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A Rare Polymorphism in the Gene for Toll-like Receptor 2 Is Associated With Systemic Sclerosis Phenotype and Increases the Production of Inflammatory Mediators

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Objective. **To investigate whether polymorphisms in Toll-like receptor (TLR) genes, previously reported to be associated with immune-mediated diseases, are involved in systemic sclerosis (SSc).**

Methods. **We genotyped 14 polymorphisms in the genes for TLRs 2, 4, 7, 8, and 9 in a discovery cohort comprising 452 SSc patients and 537 controls and a replication cohort consisting of 1,170 SSc patients and 925 controls. In addition, we analyzed 15-year followup data on 964 patients to assess the potential association of *TLR* variants with the development of disease complications. We analyzed the functional impact of the associated polymorphism on monocyte-derived dendritic cells.**

Results. **In the discovery cohort, we observed that a rare functional polymorphism in *TLR2* (Pro631His) was associated with antitopoisomerase (antitopo) positivity (odds ratio 2.24 [95% confidence interval 1.24–4.04], $P = 0.003$). This observation was validated in the replication cohort (odds ratio 2.73 [95% confidence interval 1.85–4.04], $P = 0.0001$). In addition, in the replication cohort the *TLR2* variant was associated with the diffuse subtype of the disease ($P = 0.02$) and with the development of pulmonary arterial hypertension (PAH) (Cox proportional hazards ratio 5.61 [95% confidence interval 1.53–20.58], $P = 0.003$ by log rank test). Functional analysis revealed that monocyte-derived dendritic cells carrying the Pro63His variant produced increased levels of inflammatory mediators (tumor necrosis factor α and interleukin-6) upon TLR-2–mediated stimulation (both $P < 0.0001$).**

Conclusion. **Among patients with SSc, the rare *TLR2* Pro631His variant is robustly associated with antitopoisomerase positivity, the diffuse form of the disease, and the development of PAH. In addition, this variant influences TLR-2–mediated cell responses. Further research is needed to elucidate the precise role of TLR-2 in the pathogenesis of SSc.**

Systemic sclerosis (SSc) has all of the hallmarks of an autoimmune disease, with characteristic features

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including vasculopathy, immune activation, and ultimately, extensive fibrosis of the skin and internal organs. Although research in the field of SSc has intensified over the last few years, there is still no clear view on the pathogenesis or a cure (1). It is generally accepted that genetic factors play a role in SSc; this is supported by observations of both familial and ethnic aggregation and the numerous reports describing association of specific genetic variants with SSc (2).

The family of pattern recognition receptors, and more specifically the family of Toll-like receptors (TLRs), are among the most scrutinized molecules in immunity and autoimmunity. TLRs provide a first-line pathogen recognition system and are able to direct the innate immune system toward the appropriate immune responses (3). Although the main purpose of these receptors is to identify microbial antigens and subsequently mount a proper response, there is growing evidence indicating their direct implication in various autoimmune diseases (4).

Roles of several TLRs in SSc have been demonstrated. For instance, monocyte-derived and myeloid dendritic cells (DCs) from SSc patients display an augmented response to various TLR-specific ligands, some of which have been shown to be present in SSc patient serum (5,6). In addition, subcutaneous administration of TLR ligands in an experimental model provokes a marked inflammation in the skin that partially resembles SSc skin changes (7). However, the importance of different TLR subsets in SSc has not been investigated in detail. Therefore, we investigated 14 polymorphisms in the genes for TLRs 2, 4, 7, 8, and 9, previously associated with immune-mediated disease, for their role in SSc susceptibility and corresponding clinical phenotypes. The selection of these SNPs was based on a PubMed search for TLR polymorphisms associated with immune-mediated disease, most preferably with functional impact on the protein or cell function level as well.

