune system in being a possible causative agent for RA.

SUBJECTS AND METHODS

The study was based on data and materials from 3 different groups of individuals (RA patients and controls). Ethical permits for the studies were obtained from ethical review committees at the sites where patients were recruited, and all patients and controls gave informed consent for their participation in the studies.

Individuals participating in a case–control study of gene–environment interactions in RA in Sweden (the Epidemiological Investigation of Rheumatoid Arthritis [EIRA] study). A population-based case–control study was conducted in which cases were individuals ages 18–70 years with newly diagnosed RA (5,8). Disease was determined according to the 1987 revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (19) by a rheumatologist, within 12 months after the first symptoms of joint disease. All rheumatology units within the catchment area participated in the study, assuring that almost all newly diagnosed RA cases in this area were included. A list of EIRA Study Group members (and their contributing rheumatology centers in Sweden) is provided in Appendix A.

Controls were randomly selected from the Swedish national population registry, with consideration given to age, sex, and residential area (see ref. 5). Sera and cells for serologic analysis and DNA preparation were obtained from the patients during their first visit to the rheumatology department (i.e., before institution of disease-modifying antirheumatic drugs). Sera and cells from controls were obtained from local health care units which sent blood samples to the rheumatology laboratory at Karolinska Institutet. Of the 967 identified patients with RA, 930 (96%) completed a questionnaire (654 women and 276 men) (5). The total number of identified controls was 1,357, and the response rate concerning completion of the questionnaire by these individuals was 83% overall, yielding 1,126 controls (791 women and 335 men). We received blood samples from all of the patients who answered the questionnaires and from 658 of the controls (58%) (473 women and 185 men). Data on SE and anticitrulline antibodies could be retrieved for 913 patients. Data on SE could be retrieved for 631 controls.

Information regarding environmental exposures among patients and controls was obtained by questionnaire. The detailed smoking history included information such as when in life smoking was begun and, if applicable, when it was terminated. The quantification of smoking has previously been described in detail (5) and was based on the smoking history before first symptoms of arthritis among patients and with regard to a corresponding time point among controls. Subjects who had smoked a pipe or cigars were excluded (81 patients and 48 controls), leaving cigarette smokers and those who had never smoked for analysis.

Controls from the blood bank of northern Sweden. In order to study events occurring before onset of RA, we used a population-based blood bank from northern Sweden (the Northern Sweden Health and Disease Study Cohort), which contains sera and cells together with data regarding environmental exposures from ~90,000 individuals in the county of Västerbotten. Blood samples have been included in this bank since 1989. In a study by Rantapää-Dahlqvist et al, the "pre-RA plasma samples" of individuals who developed RA in

and after 1996 were demonstrated to contain a high frequency of anticitrulline antibodies (13). In the present study, we investigated 383 controls matched to these RA patients for whom both blood samples and information regarding smoking habits were available, the latter by means of questionnaires collected at the time of plasma sampling.

Individuals subjected to bronchoalveolar lavage. Eight healthy individuals with normal findings on chest radiographs underwent bronchoscopy with bronchoalveolar lavage as previously described (20). Four (median age 46 years, 2 women) were current smokers who had smoked for a range of 16–30 pack-years. Four (median age 25 years, 2 women) were nonsmokers. In addition, bronchoalveolar lavage was performed in 3 women and 1 man (median age 40 years) diagnosed as having pulmonary inflammatory conditions (i.e., sarcoidosis, Langerhans cell histiocytosis, and respiratory bronchiolitis). All were current smokers who had smoked for a range of 10–30 pack-years.

Bronchoalveolar lavage cell preparation and investigation of citrullination in bronchoalveolar lavage cells. Bronchoalveolar lavage cells were prepared as previously described (20) and tested for viability with trypan blue (median cell viability was 95%). Cytocentrifuge slides with these cells were immunohistochemically stained using rabbit antibodies targeting chemically modified citrulline, using a previously described procedure (21,22). Slides were evaluated by microscopy. Quantification of stained cells was performed in a blinded manner by 1 experienced observer, and results were independently verified by 2 additional investigators (KL and A-KU).

