Review Article

Recent advances on the genetics of rheumatoid arthritis: current topics and the future

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Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes severe joint pain and eventually joint deformity. Recent large cohort studies and the rapid progression of genotyping platforms have enabled identification of more than 30 susceptibility genes for RA. *HLA* is the major genetic determinant for RA for which a shared epitope hypothesis (70th-74th amino acids of HLA-DR β chain determine susceptibility) has been accepted. However, recent detailed single nucleotide polymorphism (SNP) typing of the *HLA* region and imputation method revealed that the most important amino acid positions of the HLA-DR β chain are the 11th in addition to the 71st and the 74th. HLA-B (at position 9) and HLA-DPB1 (at position 9) are also important determinants. This revised shared epitope hypothesis will form a new theory for *HLA* association. Another topic is that anti-citrullinated protein antibody (ACPA)-negative RA has been shown to be genetically different from ACPA-positive RA. Many susceptibility genes including *HLA* were not associated with ACPA-negative RA; however, we have shown that some *HLA* alleles are associated with ACPA-negative RA. In this review, we present some new findings regarding *HLA* as well as some recently discovered susceptibility genes for RA.

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Introduction

Since 2003, when sequencing of the human genome was completed, there has been a burst of identification of new susceptibility genes for RA. In the last several years in particular, more than 30 genes or loci have been identified as RA-related genes[1]. This activity was supported by the development of SNP genotyping platform, which enables us to type hundreds of millions of SNPs in a few weeks, even in a relatively small lab. In addition, a growing number of large cohorts were formed to tackle the elucidation of RA pathogenesis, which provided substantial power to detect genes of significance[2].

ACPA is a specific autoantibody of RA, and its target antigens are citrullinated vimentin, filaggrin, α-enolase, and others[3]. It is a useful marker not only for diagnosis of RA, but also for predicting disease course[4]. ACPA-positive RA

is clinically severer than ACPA-negative RA. Moreover, it has been suggested that ACPA-positive RA is genetically distinct from ACPA-negative RA[5,6].

Here we present the recent advances in RA genetics and also discuss the genetic differences between ACPA-positive and ACPA-negative RA.

Human leukocyte antigen (HLA)

Genetic predisposition to RA has been investigated intensively. *HLA* is a major determinant of RA susceptibility and *HLA-DRB1**01:01, *01:02, *04:01, *04:04, *04:05, *04:08, *04:10, *04:13, *04:16, *10:01, *14:02 and *14:06 were reported to be associated with RA development. Among these *HLA-DRB1* alleles, there are common amino acid sequences at the 70th-74th residues of the HLA-DRβ chain (QKRAA, QRRAA or RRRAA), which is called a 'shared epitope' (SE)[7]. The association of *HLA-DRB1* SE with RA has been replicated in many ethnic groups[8]. However, recently the important role of Leucine at 67th position (Leu67)[9-10] and Valine at 11th position (Val11)[10] for RA development and resistant effect on RA development by Aspatic acid at 70th position (Asp70)[11] were also reported. In addition, Raychaudhuri et al. used existing genome-wide SNP data of >5,000 ACPA-positive RA cases and ~15,000 controls and imputed (expected SNP genotypes in silico from adjacent SNP gentotypes and linkage disequilibrium information) the gap SNP genotypes of HLA locus and reported the following findings. They showed that three amino acid positions (11, 71 and 74) of HLA-DRβ chain as well as single-amino acid positions in HLA-B (at position 9) and HLA-DPβ chain (at position 9) explain most of the MHC association with RA[12]. All these positions are located in peptide-binding grooves, as shown in Fig.1. Among these positions, position 11 of HLA-DRβ chain showed the strongest association with RA development ($p<10^{-581}$ for position 11). As shown in Table 1, Val11 and Leu11 are the key amino acids for susceptibility and

Table 1 Effect estimates of the 3 amino acids associated with risk of RA

HLA-DRβ1 amino acid at position			multivariate OR	95%CI	*HLA-DRB1* alleles
11	71	74			
Val	Lys	Ala	4.44	4.02-4.91	*04:01
Val	Arg	Ala	4.22	3.75-4.75	*04:08, *04:05, *04:04, *10:01
Leu	Arg	Ala	2.17	1.94-2.42	*01:02, *01:01
Pro	Arg	Ala	2.04	1.59-2.62	*16:01
Val	Arg	Glu	1.65	1.24-2.19	*04:03, *04:07
Asp	Arg	Glu	1.65	1.29-2.10	*09:01
Val	Glu	Ala	1.43	1.04-1.96	*04:02
Pro	Ala	Ala	1.00	Reference	*15:01, *15:02
Ser	Arg	Ala	0.88	0.77-1.00	*11:01, *11:04, *12:01
Ser	Arg	Leu	0.71	0.57-0.89	*08:01, *08:04
Ser	Lys	Arg	0.63	0.54-0.73	*03:01
Ser	Glu	Ala	0.59	0.51-0.68	*11:02, *11:03, *13:01, *13:02

Estimate effects for haplotypes of *HLA-DRB1*. For each haplotype, the multivariate effect is given as an odds ratio (OR), taking the most frequent haplotype (Pro-Ala-Ala) in the control samples as the reference (that is, given that the haplotype has an OR of 1). Classical shared epitope alleles are shown in bold. This table is modified from a previous report[12].

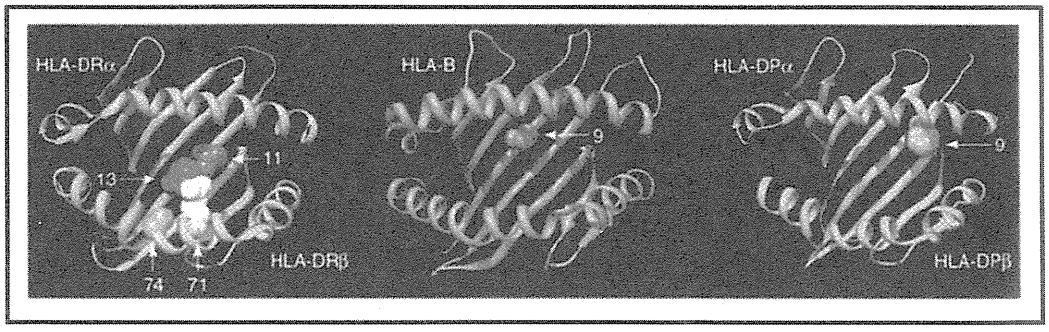

Fig.1
Three-dimensional ribbon models for the HLA-DR, HLA-B and HLA-DP proteins. Key amino acid positions identified by the association analysis are highlighted. This figure is taken from a previous report[12].

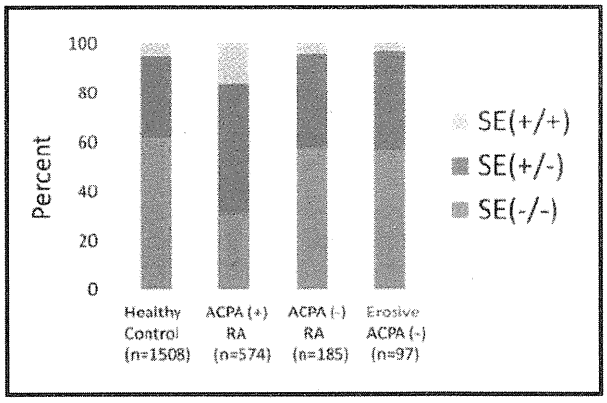

Fig.2
Prevalence of individuals carrying double SE, single SE or no SE is shown in healthy control, ACPA-positive RA, ACPA-negative RA and ACPA-negative RA with typical bone erosion as determined by X-ray. This clearly shows that ACPA-negative RA is distinct from ACPA-positive RA. This figure is illustrated based on our previous report[13].