PATIENTS AND METHODS

Patients and controls. We used a discovery cohort comprising 452 SSc patients and 537 healthy controls, matched by geographic region, age, and sex. This population was composed of 3 case–control sets of European ancestry: a Spanish cohort (188 SSc patients and 193 controls), a Dutch cohort (85 SSc patients and 255 controls), and an Italian cohort (179 SSc patients and 89 controls). As a replication cohort, we studied a second group, 1,170 SSc patients and 925 controls. This population consisted of a Swedish cohort (193 SSc patients and 167 controls), a German cohort (312 SSc patients and 247 controls), a second Italian cohort (158 patients and 231 controls), a second Dutch cohort (238 SSc patients and 196 controls), and an English cohort (269 SSc patients and 84 controls). Details on the clinical characteristics of the SSc patients in the discovery and replication cohorts are available in Supplementary Table 1 (available on the *Arthritis & Rheumatism* web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). The allele frequencies of English and Dutch controls in the replication cohort were derived from reports in the literature (8,9). When patients are studied in functional immunologic investigations, use of immunomodulatory drugs may affect the results; therefore, we accounted for the use of nonsteroidal antiinflammatory drugs and "disease-modifying antirheumatic drugs" (prednisolone, cyclophosphamide, and others).

All patients fulfilled the American College of Rheumatology classification criteria for SSc (10). The study was approved by the local ethics committee at each participating center. Written informed consent was obtained from all patients and controls before enrollment in the study. All patients were classified as having limited cutaneous SSc or diffuse cutaneous SSc (dcSSc), according to the criteria of LeRoy et al (11). Autoantibody testing was performed using either enzyme-linked immunosorbent assay or immunofluorescence microscopy. The presence of pulmonary fibrosis was investigated by high-resolution computed tomography scanning. Restrictive syndrome and reduced diffusion capacity of the lungs were defined as a forced vital capacity (FVC) $<70\%$ of predicted and a carbon monoxide diffusing capacity (DLCO) $<70\%$ of predicted. Pulmonary artery hypertension (PAH) was diagnosed by right heart catheterization and considered confirmed if the mean pulmonary artery pressure was >25 mm Hg at rest with normal left atrial wedge pressure. Furthermore, we analyzed followup data on FVC and DLCO decline, and development of pulmonary fibrosis, kidney involvement, and PAH in 964 patients, starting at the date of onset of the first non-Raynaud's symptom and ending at 15 years or at the time of death if sooner than 15 years. The patients were evaluated for these complications at least once yearly.

Genotyping. We assessed 14 functional polymorphisms in the *TLR2*, *TLR4*, *TLR7*, *TLR8*, and *TLR9* genes for their role in SSc susceptibility and clinical phenotype. Genotyping was performed using 5′-nuclease (TaqMan) assays with predesigned primers and probes (Applied Biosystems). The polymorphisms investigated and their functional properties and previous associations are displayed in Table 1.

Cell isolation, stimulation, and expression analysis. Peripheral blood mononuclear cells were isolated from heparinized venous blood by density-gradient centrifugation over Ficoll-Paque (Amersham Biosciences). Monocytes were obtained using CD14 and blood dendritic cell antigen 1 microbeads (Miltenyi Biotec) for monocytes and myeloid DCs, respectively. Generation of monocyte-derived DCs has been extensively described by our group previously (5).

Flow cytometric analysis of CD14, CD86, and major histocompatibility complex (MHC) expression on monocyte-derived DCs. Phenotypic analysis of monocyte-derived DCs was performed using standardized flow cytometry protocols as described previously (5,6). DCs were characterized by staining with monoclonal antibodies against human CD14 (Miltenyi Biotec), CD86 (BD Bioscience), and type II MHC HLA–DR/DP (clone Q1514). Cells were analyzed by fluorescence-activated cell sorting (FACSCalibur; BD Biosciences) with

Table 1. Overview of the Toll-like receptor gene polymorphisms tested*

SNP	Gene	Functional change	AB assay ID	Previous (immune system–related) association	Ref.
rs1898830	*TLR2*	−15607A>G	C_11853988_10	Bronchiolitis obliterans	20
rs5743704	*TLR2*	Pro631His	C_25607736_10	IBD, impaired membrane internalization, acute reactive arthritis	14, 21, 22
rs4986790	*TLR4*	896A>G D299G	C_11722238_20	Asthma and atopy	23
rs4986791	*TLR4*	1196C>T p.T399I	C_11722237_20	Liver cirrhosis	24
rs7873784	*TLR4*	16649G>C	C_29292008_10	Multiple sclerosis	25
rs3853839	*TLR7*	3′-UTR	C_2259573_10	SLE, increased expression	26
rs179008	*TLR7*	Gln11Leu	C_2259574_10	Asthma	27
rs2302267	*TLR7*	NA	C_15757400_10	Chronic HCV infection	28
rs5743781	*TLR7*	A448V	C_25643238_10	Newly found variant	29
rs3764879	*TLR8*	TLR8-129G>C	C_2183829_10	Fatal Crimean-Congo hemorrhagic fever	30
rs3764880	*TLR8*	Met1Val	C_2183830_10	Isoform regulating, tuberculosis	31, 32
rs5741883	*TLR8*	NA	C_29409072_10	Rheumatoid factor positivity	33
rs5744088	*TLR8*	NA	C_32184097_10	NA	
rs187084	*TLR9*	NA	C_2301952_10	Graves ophthalmopathy	34