Detection of antibodies to citrulline-containing peptides and RFs. Anticitrulline antibodies were assayed using the Immunoscan-RA Mark2 ELISA test (Euro-Diagnostica, Malmo, Sweden). All collected and frozen sera were investigated simultaneously. All samples yielding high values were further diluted to obtain definite values. A level >25 units/ml was regarded as being positive according to instructions in the kit and corroborated by validation at the clinical immunology laboratory in Uppsala, Sweden. RF in serum was determined by nephelometry. The analysis was standardized using the international standard National Institute for Biological Standards Control 64/002, and the cutoff was set to 20.

Genotyping. Genotyping for HLA–DRB1 allotypes was conducted using the sequence-specific primer–polymerase chain reaction method as previously described (8,23). Among HLA–DRB1 genes, DRB1*01, DRB1*04, and DRB1*10 genes were defined as "SE genes" (6,7). Eighty-one patients were subtyped for identification of HLA–DRB1*01 and DRB1*04 alleles. We determined a frequency of DRB1*0101 of 89% (in those with HLA–DRB1*01) and a frequency of DRB1*0401;*0404;*0405;*0408 alleles of 98% (in those with HLA–DRB1*04), and we restricted further genotyping to only DR low-resolution analysis for practical reasons (8). Any genotype with a combination of 2 of these genes was thus considered a double-SE genotype in all further analyses.

Potential confounding factors. In the case–control analysis, we adjusted for age, sex, and area of residence according to the principle of control selection. Furthermore, social class, body mass index (BMI), marital status, parity, and oral contraceptive use were considered potential confounding factors.

Statistical analysis. Odds ratios (ORs) were calculated with 95% confidence intervals (95% CIs) by means of logistic regression analysis. ORs were interpreted as relative risks (RRs), since the study was population based and the controls constituted a random sample from the study base (24). Adjustments for social class, BMI, marital status, parity, and oral contraceptive use had minor influences on the results of the study and were therefore not retained in the final analyses. Interaction between genotype and smoking was evaluated using departure from additivity of effects as the criterion of interaction, as suggested by Rothman et al (25). To quantify the amount of interaction, the attributable proportion due to interaction (AP) was calculated together with the 95% CI (26). The AP, which is assigned a value between 0 and 1, is the proportion of the incidence among persons exposed to 2 interacting factors that is attributable to the interaction per se (i.e., reflecting their joint effect beyond the sum of their independent effects). All analyses were conducted using the SAS software package, version 8.2 (SAS Institute, Cary, NC) (27).

RESULTS

Relationship between smoking and presence of anticitrulline antibodies. We investigated the relationship between different smoking habits before onset of RA and presence of anticitrulline immunity at the time of diagnosis of RA. A dose-dependent relationship was determined between extent of smoking counted as pack-years and frequency of elevated anticitrulline antibody levels at onset of RA (Table 1).

Table 1. Number and proportion of RA patients with elevated levels of anti-CCP antibodies (>25 units/ml) by amount of cigarette smoking and by SE status*

	No. of anti-CCP+ RA patients/total no. of RA patients (%)	*P*
Amount of cigarette smoking		
Never	147/275 (53)	
0–5 pack-years	59/94 (63)	0.02†
>5–20 pack-years	157/250 (63)	<0.01†
>20 pack-years	159/209 (76)	<0.01†‡
Total	522/828 (63)	
SE type		
No SE	85/248 (34)	
Single SE	292/448 (65)	0.02§
Double SE	178/217 (82)	<0.01§
Total	555/913 (61)	

* All comparisons are adjusted for age and sex. RA = rheumatoid arthritis; anti-CCP = anti–cyclic citrullinated peptide; SE = shared epitope.
† Versus patients who never smoked.
‡ Versus patients who smoked for >5–20 pack-years.
§ Versus patients with no SE.

