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The Functional Variant of the Inhibitory Fcγ Receptor IIb (CD32B) Is Associated With the Rate of Radiologic Joint Damage and Dendritic Cell Function in Rheumatoid Arthritis

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Objective. **Fcγ receptors (FcγRs) recognize immune complexes (ICs) and coordinate the immune response by modulating the functions of dendritic cells (DCs). The purpose of this study was to unravel the role of the inhibitory FcγRIIb in rheumatoid arthritis (RA) by studying the effect of the *FCGR2B* 695T>C polymorphism on susceptibility to RA, severity of the disease, and DC function.**

Methods. **Genotyping was performed in RA patients (n = 246) and healthy blood donors (n = 269). The patients' demographic data, disease severity, and disease progression were assessed over a followup of 6 years. DCs were cultured for flow cytometry to determine the expression of FcγRs. For detection of FcγRIIb (CD32B), a unique anti-FcγRIIb antibody (2B6–fluorescein isothiocyanate [FITC]) was used. The capacity for antigen uptake by DCs was studied by assessing the uptake of FITC-labeled ICs. Levels of cytokine production by DCs were measured during lipopolysaccharide-mediated cell activation in the presence and absence of ICs.**

Results. **Although no role of the *FCGR2B* variant in RA susceptibility was demonstrated, this variant was associated with a nearly doubled rate of radiologic joint damage during the first 6 years of RA. Multiple regression analysis showed that *FCGR2B* was by far the strongest predictor of joint damage identified to date. DCs from patients carrying this variant failed to display the inhibitory phenotype normally observed upon IC-mediated triggering of inflammation and displayed diminished FcγRII-mediated antigen uptake compared with wild-type DCs. However, the levels of FcγRs were not affected, suggesting that the *FCGR2B* variant alters the function rather than regulation of proteins.**

Conclusion. **This study is the first to show that a single genetic variant, the *FCGR2B* 695T>C polymorphism, is a critical determinant of disease severity in RA and radically changes DC behavior. Our results underscore the key role of DCs in the progression of RA and reveal FcγRIIb as an important potential therapeutic target in RA and other autoimmune conditions.**

Every immune response that is ignited has to be terminated to prohibit the development of chronic inflammation and subsequent breakdown of tolerance. Autoimmune diseases potentially result from an imbalance between activating and inhibitory pathways of inflammation, culminating in immune-mediated tissue injury. Dendritic cells (DCs) play a crucial role in tuning the balance between tolerance and immunity (1). Therefore, pathways that inhibit inappropriate DC activation must be present to avoid responses to self antigens (2).

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Rheumatoid arthritis (RA) is an autoimmune condition that is characterized by chronic inflammation of the synovial joints, resulting in progressive breakdown of cartilage and the underlying bone. Although the etiology of RA has not been elucidated, it is well established that a massive accumulation of B cells, macrophages, T cells, and DCs in the synovium is implicated. With the exception of T cells, all of these key players in RA highly express Fcγ receptors (FcγRs) on their surface. In humans, 3 classes of FcγRs can be distinguished, FcγRI, FcγRII, and FcγRIII (3). The FcγRII class can be further subdivided into FcγRIIa and FcγRIIb. FcγRI, FcγRIIa, and FcγRIIIa activate cellular responses upon engagement, whereas FcγRIIb has an immunoreceptor tyrosine-based inhibitory motif that regulates signaling by the inhibitory tyrosine-based activating motif, resulting in inhibition of cellular activation (4,5). FcγRs play an important role in the recognition of immune complexes (ICs), which are abundant in RA patients. FcγRs are therefore held responsible for the pathogenic consequences triggered by ICs in many autoimmune conditions (6,7).

The significance of FcγR-mediated inhibitory and activating signaling pathways during arthritis has been clearly demonstrated in animal models. Deletion of the *Fcgr2b* gene renders B6 mice susceptible to collagen-induced arthritis (CIA) and enhances the arthritic response to IgG anti–type II collagen monoclonal antibodies (8,9). Along the same line, coligation of the inhibitory FcγRIIb was found to reduce IC-mediated joint inflammation by endocytosis of ICs (10). Deficiency of FcγRIIb in DBA/1 mice increases their susceptibility to CIA (11). Importantly, the balance between activating and inhibitory FcγRs has been shown to be pivotal in the outcome of experimental arthritis (11,12).

In humans, various investigators have focused on the expression levels of activating and inhibiting FcγRs on various cell types. Highton et al demonstrated that the expression of FcγRI is increased on circulating monocytes from RA patients (13). These findings were corroborated by Shinohara and colleagues, who found an increased expression of FcγRI and FcγRII in RA patients with active disease as well as in those with disease in remission (14), an observation that was later confirmed by 2 other research groups (15–17). In contrast, the expression levels of the inhibitory FcγRIIb subtype were similar between monocytes from RA patients and those from healthy controls (17).