* SNP = single-nucleotide polymorphism; AB = Applied Biosystems; IBD = inflammatory bowel disease; 3′-UTR = 3′-untranslated region; SLE = systemic lupus erythematosus; NA = not applicable; HCV = hepatitis C virus.

FlowJo 8.7.3 (Tree Star) for the proportion of positive cells and the mean fluorescence intensity relative to cells stained with the relevant IgG isotype controls.

Stimulation of monocyte-derived DCs with TLR ligands. On day 6 of culture, monocyte-derived DCs were replated at a concentration of 0.5×10^6 cells/ml and transferred to either 24-well (1-ml) or 96-well (100-μl) culture plates. The culture medium consisted of RPMI 1640, Dutch modification (Invitrogen Life Technologies) supplemented with 10% fetal calf serum and antibiotic/antimycotic solution (Invitrogen Life Technologies) in the presence of interleukin-4 (IL-4) (500 units/ml; Schering-Plough) and granulocyte–macrophage colony-stimulating factor (800 units/ml; Schering-Plough). Cells were then stimulated with TLR-2 agonists for 24 hours, after which supernatants were collected. The concentration of the TLR-2 ligand Pam3Cys (5 μg/ml; EMC Microcollections) was similar throughout the studies.

Measurement of cytokines. Levels of tumor necrosis factor α (TNFα) and IL-6 in supernatants were measured using commercially available kits according to the instructions of the manufacturer (Bio-Rad). Cytokine levels were measured and analyzed with the Bio-Plex system (Bio-Rad). The sensitivity of the assay was <5 pg/ml for all cytokines measured.

Statistical analysis. Statistical analyses were performed using 2×2 contingency tables and Fisher's exact test, with SPSS 16.0. Homogeneity of odds ratios (ORs) among cohorts was calculated using the Breslow-Day and Woolf's Q methods; pooled ORs were calculated under a fixed-effects model (Mantel-Haenszel meta-analysis). Ninety-five percent confidence intervals (95% CIs) were determined. Candidate loci themselves were used to test for population stratification, as described previously (12). For stratification tests, in which multiple single-nucleotide polymorphisms (SNPs) were in linkage disequilibrium (LD), the SNP/LD block with the highest chi-square test value, i.e., the SNP that contributed most to differences across ethnicities, was chosen for the analysis. The TLR-7 and -8 polymorphisms were analyzed in the female population only, since both genes are situated on the X chromosome. Power analysis showed that this study had ≥80% power to detect the effect of all included polymorphisms at an OR of 1.3 in a log additive model. Survival analysis was performed using Kaplan-Meier curves, and significance levels were calculated with log rank (Mantel-Cox) statistics. Cox proportional hazards survival regression was used to determine relative risks. Statistical significance was determined using Student's *t*-test or the Mann-Whitney U test as appropriate. *P* values less than 0.05 were considered significant. We did not apply correction for multiple testing in the discovery cohort, to avoid increasing the risk of false-negative results not being thoroughly investigated in the replication cohort and hence losing sensitivity.