Table 2. Risk of developing RA in subjects exposed to different combinations of smoking and SE genes compared with subjects who have never smoked and have no SE genes*

Sex, anti-CCP status, smoking history	No SE		Single SE		Double SE	
	No. of exposed cases/ no. of exposed controls	RR (95% CI)†	No. of exposed cases/ no. of exposed controls	RR (95% CI)†	No. of exposed cases/ no. of exposed controls	RR (95% CI)†
Male and female						
Anti-CCP+						
Never smoked	20/87	Referent	72/104	3.3 (1.8–5.9)	36/31	5.4 (2.7–10.8)
Ever smoked	58/184	1.5 (0.8–2.6)	192/146	6.5 (3.8–11.4)	126/31	21.0 (11.0–40.2)
Anti-CCP−						
Never smoked	65/87	Referent	64/104	0.8 (0.5–1.3)	18/31	0.7 (0.4–1.5)
Ever smoked	84/184	0.6 (0.4–1.0)	76/146	0.8 (0.5–1.2)	18/31	0.8 (0.4–1.7)
Female						
Anti-CCP+						
Never smoked	18/74	Referent	58/75	3.6 (1.9–6.8)	30/25	5.4 (2.5–11.5)
Ever smoked	41/115	1.5 (0.8–2.9)	130/109	6.0 (3.3–10.8)	89/25	19.0 (9.3–38.5)
Anti-CCP−						
Never smoked	50/74	Referent	45/75	0.8 (0.5–1.4)	15/25	0.8 (0.4–1.8)
Ever smoked	62/115	0.8 (0.5–1.4)	52/109	0.8 (0.5–1.3)	11/25	0.7 (0.3–1.5)
Male						
Anti-CCP+						
Never smoked	2/13	Referent	14/29	3.1 (0.4–21.7)	6/6	6.1 (0.7–50.6)
Ever smoked	17/69	1.9 (0.3–13.3)	63/37	15.1 (2.3–100.0)	37/6	59.2 (7.7–457.3)
Anti-CCP−						
Never smoked	15/13	Referent	19/29	0.6 (0.2–1.6)	3/6	0.4 (0.1–2.3)
Ever smoked	24/69	0.3 (0.1–0.7)	24/37	0.5 (0.2–1.4)	7/6	0.9 (0.2–3.8)

* For males and females, the attributable proportion due to interaction was 0.4 (95% confidence interval [95% CI] 0.2–0.7) for smoking and a single copy of the SE gene and 0.7 (95% CI 0.5–0.9) for smoking and 2 copies of the SE gene. RR = relative risk (see Table 1 for other definitions).
† Adjusted for age (10 strata), sex (males and females), and area of residence.

We then investigated the possible relationship between smoking and anticitrulline immunity in healthy subjects using controls from both the EIRA study and from the northern Sweden biobank. Of those who had ever smoked in these groups, 16 of 576 (2.78%) were anticitrulline antibody positive at the time of blood sampling, compared with 6 of 435 (1.38%) among those who had never smoked. However, this difference was