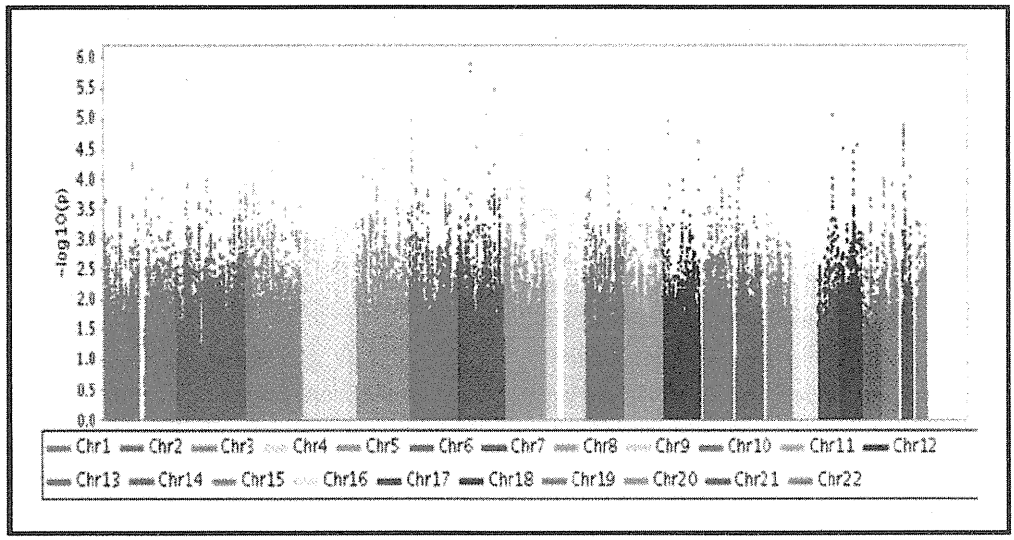

Fig 3
Probability plot for association with ACPA-negative RA (n=774) versus healthy controls (n=1079). This figure is taken from a previous report[6].

Ser11 is protective, for example, even though positions 71 and 74 are the SE types, Ser11 offsets such effects. Since most of the SE alleles have Valine or Leucine at position 11, Leucine at position 67, and do not have Serine at position 11 nor Aspatic acid at position 70, the results of previous studies using SE would not have been affected by the recent findings. Thus, key amino acid positions of HLA-DRβ chain for RA development seem to be 11th, 70th, 71st, and 74th positions and there still are some debates which positions have the primary effect. Anyway, these positions seem to be important for citrullinated peptide presentation.

HLA association with ACPA-negative RA

In 2005, a Dutch group reported that the association of SE was only exhibited with ACPA-positive RA and no association was seen with the ACPA-negative RA patients[10]. We have replicated the results in the Japanese population, and also showed that similar results were obtained even when we selected only bone-erosive ACPA-negative RA[13], which strongly suggests that this observation is not due to the contamination of non-RA arthritic diseases in ACPA-negative RA subset (Fig.2).

First of all, is there a genetic predisposition for ACPA-negative RA? From a twin study, heritability of ACPA-negative RA has been estimated and is thought to be as high as that of ACPA-positive RA[14].

Next, is *HLA* associated with ACPA-negative RA? A genome-wide association study (GWAS) meta-analysis of ACPA-negative RA showed that *HLA-DR* locus in chromosome 6 had no peak of association (see Fig.3)[6], suggest-

ing that the impact of *HLA* for development of ACPA-negative RA is not as large as that of ACPA-positive RA. In the study, the p-value of the *HLA* locus for ACPA-positive RA reached the order of 10^{-60}; in contrast, that for ACPA-negative RA reached the order of 10^{-4}. However, this does not mean that *HLA* is not associated with ACPA-negative RA, but probably means that ACPA-positive RA is a rather homogeneous subset in terms of *HLA* usage compared with ACPA-negative RA. ACPA-negative RA might have more variations of autoantigen (probably not citrullinated). In ACPA-positive RA, *HLA* usage is rather homogeneous, probably because citrullinated proteins or peptides are the common autoantigens among such patients that have SE-carrying *HLA*.

What *HLA* alleles are associated with ACPA-negative RA? In Caucasians, *HLA-DR3* and *DR13* have been reported to be associated with ACPA-negative RA[15-17]. As *HLA-DR3* association was seen in 3 independent European cohorts, it is probably true in Caucasians. In Japanese, we found that multiple *HLA-DRB1* alleles, including *12:01, *14:03 and *04:05, were associated with ACPA-negative RA susceptibility in the Japanese population[18]. *HLA-DR3* alleles were not shown because they are very rare in Japanese. We also found that *HLA-DRB1**15:02 and *13:02 were protective against ACPA-negative RA development. It is noteworthy that one of the SE alleles, *HLA-DRB1**04:05, was associated with ACPA-negative RA. Other SE alleles were not associated with ACPA-negative RA. This implies that the association of *04:05 with ACPA-negative RA is not due to the common amino acid sequence of SE because SE-carrying alleles other than *04:05 are not associated. Therefore, other mechanisms are suggested.

It seems there are two subsets in ACPA-negative RA based on RF positivity. Mackie et al. recently reported that *HLA-DRB1* SE is associated with ACPA(-)RF(+) RA but not with ACPA(-) RF(-) RA[19]. We have similar data for the Japanese population and showed that there are some specific *HLA-DRB1* alleles associated with ACPA(-) RF(+) RA or ACPA(-)RF(-) RA (Fig.4). For example, *04:05 and *09:01 were specifically associated with ACPA(-)RF(+) subset, and DR8/DR8 homozygote and DR14 were specifically associated with ACPA(-)RF(-) subset, whereas *12:01 was associated with both subsets. In contrast, ACPA(+)RA could not be separated by *HLA-DR* allelic usage.

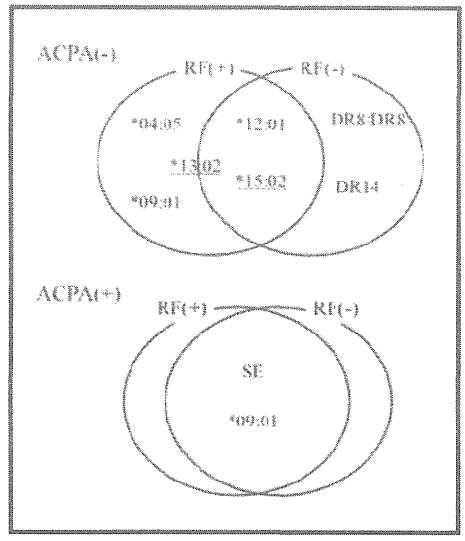

Fig.4
Scheme of HLA-DRB1 allele association with RF(+) or RF(-) subset of ACPA(-) RA or ACPA(+)RA in Japanese. Underline represents the protective allele. This figure is taken from our unpublished results.

Non-HLA genes associated with RA

A lot of genetic polymorphisms of candidate genes were tested for association with RA and reported to be associated with it, but most of them were not replicated. Perhaps the positive results are due to publication bias and relatively small sample sizes. Since 2003[20], genome-wide association studies (GWAS) have been applied to RA[21-26] and recently several meta-analyses of GWAS were performed[27-29]. Sample sizes also jumped from several hundred to tens of thousands. As a result, 30-40 genes or loci were detected to be significantly ($p<5\times10^{-8}$) associated with RA[1]. Many of these SNPs are located not in the genes (exons and introns), but near the genes, while some of the SNPs are located in exons and cause amino acid substitution (e.g. *PTPN22*). In many cases, the real causative SNPs or variants are still unknown. The list of SNPs in Table 2 shows the most strongly associated SNPs in the studies, but the real causative variants may exist somewhere else. The associated genes shown in Table 2 are classified by their main function. These genetic variants satisfied the genome-wide significance ($p<5\times10^{-8}$) or region-wide significance after Bonferroni's correction with multiple replication. Some of them are specific to Caucasians, mainly due to the absence of polymorphisms such as *PTPN22* and *RBPJ*, while some are specific to the Japanese or