RESULTS

Discovery cohort. All 14 variants investigated were in Hardy-Weinberg equilibrium, and frequencies in the control population were very similar to those reported in HapMap. There was no significant evidence of population stratification. We did not find any significant associations, except for the *TLR2* Pro631His (rs5743704) polymorphism, which was significantly associated with antitopoisomerase autoantibody (antitopo) positivity in both the Dutch and the Spanish populations in the discovery cohort (OR 4.45 [95% CI 1.36–12.54], $P = 0.01$ and OR 3.01 [95% CI 1.07–7.77], $P = 0.02$, respectively). When we performed a meta-analysis, we found a significant effect for all 3 populations combined, as well (OR 2.24 [95% CI 1.24–4.04], $P = 0.003$) (Table 2 and Supplementary Tables 2–14, available on the *Arthritis &*

Table 2. Distribution of the genotypes and alleles of the *TLR2* variant (Pro631His) in the discovery cohort

Cohort, phenotype (n)*	Genotype				Allele	
	CC	CA	AA	*P*	A	*P*
Italy						
SSc (165)	0.95	0.05	0.00	0.51	0.97	0.6
lcSSc (116)	0.94	0.06	0.00	0.51	0.97	0.79
dcSSc (40)	0.98	0.03	0.00	0.57	0.99	0.44
ACA+ (60)	0.97	0.03	0.00	0.56	0.98	0.32
Antitopo+ (74)	0.93	0.07	0.00	0.99	0.97	0.99
Controls (88)	0.93	0.06	0.01		0.96	
The Netherlands						
SSc (82)	0.89	0.11	0.00	0.35	0.95	0.52
lcSSc (63)	0.89	0.11	0.00	0.41	0.94	0.63
dcSSc (18)	0.94	0.06	0.00	0.91	0.97	0.99
ACA+ (23)	0.96	0.04	0.00	0.82	0.98	0.71
Antitopo+ (20)	0.70	0.30	0.00	0.01	0.85	0.01
Controls (249)	0.93	0.07	0.00		0.96	
Spain						
SSc (186)	0.94	0.06	0.00	0.34	0.97	0.35
lcSSc (129)	0.92	0.07	0.01	0.48	0.96	0.52
dcSSc (51)	0.90	0.08	0.02	0.15	0.94	0.24
ACA+ (74)	0.96	0.04	0.00	0.45	0.98	0.57
Antitopo+ (31)	0.81	0.16	0.03	0.01	0.89	0.02
Controls (181)	0.92	0.07	0.01		0.95	
Mantel-Haenszel meta-analysis						
SSc (433)						0.55
lcSSc (308)						0.68
dcSSc (109)						0.22
ACA+ (157)						0.21
Antitopo+ (125)						0.003
Controls (518)						

* SSc = systemic sclerosis; lcSSc = limited cutaneous SSc; dcSSc = diffuse cutaneous SSc; ACA = anticentromere antibody; antitopo = antitopoisomerase antibody.

Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

Replication cohort. Since the allele frequency of the associated polymorphism was relatively small (average 3%), we used a large replication set, consisting of an additional 1,170 SSc patients and 925 controls, to validate the findings. This replication cohort included additional Italian and Dutch populations, as well as populations of different European ethnicity encompassing German, Swedish, and English SSc patients and healthy controls. In the replication cohort we found an association of antitopo positivity with the *TLR2* Pro631His variant in the Italian, German, and Dutch populations (OR 3.15 [95% CI 1.04–8.68], $P = 0.02$; OR 3.72 [95% CI 1.64–6.62], $P = 0.001$; and OR 3.26 [95% CI 1.07–8.93], $P = 0.02$, respectively). When we combined the results from the replication cohort in a meta-analysis, we found a strong overall association (OR 2.73 [95% CI 1.85–4.04], $P = 0.0001$) (Table 3).

In addition, consistent with the observation that antitopo positivity is usually found in the dcSSc subtype of the disease, an association with dcSSc was observed (OR 1.67 [95% CI 1.08–2.58], $P = 0.02$) (Table 3). When we combined the discovery and replication cohorts, we observed a strong association of the *TLR2* Pro631His variant with antitopo-positive SSc (OR 2.55 [95% CI 1.85–3.52], $P < 0.00001$) (Figure 1). These results remained significant after Bonferroni correction for multiple testing.

