BAT1 Promoter Polymorphism Is Associated with Rheumatoid Arthritis Susceptibility

ADOLFO QUIÑONES-LOMBRAÑA, ALEJANDRO LOPEZ-SOTO, FRANCISCO JAVIER BALLINA-GARCIA, MERCEDES ALPERI-LÓPEZ, RUBEN QUEIRO-SILVA, ANTONIO LOPEZ-VAZQUEZ, CARLOS LOPEZ-LARREA, and SEGUNDO GONZALEZ

ABSTRACT. ***Objective.*** To analyze whether the polymorphisms –22 (G/C) and –348 (C/T) of the BAT1 gene are associated with susceptibility to rheumatoid arthritis (RA).

Methods. One hundred fifty-six patients with RA and 154 controls were genotyped for HLA-DRB1 and the polymorphisms –22 and –348 of the BAT1 gene.

Results. HLA-DRB1*04 alleles were associated with RA susceptibility (33.9% vs 20.1%; p_c = 0.04). Among these, HLA-DRB1*0401 (13.4% vs 5.1%; p_c = 0.04) and HLA-DRB1*0404 (5.7% vs 1.2%; p_c = 0.2) were increased in patients with RA. Additionally, carriage of BAT1 –348T polymorphism was strongly associated with RA (23.7% vs 12.1%; p_c = 0.0002). Significantly, BAT1 –348T was in linkage disequilibrium with HLA-DRB1*0404 and HLA-DRB1*0405. However, BAT1 –348 T was associated independently with HLA-DRB1 shared-epitope alleles (42.6% vs 18.9%; p = 0.001).

Conclusion. The BAT1 –348T polymorphism is associated with RA susceptibility independently of HLA-DRB1. The role of BAT1 in the regulation of tumor necrosis factor-α suggests that BAT1 may regulate the inflammatory response observed in patients with RA. (First Release Mar 15 2008; J Rheumatol 2008;35:741–4)

Key Indexing Terms:
RHEUMATOID ARTHRITIS MAJOR HISTOCOMPATIBILITY COMPLEX HLA-DR BAT1

Rheumatoid arthritis (RA) heritability accounts for ~60% of disease susceptibility[1]. The only region that has been consistently associated with RA susceptibility is the major histocompatibility complex (MHC) region[2,3]. Among the MHC genes, several HLA-DRB1 alleles containing a shared epitope (SE) of a 5-amino-acid sequence motif, QKRAA or QRRAA, from amino acid position 70 to 74 in the third hypervariable region of the DRß chain are strongly associated with susceptibility to and severity of RA[4-7]. Recent studies have reported that the MHC class III region, located telomeric to the HLA-DRB1 gene, contains an additional gene(s) that predisposes to RA[8-16]. However, strong linkage disequilibrium across the MHC has hampered the identification of the precise gene(s) involved. BAT1 is a member of the DEAD box family of ATP-dependent RNA helicases[17]. Its gene is encoded in the MHC class III RA susceptibility region[10]. Little is known about the function and expression of BAT1. However, screening of cells and tissues for BAT1 expression suggests that it is widely expressed in multiple cell types, notably in macrophages and hepatocytes[18]. Studies in monocytes and T cell lines indicate that BAT1 is involved in the production of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and IL-6, and suggest that it regulates the inflammatory response[19]. In agreement with this, BAT1 gene has been associated with susceptibility to several autoimmune and inflammatory diseases[20,21]. Recently, it has been reported that 2 polymorphisms located at –22 and –348 in the promoter of the BAT1 gene alter the binding of transcription factors and consequently affect the transcription of this gene, providing the basis for the association of BAT1 to inflammatory diseases[22]. We describe for the first time the association of BAT1 –348 T polymorphism with susceptibility to RA.

From the Department of Functional Biology, University of Oviedo, IUOPA; and Department of Rheumatology and Histocompatibility and Transplantation Unit, Hospital Universitario Central de Asturias, Oviedo, Spain.