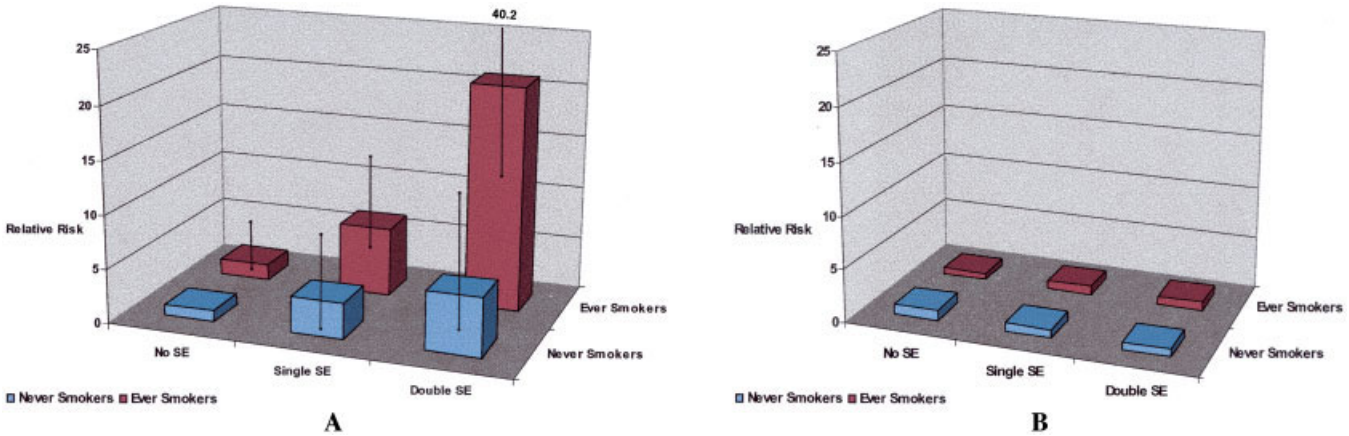

Figure 1. Relative risk of developing rheumatoid arthritis (RA) in subjects (men and women) exposed to different combinations of smoking and HLA–DR shared epitope (SE) genes (no copies, single copies, or double copies of SE genes) compared with those who never smoked and have no SE genes. **A,** Results in RA patients who are positive for anti–cyclic citrullinated peptide (anti-CCP) antibodies. **B,** Results in RA patients who are negative for anti-CCP antibodies. Error bars represent 95% confidence intervals (95% CIs). In **A,** the value of 40.2 represents the upper boundary of the 95% CI for smokers with double copies of SE genes.

Table 3. Proportion of cigarette smokers and individuals with SE genes among RA patients who were discordant regarding anti-CCP antibody status and RF status*

	Anti-CCP+, RF− (n = 68)	Anti-CCP−, RF+ (n = 109)	PR (95% CI)	*P*
Smoking	43 (63.2)	74 (67.9)	0.93 (0.75–1.16)	0.53
Single SE	40 (58.8)	45 (41.3)	1.42 (1.01–1.92)	0.02
Double SE	18 (26.5)	16 (14.7)	1.80 (0.99–3.29)	0.05
Any SE	58 (85.3)	61 (56.0)	1.52 (1.26–1.85)	0.0001

* Values are the number (%). RF = rheumatoid factor; PR = prevalence ratio comparing anti-CCP+, RF− RA patients with anti-CCP−, RF+ RA patients; 95% CI = 95% confidence interval (see Table 1 for other definitions).

not statistically significant ($P = 0.13$ by Fisher's exact test).

Relationship between carriage of HLA–DR SE genes and presence of anticitrulline antibodies. The frequency of anticitrulline antibodies was investigated in patients with no SE genes, a single SE gene copy, and double-copy SE genes. As shown in Table 1, the presence of SE genes strongly influenced the occurrence of anticitrulline antibodies.

HLA–DR SE genes and smoking are risk factors only for anticitrulline antibody–positive RA, not for anticitrulline antibody–negative RA. The presence of HLA–DR SE genes in a single copy as well as in double copies constituted a risk factor only for anticitrulline antibody–positive RA, and not for anticitrulline antibody–negative RA (Table 2 and Figure 1). Furthermore, the risk for RA conferred by smoking was entirely restricted to the anticitrulline antibody–positive subgroup of RA.

High risk of developing anticitrulline antibody–positive RA conferred by a gene–environment interaction between smoking and HLA–DR SE genes. The possible interaction between genetic and environmental risk factors was then investigated in the 2 serologically defined subsets of RA. As demonstrated in Table 2 and Figure 1A, a marked gene–environment interaction occurred between smoking and HLA–DR SE genes with regard to the risk of developing anticitrulline antibody–positive RA. Smoking conferred a small increased risk for this subtype of RA in SE-negative individuals (RR 1.5, 95% CI 0.8–2.6), while the addition of a single SE gene copy or double copies of SE genes increased the RR in smokers dramatically (i.e., to 6.5 [95% CI 3.8–11.4] in those carrying a single SE gene copy and to 21.0 [95% CI 11.0–40.2] in those carrying 2 copies of SE genes). This was compared with the RR in nonsmokers, which was 3.3 (95% CI 1.8–5.9) in those carrying a single SE gene copy and 5.4 (95% CI 2.7–10.8) in individuals carrying 2 SE gene copies.