Table 2 Candidate genes with confirmed association with rheumatoid arthritis

Gene	Best p-value	OR	Association† in		landmark SNP	SNP position	reference
			Caucasians	Japanese*			
(1)Intracellular signaling molecules and receptors							
PTPN22	9.1×10^{-74}	1.94	++	NA	rs2476601	exon	27
TRAF1-C5	4.0×10^{-14}	1.32	++	-	rs3761847	near	22
MBP	2.7×10^{-8}	1.23	-	++	rs2000811	intron	26
TNFAIP3	8.9×10^{-13}	1.22	++	++	rs6920220	near	27
BLK	5.7×10^{-9}	1.19	++	+	rs2736340	near	24
SPRED2	5.3×10^{-10}	1.13	++	+	rs934734	intron	27
TAGAP	3.8×10^{-7}	0.91	+	-	rs394581	near	44
TRAF6	3.9×10^{-8}	0.89	+	-	rs540386	intron	44
PTPRC	6.7×10^{-7}	0.88	+	-	rs10919563	intron	44
PRKCQ	4.4×10^{-8}	0.88	+	-	rs4750316	near	45
(2)Transcription factor							
REL	3.1×10^{-14}	1.25	++	-	rs13031237	intron	24
IRF5	4.2×10^{-11}	1.25	++	+	rs10488631/ rs13225818	near/near	27
STAT4	1.7×10^{-11}	1.24	++	++	rs7574865	intron	46
RBPJ	1.0×10^{-16}	1.18	++	NA	rs874040	near	27
AIRE	3.6×10^{-9}	1.18	-	++	rs2075876	intron	33
AFF3	1.0×10^{-14}	1.15	++	+	rs11676922	near	27
PRDM1	2.1×10^{-8}	1.11	++	-	rs6822844	near	44
(3)Cytokines and cytokine receptors							
CCR6	7.7×10^{-19}	1.19	++	++	rs3093024	near	25
IL2RB	4.6×10^{-8}	1.13	++	-	rs3218253	intron	47
IL2RA	1.4×10^{-11}	1.11	++	-	rs706778	intron	27
TNFRSF14	1.1×10^{-7}	0.92	+	+	rs3890745	near	45
CCL21	3.9×10^{-10}	0.87	++	-	rs951005	near	27
ANKRD55-IL6ST	9.6×10^{-12}	0.85	++	-	rs6859219	near	27
IL2-IL21	5.6×10^{-5}	0.78	+	NA	rs6822844	near	46
(4)Membrane receptors and costimulatory molecules							
HLA-DRB1	$<10^{-299}$	2.88	++	++	rs6910071	exon	27
FCRL3	8.5×10^{-7}	2.15	+	+	rs10430455	near	48
CD244	7.0×10^{-9}	1.31	-	+	rs6682654	intron	49
CD2-CD58	1.0×10^{-9}	1.13	++	-	rs11586238	near	44
CD28	1.3×10^{-8}	1.13	++	-	rs1980422	near	44
FCGR2A	1.5×10^{-5}	1.12	+	NA	rs12746613	near	44
CTLA4	6.3×10^{-9}	0.86	++	+	rs231735	near	27
CD40	2.8×10^{-9}	0.85	++	-	rs4810485	intron	27
(5)Enzymes							
PADI4	4.6×10^{-8}	1.97	+	++	rs766449	intron	20
PXK	3.1×10^{-14}	1.13	++	NA	rs13315591	near	27
DDX6	1.1×10^{-8}	0.87	++	-	rs10892279	near	28
(6)Unknown							
KIF5A-PIP4K2C	8.8×10^{-8}	0.89	+	-	rs1678542	near	45
C5orf30	4.1×10^{-8}	0.93	++	-	rs26232	intron	27

NA: not applicable due to the lack of polymorphism in Japanese

*Associations in Japanese are mainly based on our recent reports[29].

†, ++: $p<5\times10^{-8}$, +: $1\times10^{-4}<p<5\times10^{-8}$ with confirmation in other studies, -: no association

Asians, such as *AIRE*, although the reasons for this are unknown.

It is noteworthy that the list of genes includes many T cell receptor (TCR) and costimulatory signal molecules, many NFκB signal molecules and some B-cell-activation molecules, clearly indicating the importance of T and B cells and inflammatory response, especially the NFκB signal pathway. Interestingly, many molecules such as PTPN22, TNFAIP3, CTLA4 and FCRL3 are negative regulators of receptor signaling.

Here we introduce some recently discovered RA-associated genetic polymorphisms.

1)CCR6

CCR6 encodes chemokine receptor 6, which is a surface marker of Th17, a subset of T helper cells producing IL-17. We identified that genetic variation of *CCR6* is associated with RA (p=7.7x10^{-19}, OR=1.19) in Japanese by the combination of GWAS and replication studies[25]. *CCR6* genetic polymorphism is also associated with RA in Caucasians (p=1.5x10^{-11}, OR=1.11)[27]. It is interesting that not only the identified marker SNP (rs3093024) but also the functional dinucleotide polymorphism (rs968334 and the adjacent new SNP: CA, CG and TG variants, TA was not detected) was found to be associated with CCR6 expression (CA< CG<TG) and serum IL-17 level. This is quite an important finding in that Th17 involvement in the RA pathogenesis was supported genetically because there are some arguments that Th17 is not as important in human RA as in the mouse arthritis models[30, 31]. *CCR6* variant is more strongly associated with ACPA (+) RA and is also associated with Graves' disease and Crohn's disease.

2)AIRE

AIRE is a key regulatory molecule of self-antigen presentation in medullary thymic epithelial cells (mTEC). *AIRE* knockout mice lack expression of organ-specific peripheral antigens (e.g. insulin, salivary protein 1, type II collagen) in the mTEC of thymus, which leads to the development of organ-specific autoimmune diseases[32]. Combination of GWAS and replication studies in Japan revealed that genetic polymorphisms of the *AIRE* gene are associated with RA[33]. There were two SNPs with genome-wide significance, one of which is located in an intron and correlated with the decreased expression of *AIRE* gene. This is in concordance with *AIRE* knockout mice developing more rapid and severe collagen-induced arthritis[34]. The other SNP is located in exon 7, which introduces amino acid alteration (S278R) at the SAND domain, and these two SNPs are in strong linkage disequilibrium. Such altered AIRE molecule may have reduced AIRE function.

3)MBP

MBP encodes myelin basic protein, which is a constituent of the myelin sheath of peripheral nerves. We conducted GWAS and replication studies with 2 different cohorts and identified *MBP* as a susceptibility gene for RA[26]. We also found that ~70% of RA patients have anti-MBP antibody in the serum. This was surprising because MBP is an autoantigen for multiple sclerosis (MS) and RA patients do not show such neurological symptoms as MS patients do. However, soon we found that this is not so surprising. First, MBP has several isoforms and the long isoform of MBP is called Golli-MBP[35, 36]. Identified SNP is located in the intron of *Golli-MBP*. *Golli-MBP* is expressed in the hematopoietic cells and was shown to function as a negative regulator of TCR signaling through PKC[37]. *Golli-MBP* knockout T cells showed stronger reaction than the wild-type T cells[38]. Moreover, we found that anti-MBP antibody in the sera of RA recognized citrullinated MBP, but not non-citrullinated MBP. Since MBP is a well-known antigen that is physiologically citrullinated and a number of citrullinated proteins are the targets of RA autoantibodies[39], it is not surprising that MBP becomes one of the targets of RA autoimmunity. However, it has not been well studied how the *MBP* polymorphism is linked to the pathogenesis of RA. The *MBP* polymorphism is not associated with RA in Caucasians.

4)TNFAIP3

The *TNFAIP3* gene encodes a cytoplasmic zinc finger protein that possesses both ubiquitination and deubiquitination properties and is a major negative regulator of TNF-induced NF-κB signaling pathways. *TNFAIP3* polymorphism showed relatively high odds ratio for RA in both Caucasians and Japanese (odds ratios of 1.22 and 1.35, respectively). Several different polymorphisms have been associated with autoimmunity, including a nonsynonymous coding SNP (Phe127Cys), with some evidence of reduced negative regulatory ability for TNF-induced NF-κB signaling[40]. In addition to *TNFAIP3*, a number of genes related to NF-κB signaling (e.g. *TRAF1, CD40, Rel* and

NFKBIE) were reported to be associated with RA, clearly indicating the importance of NF-κB signaling in the pathogenesis of RA.