In addition to research on the cellular protein level, the role of activating FcγRs in the susceptibility to and/or outcome of autoimmune diseases has been studied in different populations, yielding variable results (18–22). However, data on the inhibitory FcγRIIb are scant, with the exception of studies showing the involvement of this receptor in the etiology of systemic lupus erythematosus (SLE), in which FcγRIIb has been linked to increased susceptibility to SLE (19,23,24). In 2 elegant studies that followed this initial observation, the Ile232Thr polymorphism was demonstrated to result in the exclusion of lipid rafts, leading to attenuated function and subsequently impaired inhibition of the proinflammatory responses suggested to underlie the pathogenesis of SLE (25,26).

A direct role for the inhibitory FcγRIIb in the pathogenesis of RA has thus far not been demonstrated. Given the hypothesis that RA is associated with decreased negative feedback of humoral and effector immune responses, the fundamental question arises as to whether FcγRIIb is designed to counteract proinflammatory responses such as chronic synovial inflammation and subsequent joint damage. The recent characterization of a polymorphism in the transmembrane region of the human inhibitory FcγRIIb, Ile232Thr, affecting B cell function (27) and membrane expression of FcγRIIb on B cells (25,26), offers a good opportunity for critical evaluation of this hypothesis.

DCs are the professional antigen-presenting cells (APCs) that are unrivaled in their capacity to regulate pivotal immunologic processes (2). Substantial recent evidence originating from various research groups supports a role for DCs in the onset and/or potentiation of synovial inflammation in RA (28–32). Likewise, Leung et al demonstrated that the transfer of collagen-pulsed DCs is sufficient for the induction of (antigen-specific) inflammation in the joint, thus supporting a role for DCs in experimental arthritis (33). The function of DCs is highly dependent on the presence and/or type of antigen–ICs, the recognition of which is largely facilitated by FcγRs (6). Consistent with this knowledge, it was recently demonstrated that the balance between activating and inhibiting FcγR systems establishes a threshold for DC activation and enables ICs to control the maturation and function of DCs (34–37). In light of such a conceptual framework, it is conceivable that functional variants of FcγRs have profound effects on DC function, and subsequently could influence the etiology or severity of RA.

In the present study, our findings show that RA patients carrying the *FCGR2B* variant experienced a nearly 2-fold increase in radiologic joint damage over 6 years. Our results also demonstrate that the functional

FcγRIIb variant markedly altered the function of APCs, suggesting that FcγRIIb plays an important role in the pathogenesis of RA.

PATIENTS AND METHODS

Ascertainment of patients. Genotyping was performed in all RA patients participating in an early RA inception cohort study that started in 1985. The present study included only those patients who met the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for RA (38), had a disease duration of <1 year, and had not been treated with disease-modifying antirheumatic drugs (DMARDs) or biologic agents. All patients were monitored regularly for disease phenotype, severity, and outcome. Healthy controls were recruited from among blood donors living in the same geographic area. The local ethics committee provided approval for the study.

For in vitro experiments, blood was drawn from RA patients who were considered to have active disease (based on a Disease Activity Score in 28 joints [DAS28] [39] >5.2) and who were receiving a steady dose of methotrexate (n = 16). The use of biologic agents (tumor necrosis factor α [TNFα] blockers, interleukin-1 [IL-1] receptor antagonists) and high doses of prednisolone (>5 mg daily) was prohibited. Synovial fluid (SF) was isolated from 3 RA patients with active disease (later pooled) who were not receiving biologic agents or prednisolone.

Characterization of disease activity and outcome. Demographic data such as sex, age at disease onset, and the presence of rheumatoid factor, HLA–DR4, and the shared epitope (SE) were included in the analysis. We used the DAS28 (39) and the modified Sharp score (40) at baseline and after 3 and 6 years of followup to determine the disease course and radiologic joint progression, respectively. The use of DMARDs was analyzed using essentially the same protocols as previously described (41).

