

FULL PAPER

Genetic risk factors for infection in patients with early rheumatoid arthritis

LB Hughes¹, LA Criswell², TM Beasley¹, JC Edberg¹, RP Kimberly¹, LW Moreland¹, MF Seldin³ and SL Bridges Jr¹

¹University of Alabama at Birmingham, Birmingham, AL, USA; ²University of California, San Francisco, USA; ³University of California, Davis, USA

We analyzed clinical and genetic factors contributing to infections in 457 subjects with early rheumatoid arthritis (RA) enrolled in a prospective, 1-year clinical trial of methotrexate and the TNF inhibitor etanercept. Subjects were genotyped for the following single nucleotide polymorphisms (SNPs): (TNF -308, -238, and +488); lymphotoxin- α (LTA) (LTA +249, +365, and +720); and Fc gamma receptors FCGR2A 131 H/R; FCGR3A 176 F/V; and FCGR3B NA 1/2 and genotypes were correlated with infections. At least one URI was noted in 52% of subjects (99/191) with the NA2/NA2 genotype of the neutrophil-specific FCGR3B gene, compared to 42% (77/181) of those with the NA1/NA2 genotype and 39% (23/59) of those with the NA1/NA1 genotype ($P=0.038$). Urinary tract infection (UTI) was associated with the TNF -238 A (odds ratio(OR) 2.56, 95% confidence interval (CI) 1.05–6.25) and LTA +365 C (OR 1.73, 95% CI 1.07–2.79) alleles, and marginally with the FCGR3A F allele (OR 1.72, 95% CI 0.99–3.00). There was a striking linear correlation between UTI and the number of risk alleles defined by these three SNPs ($P<0.001$), suggesting an additive effect on susceptibility. These findings have important implications for the role of genetics in susceptibility to bacterial and viral infections.

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Introduction

Patients with rheumatoid arthritis (RA), a common chronic inflammatory disease, have been shown to have risk of infection twice that of age- and sex-matched controls.¹ The most common infections reported in patients with established RA include urinary tract infections (UTI), respiratory tract infections (URI), skin and soft tissue infections, pneumonia, and joint infections.¹ Increased susceptibility to infections in RA is likely multifactorial resulting from the underlying immunologic disturbances associated with the disease, the use of immunosuppressive agents, and genetic predisposition. Population-based studies have identified clinical factors associated with infections in patients with RA, including increased age, comorbid diseases, corticosteroid use, and markers of disease severity such as serum rheumatoid factor (RF), the presence of rheumatoid nodules, elevated Westergren sedimentation rate (ESR), and reduced functional capacity.¹

Methotrexate (MTX) is one of the most commonly used and effective therapies for RA.² RA patients receiving

MTX are more likely to have infections of the respiratory tract and skin and to receive prescriptions for antibiotics than RA patients receiving no disease-modifying anti-rheumatic drugs (DMARDs) or DMARDs other than MTX (not including biologic agents).³ In clinical trials, infection rates among subjects taking etanercept are no higher than that of MTX,⁴ although opportunistic infections have been reported (reviewed in Cunnane *et al*⁵). The use of biologic agents in RA has raised concern over risk of serious and opportunistic infections, but common infections have not been evaluated in large, prospective studies. Infections such as URI and UTI contribute substantially to health-care costs and patient morbidity.

The proinflammatory cytokines TNF and LTA play important roles in infectious and autoimmune diseases. TNF blockade *ex vivo* inhibits expression of Toll-like receptor 4 (TLR-4) on dendritic cells from RA patients and controls,⁶ which has important implications with regard to susceptibility to multiple infectious organisms. LTA shares many of the same biological and structural characteristics of TNF, and their genes lie in tandem in the human MHC region on chromosome 6p21. Murine studies have shown LTA to be important in tuberculosis, cerebral malaria, and cerebral *Toxoplasmosis gondii*.^{7–9}

Single nucleotide polymorphisms (SNPs) in TNF, and to a lesser extent LTA, are associated with susceptibility to, or severity of various autoimmune and infectious diseases. Since the report of association between the TNF -308 SNP and susceptibility to cerebral malaria,¹⁰ the