Supported in part by grant FIS PI060841, from the Fondo de Investigaciones Sanitarias from the Spanish Ministry of Health. Mr. Lopez-Soto holds a predoctoral fellowship from FICYT of Asturias (ref. BP06-99).

A. Quiñones-Lombraña, BSc; A. Lopez-Soto, BSc; S. Gonzalez, MD, PhD, Department of Functional Biology, University of Oviedo; F.J. Ballina-Garcia, MD, PhD; M. Alperi-López, MD; R. Queiro-Silva, MD, PhD, Department of Rheumatology; A. Lopez-Vazquez, MD; C. Lopez-Larrea, PhD, Histocompatibility and Transplantation Unit, Hospital Universitario Central de Asturias, Fundación Renal "Iñigo Alvarez de Toledo".

Address reprint requests to Dr. S. González, Department of Functional Biology, IUOPA, Universidad de Oviedo, Facultad de Medicina, Julián Clavería sn, 33006, Oviedo, Spain. E-mail: segundog@uniovi.es

Accepted for publication December 3, 2007.

MATERIALS AND METHODS

Patients and controls. We analyzed 156 consecutive Caucasian patients with RA and 154 ethnically matched controls in this study. All patients fulfilled the American College of Rheumatology revised criteria for RA[23]. One hundred twelve patients were women (71%) and 44 men (29%) and the mean age of the patients was 54.3 years (range 18–81). The laboratory examination included an enzyme linked immunosorbent assay (ELISA) for IgM anti-rheumatoid factor and an anti-cyclic citrullinated peptide (CCP) antibody ELISA (Euro-Diagnostica AB). The control population was obtained from bone marrow

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

donors who had no history of any rheumatic disease. Our study was approved by the ethics committee of Hospital Central de Asturias, and informed consent was obtained from all patients.

HLA-DRB1 and BAT1 typing. HLA-DRB1 alleles were typed by performing polymerase chain reaction using specific primers (PCR-SSP)[24] and by sequence-specific oligonucleotide probes (PCR-SSOP; INNO-LiPA, Innogenetics, NV Ghent, Belgium). The polymorphisms at position –22 and –348 in the promoter of the BAT1 gene were typed by PCR-SSP. To type the polymorphisms G/C at –22, the antisense primer 5'-AAG GAA ATA GCG AAC CAA CTA -3' was used in combination with the sense primers: 5'-AAC CGG AAG TGA AGG CAG-3' or 5'-AAC CGG AAG TGA AGG CAC-3'. For typing the polymorphism C/T at –348, the same antisense primer was used in combination with the sense primers 5'-GTT CCT CGC GCA TCC AC-3' or 5'-GTT CCT CGC GCA TCC AT-3'. The PCR conditions and profile have been described[25].

Statistical analysis. Allelic frequencies were calculated by direct counting and the significance of the association was determined using the chi-square test. The odds ratio (OR) was calculated by the cross-product ratio. Ninety-five percent confidence intervals (95% CI) were calculated for the OR. The p values were corrected (p_c) by multiplying these by the number of comparisons at every locus. Fisher's exact test was used when the expected frequencies were $\leq$ 5. The extent of linkage disequilibrium between the 2 loci is expressed as the observed disequilibrium value (λs), that is, a proportion of the theoretical maximum disequilibrium value (λ_{max}) achievable for this combination of alleles. The λs was calculated using the formula: $\lambda s = \lambda/\lambda_{max} = Pab - (Pa.Pb)/Pa.(1 - Pb)$.

RESULTS

DNA from 156 patients with RA and 154 controls was genotyped for HLA-DRB1 alleles and the polymorphism –22 and –348 of BAT1 gene. All alleles analyzed in our patient and control populations were in Hardy-Weinberg disequilibrium (data not shown). A significant association of HLA-DRB1*04 (33.9% vs 20.1%; p_c = 0.04, OR 2.04, 95% CI 1.2–3.4) with RA susceptibility was observed[16]. This was mainly due to the increase of HLA-DRB1*0401 (13.4% vs 5.1%; p_c = 0.04, OR 3.2, 95% CI 1.3–7.9) and HLA-DRB1*0404 (5.7% vs 1.2%; p = 0.03, p_c = 0.2, OR 4.6, 95% CI 0.9–21.8).