***FCGR2B* genotyping.** Genotyping of the 695T>C (Ile232Thr) polymorphism was based on restriction fragment-length polymorphism (RFLP) analysis and direct sequencing. We started with a long-range polymerase chain reaction (PCR), essentially as described by Kyogoku et al (19) (forward primer 5′-AAGGACAAGCCTCTGGTCAA-3′; reverse primer 5′-CAACTTTGTCAGCCTCAT-3′), followed by a nested PCR (forward primer 5′-TGTGACCATCACTGTC-CAAG-3′; reverse primer 5′-CTGAAATCCGCTTTTT-CCTG-3′). First, amplification of fragments was carried out in a 50-μl volume using the Expand Long Template PCR System (Roche, Indianapolis, IN) according to protocol in a PTC-200 thermal cycler (MJ Research, San Francisco, CA). The nested PCR was carried out on the 4,323-bp product of the XL (extra long) PCR after the product was checked by direct sequencing. Amplification was performed in a 50-μl volume containing 5 μl XL PCR product (diluted 10,000×), 300 μ*M* of dNTPs, 250 ng of each primer, buffer containing 10 m*M* Tris HCl, pH 9.0, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% (weight/volume) gelatin, and 2 units of DNA polymerase (Invitrogen, Carlsbad, CA). Amplification was carried out in a PTC-200 thermal cycler, with conditions as follows: 92°C for 3 minutes, 30 cycles of 92°C for 1 minute, 54.8°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

The product, 863 bp in length, was purified on Multiscreen PCR plates (Millipore, Bedford, MA) and genotyped by RFLP analysis using *Bsm* F1 (Westburg, Leiden, The Netherlands). Since direct sequencing of genotypes for validation showed evidence of *FCGR2C* in the product of the nested PCR, which might hamper the identification of homozygous carriers of the variant allele, we performed a nested-over-nested PCR on the purified 863-bp template from carriers of the variant allele (after 10,000× dilution) with a forward primer that distinguishes between *FCGR2B* and *FCGR2C* (5′-GGGAGCCCTTCCCTCTGT-3′) and the reverse primer used in the nested PCR. Amplification conditions were comparable with those in the nested PCR (annealing at 64°C). The resulting 359-bp fragment was genotyped by direct sequencing (primer 5′-GGAGGCATAAGTCCAGCCAC-3′).

Culture of monocyte-derived DCs. Monocyte-derived DCs were cultured using standardized protocols as previously described (32). Briefly, peripheral blood mononuclear cells were isolated from heparinized venous blood by density-gradient centrifugation over Ficoll-Paque (Amersham Biosciences, Roosendaal, The Netherlands). After isolation, the cells were allowed to adhere for 1 hour at 37°C in RPMI 1640, Dutch modification (Invitrogen) supplemented with 2% human serum. Adherent monocytes were cultured in RPMI 1640, Dutch modification supplemented with 10% fetal calf serum (FCS) and antibiotic-antimycotic (Life Technologies, Gaithersburg, MD) in the presence of IL-4 (500 units/ml; Schering-Plough, Kenilworth, NJ) and granulocyte–macrophage colony-stimulating factor (800 units/ml; Schering-Plough) for 6 days.

Phenotype analysis of monocyte-derived DCs. The phenotype analysis of monocyte-derived DCs was performed using standardized flow cytometry protocols as described previously. Briefly, 1×10^5 DCs were incubated with monoclonal antibodies against human CD14 (DakoCytomation, Glostrup, Denmark), CD80 (Becton-Dickinson, Mountain View, CA), CD83 (Beckman Coulter, Mijdrecht, The Netherlands), CD86 (PharMingen, San Diego, CA), class I major histocompatibility complex (clone W6/32), and class II MHC DR/DP (clone Q1514) for 30 minutes at 4°C. Cells were then washed and incubated with fluorescein isothiocyanate (FITC)–conjugated goat anti-mouse IgG (Zymed, South San Francisco, CA) for 30 minutes at 4°C in complete darkness. Subsequently, cells were washed and analyzed by fluorescence-activated cell sorting (FACS) analysis (FACSCalibur; Becton-Dickinson, San Jose, CA) for the proportion of positive cells relative to cells stained with relevant IgG isotypes. Cells were gated according to their forward- and side-scatter patterns to determine the expression of CD14 and CD83 on immature and mature DCs, respectively. For each marker, 10^4 cells were counted in the gate.

Flow cytometry was performed to determine the expression of FcγRI (CD64, clone 10.1, isotype IgG1κ), FcγIIa (CD32, clone IV.3), and FcγIII (CD16, clone DJ130c, isotype IgG1κ) and the expression of fluorescent ICs. First, DCs were incubated with human monoclonal antibodies against CD16, CD32, and CD64 (all from DakoCytomation) and were then washed and incubated with FITC-labeled goat anti-mouse IgG (Zymed) for 30 minutes at 4°C. Subsequently, cells were analyzed by FACS analysis (FACSCalibur; Becton-Dickinson).

