Genetic contribution of the CD14 -159C/T dimorphism in the promoter region in Japanese RA

F. Takeuchi[1], N. Nakaue[1], N. Kobayashi[1], S. Kuwata[2], T. Murayama[3], K. Kawasugi[1], M. Mori[1], K. Matsuta[4]

[1]Department of Internal Medicine (Allergy and Rheumatology), Faculty of Medicine, University of Tokyo, Tokyo, Japan; [2]3rd Department of Internal Medicine, Faculty of Medicine, Teikyo University School of Medicine, Ichihara, Japan; [3]Johoku Clinic, Kanazawa, Japan; [4]Matsuta Clinic, Tokyo, Japan.

Fujio Takeuchi, MD; Naoko Nakaue; Natsuko Kobayashi; Shoji Kuwata, MD, Associate Professor; Takashi Murayama, MD; Kaname Kawasugi, MD; Masaki Mori, MD; Kunio Matsuta, MD.

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Please address correspondence and reprints requests to: Fujio Takeuchi, MD, 504 Laboratory, Department of Internal Medicine (Allergy and Rheumatology), Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: fujio-tky@umin.ac.jp

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ABSTRACT

Objective. *To study the contribution of the CD14 gene to the pathogenesis of rheumatoid arthritis (RA) in Japanese patients.*

Methods. *CD14 genotyping was carried out at the -159C/T dimorphic site in 97 RA patients and 104 normal subjects by the PCR-RFLP (restriction fragment length polymorphism) method. HLA-DRB1 genotyping was performed by the PCR-SSCP (sequence specific conformational polymorphism) method.*

Results. *The -159C/T dimorphism is not associated with whole RA or with female RA, and the results were compatible with a previous report from Germany. The -159C/T dimorphism was not associated with rheumatoid factor (RF)-positive RA, although the -159T allele tended to be associated with RF in the German report. The -159C/T dimorphism showed no association even in RA patients with the RA-susceptibility HLA-DRB1*0405. The -159T allele was prevalent in Japanese controls.*

Conclusion. *The CD14 gene is very unlikely to be genetically involved in the pathogenesis of Japanese RA.*

Introduction

Rheumatoid arthritis (RA) is a well-known autoimmune disease of obscure etiology. Many investigators have discussed the involvement of genetic factor(s) in the pathogenesis of RA. A polygenic trait has been deduced from the results of family studies on RA, and associations with HLA antigens have been reported in various ethnic groups. After the proposal of the shared epitope (SE) hypothesis in 1987 (1), the amino acid sequence, ^{70}QRRA^{74}A was confirmed to contribute pathogenically on the HLA DR β1 domain in Japanese subjects. DRB1*0405 allele is a predominant allele in Japanese RA (2). Though the SE is widely known to contribute to the pathogenesis of RA, the odds ratio of SE has been relatively low in various studies. It is also well known that a relatively high frequency of RA patients do not have SE and the features of RA manifested by those patients are often typical. The quantitative contribution of genetic factors was estimated at 60%, and the contribution of the major histocompatibility complex was suggested to be one-third of the genetic risk in RA (3). These observations raise the possibility the SE is only one of several genetic factors that contribute to the pathogenesis of RA. Studies of several genetic polymorphisms in Japanese subjects have demonstrated possible contributions of non-HLA genes to the pathogenesis of the disease (4, 5).

CD14 on monocytes, macrophages, and neutrophils is a receptor for complexes of lipopolysaccharides (LPS) and LPS binding protein (LBP) (6), as well as other bacterial components. The signal transduction of the LPS/LBP/CD14 complex is mediated by the Toll-like receptor 4 (TLR4) and induces the activation and release of pro-inflammatory cytokines and mediators (7).

The CD14 gene is located on chromosome 5q31.1. A dimorphism has been identified at position -159 (-159C/T) in the promoter region, and an association of -159TT homozygotes with a high level expression in monocytes has been observed (8). High levels of soluble CD14 have been identified in -159TT homozygotes (9) and carriers of the T allele (10). Macrophages of RA synovial tissue possess broad pro-inflammatory functions and are assumed to play an important role in the pathogenesis of RA (11).

Considering the immune-regulatory and pro-inflammatory functions of CD14, the gene is an interesting candidate as a disease-susceptible gene or a genetic marker of RA. Fontaine *et al*. recently reported a lack of any association of the CD14 -159C/T dimorphism with RA in Caucasians (12). The contribution of the CD14 -159C/T dimorphism as a genetic factor of RA, especially the influence of the susceptibility HLA-DR on CD14 dimorphisms is, however, still unclear.