The association of 2 functional polymorphisms located at –22 and –348 in the promoter of BAT1 gene with RA susceptibility was also studied. No statistical differences were observed in the distribution of genotypic or allelic frequencies of BAT1 polymorphism at –22 between patients and controls (BAT1–22C was 34.3% vs 27%; Table 1). However, patients with RA had a higher prevalence of –348T polymorphism (23.7% vs 12.1%; p_c = 0.0002, OR 2.2, 95% CI 1.4–3.5) and –348 CT genotype (39.8% vs 18.8%; p_c = 0.0001) (Table 1). We did not detect an association of BAT1 polymorphism with the presence of antibodies against CCP or rheumatoid factor (data not shown).

BAT1 gene is located in the MHC class III region telomeric to HLA-DRB1 gene. The analysis of linkage disequilibrium indicated that BAT–22G/–348T was in linkage disequilibrium with HLA-DRB1*0404 (λs = 0.43) and HLA-DRB1*0405 (λs = 0.6) in patients. No significant linkage disequilibrium with other HLA-DRB1 alleles was observed. However, although BAT1–348T was in linkage disequilibrium with some SE HLA-DRB1 alleles, it was also increased in SE-negative patients (42.6% vs 18.9%; p = 0.001, OR 3.1, 95% CI 1.5–6.6), suggesting that BAT–348T is associated with RA susceptibility independently of HLA-DRB1 (Table 2).

DISCUSSION

We analyzed the association of BAT1 gene with RA susceptibility. Several recent data suggest that BAT1 may be associated with RA. Different studies have shown that the telomeric region of the MHC contains an additional genetic factor to the HLA-DRB1 gene that predisposes to RA[8-16], and linkage analysis in British Caucasian families with RA indicates that at least 1 non-HLA-DRB1 susceptibility locus for RA exists in the vicinity of BAT1 locus[15]. Moreover, although the function of BAT1 has been poorly characterized, it has been reported that BAT1 is expressed on monocytes and may down-regulate the production of inflammatory cytokines such as TNF-α[19], which plays a central role in the pathogenesis of RA. Additionally, BAT1 promoter is polymorphic and 2 functional polymorphisms located at –22 and –348 have recently been described[22]. These polymorphisms affect the binding of transcription factor and alter the transcription and expression of BAT1, and consequently, they regulate the intensity of the inflammatory response. In agreement with this, the region spanning BAT1 gene has been implicated in susceptibility to numerous immunological diseases such as type I diabetes, multiple sclerosis, ulcerative colitis, and RA.

We report the strong association of –348T polymorphism in the promoter of BAT1 gene with RA susceptibility. The functional significance of this polymorphism remains unclear. It has been reported that the polymorphism of BAT1–348T is part of the 7.1 ancestral haplotype (AH) (HLA-A3, B7, DR15). Previous studies have reported that the cells carrying the diabetogenic 8.1AH (HLA-A1, B8, DR3) that presents a BAT1–348G polymorphism may generate lower BAT1 protein than cells carrying 7.1AH, which has a BAT1–348T polymorphism, providing a possible explanation for why 8.1AH is associated with diabetes[21]. Nevertheless, the association of BAT1 to RA in the Spanish population seems to be more complicated, since we did not observe a strong conservation of AH7.1 haploptype. Indeed, only 8 of 32 of the BAT1–348T Spanish controls were HLA-B*0702. However, BAT1–348T is also present in the AH60.1 haplotype, which also carries HLA-DRB1*0404[26]. This is consistent with our finding of significant linkage disequilibrium of BAT1–348T HLA-DRB*0404 and HLA-DRB*0405, which have been implicated in RA susceptibility[4-7]. No data exist concerning the functional significance of these haplotypes or the expression of BAT1 in patients with RA. Nevertheless, the role of BAT1 polymorphisms in the regulation of transcription suggests that they may modulate BAT1 transcription and that this may modify the inflammatory response in patients with RA. These findings suggest that the presence of BAT1–348T on RA susceptibility haplotypes may modify the susceptibility to or severity of RA conferred by the HLA-DRB1 gene.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

Table 1. Distribution of the BAT1 –22 and –348 genotypes and alleles in RA patients and controls.