Table 1. Frequency of the *FCGR2B* 695T>C (Ile232Thr) polymorphism in white patients with rheumatoid arthritis (RA) and healthy controls*

Genotype or allele	RA patients (n = 246)	Controls (n = 269)	χ^2	*P*†	OR‡	95% CI‡
Individual genotype analysis						
695 TT	197 (80.1)	205 (76.2)				
695 TC	47 (19.1)	58 (21.6)				
695 CC	2 (0.8)	6 (2.2)				
Pooled genotype analysis						
695 TT	197 (80.1)	205 (76.2)	1.13	0.29	0.80	0.41–1.57
695 TC + CC	49 (19.9)	64 (23.8)				
Allele analysis						
T	441 (89.6)	468 (87.0)	1.73	0.19	0.78	0.33–1.86
C	51 (10.4)	70 (13.0)				

* Except where indicated otherwise, values are the number (%) of subjects.
† Calculated using the chi-square test for 2 × 2 contingency tables (df 1).
‡ The odds ratio (OR) and 95% confidence interval (95% CI) were calculated in comparison with the wild type.

The expression of FcγRIIb and FcγRIIa was tested using anti-FcγRIIb antibody 2B6-FITC and FcγRIIa antibody IV.3, respectively (kind gifts from Dr. J. Rönnelid, University Hospital, Uppsala, Sweden). The 2B6 antibody selectively binds and blocks human FcγRIIb and was obtained from MacroGenics (Rockville, MD) (35). This antibody reacts specifically with CD32B and not with CD32A, as has been shown by enzyme-linked immunosorbent assay, surface plasmon resonance, and FACS staining of cell lines and transfectants (Veri MC, et al: unpublished observations).

Endocytosis of heat-aggregated gamma globulins by DCs. The capacity for antigen uptake by DCs was studied as described previously (42). Briefly, human gamma globulins (Sigma-Aldrich, Bornem, Belgium) at a concentration of 10 mg/ml were labeled with FITC using the FluoReporter FITC protein-labeling kit (Molecular Probes, Eugene, OR) and subsequently heated to 63°C for 30 minutes. One hundred microliters of cell suspension (1×10^6 cells/ml) was added to FACS tubes containing FITC-labeled heat-aggregated gamma globulins and incubated for different time points at 37°C. Trypan blue (0.4% [w/v]; Sigma-Aldrich), which quenches extracellular but not intracellular fluorescence, was added (43). In each experiment, 100 μg/ml of FITC-labeled heat-aggregated gamma globulins was used. This concentration was defined based on the results of testing various concentration ranges. At a concentration of 100 μg/ml, self-quenching did not occur.

Flow cytometry was used to quantify antigen uptake by DCs. The DCs from RA patients carrying (n = 8) and those not carrying (n = 8) the FcγRIIb variant were used in 3 different experiments. In all experiments, similar ICs that all originated from the same batch were used.

Measurement of cytokines in culture supernatant. Levels of TNFα, IL-6, IL-10, and IL-12 were measured in the supernatant of the monocyte/DC cultures using commercially available kits (Bio-Rad, Richmond, CA) according to the manufacturer's instructions. Cytokine levels were measured and analyzed with the Bio-Plex system (Bio-Rad).

Statistical analysis. The genotype frequencies of the 695T>C polymorphism in *FCGR2B* were tested for Hardy-Weinberg equilibrium using the standard goodness-of-fit test. Similarity of genotype and allele distribution between patients and controls was tested with chi-square tests for 2 × 2 contingency tables. In all cases, homozygous (CC) genotypes were analyzed together with the heterozygous (TC) genotypes. Differences in the disease characteristics between patients were analyzed using Student's *t*-test or the Mann-Whitney U test. *P* values less than or equal to 0.05 were considered significant.

Multiple linear regression analysis was performed to assess whether the FcγRIIb functional genetic variant is an independent explanatory factor for the level of disease activity and/or extent of radiologic joint damage after 3 and 6 years of RA. To this aim, a staged approach to model building was used, first by entering the known prognostic factors individually, and then by adding the *FCGR2B* variant. Based on the analyses from earlier studies, the following prognostic factors (independent variables) were included in the model: presence/absence of rheumatoid factor, HLA–DR4, and the SE and radiologic damage at baseline (41,44).

RESULTS

Distribution of the *FCGR2B* polymorphism among RA patients and controls. As shown in Table 1, 246 white patients with RA and 269 healthy individuals were included in the present study and genotyped for the *FCGR2B* 695T>C (Ile232Thr) polymorphism. Neither the patient group's genotype data nor those of the control group showed significant deviations from Hardy-Weinberg equilibrium ($P = 0.44$ and $P = 0.66$, respectively). The distribution of genotypes and the frequencies of alleles among the RA patients and healthy controls were found to be similar (in pooled analysis, $P = 0.29$ for TT versus TC + CC), suggesting that the *FCGR2B* 695T>C variant is not associated with suscep-

Table 2. Multiple regression analysis of radiologic joint damage according to the modified Sharp score after 3 and 6 years of disease followup in patients with rheumatoid arthritis

	Sharp score 0–3 years		Sharp score 0–6 years	
	β	P	β	P
Explanatory variable				
FCGR2B variant	1.50	<0.0001	1.85	<0.0001
Rheumatoid factor	1.32	0.001	1.74	0.002
Mean DAS28*	0.79	0.001	1.10	0.002
High baseline Sharp score	0.09	<0.001	0.08	<0.001
R^2	0.46		0.45	

* DAS28 = Disease Activity Score in 28 joints.

tibility to RA. Frequencies of alleles of the *FCGR2B* variant were comparable with those reported in earlier studies of healthy Dutch individuals (22).