Correspondence: Dr SL Bridges Jr, Division of Clinical Immunology and Rheumatology, 412 Lyons-Harrison Research Building, University of Alabama at Birmingham, Birmingham, AL, 35294-0007, USA.
E-mail: LBridges@uab.edu
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role of *TNF* polymorphisms have been studied in many other diseases, including RA, psoriatic arthritis, leishmaniasis, hepatitis C, and ankylosing spondylitis.^{11–15} Although the roles of *TNF* and *LTA* SNPs in infections and in susceptibility to and severity of RA are controversial, their roles in susceptibility to infections may be accentuated in RA patients receiving MTX or etanercept.

In addition to cytokine-mediated immune processes, antibody-mediated pathways are integral to the pathogenesis of infectious and autoimmune diseases. The binding of IgG to Fc γ receptors can trigger numerous important effector responses including macrophage phagocytosis, natural killer cell antibody-dependent cellular cytotoxicity, and neutrophil activation.¹⁶ Alteration in the function of Fc γ receptors has been associated with susceptibility to autoimmune and infectious diseases.¹⁷ In turn, allelic variants that influence receptor function have been identified in the *FCGR2A*, *FCGR3A*, and *FCGR3B* genes.¹⁸ These differences and their predicted effect on infections are shown in Table 1. Kimberly *et al*^{19,20} have presented preliminary evidence for roles of Fc γ receptor and *TNF* polymorphisms in infection among subjects with RA.

The goal of the current study was to delineate influences of clinical factors and polymorphisms in *TNF*, *LTA*, and Fc γ R genes on infections in subjects with early RA treated with etanercept or MTX.

Results

Baseline characteristics, measures of disease activity, and number of subjects taking low dose prednisone were similar in the three treatment groups (Table 2). Approximately 40% of subjects were taking low-dose glucocorticoids (prednisone \leq 10 mg/day) at study entry.⁴ Among subjects in the MTX group, the mean dose of MTX was 19 mg per week.⁴ The number of patients with one or more infections was similar among the treatment groups.⁴ In all, 61.5% of the subjects had at least one reported infection during the study period. The most common infections are listed in Table 3. The number of patients with one or more infections was similar in all treatment groups,⁴ except for increased frequency of URI in the MTX group compared to the ET10 and ET25 groups. Infections requiring hospitalization or intravenous antibiotics occurred in less than 3% of patients in each group.⁴ There were no documented opportunistic

infections or deaths from infections during the study period.⁴ Importantly, neither the univariate nor multivariable analysis found association between the use of

Table 2 Baseline characteristics of 457 patients with early RA according to treatment group

Baseline characteristic*	Methotrexate (n = 156)	Etanercept 10 mg (n = 150)	Etanercept 25 mg (n = 151)
Age (mean)	49	51	50
Female (%)	72	73	74
Caucasian (%)	88	87	87
Disease duration (mean no. months)	11	12	13
RF positive (%)	89	88	87
Elevated ESR (%)	79	80	77
No. tender joints (mean)	30	32	30
No. swollen joints (mean)	24	24	23
Previous DMARD (%)	45	41	41
No. of erosions (mean)	7	6	7
Low-dose prednisone (%)	45	42	38

*All comparisons were nonsignificant (i.e., $P > 0.05$) based on χ^2 (for dichotomous variables) or Student's *t*-tests (for continuous variables).

RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; DMARD = disease-modifying antirheumatic drugs.

Table 3 Number and type of most common infections in the parent clinical trial

Infection type	No. infections	% of all infections	No. individuals with infection	No. of subjects genotyped ^a
URI ^b	533	45.2	275	199
Influenza	82	7.0	73	64
UTI	66	5.7	51	48
Dental infection	33	3.0	29	26
Gastroenteritis	24	2.1	21	19
Pneumonia	23	2.0	19	18
Other	52	4.8	45	39

^aGenotyping was successful in all subjects at the three *TNF* SNPs and the three *LTA* SNPs, and in >95% of subjects at the three Fc receptor SNPs.

^bURI = There was a significant difference in the number of URIs among the treatment groups, MTX: 109 URIs, ET10: 80 URIs, ET25: 86 URIs ($P = 0.004$).