Table 3. Distribution of the genotypes and alleles of the *TLR2* variant (Pro631His) in the replication cohort

Cohort, phenotype (n)*	CC	CA	AA	A	*P*
Italy					
SSc (158)	0.94	0.06	0.00	0.03	0.99
lcSSc (97)	0.95	0.05	0.00	0.03	0.99
dcSSc (44)	0.89	0.11	0.00	0.06	0.15
ACA+ (88)	0.95	0.05	0.00	0.03	0.79
Antitopo+ (39)	0.85	0.13	0.03	0.09	0.02
Controls (231)	0.94	0.06	0.00	0.03	
Sweden					
SSc (193)	0.92	0.08	0.00	0.04	0.55
lcSSc (117)	0.94	0.06	0.00	0.03	0.99
dcSSc (50)	0.86	0.14	0.00	0.07	0.08
ACA+ (109)	0.95	0.05	0.00	0.03	0.79
Antitopo+ (48)	0.88	0.10	0.02	0.07	0.07
Controls (167)	0.94	0.06	0.00	0.03	
Germany					
SSc (312)	0.94	0.06	0.00	0.03	0.74
lcSSc (163)	0.93	0.07	0.00	0.04	0.99
dcSSc (121)	0.89	0.11	0.00	0.06	0.24
ACA+ (146)	0.93	0.07	0.00	0.04	0.99
Antitopo+ (116)	0.79	0.19	0.02	0.10	0.001
Controls (247)	0.93	0.07	0.00	0.04	
The Netherlands					
SSc (238)	0.97	0.03	0.00	0.02	0.05
lcSSc (66)	0.95	0.05	0.00	0.03	0.58
dcSSc (31)	0.88	0.12	0.00	0.06	0.31
ACA+ (61)	0.95	0.05	0.00	0.03	0.58
Antitopo+ (29)	0.86	0.14	0.00	0.11	0.02
Controls (196)	0.92	0.08	0.00	0.04	
United Kingdom					
SSc (269)	0.94	0.06	0.00	0.03	0.33
lcSSc (172)	0.96	0.04	0.00	0.02	0.1
dcSSc (59)	0.89	0.11	0.00	0.06	0.99
ACA+ (169)	0.95	0.05	0.00	0.03	0.18
Antitopo+ (54)	0.87	0.13	0.00	0.07	0.27
Controls (84)	0.99	0.01	0.00	0.05	
Mantel-Haenszel meta-analysis					
SSc (1,170)					0.22
lcSSc (615)					0.24
dcSSc (305)					0.02
ACA+ (573)					0.19
Antitopo+ (286)					0.0001
Controls (925)					

* SSc = systemic sclerosis; lcSSc = limited cutaneous SSc; dcSSc = diffuse cutaneous SSc; ACA = anticentromere antibody; antitopo = antitopoisomerase antibody.

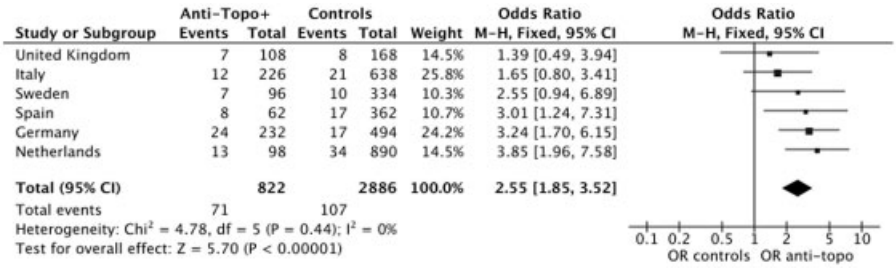

Figure 1. Effect of the *TLR2* Pro631His variant on susceptibility to antitopoisomerase (antitopo)-positive systemic sclerosis. Meta-analysis was performed using allele frequencies. M-H = Mantel-Haenszel; 95% CI = 95% confidence interval; OR = odds ratio.

Role of the *TLR2* variant in SSc disease severity. Since we were interested in whether this functional polymorphism, which seemed to promote a proinflammatory environment, could have a role in the severity of SSc, we used a followup cohort consisting of 964 SSc patients, who were evaluated at least once a year for development of complications. Patients were followed up for either 15 years or until the time of death if that occurred sooner; the followup period started at the onset of the first symptom after the development of Raynaud's phenomenon. We evaluated the decrease of both FVC and DLco to <70% of predicted, the development of PAH as measured by right heart catheterization, the development of pulmonary fibrosis as assessed by high-resolution computed tomography, and disease duration until time of death. Although the TLR-2 ligands did not influence patient survival, pulmonary fibrosis, or kidney involvement, patients carrying the variant progressed to develop right heart catheterization–proven PAH significantly sooner compared with patients not carrying the risk allele (Cox proportional hazard ratio 5.61 [95% CI 1.53–20.58], $P = 0.003$ by log rank test) (Figure 2) (PAH developed in a total of 40 patients). There was no significant effect of antitopo titers on development of PAH, which might have biased the results.