In the near future: rare variants

The genetic influence of each polymorphism is very modest (OR mostly ranging from 1.1 to 1.5). Therefore, there is no obvious clinical utility to predict the development of RA with such polymorphisms. This may change as the obtained knowledge becomes more complete, but currently all the known genetic variants can explain only ~15% of the genetic component[41]. This will not change very much even though we have found >100 associated genes with common variants (SNPs). Since most of the GWASs adopt common SNPs with a population prevalence of >3-5%, there may be some rare genetic variants with high genetic impacts. Sialic acid acetylesterase (*SIAE*) is an enzyme that negatively regulates B lymphocyte antigen receptor signaling and is required for the maintenance of immunological tolerance. By sequencing the *SIAE* exons, various defective variants were found in various autoimmune diseases including RA[42]. Defective variants were found in only 2 out of 648 (0.3%) healthy European subjects, whereas 24 out of 923 (2.6%) autoimmune disease patients had defective variants (OR=8.62). The odds ratio for RA was 8.31. Although this result was not successfully replicated in a larger study[43], some unknown rare variants may have strong impacts on the development of RA.

Now that the sequencing technology has developed markedly and is becoming less expensive, finding rare genetic variants associated with RA by whole-genome sequencing is realistic. As a first step, researchers started sequencing only exons of the whole genome, which is called the exome sequence, because it is much more economical than whole-genome sequencing. However, in the very near future, it is announced that the whole-genome sequence of one person can be read for $1,000 in a day. From this point onwards, it will be more realistic to understand completely the impact of genetic variants on the development of RA.

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ORIGINAL ARTICLE

Time-Dependent Increased Risk for Serious Infection From Continuous Use of Tumor Necrosis Factor Antagonists Over Three Years in Patients With Rheumatoid Arthritis

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Objective. To investigate associations between continuous treatments with tumor necrosis factor (TNF) antagonists and risk for developing serious infections (SIs) over 3 years in Japanese patients with rheumatoid arthritis (RA) enrolled in the Registry of Japanese RA Patients for Long-Term Safety (REAL) database.

Methods. We analyzed 727 RA patients who had started either infliximab or etanercept (the anti-TNF group; 1,480.1 patient-years [PY]) and 571 RA patients who had started conventional nonbiologic disease-modifying antirheumatic drugs (the unexposed group; 1,104.1 PY) at the time of enrollment in the REAL. We assessed the occurrence of SIs within a 3-year observation period, including the period after switching to other TNF antagonists, and all SIs, unlimited to the first one in each patient as reported in other studies, to evaluate the real safety of TNF antagonists in daily practice.

Results. The incidence rate of SIs per 100 PY was 5.54 (95% confidence interval [95% CI] 4.44–6.84) in the anti-TNF group and 2.72 (95% CI 1.87–3.83) in the unexposed group. Poisson regression analysis revealed that the relative risk (RR) of continuous use of TNF antagonists for SIs after adjusting for baseline and time-dependent covariates was significantly elevated both overall (1.97, 95% CI 1.25–3.19) and for the first year (2.40, 95% CI 1.20–5.03), but not for the second and third years combined (1.38, 95% CI 0.80–2.43). The adjusted RR for SIs of etanercept compared to infliximab was not significantly elevated.

Conclusion. Continuous anti-TNF therapy was significantly associated with increased risks for developing SIs during, but not after, the first year.

INTRODUCTION

Biologic disease-modifying antirheumatic drugs (DMARDs) have been widely used to treat patients with rheumatoid arthritis (RA) whose response to conventional DMARD therapy was inadequate (1–4). In Japan, 6 biologic DMARDs (infliximab, etanercept, adalimumab, tocilizumab, abatacept, and golimumab) have been approved and widely used in clinical practice. The criterion for indication for

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Significance & Innovations

- Using a Japanese rheumatoid arthritis (RA) patient registry, we show for the first time in Asia that the continuous use of tumor necrosis factor (TNF) antagonists over a 3-year observation period was associated with a 2-fold increased risk for serious infections (SIs) compared to nonbiologic disease-modifying antirheumatic drugs (DMARDs). This elevation, however, was time dependent and significant only for the first year, not for the second and third years combined.
- To redeem methodologic shortcomings in previous reports, we examined all SIs occurring during treatment with TNF antagonists, including those after switching to other TNF antagonists. We used not only baseline but also time-dependent variables as candidates for risk factors for SIs in multivariate analysis because disease activity of RA and the dose of drugs such as corticosteroids and methotrexate are subject to change during treatment.
- Over 3 years, the incidence rate of SIs in the etanercept group was numerically higher than that of the infliximab group, but the risk for SIs from treatment with etanercept was not significantly different from that of infliximab after adjusting for covariates.

infliximab or the other 5 biologic DMARDs, according to Japanese labeling, consists of inadequate response to methotrexate (MTX) or nonbiologic DMARDs, respectively. In addition, Japanese rheumatologists follow the guidelines proposed by the Japan College of Rheumatology (5,6).

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Although biologic DMARDs have superior clinical efficacy for patients with RA, there are concerns about increased risk for infection (7). Prevention of infections in RA patients who are treated with immunosuppressive drugs is relevant because the incidence rate (IR) of infections is already higher in patients with RA than in the general population, and infection is a major factor hampering proper management of the disease and influencing prognosis (8–10). Infection was the most frequent serious adverse event (SAE) reported in postmarketing surveillance programs for infliximab and etanercept in Japan: the most prevalent infectious disease was pneumonia, and higher IRs of tuberculosis and *Pneumocystis jiroveci* pneumonia (PCP) were reported compared to Western countries (11–13). We established the Registry of Japanese RA Patients for Long-Term Safety (REAL) in 2005 and, utilizing this database, recently reported that treatment with either tumor necrosis factor (TNF) antagonist infliximab or etanercept for up to 1 year was associated with increased risk for serious infections (SIs) compared to treatment with nonbiologic DMARDs (14). Recent data from prospective observational studies in Europe and the US also suggest that the risk for infection was higher in RA patients treated with biologic DMARDs, at least in the short term (15–18), and disappeared with increasing treatment duration (15,16,18–20).

In clinical practice, rheumatologists often switch from the initial TNF antagonist to an alternative TNF antagonist when the patient shows insufficient efficacy or develops an adverse event. Some patients also experience more than one adverse event during treatment with TNF antagonists. In previous reports from prospective cohort studies, observation was stopped after switching to another TNF antagonist or after the first adverse event (18,21–23); therefore, second or third adverse events and those occurring after switching TNF antagonists were not analyzed (18,21–23). In addition, the time dependency of covariates such as corticosteroid dose and disease activity was not included in some studies (14,15,19–24). To understand the real safety of TNF antagonists for patients with RA, it is essential to design an epidemiologic study that evaluates all adverse events during continuous treatment with these agents. However, in Japan, as well as in Asia overall, there are no safety data from prospective cohort studies with an observation period longer than 1 year in RA patients receiving TNF antagonists. Because differences in genetic, environmental, and medical factors in each geographic region may influence the safety of biologic DMARDs (25), it is prudent to compare the safety of biologic DMARDs from various countries or regions. The primary purpose of this study was to assess the risk for SIs associated with continuous use of infliximab or etanercept for 3 years, including the period after switching to other TNF antagonists, and its trend over time, and to identify independent risk factors after adjusting for time-dependent covariates. In a secondary analysis, we focused on the first TNF antagonist used in each patient to investigate differences in the risk for SIs among the agents.

PATIENTS AND METHODS

Database. The REAL is an ongoing prospective cohort established to investigate the long-term safety of biologic DMARDs in patients with RA. Details of the REAL have been previously described (14,26). In brief, 27 institutions participated in the REAL, including 16 university hospitals and 11 referring hospitals. The criteria for enrollment in the REAL include those patients meeting the 1987 American College of Rheumatology criteria for RA (27) with written informed consent and starting or switching treatment with biologic DMARDs (the biologics exposed group) or starting, adding, or switching nonbiologic DMARDs (the biologics unexposed group) at the time of study entry. Until the end of 2007, patients already receiving treatment with nonbiologic DMARDs at the time of study entry were also enrolled in the unexposed group. To facilitate enrollment in the REAL, participating physicians were asked to enroll their patients already registered to postmarketing surveillance programs previously implemented by pharmaceutical companies for biologic DMARDs (11,12). In addition, our investigators were also encouraged to enroll as many patients as possible who fulfilled the inclusion criteria (14). For this study, data were retrieved from the REAL database on November 30, 2009. This study was in compliance with the Declaration of Helsinki (revised in 2008). The REAL study was approved by the ethics committees of the Tokyo Medical and Dental University Hospital and the other participating institutions (see Appendix A for members of the REAL Study Group and their affiliates).