Strong predictive capacity of the *FCGR2B* variant for radiologic joint damage in RA. We next investigated the relationship between the *FCGR2B* 695T>C (Ile232Thr) polymorphism and disease characteristics in patients with RA. Data on 150 patients (45 with the TC genotype and 105 with TT) and 123 patients (35 with TC and 88 with TT) were available after 3 and 6 years of followup, respectively. No differences in the demographic characteristics, clinical features, or disease phenotype were observed between the homozygous and heterozygous individuals.

Intriguingly, however, patients carrying the *FCGR2B* functional variant were receiving significantly more DMARDs (per patient per year) and these DMARDs were significantly more aggressive, suggesting that patients with this variant had a more severe disease phenotype. Indeed, carriership of the *FCGR2B* variant was associated with significantly higher levels of radiologic joint damage after 3 years (mean ± SEM Sharp score 95.0 ± 13 versus 50.1 ± 6; $P < 0.0001$) and 6 years (125.0 ± 23 versus 76.0 ± 9; $P < 0.0001$) of disease followup compared with patients carrying the wild-type alleles (Figure 1). In contrast, the *FCGR2B* variant was not associated with extent of disease activity (the DAS28), presence of rheumatoid factor, level of anti–cyclic citrullinated peptide IgG, or HLA–DR subtypes, which are disease features often associated with joint damage.

Consistent with this finding, multivariate analysis revealed that the *FCGR2B* variant was an independent and by far the strongest predictor of radiologic joint damage. In accordance with the findings of previous studies, the only other significant contributors in this model were the presence of rheumatoid factor ($P < 0.005$), the level of radiologic damage at inclusion into the study ($P = 0.003$), and the mean level of disease activity (the DAS) ($P = 0.001$) (22,45).

Shift of DC function toward a proinflammatory phenotype in the presence of the FcγRIIb variant. We have recently shown that FcγR-driven DC function in RA is altered and heavily depends on the expression of the inhibitory FcγRIIb (36). In all experiments, the phenotype of DCs was studied using flow cytometry techniques. All immature DCs were negative for the membrane marker CD14 and showed a low expression of CD80, CD83, and CD86. Class I MHC and class II MHC were expressed on an intermediate level, which is consistent with evidence in the literature and previous results from our group. No differences in any marker

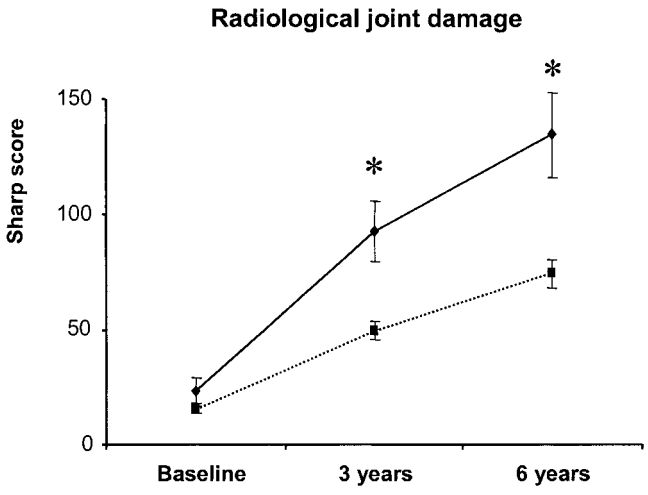

Figure 1. Rate of radiologic joint damage over time in patients carrying 1 allele of the *FCGR2B* genetic variant (TC; diamonds) and those carrying only wild-type alleles (TT; squares). After 3 years (TC, n = 45; TT, n = 105) and 6 years (TC, n = 35; TT, n = 88) of disease followup, patients with the *FCGR2B* variant had significantly more radiologic joint damage compared with those carrying the *FCGR2B* wild-type alleles. * = $P < 0.0001$. Bars show the mean ± SD.