Table 1 Selected characteristics of human Fc γ receptors

Fc receptor	Gene	Cell expression	Fc binding specificity	Genotype	Fc binding affinity	Predicted effect on infections
Fc γ RIIa	<i>FCGR2A</i>	Most cell types	IgG ₁ , IgG ₃ , IgG ₂ (H allele only)	131 HH HR RR	High for IgG ₂ Intermediate Low for IgG ₂	Fewest infections Intermediate Most infections
Fc γ RIIIa	<i>FCGR3A</i>	NK cells, macrophages	IgG ₁ , IgG ₃ , IgG ₄	176 VV FV FF	Highest Intermediate Lowest	Fewest infections Intermediate Most infections
Fc γ RIIIb	<i>FCGR3B</i>	Neutrophils	IgG ₁ , IgG ₃	NA1/NA1 NA1/NA2 NA2/NA2	Highest ^a Intermediate Lowest	Fewest infections Intermediate Most infections

^a*FCGR3B* NA alleles differ in their quantitative ability to activate neutrophils; ligand binding is comparable.

low-dose glucocorticoids and susceptibility to total infections, URI, or UTI.

Genotype frequencies for the nine polymorphisms are shown in Table 4. There were no statistically significant differences in the allele frequencies between the Caucasian and non-Caucasian subjects, although the number of non-Caucasians was small.⁴ Univariate analysis revealed no significant association between any individual SNP allele and presence or absence of any infection in the entire group of subjects or in subjects in each treatment group. We then analyzed the three largest groups of specific infections (URI, influenza, and UTI) individually. There were no significant SNP associations with susceptibility to influenza, which was the second most common reported infection. Univariate analysis revealed significant associations between URI and age ≥ 65 years, treatment with MTX, and the presence of the *FCGR3B* NA2 allele (Table 5). Presence of serum RF and elevated ESR were of marginal significance ($P = 0.055$ and 0.080 , respectively), while corticosteroid use was not signifi-

cant. The NA2 allele was associated with URI (odds ratio(OR) 1.34, 95% confidence interval(CI) 1.02–1.77). The proportions of subjects with at least one URI were: 52% (99/191) of those with the NA2/NA2 genotype; 42% (77/181) of those with NA1/NA2; and 39% (23/59) of those with NA1/NA1. In the multivariable model, age ≥ 65 years, and MTX treatment remained significant, and elevated baseline ESR achieved statistical significance (OR 1.70, 95% CI 1.12–2.58) (Table 5). The *FCGR3B* NA2 allele remained significantly associated with URI in this model (OR 1.34, 95% CI 1.01–1.78) (Table 5). We found no associations with *FCGR* haplotypes and URI, suggesting that NA2 allele is the biologically relevant polymorphism playing a role in susceptibility to URI.

When analyzing risk alleles for UTI, univariate analysis revealed statistically significant associations with the *TNF* -238 A, *LTA* +365 C, and *FCGR3A* F alleles, but with none of the clinical variables (Table 6). Seven of 42 (17%) of individuals with the *TNF* -238 AG genotype had at least one UTI during the study period, compared to 30 of 415 (7%) of subjects with the *TNF* -238 GG genotype, (OR 2.56, 95% CI 1.05–6.25) (Table 6). No individuals in the study had the *TNF* -238 AA genotype (Table 4). In all, 13% (17/132) of individuals with the *LTA* +365 CC genotype had at least one UTI during the study period, compared to 7% (14/209) of subjects with the *LTA* +365 CG genotype and 5% (6/116) of those with the *LTA* +365 GG genotype (OR 1.73, 95% CI 1.07–2.79) (Table 6). The *FCGR3A* F was marginally associated with UTI, as 10% (21/201) of those with the FF genotype, 7% (13/196) of those with FV genotype, and 3% (2/58) of those with the VV genotype had at least one UTI during the study period (OR 1.72, 95% CI 0.99–3.00) (Table 6). In the multivariable model, the *TNF* -238 A allele showed a trend towards increased UTI but no longer achieved