	Patients, n = 156	%	Controls, n = 154	%	p	p_c	OR (95% CI)
–22 genotypes							
GG	70	44.8	82	53.2	NS		0.7 (0.4–1.1)
GC	65	41.6	61	39.6	NS		1 (0.6–1.7)
CC	21	13.4	11	7.1	NS		2 (0.9–4.3)
–22 alleles							
G	205	65.7	225	73	NS		0.7 (0.5–0.9)
C	107	34.3	83	27	NS		1.4 (1–1.9)
–348 genotypes							
CC	88	56.4	121	78.5	0.00003	0.00006	0.3 (0.2–0.5)
CT	62	39.7	29	18.8	0.00005	0.0001	2.8 (1.6–4.7)
TT	6	3.8	4	2.5	NS		1.5 (0.4–5.4)
–348 alleles							
C	238	76.2	271	87.9	0.0001	0.0002	0.4 (0.2–0.6)
T	74	23.7	37	12.1	0.0001	0.0002	2.2 (1.4–3.5)

NS: not significant.

Table 2. Distribution of the BAT1 –348 alleles in shared-epitope HLA-DRB1-negative patients and controls.

	Patients, n = 61	%	Controls, n = 90	%	p	OR (95% CI)
–348C	35	57.3	73	81.1	0.001	0.3 (0.1–0.6)
–348T	26	42.6	17	18.8	0.001	3.1 (1.5–6.6)

Additionally, BAT1–348T was also found to be associated with RA susceptibility in SE HLA-DRB1-negative patients, suggesting that BAT1 may also increase the risk of RA susceptibility independently of HLA-DRB1 in the Spanish population.

We recently reported that the MICB gene located in the MHC class III region is also associated with RA susceptibility; however, other genes analyzed were not associated[16]. Nevertheless, the characterization of the precise gene(s) responsible for the susceptibility remains elusive. This is due to the fact that in the MHC class III region, the recombination is very rare and blocks of genes with potential immune function are strongly linked, making characterization of the susceptibility gene for these diseases difficult[26]. It is also possible that more than one gene located in this region may be associated with RA susceptibility. Thus, to confirm the association of BAT1 with RA susceptibility, it is necessary to replicate this study in other populations with different genetic profiles. Additionally, to further elucidate the role of BAT1 in the pathogenesis of RA, the expression and function of BAT1 in patients with RA should be analyzed.