Data collection. Each patient's recorded baseline data included demography, disease activity, comorbidities, treatments, and laboratory data at the start of the observation period. A followup form was submitted every 6 months by the participating physicians to the REAL Data Center at the Department of Pharmacovigilance of Tokyo Medical and Dental University to report the occurrence of SAEs, current RA disease activity, treatments, and clinical laboratory data. We collected the Steinbrocker class (28) as the measurement for patient physical disability instead of the Health Assessment Questionnaire disability index at baseline (29). Using this protocol, SAEs were reported at regular followup times every 6 months. The REAL Data Center checked all of the data sent by attending physicians to improve the quality of data, and the participating physicians in each hospital confirmed them on the web site of the REAL.

Anti-TNF group. In the biologics exposed group, there were 727 patients with RA who started infliximab or etanercept at enrollment in the REAL (anti-TNF group; 1,480.1 patient-years [PY]); 335 started infliximab (infliximab group) and 392 started etanercept (etanercept group). In the infliximab group, 67 patients were switched to either etanercept (58 patients), tocilizumab (8 patients), or adalimumab (1 patient), and 74 patients stopped treatment with infliximab during the study observation period. The remaining patients continued treatment with infliximab

throughout. In the etanercept group, 60 patients were switched to either infliximab (27 patients), tocilizumab (25 patients), or adalimumab (8 patients), and 62 patients stopped administration of etanercept during the study observation period. The remaining patients continued etanercept treatment throughout. The overall survival rates of the first biologic agent at year 3 were 0.48 (95% confidence interval [95% CI] 0.41–0.55) for infliximab and 0.61 (95% CI 0.55–0.66) for etanercept. Our analysis was restricted to infliximab or etanercept because few patients receiving adalimumab or tocilizumab were registered in the REAL database and golimumab and certolizumab pegol were not approved in Japan at the time this study was conducted.

Unexposed group. Among 574 RA patients in the biologics unexposed group, 3 patients had received biologic DMARDs within 90 days before their enrollment in the REAL. These 3 patients were excluded from our analysis in consideration of the pharmacokinetic and pharmacodynamic property of biologic DMARDs and their possible effects on development of infection. Fifteen patients who had received biologic DMARDs and stopped them over 90 days before their enrollment in the REAL were included in this analysis. Therefore, 571 RA patients who initiated or were receiving nonbiologic DMARDs and not receiving biologic DMARDs at enrollment in the REAL were included in the unexposed group (1,104.1 PY). At enrollment, 347 patients (60.8%) of the patients in the unexposed group were being treated with MTX, 127 patients (22.4%) with sulfasalazine, 103 patients (18.0%) with tacrolimus, 95 patients (16.6%) with bucillamine, and 29 patients (5%) with other nonbiologic DMARDs.

Followup. For those patients who initiated nonbiologic DMARDs or biologic DMARDs at entry, the start of the observation period was the date these agents were first administered. For those patients enrolled in the unexposed group already receiving treatment with nonbiologic DMARDS at the time of study entry, the observation period started from the date of their enrollment in the REAL database.

Observation was stopped either 3 years after the start of the observation period, the day a patient died or met the exclusion criteria (14), or on November 30, 2009, whichever came first. For the unexposed group, stopping all nonbiologic DMARDs or starting any biologic DMARDs stopped followup. For the anti-TNF group, stopping therapy with either infliximab or etanercept ended observation. Patients were followed even after development of SAEs, as long as they did not meet the above criteria for stopping observation. The date of the last administration of infliximab or etanercept was retrieved from medical records and reported by the participating physicians. The mean ± SE followup was 2.04 ± 0.92 years for the anti-TNF group and 1.93 ± 0.99 years for the unexposed group. Figure 1 shows the number of patients for each year and the number who dropped out from each group during observation. Four hundred forty-two patients (34%) of all patients (n = 1,298) were followed up for 3 years.

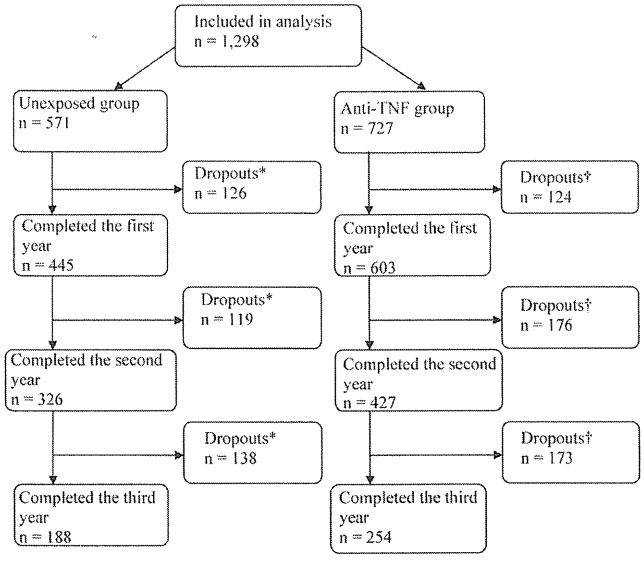

Figure 1. Distribution of numbers of patients with rheumatoid arthritis during the 3-year observation period. * = dropouts from the unexposed group include patients who started biologic disease-modifying antirheumatic drugs (DMARDs) or patients whose observation did not complete the next 1 year; † = dropouts from the anti–tumor necrosis factor (anti-TNF) group include patients who stopped infliximab or etanercept or switched to biologic DMARDs, except infliximab and etanercept, or patients whose observation did not complete the next 1 year.

Definition of SAEs. Our definition of an SAE, including an SI, was based on the report by the International Conference on Harmonisation (30). In addition, bacterial infections that required intravenous administration of antibiotics, as well as opportunistic infections, were also regarded as SAEs (14) (see Supplementary Table 1, available in the online version of this article at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)2151-4658).

Statistical analysis. Crude IRs per 100 PY and crude IR ratios (IRRs) with their 95% CIs were calculated. We conducted 2 analyses in this study. In the primary analysis (analysis 1), risk factors for SIs during continuous treatment with infliximab or etanercept for up to 3 years were identified. We also calculated the risk of TNF antagonists for SIs in the first year and in the second and third years combined to investigate time dependence of the risk. In the secondary analysis (analysis 2), the risks for SIs were compared between treatment with infliximab and etanercept.

Analysis 1. We included both patient groups and the entire observation period for each patient as described above for analysis 1 and added risk windows as follows. When a patient no longer received either infliximab or etanercept, the patient was excluded from the study on the day of the last administration of the agents and a 90-day postdiscontinuation risk window was applied (14). Any SAEs occurring within the risk window were attributed to the effects of the TNF antagonists. No risk window was needed for the unexposed group. For multivariate analysis, Poisson regression models were employed to estimate

Table 1. Comparison of RA patients treated with or without TNF antagonists infliximab or etanercept at the start of the observation period*

	Unexposed group (n = 571)	All anti-TNF groups (n = 727)	Infliximab group (n = 335)	Etanercept group (n = 392)	P†
Age, years	59.3 ± 13.1	56.3 ± 13.4‡	53.7 ± 13.9	58.5 ± 12.7	< 0.001
Women, %	83.2	82.0	79.3	85.1	0.045
Disease duration, years	8.9 ± 9.3	9.5 ± 8.6‡	8.1 ± 8.0	10.6 ± 9.0	< 0.001
Steinbrocker class 3 or 4, %§	10.7	30.7‡	28.4	32.7	0.211
Steinbrocker stage III or IV, %§	39.6	53.0‡	45.1	59.7	< 0.001
DAS28-CRP	3.4 ± 1.2	4.5 ± 1.2‡	4.6 ± 1.1	4.5 ± 1.3	0.197
N	567	723	335	388	
MTX use, %	60.8	68.8‡	99.1	42.9	< 0.001
MTX dosage, mg/week	6.4 ± 2.0	7.6 ± 2.2‡	7.9 ± 2.2	7.0 ± 2.1	< 0.001
MTX dosage >8 mg/week, %	4.4	10.6‡	18.2	4.1	< 0.001
Use of immunosuppressive drugs except for MTX, %¶	20.1	4.3‡	1.2	6.9	< 0.001
Oral corticosteroid use, %	58.3	71.5‡	69.0	73.7	0.16
Prednisolone or equivalent dosage of corticosteroids, mg/day	4.6 ± 2.1	5.7 ± 3.0‡	5.3 ± 2.7	6.0 ± 3.2	0.006
Prednisolone or equivalent dosage of corticosteroids ≥10 mg/day, %	1.9	9.1‡	5.7	12.0	0.003
No. of previous DMARDs	2.2 ± 1.2	2.5 ± 1.2‡	2.3 ± 1.1	2.7 ± 1.2	< 0.001
Chronic pulmonary disease, %#	18.7	21.6	11.9	29.8	< 0.001
Diabetes mellitus, %	5.8	12.0‡	8.7	14.8	0.011