Table 4 SNP genotypes of all subjects and the subset of Caucasians

	Total ^a	Caucasian
<i>TNF</i> -308 GG	284 (0.62)	250 (0.63)
GA	168 (0.37)	145 (0.36)
AA	5 (0.01)	3 (0.01)
<i>TNF</i> -238 GG	415 (0.91)	365 (0.92)
GA	42 (0.09)	33 (0.08)
AA	0	0
<i>TNF</i> +488 GG	387 (0.85)	338 (0.85)
GA	63 (0.14)	55 (0.14)
AA	7 (0.01)	5 (0.01)
<i>LTA</i> +249 AA	162 (0.35)	140 (0.35)
AG	282 (0.62)	248 (0.62)
GG	13 (0.03)	10 (0.03)
<i>LTA</i> +365 GG	146 (0.32)	123 (0.31)
GC	247 (0.54)	215 (0.54)
CC	64 (0.14)	60 (0.15)
<i>LTA</i> +720 CC	162 (0.35)	141 (0.35)
AC	251 (0.55)	222 (0.56)
AA	44 (0.10)	35 (0.09)
<i>FCGR2A</i> 131 HH	108 (0.24)	93 (0.24)
HR	224 (0.50)	187 (0.49)
RR	114 (0.26)	101 (0.27)
<i>FCGR3A</i> 176 FF	201 (0.44)	165 (0.43)
FV	197 (0.43)	175 (0.45)
VV	58 (0.13)	47 (0.12)
<i>FCGR3B</i> NA2/NA2	191 (0.44)	167 (0.45)
NA1/NA2	183 (0.42)	157 (0.43)
NA1/NA1	59 (0.14)	46 (0.12)

^aThe number of subjects successfully genotyped for the three *TNF* SNPs and the three *LTA* SNPs was 457 (398 Caucasian). The total number of subjects genotyped for the *FCGR2A*, *FCGR3A*, *FCGR3B* polymorphisms were 446, 456, and 433, respectively. The number of Caucasian subjects genotyped for the *FCGR2A*, *FCGR3A*, *FCGR3B* polymorphisms were 381, 387, and 370, respectively.

Table 5 Factors associated with URI in univariate and multivariable analyses

Parameter	OR	95% CI	P
<i>(a) Univariate analysis</i>			
Age (≥ 65 years)	2.42	1.50–3.91	<0.001
Elevated ESR	1.34	0.97–1.85	0.080
Positive RF	1.63	0.98–2.71	0.055
Corticosteroid use	1.12	0.81–1.54	0.486
Treatment group ^a	1.34	1.02–1.77	0.040
<i>TNF</i> -308 A	1.06	0.74–1.53	0.741
<i>TNF</i> -238 A	1.28	0.67–2.44	0.456
<i>TNF</i> +488 A	0.83	0.53–1.30	0.412
<i>LTA</i> +249 G	0.94	0.66–1.33	0.710
<i>LTA</i> +365 C	0.90	0.70–1.15	0.397
<i>LTA</i> +720 A	1.04	0.77–1.40	0.788
<i>FCGR2A</i> R	1.08	0.83–1.41	0.566
<i>FCGR3A</i> F	1.29	0.99–1.70	0.061
<i>FCGR3B</i> NA2	1.34	1.02–1.77	0.038
<i>(b) Multivariable analysis</i>			
Age (≥ 65 years)	2.29	1.26–4.15	0.007
Elevated ESR	1.70	1.12–2.58	0.013
Positive RF	1.42	0.74–2.75	0.293
Corticosteroid Use	1.22	0.81–1.82	0.341
Treatment Group ^a	1.51	1.09–2.11	0.014
<i>FCGR3B</i> NA2	1.34	1.01–1.78	0.043

OR = odds ratio, 95% CI = 95% confidence intervals. ^aTreatment group was MTX *vs* etanercept (either 10 or 25 mg twice weekly).