In this study, we use the PCR-RFLP method to investigate the contribution of the CD14 -159 dimorphisms to RA in a population of 97 Japanese RA patients (9). Associations of CD14 in RA patients with the susceptible DRB1*0405 are also examined and discussed.

Patients and methods

Patients

The association of RA with the CD14 dimorphisms was investigated in 97 unrelated Japanese patients (87 women and 10 men) aged 55.6±11.6 years old (mean±SD). All of the patients fulfilled the American Rheumatism Association diagnostic criteria for RA. Eighty-seven of the 97 patients were positive for rheumatoid factor (RF). A group of 104 randomly selected, unrelated, healthy subjects was compared with the RA group as control. All participants gave their informed consent. The work was approved by the Ethnical Research Panel of Tokyo University Hospital.

CD14 genotyping

The dimorphism at position -159 in the promoter region (-159C/T) was detected by the PCR-RFLP method using the specific oligonucleotide primers, 5'-GTGCCAACAGATGAGGTTCAC-3' and 5'-GCCTCTGACAGTTTATGTAATC-3' (9). In brief, PCR amplification was accompanied by 30 cycles of denaturation at 95°C for 1 min., annealing at 57°C for 1 min., and extension at 72°C for 1 min. using specific primers. The PCR product was then digested with Ava II.

HLA DR typing

HLA-DRB1 typing was performed on the second exon of HLA-DRB1 by PCR using the DR group specific primers described previously (2). HLA-DR4 was then genotyped by the PCR-SSCP (sequence specific conformational polymorphism) method, essentially by the method previously described (2).

Statistics

Fisher's exact test was used for comparisons. The probability value (p) is shown after corrections by the number of comparisons indicated in Table I.

Results

The CD14 -159C/T alleles in the Japanese normal controls are shown in Table I. The -159T allele was slightly predominant among the Japanese subjects, as demonstrated previously by Ito *et al*. (13)

The genotype and allele frequencies of the -159C/T dimorphism in RA are shown in Table I. No CD14 -159C/T genotypes were found to be significantly associated with RA. The allele frequency of -159T was 56.2% in whole RA, which was more or less equal to that in the controls (57.2%). No -159C/T genotypes or alleles showed any significant association in female RA or RF-positive RA.

Table I. CD14 –159C/T dimorphisms in Japanese RA.

	Control	RA	RA-Female	RA RF +	RA *0405+
N	104	97	87	87	50
-159CC	20 (19.2)	20 (20.6)	16 (18.4)	18 (20.7)	11 (22.0)
CT	49 (47.1)	45 (46.4)	41 (47.1)	41 (47.1)	24 (48.0)
TT	35 (33.7)	32 (33.0)	30 (34.5)	28 (32.2)	15 (30.0)
allele-C	89 (42.8)	85 (43.8)	73 (42.0)	77 (44.3)	46 (46.0)
T	119 (57.2)	109 (56.2)	101 (58.0)	97 (55.7)	54 (54.0)

Frequencies are compared between controls and RA. *P*-values are corrected by 3 for genotype frequency. No statistical significance was observed.

The HLA-DRB1 study revealed that 50 RA patients had HLA-DRB1*0405. Genotype and allele frequencies of the -159C/T dimorphism were compared in the RA groups with the DRB1*0405 allele. None of the -159C/T genotype or allele frequencies in the DRB1*0405-positive RA group were higher than those in the control group or in the DRB1*0405-negative RA group. HLA-DR*0405 had no significant influence on the dimorphism of -159C/T.

Discussion

Though various immunological abnormalities are known to contribute to the pathogenesis of RA, the contribution of the CD14 gene is not confirmed. The CD 14 gene dimorphism might influence the susceptibility of RA because the enhanced transcription of the CD14 by a C to T change at position -159 (8, 9, 14) would be associated with the increased level of cytokines (15). The association of this CD14 -159 dimorphism has been reported in chronic spondyroarthropathy, ulcerative colitis, Crohn's disease, asthma, and myocardial infarction (8, 16), though in several cases these associations are controversial. In our experiment, we found no association with the CD14 -159C/T dimorphism in Japanese RA. Our observations strongly support the previous results from Fontaine *et al*. using German RA patients (12), though our population of 97 patients was rather small to reach a definitive conclusion or to refute the type II error completely.

Fontaine *et al*. also found a trend for an association of the -159T allele with RF in their population (12), whereas, no significant association of this type was observed in our study. No other clinical parameters were shown in our patients, as the population was relatively small for meaningful analyses.