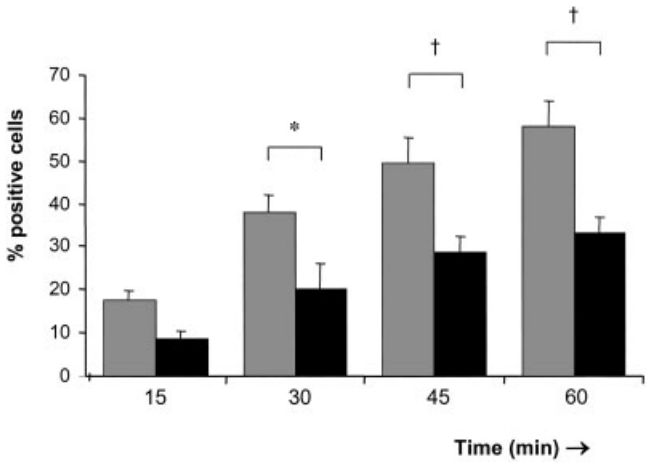

Figure 2. Percentage of dendritic cells (DCs) that have taken up fluorescein isothiocyanate–labeled immune complexes (FITC-ICs) by endocytosis over time. After 30, 45, and 60 minutes, DCs from patients expressing the *FCGR2B* variant (n = 4; solid bars) took up significantly less FITC-ICs compared with DCs from patients not expressing the variant (n = 4; shaded bars). Results are the mean and SD. ∗ = P = 0.0005; † = $P < 0.00001$.

were observed in DCs from RA patients carrying the FcγRIIb variant and those not carrying the FcγRIIb variant.

The results (shown in Figure 2) demonstrate that carriership of the *FCGR2B* variant allele led to alterations in DC behavior in terms of the IC uptake capacity and the cytokine profile of DCs from patients with RA. The uptake of fluorescent ICs by immature DCs from RA patients carrying the FcγRIIb functional variant as compared with DCs from those carrying the wild-type alleles was consistently and strikingly lower after 30 minutes (mean ± SD 20 ± 6% versus 38 ± 7%; P = 0.0005), 45 minutes (29 ± 6% versus 49 ± 6%; $P <$ 0.00001), and 60 minutes (33 ± 4% versus 58 ± 6%; $P <$ 0.00001) of incubation in 4 separate experiments, each comparing DCs from 1 patient carrying and 1 not carrying the functional variant (Figure 2).

We also compared the production of TNFα, IL-6, IL-10, and IL-12 by DCs between RA patients with and those without the FcγRIIb variant upon stimulation with either lipopolysaccharide (LPS) alone or LPS in combination with ICs. DCs from patients carrying 2 wild-type *FCGR2B* alleles (n = 8) effectively inhibited the LPS-mediated proinflammatory response elicited with co-stimulation of FcγR-mediated pathways, which was reflected by a significantly decreased production of TNFα (32% compared with LPS alone; $P < 0.001$), IL-6 (22% compared with LPS alone; P = 0.003), and IL-12 (55%; $P < 0.001$) (Figure 3). The production of the antiinflammatory cytokine IL-10 after stimulation with LPS and ICs in these patients seemed to be increased (15% compared with LPS alone), although this difference did not reach statistical significance (P = 0.01). In contrast, LPS plus IC–mediated triggering of DCs from RA patients carrying the *FCGR2B* variant (n = 8) resulted in a clear potentiation of the proinflammatory response, reflected by an increased production of TNFα (30% compared with LPS alone; $P < 0.001$), IL-6 (17% compared with LPS alone; P = 0.004), and IL-12 (84% compared with LPS alone; $P < 0.001$) and somewhat increased secretion of IL-10 (16% compared with LPS alone; P = 0.001).

The expression of all FcγR subtypes was comparable during steady-state conditions between DCs from patients with (n = 8) and those without (n = 8) the *FCGR2B* variant (Figures 4A and B). In contrast, upon incubation with SF from RA patients, DCs from patients with the *FCGR2B* variant (n = 4) failed to increase the expression of the inhibitory FcγRIIb compared with DCs from the same patients that were stimulated with FCS (Figure 4C). DCs from RA patients not carrying the *FCGR2B* variant (n = 4) were able to increase the