The magnitude of the gene–environment interactions regarding anticitrulline-positive RA was demonstrated by the calculated APs, which were 0.4 (95% CI 0.2–0.7) for smoking and a single SE gene copy and 0.7 (95% CI 0.5–0.9) for smoking and 2 SE gene copies. When women and men were investigated separately with regard to the risk of developing anticitrulline antibody–positive RA (Table 2), the RRs were, in general, somewhat higher among men, but the 95% CIs were quite broad (especially due to the small number of exposed cases among those who had never smoked and were without SE genes).

Relationship of smoking and SE genes to anticitrulline antibodies and RF. The distribution of smokers and individuals with SE genes was investigated among RA patients who were discordant for anticitrulline antibodies and RF positivity. The presence of SE

Table 4. Percentage of citrulline-expressing bronchoalveolar lavage cells in cigarette smokers with pulmonary inflammation, healthy smokers, and healthy nonsmokers*

Smoking status	% citrulline cells
Smokers with pulmonary inflammation	
1	29.0
2	0.0
3	21.0
4	64.0
Mean	28.5
Healthy smokers	
1	0.0
2	15.0
3	14.0
4	26.0
Mean	13.75
Healthy nonsmokers	
1	0.0
2	0.0
3	0.0
4	0.0
Mean	0.0

* Bronchoalveolar lavage cells were analyzed by immunohistochemical staining using a rabbit anti–modified citrulline antibody. A minimum of 1,000 cells were evaluated in a blinded manner.