The *TLR2* variant alters the level of cytokine response upon stimulation. Since the polymorphism has previously been shown to influence the expression and function of the TLR-2 receptor with regard to antigen uptake (13), we were interested in whether it influenced the inflammatory response evoked by the TLR-2 ligand Pam3Cys. To investigate this, we isolated DCs from 12 patients with or without the rare *TLR2* allele and compared the response upon stimulation. These experiments were carried out on 3 separate occasions, always including 1 patient carrying the rare variant compared with 2 or more patients carrying the wild type. After 24 hours of stimulation, we found that cells from patients carrying the *TLR2* variant exhibited a marked increase in production of IL-6 and TNFα upon stimulation with

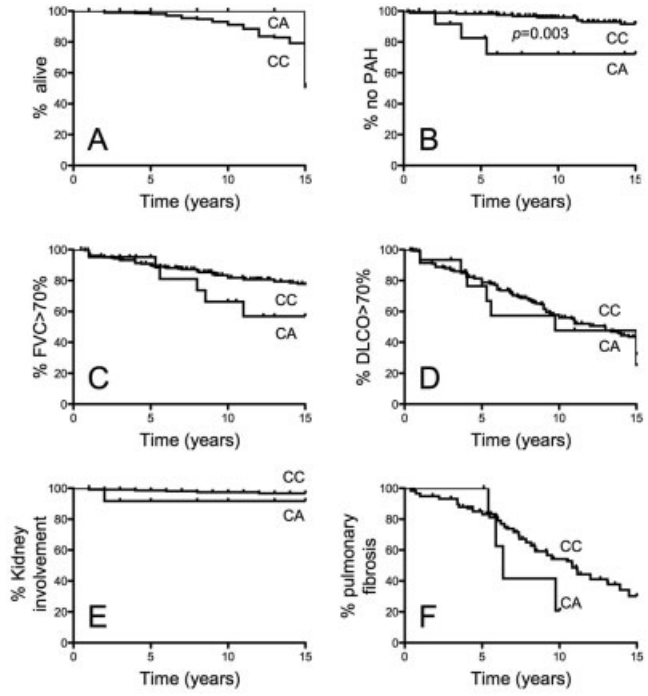

Figure 2. Effect of the *TLR2* Pro631His variant on development of complications of systemic sclerosis. No significant effects on mortality (**A**), deterioration of forced vital capacity (FVC) (**C**) or carbon monoxide diffusing capacity (DLco) (**D**) to <70% of predicted, development of pulmonary fibrosis (**F**), or development of kidney involvement (**E**) were observed. However, we found an association with development of pulmonary arterial hypertension (PAH) (Cox proportional hazard ratio 5.61 [95% confidence interval 1.53–20.58]) (**B**). Patients (n = 964) were followed up starting at the onset of the first non-Raynaud's symptom, for 15 years or until the time of death if death occurred before 15 years of followup, and Kaplan-Meier curves were created.