Table 6 Factors associated with UTI in the univariate and multivariable analyses

Parameter	OR	95% CI	P
<i>(a) Univariate analysis</i>			
Age (≥ 65 years)	1.77	0.89–3.52	0.118
Female	1.07	0.53–2.14	0.855
Elevated ESR	1.27	0.71–2.27	0.417
Positive RF	1.64	0.57–4.68	0.329
Corticosteroid use	1.21	0.68–2.151	0.519
Treatment group ^a	1.01	0.51–2.02	0.970
TNF –308 A	1.05	0.54–2.04	0.890
TNF –238 A	2.56	1.05–6.25	0.038
TNF +488 A	1.04	0.46–2.38	0.923
LTA +249 G	1.87	0.98–3.57	0.057
LTA +365 C	1.73	1.07–2.79	0.026
FCGR2A R	1.30	0.81–2.13	0.275
FCGR3A F	1.72	0.99–3.00	0.055
FCGR3B NA2	1.28	0.79–2.05	0.316
<i>(b) Multivariable analysis</i>			
Age (≥ 65 years)	1.49	0.63–3.52	0.363
Elevated ESR	1.26	0.62–2.56	0.518
Positive RF	1.32	0.43–4.06	0.631
Corticosteroid Use	1.15	0.58–2.27	0.695
Treatment Group ^a	1.05	0.58–1.91	0.881
TNF –238 A	2.45	0.99–6.13	0.054
LTA +365 C	1.70	1.04–2.77	0.033
FCGR3A F	1.76	1.01–3.10	0.048

^aMTX *vs* etanercept (10 or 25 mg twice weekly).

statistical significance (OR 2.45, 95% CI 0.99–6.13) (Table 6). The LTA +365 C allele association with UTI remained significant in the multivariable analysis (OR 1.70, 95% CI 1.04–2.77), while the association with the FCGR3A F allele achieved significance (OR 1.76, 95% CI 1.01–3.10) (Table 6). There were no interactions between factors in the multivariable analysis.

If these three polymorphisms each have independent biologic effects leading to increased susceptibility to UTI, there may be an additive or synergistic effect of having multiple risk alleles. Thus, we examined associations between UTI and the number of risk alleles, defined as the total number of the TNF –238 A, LTA +365 C, and FCGR3A F alleles. Since no individuals were homozygous for the TNF –238 A allele, subjects had a range of 0 to 5 UTI risk alleles. Although there was a normal distribution of UTI risk alleles among all individuals in the study (Figure 1a), there was a striking correlation between the number of risk alleles and presence of at least one UTI during the study period. As shown in Figure 1b, the percentage of subjects with at least one UTI were 0% (0 of 20 subjects), 1.7% (1 of 60 subjects), 6.7% (11 of 164 subjects), 8.5% (11 of 130 subjects), 15.7% (11 of 70 subjects), and 18.2% (2 of 11 subjects) for those with 0, 1, 2, 3, 4, and 5 risk alleles, respectively (Pearson correlation coefficient $r = 0.161$, $P < 0.001$).

There were no significant associations between UTI and various combinations of haplotypes defined by the three TNF SNPs, the three LTA SNPs, all six TNF/LTA SNPs, or the Fc γ R SNPs. Again, these results suggest that associations with UTI are either specific to the TNF –238 A, LTA +365 C, and FCGR2A F alleles, or to haplotypes in linkage disequilibrium with these SNPs but not examined in this study.