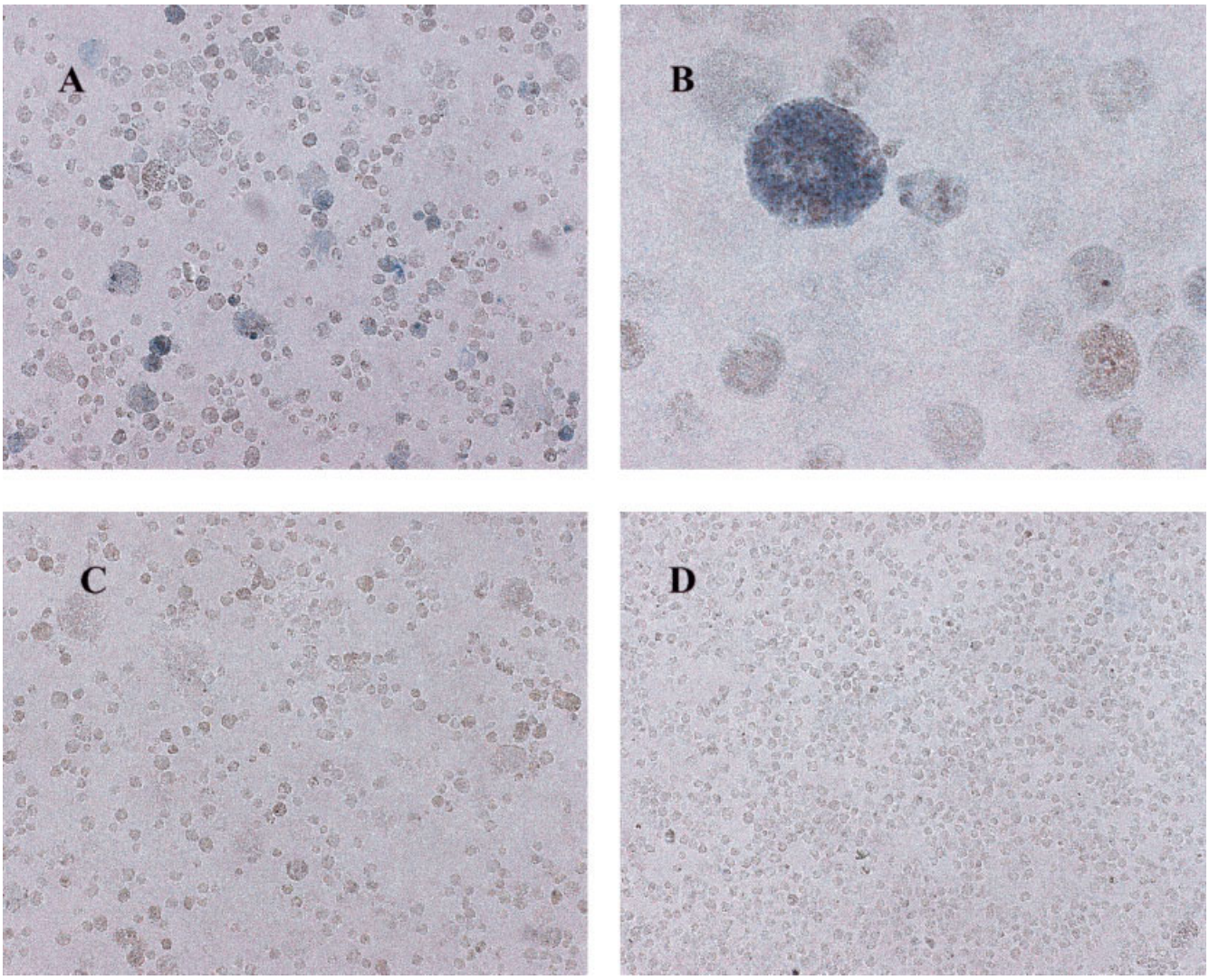

Figure 2. Presence of citrulline in bronchoalveolar lavage cells, analyzed by immunocytochemical staining using a rabbit anti–modified citrulline antibody on chemically modified cells from healthy smokers (**A** and **B**) and from a nonsmoker (**D**). Control stainings for **A** and **B** were performed in parallel on unmodified cells (**C**). (Original magnification × 10 in **A**, **C**, and **D**; × 40 in **B**.)

genes was primarily associated with the occurrence of anticitrulline antibodies rather than with RF positivity ($P = 0.0001$), while no such association was evident for smoking ($P = 0.53$) (Table 3).

Induction by smoking of citrullination in cells from bronchoalveolar lavage fluid. Cells were retrieved from bronchoalveolar lavage fluid from nonsmokers (healthy individuals) and smokers (healthy individuals and patients with pulmonary inflammatory conditions) and investigated for the presence of citrullinated proteins. Alveolar macrophages constituted 94% and 89% (mean values) of the bronchoalveolar lavage cells in smokers and nonsmokers, respectively. An average of 13.75% citrulline-positive bronchoalveolar lavage cells were detected in healthy smokers, while the average was even higher in smokers with pulmonary inflammation (28.5%). In none of the nonsmokers did we find any bronchoalveolar lavage cells that stained positively with antibodies to citrullinated proteins (Table 4 and Figure 2).

DISCUSSION

We present 3 new sets of data concerning the etiology of RA. First, we report that the classic genetic

risk factor, the presence of the HLA–DR SE, confers a risk only in the subgroup of RA patients who are positive for anticitrulline antibodies. Second, we show that the same restriction applies to the most well-known environmental risk factor, smoking. When these factors were combined, a major gene–environment interaction between HLA–DR SE genes and smoking was observed, with a 21-fold increased relative risk in smokers carrying 2 copies of the risk gene compared with nonsmokers without the risk gene. The third new observation is related to possible biologic mechanisms which may explain these findings from genetic epidemiology (i.e., that smoking may be associated with increased presence of citrulline-modified proteins in the lungs). With these findings taken together, an etiologic hypothesis can thus be formulated that involves genes, environment, and immunity to self molecules made immunogenic (and possibly arthritogenic) through posttranslational modifications induced by the environmental agent. Notably, the components of this putative series of events are present in one distinct subpopulation of RA patients, but not at all in another.

We used a case–control methodology to generate data regarding risk for disease as well as presence of anticitrulline antibodies in relation to HLA–DR SE genes and smoking. The problem of selection bias always encountered in case–control studies was minimized by the population-based design of the current study, by the very high frequency of participation among cases as well as among controls, and by the observation that smoking habits did not differ between controls with and without blood samples. This, and the fact that identification of controls was made by matching for age, sex, and area of residence in the same population that generated the cases, makes us confident that the results are highly reliable from a methodologic standpoint.