REFERENCES

1. MacGregor AJ, Bamber S, Silman AJ. A comparison of the performance of different methods of disease classification for rheumatoid arthritis. Results of an analysis from a nationwide twin study. J Rheumatol 1994;21:1420-6.
2. Barton A, Ollier W. Genetic approaches to the investigation of rheumatoid arthritis. Curr Opin Rheumatol 2002;14:260-9.
3. Gregersen PK. Teasing apart the complex genetics of human autoimmunity: lessons from rheumatoid arthritis. Clin Immunol 2003;107:1-9.
4. Stasny P. HLA-D and Ia antigens in rheumatoid arthritis and systemic lupus erythematosus. Arthritis Rheum 1978; Suppl 21:139-43.
5. Woodrow JC, Nichol FE, Zaphiropoulos G. DR antigens and rheumatoid arthritis: a study of two populations. Br Med J (Clin Res Ed) 1981;283:1287-8.
6. Wordsworth BP, Lanchbury JS, Sakkas LI, Welsh KI, Panayi GS, Bell JI. HLA-DR4 subtype frequencies in rheumatoid arthritis indicate that DRB1 is the major susceptibility locus within the HLA class II region. Proc Natl Acad Sci USA 1989;86:10049-53.
7. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205-13.
8. Jawaheer D, Li W, Graham RR, et al. Dissecting the genetic complexity of the association between human leukocyte antigens and rheumatoid arthritis. Am J Hum Genet 2002;71:585–94.
9. Zanelli E, Jones G, Pascual M, et al. The telomeric part of the HLA region predisposes to rheumatoid arthritis independently of the class II loci. Hum Immunol 2001;62:75-84.
10. Ota M, Katsuyama Y, Kimura A, et al. A second susceptibility gene for developing rheumatoid arthritis in the human MHC is localized within a 70-kb interval telomeric of the TNF genes in the HLA class III region. Genomics 2001;71:263-70.
11. Pascual M, Mataran L, Jones G, et al. HLA haplotypes and susceptibility to rheumatoid arthritis. More than class II genes. Scand J Rheumatol 2002;31:275-8.
12. Kilding R, Iles MM, Timms JM, Worthington J, Wilson AG.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

Additional genetic susceptibility for rheumatoid arthritis telomeric of the DRB1 locus. Arthritis Rheum 2004;50:763-9.

13. Newton JL, Harney SM, Timms AE, et al. Dissection of class III major histocompatibility complex haplotypes associated with rheumatoid arthritis. Arthritis Rheum 2004;50:2122-9.
14. Tamiya G, Shinya M, Imanishi T, et al. Whole genome association study of rheumatoid arthritis using 27039 microsatellites. Hum Mol Genet 2005;14:2305-21.
15. Kilding R, Iles MM, Timms JM, Worthington J, Wilson AG. Additional genetic susceptibility for rheumatoid arthritis telomeric of the DRB1 locus. Arthritis Rheum 2004;50:763-9.
16. Lopez-Arbesu R, Ballina-García FJ, Alperi-Lopez M, et al. Association of MHC class I chain-related gene B (MICB) with rheumatoid arthritis susceptibility. Rheumatology Oxford 2007;46:426-30.
17. Peelman LJ, Chardon P, Nunes M, et al. The BAT1 gene in the MHC encodes an evolutionarily conserved putative nuclear RNA helicase of the DEAD family. Genomics 1995;26:210-8.
18. Allcock RJ, Price P, Gaudieri S, Leelayuwat C, Witt CS, Dawkins RL. Characterisation of the human central MHC gene, BAT1: genomic structure and expression. Exp Clin Immunogenet 1999;16:98-106.
19. Allcock RJ, Williams JH, Price P. The central MHC gene, BAT1, may encode a protein that down-regulates cytokine production. Genes Cells 2001;6:487-94.
20. Mewar D, Marinou I, Lee ME, et al. Haplotype-specific gene expression profiles in a telomeric major histocompatibility complex gene cluster and susceptibility to autoimmune diseases. Genes Immun 2006;7:625-31.
21. Cheong KY, Allcock RJ, Eerligh P, et al. Localization of central MHC genes influencing type I diabetes. Hum Immunol 2001;62:1363-70.
22. Price P, Wong AM, Williamson D, et al. Polymorphisms at positions -22 and -348 in the promoter of the BAT1 gene affect transcription and the binding of nuclear factors. Hum Mol Genet 2004;13:967-74.
23. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
24. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992;39:225-35.
25. Gonzalez S, Rodriguez-Rodero S, Martinez-Borra J, Lopez-Vazquez A, Rodrigo L, Lopez-Larrea C. MICB typing by PCR amplification with sequence specific primers. Immunogenetics 2003;54:850-5.
26. Allcock RJ, Windsor L, Gut IG, et al. High-density SNP genotyping defines 17 distinct haplotypes of the TNF block in the Caucasian population: implications for haplotype tagging. Hum Mutat 2004;24:517-25.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org