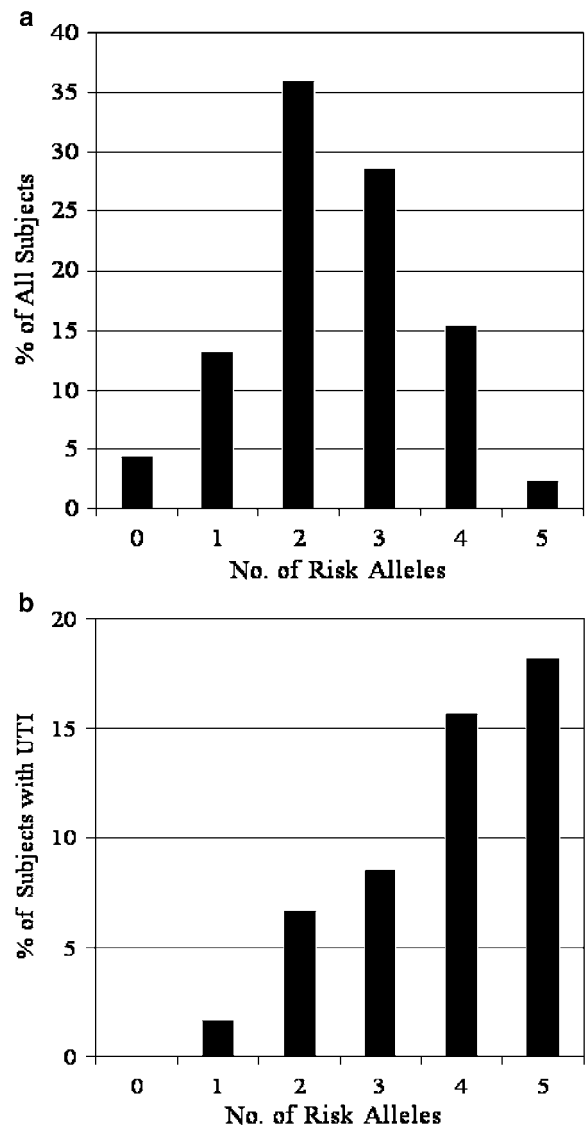


Figure 1 (a) Distribution of 455 study subjects according to number of risk alleles. Of the original 457 subjects, two were excluded because of lack of complete genotypes. Risk alleles were defined as number of TNF –238 A, LTA +365 C, and FCGR3A 176 F alleles. Since no individuals were homozygous for the TNF –238 A allele, subjects could have a maximum of five risk alleles. (b) Percentage of subjects with given number of risk alleles who had at least one UTI during the study period.

Discussion

The current study is the largest reported analysis (457 subjects) of the impact of clinical and genetic risk factors for common infections. A major strength of the study is that infections were identified systematically as part of a prospective double-blind clinical trial. We identified several clinical and genetic factors that are associated with susceptibility to URI and UTI in RA patients receiving MTX or etanercept. Of the polymorphisms examined, the functional properties of the nonsynonymous alleles in the coding regions of the Fc receptor genes are best characterized. It is known that the isoform

containing the *FCGR3B* NA1 allele produces a larger phagocytic response, oxidative burst, and degranulation response than the *FCGR3B* NA2 allele,¹⁸ which is consonant with our finding of increased URI in subjects with the NA2 allele. The FcγIIIb receptor is expressed exclusively on neutrophils and eosinophils; depending on the causative organism, these cells may be critical for host defense against URI. The *FCGR3B* NA2 allele may play a role in susceptibility to other infections, as the combination of *FCGR2A* 131 R/R and *FCGR3B* NA2/NA2 genotypes is associated with meningococcal disease in subjects with late complement component (C5–C9) deficiency.²¹ The current study is the first to demonstrate a role for the *FCGR3B* NA2 allele in a common form of infection, URI.

FcγIIIa, encoded by *FCGR3A*, is expressed predominantly on NK cells and macrophages. Previous reports of the functional significance of the *FCGR3A* F allele have shown decreased *FCGR3A* receptor activity compared to the V allele¹⁸ consistent with our finding of increased UTI in subjects with the *FCGR3A* F allele. Few reports exist regarding FcγRIIIa polymorphisms and risk of infection. In one recent study, the FF genotype was associated with increased susceptibility to poliomyelitis.²² This effect has been hypothesized to be due to less effective clearance of the poliovirus by the *FCGR3A* F allele compared to the *FCGR3A* V allele. Thus, our findings of increased susceptibility to URI in relation to the *FCGR3B* NA2 allele and increased susceptibility to UTI in relation to the *FCGR3A* 176 F allele are consistent with the biologic effects of these polymorphisms.

Several studies have found associations of *FCGR2A* polymorphisms with susceptibility to meningococcal disease,²³ periodontal disease²⁴ recurrent bacterial respiratory tract infection in children with low IgG2 anti-carbohydrate antibodies²⁵ bacteremic pneumococcal pneumonia,²⁶ and invasive pneumococcal infections in patients with SLE.²⁷ The *FCGR2A* 131 H/R SNP strongly influences the ability of the receptor to bind human IgG₂, the isotype often made in response to encapsulated bacteria. In the current analysis, we did not find association between *FCGR2A* alleles and infections. One possible explanation for the absence of such an association is that most previous reports focused on serious infections requiring hospitalization, in which cases were identified in retrospective reviews of extended periods of hospital records. Our data were collected among outpatients over a 1-year period and there were few infections requiring hospitalization.