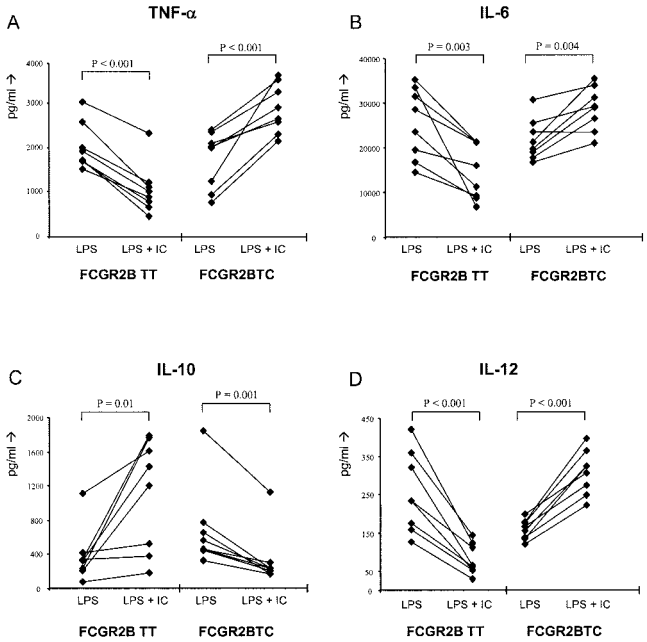

Figure 3. Secretion of tumor necrosis factor α (TNFα) (**A**), interleukin-6 (IL-6) (**B**), IL-10 (**C**), and IL-12 (**D**) upon lipopolysaccharide (LPS)–mediated triggering of dendritic cells in the absence or presence of immune complexes (ICs), from patients without (TT) and with (TC) the *FCGR2B* variant. Results (squares) are the mean (n = 8 patients in each group).

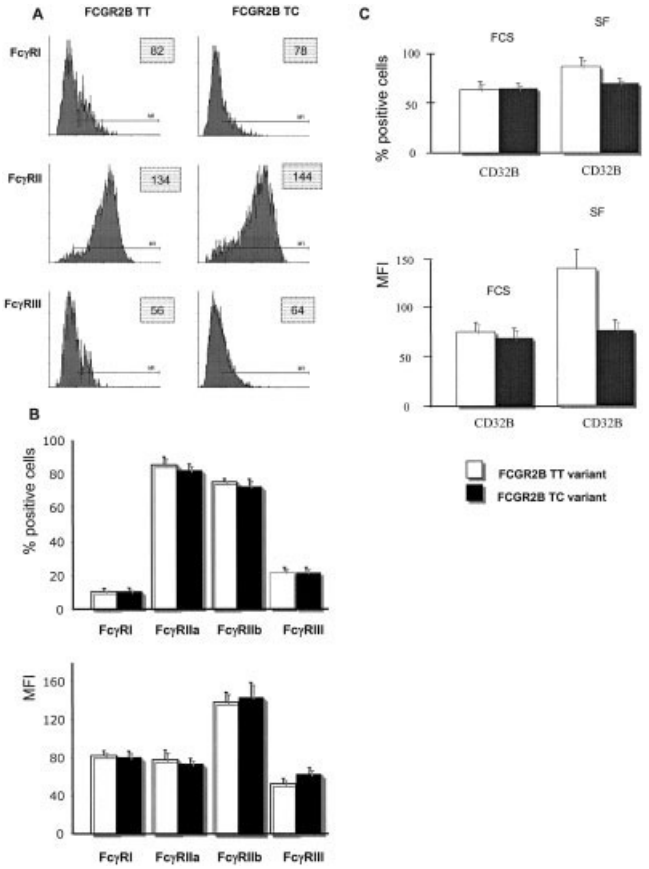

Figure 4. Expression of Fcγ receptor (FcγR) subtypes by dendritic cells (DCs) from rheumatoid arthritis (RA) patients with and those without the *FCGR2B* variant. **A,** Flow cytometry revealed no differences in expression of FcγRI, FcγRIIa, and FcγRIIIa between patients carrying (n = 8) and those not carrying (n = 8) the *FCGR2B* variant; results are from 1 representative patient. Values in the shaded boxes are the mean fluorescence intensity (MFI). **B,** The percentage of positive cells and the MFI values for the whole group for FcγRI, FcγRIIa, FcγRIIb, and FcγRIII were also assessed. **C,** The expression of FcγRIIb in RA patients with or without the *FCGR2B* variant was assessed both under steady-state conditions (incubation with fetal calf serum [FCS]) and upon incubation of DCs with synovial fluid (SF) from RA patients with the genetic variant (n = 4). In **A,** the bar marked M1 represents the cutoff point for the isotype control (per marker investigated) in which fewer than 5% of the cells were positive; thus, flow cytometry results appearing to the right of the M1 cutoff indicate positivity for the actual antibody and thus represent the level of expression of that given marker. Bars show the mean and SD.

expression of FcγRIIb significantly in response to incubation with RA SF. This latter finding suggests that DCs in RA patients carrying the *FCGR2B* variant have a diminished capacity to increase the expression of FcγRIIb after incubation with certain stimuli.

As a control in all experiments, the effect of non–heat-treated IgG was compared with that of heat-aggregated gamma globulins. Non–heat-treated IgG elicited very low responses with regard to cytokine production by DCs, but these responses were similar between individuals carrying and those not carrying the FcγRIIb variant (results not shown).