* Values are the mean ± SD unless otherwise indicated. For univariate analysis, the chi-square test for categorical variables and the Student's *t*-test or Mann-Whitney test were used to compare continuous variables among groups. RA = rheumatoid arthritis; TNF = tumor necrosis factor; DAS28-CRP = 3-variable Disease Activity Score including 28-joint counts using the C-reactive protein level; MTX = methotrexate; DMARDs = disease-modifying antirheumatic drugs.
† Between the 2 anti-TNF antagonists.
‡ $P < 0.05$ versus the unexposed group.
§ Steinbrocker classification (28) was used to define RA disease stages and classes.
¶ Immunosuppressive drugs include tacrolimus, leflunomide, mizoribine, and cyclosporine.
# Chronic pulmonary diseases include interstitial pneumonia, chronic obstructive pulmonary disease, bronchial asthma, prior pulmonary tuberculosis, and bronchiectasis.

the risk for SIs with TNF antagonist treatment. To analyze the time-dependent risk for SIs, observation periods were divided into the first year and the second and third years combined.

Analysis 2. To compare the risk for SIs between the use of infliximab and etanercept in the anti-TNF group, the treatment period with the first TNF inhibitor for each patient was evaluated without setting a risk window because most of the patients who had stopped the first biologic agent started treatment with the second one immediately. We applied propensity score (PS) methodology to calculate the likelihood of being treated with TNF antagonists. First, we made a multivariate logistic regression model with the use of TNF antagonists as the dependent variable and the following as independent variables: age, sex, the 3-variable Disease Activity Score including 28-joint counts using the C-reactive protein level (DAS28-CRP), the presence of chronic pulmonary comorbidity, diabetes mellitus, calendar year of entry in the REAL, Steinbrocker stage (III or IV), MTX (≤8 or >8 mg/week), and oral corticosteroid (prednisolone or equivalent dosage <10 or ≥10 mg/day) at enrollment. We applied the Hosmer-Lemeshow goodness-of-fit test to assess how effectively the model described the outcome variable (i.e., the use of TNF antagonist: yes/no). We used the PS to select representative patients receiving TNF antagonist treatment: the patients with a PS >0.4 were included in analysis 2 and different cutoff values for PS were used for sensitivity analyses (31). To compare the risk for SIs between etanercept and infliximab, we employed Poisson regression models in the anti-TNF group patients with various combinations of adjusting factors, including the PS, to calculate the relative risks (RRs) of etanercept with 95% CIs, using infliximab as the reference.

These statistical analyses were conducted using SPSS, version 16.0, and R statistical language software, version 2.8.1. All *P* values were 2-tailed and *P* values less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics of patients. This study included a total of 1,298 patients: 727 in the anti-TNF group and 571 in the unexposed group. Baseline data for the patients are shown in Table 1. Compared to the unexposed group, the anti-TNF group was younger ($P < 0.001$), had more severe disease activity ($P < 0.001$), and was treated with higher doses of MTX ($P < 0.001$) and oral corticosteroids ($P < 0.001$). Significantly more patients with diabe-

Table 2. Number and IRs of SAEs in RA patients treated with and without the TNF antagonists infliximab or etanercept*

	Unexposed group (n = 571)	Anti-TNF group			Etanercept vs. infliximab, crude IRR (95% CI)	Anti-TNF vs. unexposed group, crude IRR (95% CI)
		All (n = 727)†	Infliximab (n = 335)‡	Etanercept (n = 392)§		
Patient-years (PY)	1,104.1	1,480.1	583.31	787.94		
All SAEs					1.49 (1.10–2.03)	1.67 (1.31–2.13)
No. of events	95	213	61	123		
IR/100 PY (95% CI)	8.60 (7.00–10.47)	14.39 (12.55–16.42)	10.46 (8.07–13.34)	15.61 (13.03–18.56)		
Serious infection					1.16 (0.72–1.87)	2.04 (1.34–3.10)
No. of events	30	82	28	44		
IR/100 PY (95% CI)	2.72 (1.87–3.83)	5.54 (4.44–6.84)	4.80 (3.26–6.84)	5.58 (4.11–7.42)		
Serious respiratory tract infection					1.20 (0.65–2.24)	1.96 (1.10–3.48)
No. of events	17	42	16	26		
IR/100 PY (95% CI)	1.45 (0.86–2.30)	2.84 (2.07–3.80)	2.74 (1.63–4.35)	3.30 (2.21–4.76)		
Serious infection leading to death					NA	0.75 (0.15–3.69)
No. of events	3	3	0	3		
IR/100 PY (95% CI)	0.27 (0.08–0.72)	0.20 (0.06–0.54)	0	0.38 (0.11–1.02)		

* Note that the number of severe adverse events (SAEs) in the All column is not the sum of the Infliximab and Etanercept columns. IRs = incidence rates; TNF = tumor necrosis factor; IRR = IR ratio; 95% CI = 95% confidence interval; NA = not applicable.
† The continuous treatment period with infliximab or etanercept for each patient was evaluated.
‡ Patients with rheumatoid arthritis (RA) given infliximab as the first TNF inhibitor in the Registry of Japanese RA Patients for Long-Term Safety (REAL) were included. The treatment period with infliximab for each patient was evaluated.
§ Patients with RA given etanercept as the first TNF inhibitor in the REAL were included. The treatment period with etanercept for each patient was evaluated.

tes mellitus ($P < 0.001$) were seen in the anti-TNF group compared to the unexposed group. In the anti-TNF group, the etanercept group compared to the infliximab group was older ($P < 0.001$), had a longer disease duration ($P < 0.001$), used MTX less frequently ($P < 0.001$), was treated with higher doses of oral corticosteroids ($P = 0.006$), and had higher percentages of chronic pulmonary comorbidity ($P < 0.001$) (see Table 1 for definition) and diabetes mellitus ($P = 0.011$) (Table 1).

Types and occurrence of SAEs. Among the 1,298 patients, 308 SAEs were reported during the observation period, 95 in the unexposed group and 213 in the anti-TNF group. The crude IRR comparing the anti-TNF group with the unexposed group for SAEs was 1.67 (95% CI 1.31–2.13) and for SIs was 2.04 (95% CI 1.34–3.10); both of these IRRs were significantly elevated. The IRs of SAEs, SIs, and serious respiratory tract infections in the infliximab group and the etanercept group are shown in Table 2. The crude IRR comparing the infliximab group with the etanercept group for SAEs was 1.49 (95% CI 1.10–2.03) and for SIs was 1.16 (95% CI 0.72–1.87). The IRs of SAEs, SIs, serious respiratory tract infections, and SIs leading to death are summarized in Table 2.

In the anti-TNF group, there were 82 SIs, including 21 opportunistic (14 cases of herpes zoster, 4 PCP, 3 pulmonary cryptococcosis, and 1 pulmonary nontuberculous mycobacterial infection) and 61 other infections. In the unexposed group, 30 SIs occurred, including 12 opportunistic (4 cases of herpes zoster, 3 PCP, 2 pulmonary tuberculosis, and 3 pulmonary nontuberculous mycobacterial infections) and 18 other infections. The names of the SIs in each site of infection are listed in Table 3. The respiratory system was the most frequent site of infection (n = 59), followed by skin and subcutaneous tissue (n = 24), gastrointestinal (n = 6), urinary tract (n = 5), and bone and joints (n = 5). Four of the latter 5 patients had histories of joint surgery. Three patients in each group died from SIs.