The remarkable gene–environment interaction observed in the case–control study, together with the findings of immunostaining of bronchoalveolar lavage cells, might now provide a clue to the molecular mechanisms of importance for disease development in a subset of RA patients. We would like to propose the following working hypothesis: Long-term exposure to cigarette smoke, and probably also to other environmental stimuli, may induce mechanisms that accelerate deimination of arginine to citrulline in autoantigens present in the lungs, possibly via up-regulation of peptidylarginine–deiminase activity in macrophages that are activated or undergoing apoptosis (28). An immune response to the citrullinated proteins might then be preferentially induced in individuals carrying the HLA–DR SE genes, since citrullination has been demonstrated to increase the binding of modified peptides to SE-containing HLA–DR antigens and thereby to enhance the immunogenicity of the protein (29). As a consequence of long-term smoking as well as of other stimuli, this activation of the adaptive immune response to citrullinated proteins may occur years before clinical onset of disease (12–14), particularly in individuals carrying the HLA–DR SE genes (10,15).

The additional events needed to trigger clinical signs of arthritis are not known, but it has been demonstrated that citrullination is not specific for RA, but may also occur in other types of arthritis including undifferentiated arthritis (30). Taking this finding into account as well, one may suggest a 2-step model for the pathogenesis of a subset of RA, in which induction of systemic immunity to citrullinated proteins precedes the disease, and in which a second event may trigger an undifferentiated arthritis, which is accompanied by citrullination of proteins in the synovium. This synovitis may be transient in individuals without preceding anticitrulline immunity, while development toward the chronic disease we call RA is more likely in individuals with preexisting anticitrulline immunity, which may then enhance joint inflammation (see ref. 31).

The likelihood of this scenario is concordant with recent studies in rodents in which citrullination was reported to increase the arthritogenicity of at least 2 different autoantigens (fibrinogen and collagen) (18,32), and in which citrullination of joint proteins occurred in parallel with the initial accumulation of activated inflammatory cells in the joints (18). In order to further investigate this possible mechanism of disease, it will be important to characterize which proteins are citrullinated in the lungs and joints and to study the triggering, specificity, and regulation of immune reactions to such modified molecules before and after onset of RA. So far, citrullinated fibrinogen and vimentin have both been shown to be present in RA joints and to be targets of anticitrulline antibodies from RA patients (33,34), but it is likely that citrullination also occurs in other proteins, which may become further targets for the autoimmune reactions.

Many additional genes, environmental stimuli, and immune reactions apart from those mentioned here may be involved in the pathogenetic process in different subgroups of RA (35–39). RFs deserve special attention, since they are part of the definition of RA (19), since the presence of both HLA–DR SE genes and smoking has previously been linked to RF-positive RA (8), and since the presence of anticitrulline antibodies correlates with,

but does not completely coincide with, the presence of RF (9). We suggest that anticitrulline immunity in the present context represents a primary event, since a subanalysis of RA patients discordant for RF and anti-citrulline antibody positivity primarily revealed an association of HLA–DR SE with anticitrulline immunity rather than with RF positivity. In addition, anticitrulline antibodies were observed prior to the occurrence of RF in 2 studies of sera obtained before onset of RA (13,14), suggesting that the increase in RF titers may in some of these cases be an event secondary to anticitrulline immunity and immune complex formation involving citrullinated antigens (40). These observations, together with the fact that there is a high concordance between RF positivity and the presence of antibodies to citrullinated proteins, suggest that the interactions between smoking and HLA–DR SE genes as risk factors for RF-positive but not RF-negative RA, previously reported by us (8), might in fact be an event secondary to the now-described relationship between smoking, HLA–DR, and immunity to citrulline-modified proteins.

In summary, we can now define a prototype environmental stimulus that may induce mechanisms that accelerate posttranslational modification of proteins as well as activation of the immune system; further, a prolonged action of this stimulus in a specific genetic context can cause the emergence of potentially arthritis-inducing immunoreactivity. These findings may provide new opportunities to both predict and understand onset of RA and to interfere with RA-inducing events before clinical symptoms are apparent.

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APPENDIX A: THE EPIDEMIOLOGICAL INVESTIGATION OF RHEUMATOID ARTHRITIS STUDY GROUP

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