DISCUSSION

The role of activating FcγRs in RA has been studied thoroughly. However, the potential role of the inhibitory FcγRIIb has not been subjected to similar scrutiny. The present study is the first to show that the *FCGR2B* variant is associated with a strongly increased rate of radiologic joint damage in patients with RA, despite the fact that these patients received a higher number of DMARDs that were more potent. In addition, we provide firm evidence that the function of DCs in RA after FcγR-mediated triggering is severely affected by the FcγRIIb functional variant. This suggests that the balance between activating and inhibiting FcγRs expressed by DCs, and likely other FcγR-bearing immune effector cells such as macrophages and B cells, is a strong determinant of disease progression in RA and possibly other autoimmune diseases.

It is well documented that the balance between triggering of activating and inhibitory FcγRs determines the outcome of immune responses. Kalergis and Ravetch demonstrated that selective engagement of activating FcγRs by ICs led to potent T cell activation, whereas stimulation with ICs in the presence of the inhibitory FcγRIIb resulted in T cell tolerance (46). More recently, Dhodapkar et al (35) demonstrated that FcγRIIb blockade of DCs loaded with tumor cells led to increased tumor-specific T cell immunity without the need for exogenous stimuli. These findings suggest that the inhibitory FcγR is a pivotal component of the regulatory network that prevents DC maturation in response to ICs during health and disease. Consistent with this possibility, the balance between activating and inhibitory FcγRs was found to be strongly associated with the phenotype and cytokine response of DCs and monocytes from RA patients (17,36,37).

Since antigen–ICs are abundant in RA patients, it is tempting to speculate that signaling through the inhibitory FcγRIIb functions as a counteractive mechanism in RA to dampen the Th1-driven immune response. The finding that RA patients carrying the *FCGR2B* variant experience significantly more radiologic joint damage underscores our hypothesis that the inhibitory FcγR is involved in the dampening of the

immune response via IC-mediated pathways in RA. Recently, the existence of such a mechanism was further substantiated by the observation that FcγRIIb deficiency in mice renders them susceptible to experimental arthritis (8,9) and other autoimmune diseases, including SLE (47).

Interestingly, our results provide support for the recent discussions in which it was postulated that inflammation and destruction in RA might be uncoupled processes (48,49). In the present study, RA patients carrying the *FCGR2B* variant had more radiologic joint damage after 3 and 6 years of disease, despite being treated with more aggressive DMARDs. This underscores the possibility that although the disease activity may be controlled, structural joint damage may continue.

Thus far, only a small number of studies have addressed the potential role of *FCGR2B* polymorphisms in autoimmune diseases. SLE is an exception, since the 695T>C variant coding for the Ile232Thr substitution in the transmembrane domain of FcγRIIb was found to be associated with this disorder in Japanese (19), Chinese (23), and Thai (24) populations. On the functional level, Li et al demonstrated that coengagement of the FcγRIIb Ile232Thr genetic variant with the B cell antigen receptor resulted in an enhanced capacity to dephosphorylate tyrosine residues in CD19 and to inhibit the calcium influx (27). These findings suggest that the FcγRIIb variant allele codes for a more active receptor on B cells, at least in some aspects of signaling. The effect of the variant on the functionality of the receptor on immature B cells or DCs, in which the role of FcγRIIb is different from that on mature B cells, has not been investigated. By means of a highly selective monoclonal antibody directed against FcγRIIb (35), the present study demonstrates that the *FCGR2B* variant might result in both a diminished function and/or a reduced induction of expression of the receptor upon certain triggers. Interestingly, the latter is consistent with recent findings from 2 research groups demonstrating that the FcγRIIb polymorphism in SLE results in loss of function by exclusion from lipid rafts, suggesting that in SLE the FcγRIIb system is also up-regulated by circulating factors (25,26).

FcγRs are expressed on a wide variety of immunologic effector cells, including macrophages, DCs, B cells, and chondrocytes, all of which are implicated in the inflammatory circle of synovitis and joint destruction. Herein we focused on the effect of the *FCGR2B* variant on DC function. Since DCs are important in controlling the balance between immunity and tolerance, the observed loss of IC-mediated inhibition of DC function due to the *FCGR2B* variant could lead to a potentiated immune response, resulting in an ongoing loop of joint inflammation and destruction. Despite the fact that our focus was on DCs, it is conceivable that the function of other effector cells that express FcγRs, such as macrophages, B cells, and chondrocytes, is also influenced by the *FCGR2B* variant. Further research that deciphers the potential role of FcγRIIb in the function of these cells is therefore likely to increase our knowledge of the role of the inhibitory FcγRIIb in many autoimmune conditions.

Our present study thus provides compelling evidence for the critical role of FcγRIIb in the severity of RA. These findings warrant further in-depth research to reveal the potential of targeting FcγRIIb as a novel therapeutic approach in RA and other IC-driven autoimmune conditions.

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