Continuous treatment with TNF antagonists and other risk factors contributing to the development of SIs (analysis 1). We initially performed univariate analyses to compare patients who did and did not develop SIs (data not shown) and selected the following variables for multivariate analysis: age, sex, chronic pulmonary comorbidity, diabetes mellitus, disease duration, calendar year, the number of previous DMARDs, Steinbrocker class, the use of immunosuppressive drugs, mean DAS28-CRP, and the mean dose of MTX and oral corticosteroids during the observation period. We used Poisson regression models and identified continuous use of TNF inhibitors as an independent risk factor for the development of SIs (RR 1.97, 95% CI 1.25–3.19; $P = 0.0045$) (Table 4). Among the confounding factors, we found that increasing age (RR 1.45 per 10-year increment, 95% CI 1.20–1.77; $P < 0.001$), chronic pulmonary comorbidity (RR 1.77, 95% CI 1.15–2.70; $P = 0.009$), mean DAS28-CRP score (RR 1.33, 95% CI 1.05–1.66; $P = 0.015$), mean dosage of MTX >8 mg/week (RR 2.14, 95% CI 1.15–3.87; $P = 0.013$), and mean dosage of oral prednisolone ≥10 mg/day (RR 2.49, 95% CI 1.08–5.50; $P = 0.027$) were significantly associated with SIs. The

Table 3. Classification of serious infections*

Site and name of infection	No. of infections		No. of deaths	
	Anti-TNF group	Unexposed group	Anti-TNF group	Unexposed group
Pulmonary				
Bacterial pneumonia	27	9	1	2
Fungal pneumonia†	7	3	0	1
Bronchitis	4	0	0	0
Nontuberculous mycobacterial infection	1	3	0	0
Empyema	1	0	0	0
Tuberculosis	0	2	0	0
Aspiration pneumonia	1	0	1	0
Infectious pneumatocele	1	0	0	0
Total	42	17	2	3
Skin				
Herpes zoster	14	4	0	0
Cellulitis	4	2	0	0
Total	18	6	0	0
Gastrointestinal				
Infectious gastroenteritis	3	0	0	0
Acute suppurative cholangitis	1	0	0	0
Appendicitis	1	0	0	0
Infection due to drain replacement‡	0	1	0	0
Total	5	1	0	0
Urinary				
Pyelonephritis	3	1	0	0
Urinary tract infection	1	0	0	0
Total	4	1	0	0
Bone and joints				
Infectious arthritis	3	1	0	0
Osteomyelitis	0	1	0	0
Total	3	2	0	0
Others				
Sepsis	4	1	0	0
Surgical wound infection	0	2	0	0
Bacteremia	1	0	0	0
Bacterial meningitis	1	0	1	0
Sinusitis	1	0	0	0
Viral meningitis	1	0	0	0
Unidentified	2	0	0	0
Total	10	3	1	0

* Anti-TNF = anti–tumor necrosis factor.
† Fungal pneumonia included *Pneumocystis jiroveci* pneumonia and cryptococcal pneumonia.
‡ For the treatment of cholangiocellular carcinoma.

Poisson regression analysis also revealed that the RR of TNF inhibitors in the first year was significantly elevated (RR 2.40, 95% CI 1.20–5.03), but not in the second and third years combined (RR 1.38, 95% CI 0.80–2.43).

Comparison of risk for SIs between infliximab and etanercept (analysis 2). We next investigated possible differences between the TNF inhibitors in their contribution to risk for development of SIs. The PS of each patient was calculated by logistic regression model as described in the Methods. The model fit well; the Hosmer-Lemeshow goodness-of-fit statistics did not show a significant difference between observed and predicted frequencies (P = 0.164). The patients with a PS of <0.4 (17.6% of the infliximab group and 20.9% of the etanercept group) were considered not representing those receiving TNF antagonists and we excluded them from the following analysis. We constructed 3 Poison regression models to calculate the RR from the use of etanercept for the development of SIs compared to infliximab. In the first model, we adjusted for age, sex, Steinbrocker class, chronic pulmonary comorbidity, diabetes mellitus, observation period, and the PS. The second model added the mean dosage of MTX (≤8 or >8 mg/week) and the mean dosage of oral corticosteroids (<10 or ≥10 mg prednisolone or equivalent/day) to the adjusting factors in the first model. The third model added the calendar year and the number of previous nonbiologic DMARDs to the adjusting factors in the second

Table 4. Multivariate analysis of independent risk factors for serious infections during continuous use of TNF antagonists in the Registry of Japanese Rheumatoid Arthritis Patients for Long-Term Safety database*

	RR (95% CI)†	*P*
TNF antagonist (infliximab or etanercept)	1.97 (1.25–3.19)	0.0045
Age by decade	1.45 (1.20–1.77)	< 0.001
Chronic pulmonary disease	1.77 (1.15–2.70)	0.009
Diabetes mellitus	1.20 (0.69–1.97)	0.49
Mean DAS28-CRP (per 1.0 increment)	1.33 (1.05–1.66)	0.015
Mean MTX dosage >8.0 mg/week‡	2.14 (1.15–3.87)	0.013
Mean prednisolone dosage ≥10 mg/day‡	2.49 (1.08–5.50)	0.027

* TNF = tumor necrosis factor; RR = relative risk; 95% CI = 95% confidence interval; DAS28-CRP = 3-variable Disease Activity Score including 28-joint counts using the C-reactive protein level; MTX = methotrexate.
† The RRs of biologic agents for development of serious infection for up to 3 years of the observation period were calculated using the Poisson regression model after adjusting for confounding factors of age, sex, disease duration, chronic pulmonary disease, diabetes mellitus, Steinbrocker class (28), calendar year, number of previous disease-modifying antirheumatic drugs, observation period, disease activity, immunosuppressive drugs, corticosteroid dose, and MTX dose.
‡ Mean dosage during the observation period.

model. The RR for using etanercept compared to infliximab in the first model was 1.28 (95% CI 0.73–2.30, P = 0.41), for the second model was 1.39 (95% CI 0.69–2.76, P = 0.35), and for the third model was 1.32 (95% CI 0.65–2.66, P = 0.44). We performed sensitivity analyses using different cutoffs for PS and observed essentially the same results.

DISCUSSION

This is the first epidemiologic study of patients with RA that uses a prospective cohort from an Asian country to investigate the association of SIs and use of TNF antagonists during 3 years and includes patients that changed to a second agent. In addition, we performed a head-to-head comparison of the risk for SIs between infliximab and etanercept. We demonstrated that the continuous use of TNF antagonists for up to 3 years was an independent risk factor for SIs (RR 1.97, 95% CI 1.25–3.19), but the risk was time dependent. We also revealed that the RR for SIs comparing the etanercept group with the infliximab group after adjusting for covariates was not significantly different.

Studies from European biologics registries analyzed the association of TNF antagonists with infections in patients with RA (32,33). There are some reports indicating that the risk for SIs was not increased by TNF antagonists (21–24), but other studies show significant associations between the use of these agents and development of SIs (14–20,34–36). Several of the latter studies revealed time dependence of the risk for SIs (15,16,18–20,34), which is compatible with our results where the risk for SIs was significantly elevated only in the first year and declined in the second and third years combined. The decrease in risk might be explained in part by the effect of dropout patients who developed SIs and stopped the TNF antagonist (34). Of 68 patients who developed SIs in the anti-TNF group, 22 discontinued the biologic agents. Patients who were not susceptible to SIs were more likely to remain in the cohort, which could contribute to reduced risk with increasing observation period.

Increasing age, presence of chronic pulmonary comorbidity, higher mean DAS28-CRP, mean dosage of MTX >8 mg/week, and mean dosage of oral prednisolone ≥10 mg/day were identified as independent risk factors for SIs in this study. Most previous studies have reported that increasing age, pulmonary comorbidity, and use of oral prednisolone were risk factors for infections (14,21–23,35,36) and for PCP (37) in RA patients treated with TNF antagonists. Conflicting results, however, have been reported regarding the association of disease activity and risk for SIs (23,36). Because disease activity is often improved rapidly and significantly by treatment with biologic agents, including TNF antagonists, it seems reasonable that baseline disease activity may not accurately predict infectious events. Mean disease activity during the observation period may serve as a better predictor, as our study indicates.