Our understanding of the biological relevance of the *TNF* –238 and *LTA* +365 SNPs is less clear than that of the FcγR polymorphisms. The *TNF* –308 A allele, for example, has been reported to be associated with increased *TNF* production *in vitro* and increased transcription of *TNF*.²⁸ Other investigators, however, have found no significant effect of this polymorphism on *TNF* expression.^{11,29} Studies using chromatin immunoprecipitation and mass spectroscopy showed, somewhat surprisingly, that the *TNF* –308 SNP influences transcription of the *LTA* gene, but not the *TNF* gene.³⁰

In vitro transfection assays indicate that *TNF* –238 A allele does not appear to have a direct effect on transcriptional activation of the *TNF* gene.³¹ A study of subjects with chronic active hepatitis C, however, found

that the frequency of the *TNF* –238 A allele was significantly higher in subjects than controls.¹⁴ This finding, which was not explained by linkage disequilibrium (LD) to HLA-B or HLA-DR genes, suggests that the *TNF* –238 A allele predisposes to less efficient host defense to this viral infection.

There are several potential explanations for the observed association of infections with individual *TNF* and *LTA* SNP alleles rather than SNP haplotypes. First, the number of subjects may be too small to allow for analysis of multiple haplotypes. Second, the individual *TNF* and *LTA* SNP alleles themselves may be more biologically relevant than haplotypes, as appears to be the case with the Fcγ receptor SNPs analyzed in this study. Finally, these genetic associations may reflect the effect of other SNPs in LD with the SNPs genotyped in this study. These SNPs may lie in the promoter or other regulatory regions and may influence gene transcription or mRNA stability.

It is unclear whether the results of our analysis can be extrapolated to individuals without RA, to subjects with RA on DMARDs other than MTX or etanercept, or to RA patients on no DMARDs. The majority of the subjects in the parent clinical trial were Caucasian,⁴ so the role of racial/ethnic differences in genetic influences on infection could not be examined. Because serious infections were rare in the 1-year study period, we were unable to analyze genetic influences on serious infections such as opportunistic infections, or those requiring hospitalization.

Our multivariable analysis found that three clinical variables, age >65 years, elevated baseline ESR, and MTX treatment, were associated with increased risk of URI. No clinical variables were associated with UTI or total infections. In a population-based study of 609 subjects with established RA, Doran *et al*¹ also found that increased age and elevated ESR were important clinical predictors of infection. In addition, they found that comorbid diseases, extra-articular manifestations of RA, positive RF, rheumatoid nodules, reduced functional capacity, and corticosteroid use were associated with infections. There are several factors that likely contribute to the lack of significance of these additional variables in our study. First, the patients in our study had early RA, in which extra-articular manifestations and reduced functional capacity are uncommon. In addition, patients with important concurrent illnesses were excluded from the parent clinical trial, whereas subjects with chronic lung disease, alcoholism, leukopenia, organic brain disease, and other serious conditions were included in the population-based study of Doran *et al*. Approximately ~40% of our patients were taking low dose corticosteroids (prednisone ≤10 mg/day) at baseline. Since subjects had mean disease duration of ~12 months, the duration of corticosteroid use was probably limited. In contrast, ~47% of subjects in the study by Doran *et al* had received i.m. or i.v. corticosteroids, and the median number of days corticosteroids were received was 798 (~2.2 years). Thus, major differences in study design probably account for the differences in results.

In summary, using prospectively collected data from 457 subjects with early RA treated with MTX or etanercept, we identified associations between common infections and polymorphisms in genes relevant to the

pathogenesis of infectious and autoimmune diseases. The association between the *FCGR3B* NA 2 allele and susceptibility to URI, and between the *FCGR3A* F, *TNF* -238 A, and *LTA* +365 C alleles and UTI have important implications regarding the pathogenesis of infectious diseases and mechanisms underlying susceptibility to infection in subjects with RA and perhaps normal individuals.