Noting earlier reports that other genes contribute to the pathogenesis of RA (5, 17) and other diseases together with HLA, we examined the synergistic effect of the RA-susceptibility HLA-DRB1*0405 on the CD14 -159C/T dimorphism. Singal *et al*., for example, assumed that the TAP gene and HLA-DR contributed to the pathogenesis of RA together (17). Our results revealed no significant association of the CD14 -159C/T dimorphism with Japanese RA containing the RA-susceptibility HLA-DRB1*0405.

On the other hand, the prevalence of the -159 alleles in our Japanese controls significantly differed from that in German controls reported earlier (12). The -159C allele was predominant in German controls, whereas TT homozygotes constituted only 18.5% of that population. It indicates that the genetic background of the CD14 gene (region) clearly differs between Japanese and Germans. The absence of any association of the CD14 -159C/T dimorphism with Japanese or South German RA (12) suggests that a new susceptibility gene near the CD14 gene is very unlikely.

Our observations confirm that there is no association of the CD14 -159C/T dimorphism with Japanese RA. These

results are compatible with a previous report (12). The CD14 dimorphism was not even associated with RA containing the RA-susceptibility HLA-DR epitope.

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References

1. GREGERSEN PK, SILVER J, WINCHESTER RJ: The shared epitope hypothesis: An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Art*hritis Rheum* 1987; 30: 1205-13.
2. TAKEUCHI F, NAKANO K, MATSUTA K *et al.*: Positive and negative association of HLA-DR genotypes with Japanese rheumatoid arthritis. *Clin Exp Rheumatol* 1996; 14: 17-22.
3. DEIGHTON CM, WALKER DJ, GRIFFITHS ID, ROBERTS DF: The contribution of HLA to rheumatoid arthritis. *Clin Genet* 1989; 36: 178-82.
4. MORINOBU S, MORINOBU A, KANAGAWA S, HAYASHI N, NISHIMURA K, KUMAGAI S: Glutathione S-transferase gene polymorphisms in Japanese patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2006; 24: 268-73.
5. TAKEUCHI F, NABETA H, HONG GH *et al.*: Genetic contribution of the TNFα11 microsatellite allele and the TNFB+252*2 allele in Japanese RA. *Clin Exp Rheumatol* 2005; 23: 494-8.
6. WRIGHT SD, RAMOS RA, TOBIAS PS, ULEVITCH RJ, MATHISON JC: CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990; 249: 1431-3.
7. ADEREN A, ULEVITCH RJ: Toll-like receptors in the induction of the innate immune response. *Nature* 2000; 406: 782-7.
8. HUBACEK JA, ROTHE G, PIT'HA J, SKODOVA Z, STANEK V, POLEDNE R: C(-260)→T polymorphism in the promoter of CD14 monocyte receptor gene as a risk factor for myocardial infarction *Circulation* 1999; 99: 3218-20.
9. BALDINI M, LOHMAN IC, HALONEN M, ERICKSON RP, HOLT PG, MARTINEZ FD: A polymorphism in the 5'franking region of CD14 gene is associated with circulation soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; 20: 976-83.
10. KARHUKORPI J, YAN Y, NIEMELA S *et al.*: Effect of CD 14 promoter polymorphism and H. pylori infection and its clinical outcomes on circulating CD14. *Clin Exp Immunol* 2002; 128: 326-32.
11. KINNE RW, BRAUER R, STUHLMULLER B, PALOMBO-KINNE E, BURMESTER GR: Macrophages in rheumatoid arthritis. *Arthritis Res* 2000; 2: 189-202.
12. FONTAINE L DE L, SCHWARTZ M, PLISCHKE H, KLEINDIENST N, GRUBER R: Lack of association of the CD14/C-159T polymorphism with susceptibility and serological activity parameters of rheumatoid arthritis. *Scand J Rheumatol* 2006; 35: 20-2.
13. ITO D, MURATA M, TANAHASHI N *et al.*: Polymorphism in the promoter of lipopolysaccharide receptor CD14 and ischemic cerebrovascular disease. *Stroke* 2000; 31: 2661-4.
14. LEVAN TD, BLOOM JW, BAILEY TJ *et al.*: A common single nucleotide polymorphism in the CD14 promoter decrease the affinity of Sp protein binding and enhances transcriptional activity. *J Immunol* 2001; 167: 5838-44.
15. DOBROVALSKAIA MA, VOGEL SN: Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes Infect* 2002; 4: 903-14.
16. REPO H, ANTTONEN K, KILPINEN SK *et al.*: CD14 and TNFα promoter polymorphisms in patients with acute arthritis. *Scand J Rheumatol* 2002; 31: 355-61.
17. SINGAL DP, YE M, QIU X, D'SOUZA M: Polymorphisms in the TAP2 gene and their association with rheumatoid arthritis. *Clin Exp Rheumatol* 1994; 12: 29-33.