In Japan, the data from postmarketing surveillance programs conducted by pharmaceutical companies showed that the IRs of pneumonia, PCP, and tuberculosis occurring during the first 6 months of treatment with infliximab were numerically higher than those of etanercept (11–13). In the present study, however, we show that the risk for SIs of treatment with etanercept during the longer observation period was not significantly different from that of infliximab after adjusting for covariates. Some observational studies directly (23) or indirectly (17,20) compared the risk for SIs between treatment with infliximab and etanercept, and found no statistically significant difference. A recent meta-analysis including randomized controlled trials and their extension studies also supports the results of our study; the odds ratio of etanercept treatment for SIs indirectly compared with infliximab was 0.73 (95% CI 0.46–1.15), which was not statistically significant (38).

There are a number of limitations to our study. First, we have to consider possible selection bias in our study. All of

the patients were enrolled from university hospitals or referral hospitals that are dedicated to the treatment of RA. The number of the unexposed group was smaller than that of the anti-TNF group in this study, which did not reflect the real world and may indicate unidentified selection bias. Although we estimated the risk of SIs after adjusting for variables that were clinically important, we had to interpret our data under these conditions. A second limitation is the effect of prevalent users on the analyses. In the exposed group, there were 273 prevalent nonbiologic DMARD users who had already been receiving the nonbiologic DMARDs at enrollment in the REAL database, and the rest were incident nonbiologic DMARD users. Inclusion of these prevalent nonbiologic DMARD users in our cohort might lead to the underestimation of the incidence of SIs. However, the majority of these patients started new nonbiologic DMARDs or underwent dose escalations of nonbiologic DMARDs during the observation period (data not shown), reducing the degree of underestimation. Third, the mean observation periods for both groups were approximately 2 years; it is possible that we underestimated the rate of SIs in the third year. Fourth, the mean dose of MTX of our database is lower than those of Western cohorts. In Japan, the maximum approved dosage of MTX for RA has been increased since February 2011 and Japanese rheumatologists can now officially prescribe MTX up to 16 mg/week for patients with RA. Therefore, in the future, we will be able to conduct further studies to examine the risk of TNF antagonists in patients receiving a higher dose of MTX.

In conclusion, we have shown that the continuous use of TNF therapy for up to 3 years in Japanese patients with RA, including cases where a clinical switch to a second TNF antagonist was employed, time dependently increased the risk for SIs compared to treatment with nonbiologic conventional DMARDs. A comparison of actual long-term safety among different classes of biologic DMARDs using registry data will be necessary for choosing the appropriate treatment of RA and needs to be performed.

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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. Harigai had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Sakai, Komano, Michi Tanaka, Nanki, Ryuji Koike, Miyasaka, Harigai.

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Analysis and interpretation of data. Sakai, Komano, Michi Tanaka, Nanki, Ryuji Koike, Miyasaka, Harigai.

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APPENDIX A: MEMBERS OF THE REAL STUDY GROUP AND THEIR AFFILIATES

Members of the Registry of Japanese Rheumatoid Arthritis Patients for Long-Term Safety (REAL) Study Group and their affiliates who contributed to this work were as follows: Hideto Kameda (Saitama Medical University), Shinsuke Yasuda (Hokkaido University), Mitsuhiro Takeno (Yokohama City University), Shintaro Hirata (University of Occupational and Environmental Health), Taichi Hayashi (University of Tsukuba), Yoshinari Takasaki (Juntendo University), Tsuneyo Mimori (Kyoto University), Hiroaki Ida, Katsumi Eguchi (Nagasaki University), Kazuhiko Yamamoto (University of Tokyo), Shunichi Shiozawa, Yasushi Miura (Kobe University), Tetsuji Sawada (Tokyo Medical University Hospital), Hiroaki Dobashi (Kagawa University Hospital), Sae Ochi (Tokyo Metropolitan Bokutoh Hospital), Ayako Nakajima, Hisashi Yamanaka (Tokyo Women's Medical University), Kiyoshi Migita (National Hospital Organization Nagasaki Medical Center), and Hayato Yamazaki, Kaori Watanabe (Tokyo Medical and Dental University).

The following university and hospitals are also members of the REAL Study Group, but were not involved in the present study: Keio University, Kurashiki Kohsai Hospital, Tokyo Kyosai Hospital, and Yokohama City Minato Red Cross Hospital.

EXTENDED REPORT

Drug retention rates and relevant risk factors for drug discontinuation due to adverse events in rheumatoid arthritis patients receiving anticytokine therapy with different target molecules

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ABSTRACT

Objective To compare reasons for discontinuation and drug retention rates per reason among anticytokine therapies, infliximab, etanercept and tocilizumab, and the risk of discontinuation of biological agents due to adverse events (AE) in patients with rheumatoid arthritis (RA).

Method This prospective cohort study included Japanese RA patients who started infliximab (n=412, 636.0 patient-years (PY)), etanercept (n=442, 765.3 PY), or tocilizumab (n=168, 206.5 PY) as the first biological therapy after their enrolment in the Registry of Japanese Rheumatoid Arthritis Patients for Long-term Safety (REAL) database. Drug retention rates were calculated using the Kaplan–Meier method. To compare risks of drug discontinuation due to AE for patients treated with these biological agents, the Cox proportional hazard model was applied.

Results The authors found significant differences among the three therapeutic groups in demography, clinical status, comorbidities and usage of concomitant drugs. Development of AE was the most frequent reason for discontinuation of biological agents in the etanercept and tocilizumab groups, and the second most frequent reason in the infliximab group. Discontinuation due to good control was observed most frequently in the infliximab group. Compared with etanercept, the use of infliximab (HR 1.69; 95% CI 1.14 to 2.51) and tocilizumab (HR 1.98; 95% CI 1.04 to 3.76) was significantly associated with a higher risk of discontinuation of biological agents due to AE.

Conclusions Reasons for discontinuation are significantly different among biological agents. The use of infliximab and tocilizumab was significantly associated with treatment discontinuation due to AE compared with etanercept.

Biological disease-modifying antirheumatic drugs (biological agents) are a standard treatment for rheumatoid arthritis (RA).[1 2] A number of clinical trials have demonstrated that biological agents significantly improve signs and symptoms of RA patients with both early and established disease, and that remission of RA can be achieved with biological agents not only in early RA patients, but also in established RA patients who have shown inadequate responses to conventional non-biological disease-modifying antirheumatic drugs (DMARD).

In Japan, six biological agents have been approved for the treatment of RA, infliximab in 2002, etanercept in 2005, tocilizumab and adalimumab in 2008, abatacept in 2010 and golimumab in 2011. These drugs are widely used in clinical practice according to treatment guidelines for biological agents by the Japan College of Rheumatology[3 4] and Japanese drug package inserts. Postmarketing surveillance and some clinical studies have shown short-term effectiveness and safety of these biological agents for Japanese RA patients.[5–8] The European League Against Rheumatism recommendations for the management of RA state that a tumour necrosis factor (TNF) antagonist should be administered as the first biological DMARD for patients who fail to respond to non-biological DMARD, including methotrexate,[9] whereas Japanese guidelines do not clearly specify the precedence of biological agents.

Some RA patients treated with biological agents are compelled to stop the administration of these drugs because of lack of efficacy (LOE), adverse events (AE), or financial reasons. In addition, some RA patients discontinue biological agents in the hope of a biological-free remission or biological-free low disease activity status.[10–12] In general, drugs with high retention rates have a good balance between long-term effectiveness and tolerability, reflecting the satisfaction of patients and doctors with the treatment. Because treatment for RA continues for many years or is life-long in the majority of patients, the examination of long-term drug retention rates using a prospective cohort study is important for the evaluation of biological agents.

To establish better treatment strategies for RA, it is important to identify reasons and risk factors causing the discontinuation of a drug, especially for biological agents. Several studies have shown that