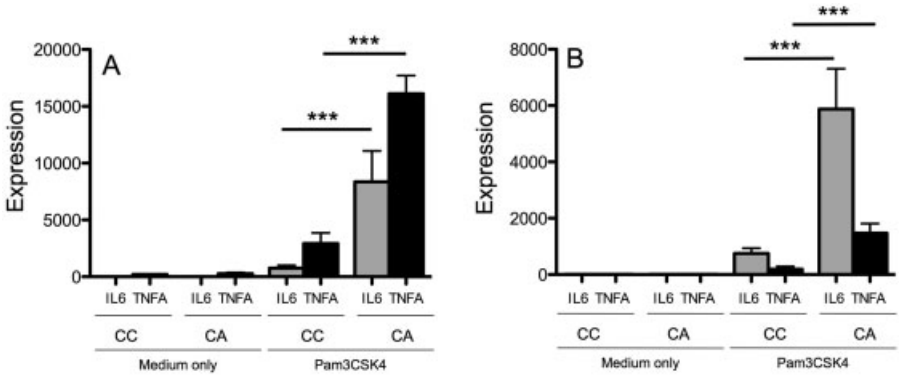

Figure 3. A, Expression of interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) in carriers of the CC genotype (n = 9) and carriers of the CA genotype (n = 3) after stimulation of monocyte-derived dendritic cells with medium or with the Toll-like receptor 2 agonist Pam3CSK4 was determined. The expression of both IL-6 and TNFα was significantly increased in carriers of the CA variant. **B,** The same experiment was performed with myeloid dendritic cells from carriers of the CC genotype (n = 9) and carriers of the CA genotype (n = 3). Similar results were obtained in studies of monocyte-derived dendritic cells and of myeloid dendritic cells. Values are the mean ± SEM. *** = $P < 0.0001$.

the ligand (both $P < 0.0001$) (Figure 3A). Results obtained in studies of myeloid DCs from patients were similar to those observed with monocyte-derived DCs ($P < 0.0001$ for both IL-6 and TNFα) (Figure 3B). There were no significant differences between the group of patients with the variant and the group without the variant, in terms of sex distribution, clinical phenotype, or medication. Patients who had received prednisolone or cyclophosphamide therapy within 4 weeks prior to the study were excluded from this analysis.

DISCUSSION

Due to the accumulating evidence suggesting a role of TLRs in SSc, we investigated whether genetic variants in 5 different *TLR* genes influenced SSc susceptibility, severity, and cellular function. After an initial study with 3 different ethnic cohorts we found that only 1 of 14 polymorphisms investigated was associated with SSc. More specifically, a rare variant in *TLR2* was found to be associated with antitopo-positive SSc. This association was validated in a large replication cohort consisting of subjects of multiple European nationalities. Considering the size and power of the study it is unlikely that the observed effects are due to a Type I error. In a recent genome-wide association study in SSc, possible associations of tag-SNPs with SSc and clinical phenotypes were investigated. However, possible associations with autoantibody profiles were not investigated, and the study did not include the Pro631His (rs5743704) SNP as a haplotype marker, explaining why it was not identified as an associated marker in that effort (14). Furthermore, this SNP is located in a region with very low linkage disequilibrium, making it less likely that another SNP underlies the association.

The associated polymorphism *TLR2* Pro631His confers an amino acid change that has previously been functionally investigated. HEK cells transfected with the variant exhibit slowed internal trafficking of TLR-2, which seems to not follow conventional pathways. In addition, in carriers of the variant, more *TLR2* variant than wild-type *TLR2* is expressed on cell membrane (13). This could explain our observation that DCs from patients with the variant displayed a more potentiated cytokine response. Although the number of donors with the minor variant was relatively small compared to the number of donors investigated who did not carry the variant, these results seem to be consistent across monocyte-derived and myeloid DCs. Moreover, the level of IL-6 and TNFα production varied little within the group carrying the minor *TLR2* variant, or within the group not carrying the variant.

Interestingly, antitopo antibodies are able to bind to fibroblasts and subsequently attract monocytes in vitro (15). These antibodies are present in the earliest stages of SSc, but also in healthy individuals (16,17). It could be hypothesized that when monocytes attracted by antitopo carry the proinflammatory *TLR2* variant, both fibroblasts and monocytes might contribute to a more pronounced inflammatory response and subsequently to a higher risk of developing full-blown disease. Interestingly, several autologous TLR-2 ligands can develop during tissue injury (18,19). In light of this, it is interesting to note that endothelial cells have been shown to express TLR-2, which, in individuals carrying the rare

TLR2 variant, might result in an augmented inflammatory response upon stimulation, contributing to the development of PAH. However, these hypotheses remain to be further investigated.

In conclusion, we have shown that TLR-2 is involved in the phenotype of SSc, which might be explained by its altered function upon binding of its ligands. Further research is warranted to better determine the precise role of TLR-2 in disease onset and/or perpetuation. Since neutralizing antibodies against multiple TLRs are about to enter the clinical arena, more knowledge on the role of TLR-2 in SSc may lead to a broadening of the therapeutic options for this serious condition.

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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 to be published. Dr. Radstake 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.

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