Patients and methods

Study subjects

In the Immunex Early RA trial,⁴ a total of 632 patients with early RA (≤ 3 years) were randomized to receive either MTX (initial dose of 7.5 mg with rapid escalation to 20 mg per week by week 8), low-dose etanercept (10 mg twice weekly) (ET10), or standard dose etanercept (25 mg twice weekly) (ET25). DMARDs, including hydroxychloroquine and sulfasalazine, were discontinued at least 4 weeks before the study began. Stable doses of nonsteroidal anti-inflammatory drugs and prednisone (≤ 10 mg daily) were allowed. Of the 632 subjects, 457 (72.3%) consented to participate in the current genetic study.

During the trial, clinical and laboratory assessments were performed at baseline, week 2, and at 1, 6, 8, 10, and 12 months. All infections were recorded by study coordinators using a standardized protocol that characterized the site and intensity of the infection, infection onset, treatment given, requirement for hospitalization, and resolution of the infection.

Genetic polymorphisms

Candidate genes for this study and associated polymorphisms were carefully chosen based on their possible association with susceptibility to infections and their likelihood of being affected by immunomodulatory agents. We analyzed SNPs at positions -308, -238, and +488 of the *TNF* gene; and three SNPs at positions +249, +365, and +720 of the lymphotoxin-alpha (*LTA*) gene, which have been reported to define haplotypes.³² Because of the importance of Fc receptors in infectious diseases, and because etanercept-TNF complexes are thought to be degraded through the Fc receptor (FcR) pathway, we genotyped each subject for the following FcR polymorphisms: *FCGR2A* 131 H/R, *FCGR3A* 176 F/V, and *FCGR3B* NA 1/2.

Genotyping methods

***TNF/LTA* SNPs.** *TNF* and *LTA* SNPs were genotyped by PCR amplification of genomic DNA and restriction fragment length polymorphism (RFLP) analysis based in part on the methods described by Mullighan *et al.*³²

Fc receptor polymorphisms. The 131 H/R alleles of *FCGR2A*, the 176 F/V alleles of *FCGR3A*, and the NA1/NA2 alleles of *FCGR3B* were determined as previously described.³³ The genotyping approach included both allele-specific PCR and direct sequencing optimized for heterozygote detection on an ABI 377 automated sequencer.

Haplotypes defined by the six *TNF-LTA* polymorphisms and by the three Fc γ R alleles were constructed using the PHASE program.³⁴

Statistical methods

Baseline clinical characteristics potentially affecting susceptibility to infections and SNP alleles were analyzed for association with (1) presence or absence of any infection and (2) presence or absence of the three most common reported specific infections (URI, influenza, and UTI). Each potential predictor variable was analyzed using univariate logistic regression and the OR, 95% CI, and *P*-values were reported. A stepwise selection process was used to create a multivariable model of potential predictors of infection, including examination of two-way interaction among all variables in the model. The following baseline clinical and laboratory variables were included in the analyses: (1) advanced age (≥ 65 years of age *vs* < 65 years of age); (2) elevated ESR (females > 30 mm/hr, males > 13 mm/hr), presence of rheumatoid factor (baseline RF ≥ 20 IU/ml, use of corticosteroids (prednisone ≤ 10 mg/day at baseline), and treatment group (MTX *vs* ET10 *vs* ET25). Because UTI is more common in women than in men, sex (female *vs* male) was included in the model used to analyze UTI. Specific SNP alleles were analyzed using an additive mode of inheritance. All analyses were performed using SPSS version 11.0 statistical software.³⁵

Although the genes examined in the current study were chosen *a priori* based on evidence of association with infection, the number of comparisons increases the likelihood of false-positive results. It is difficult to determine the appropriate level of statistical adjustment for these analyses since many of the polymorphisms (eg, those corresponding to the same gene or genomic region) are not independent. Therefore, all *P*-values shown are nominal (ie, uncorrected) and *P*-values < 0.05 were considered significant.

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