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Regulation of Anti–Cyclic Citrullinated Peptide Antibodies in Rheumatoid Arthritis

Contrasting Effects of HLA–DR3 and the Shared Epitope Alleles

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Objective. **To examine the association between HLA–DRB1 alleles and the production of anti–cyclic citrullinated peptide (anti-CCP) and rheumatoid factor (RF) autoantibodies in patients with rheumatoid arthritis (RA).**

Methods. **We studied 1,723 Caucasian RA patients enrolled in the North American Rheumatoid Arthritis Consortium (NARAC) family cohort and the Study of New Onset Rheumatoid Arthritis (SONORA) cohort. All patients were tested for anti-CCP antibodies (by enzyme-linked immunosorbent assay), RF (by nephelometry), and HLA–DR genotype (by polymerase chain reaction and sequence-specific oligonucleotide hybridization).**

Results. **When controlled for the presence of RF, anti-CCP positivity was strongly associated with the HLA–DRB1 shared epitope (SE). In RF+ patients, the presence of the SE was very significantly associated with anti-CCP positivity, with an odds ratio (OR) of 5.8 and a 95% confidence interval (95% CI) of 4.1–8.3. This relationship was also seen in RF– patients (OR 3.1 [95% CI 1.8–5.3]). In contrast, RF positivity was not significantly associated with presence of the SE independently of anti-CCP antibodies. Strikingly, HLA–DRB1*03 was strongly associated with reduced anti-CCP titers, even after controlling for the presence of the SE and restricting the analysis to anti-CCP+ patients. HLA–DR3 was also associated with anti-CCP– RA in our population.**

Conclusion. **The HLA–DRB1 SE is strongly associated with the production of anti-CCP antibodies, but not RF. In contrast, HLA–DR3 alleles are associated with anti-CCP– disease and with lower levels of anti-CCP antibodies, even when controlling for the SE. These data emphasize the complexity of the genetic effects of the major histocompatibility complex on the RA phenotype.**

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A complex group of HLA class II alleles are associated with an increased risk of developing rheumatoid arthritis (RA), but their exact role in its pathogenesis remains unclear (1–4). Since the time it was originally proposed, many studies have examined the hypothesis that shared epitope (SE)–containing DRB1 alleles can account for these associations, and this remains a useful paradigm (5–8). There have been conflicting reports associating the SE alleles with rheumatoid factor (RF) positivity (9,10). More recently, associations between the different SE alleles and the production of anti–cyclic citrullinated peptide (anti-CCP) antibody have been described (11,12). Since RF and CCP autoantibodies are usually highly correlated, it is unclear whether they are each independently associated with SE alleles in patients with RA.

We have recently shown that a particular Caucasian HLA haplotype, commonly known as the A1;B8; DR3 haplotype (also known as the 8.1 haplotype), carries risk genes for RA (1). The relevant risk alleles appear to be encoded within the central major histocompatibility complex (MHC) and do not involve the HLA–DRB1 locus that encodes for the DR3 (DRB1*0301) allele. Indeed, DR3 itself is not associated with RA.

In the present study, we show that DR3 is associated with lower levels of anti-CCP antibodies, suggesting that the A1;B8;DR3 haplotype may have both negative and positive effects on the risk of RA. This is reminiscent of recent findings on the role of the A1;B8; DR3 haplotype in myasthenia gravis (13) and emphasizes the complexity of the MHC associations with autoimmunity.

PATIENTS AND METHODS

Patient population. We studied a total of 1,723 Caucasian patients with RA, 1,105 from the North American Rheumatoid Arthritis Consortium (NARAC) collection of families and 618 from the Study of New Onset Rheumatoid Arthritis (SONORA) cohort. For the current study, we examined all patients enrolled in each study in whom anti-CCP, RF, and HLA typing had been successfully completed. Overall, our patient population was 75% female (n = 1,288).

We studied DNA and serum samples from patients who met the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria for RA (14) at the time of enrollment in the SONORA cohort. These patients were enrolled within 12 months of diagnosis (15). The NARAC was established to create a resource for RA gene mapping studies in affected sibling pair families. The clinical characteristics of the first 512 multicase families have been described previously (16). Briefly, in order to be eligible for entry into the NARAC family collection, families had to meet the following 3 criteria: ≥2 siblings satisfying the ACR 1987 criteria for RA, at least 1 sibling having documented erosions on hand radiographs, and at least 1 sibling having disease onset between the ages of 18 years and 60 years. Exclusion criteria were the presence of psoriasis, inflammatory bowel disease, or systemic lupus erythematosus in the families.

Autoantibody determinations. RF was measured with the Behring BN2 nephelometer test (Dade-Behring, Deerfield, IL). This method uses human and sheep IgG as target antigens coated on latex beads and primarily detects IgM-RF (17). The upper limit of the reference range is 12 IU/ml. Antibodies to CCP were measured using a second-generation commercial anti-CCP enzyme immunoassay (Inova Diagnostics, San Diego, CA) and was performed as recommended by the vendor, using a serum dilution of 1:100. This assay has a coefficient of variation of 10–15% in our laboratory, and the upper limit of the reference range is 20 units. Specimens were assayed in a blinded manner.

Genotyping of HLA–DRB1. Broad-level HLA–DRB1 typing and high-resolution DRB1*04 typing were accomplished by initial polymerase chain reaction (PCR) amplification of groups of alleles (all DRB1 alleles for broad-level typing and group-specific amplification for DRB1*04 alleles) using biotinylated PCR primers, followed by hybridization to immobilized sequence-specific oligonucleotide (SSO) probes in a linear array format (18). Positive hybridization reactions were detected using a streptavidin–horseradish peroxidase conjugate and a soluble colorless substrate, 3,3′,5,5′-tetramethylbenzidine. A computer algorithm, based on the SSO–probe hybridization pattern, and the A. Nolan Immunogenetics/HLA Sequence Database (available at http://www.ebi.ac.uk/imgt/hla/) were used to assign genotypes to each sample. SE alleles were defined as DRB1*0101, 0401, 0404, 0405, 0408, or 1001.

Statistical analysis. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using chi-square statistics. In order to compare antibody titers between groups, the Mann-Whitney U test was applied to the distribution of antibody titers (anti-CCP or RF) in each group, and correlations between anti-CCP and RF titers were analyzed using Spearman's rank test. Since the upper range of anti-CCP titers was not fully established for all subjects, we truncated the upper range to a standard value of anti-CCP for some nonparametric comparisons. Multivariate analysis was also used to examine the relationships between RF, anti-CCP antibodies, and the presence of various HLA alleles.

RESULTS

The distribution of our patient population with respect to autoantibody status (anti-CCP and RF) is shown in Table 1. Anti-CCP positivity was strongly associated with RF in our population (OR 22.7 [95% CI 17.3–29.8]). When anti-CCP and RF titers for each patient were compared, we observed a strong correlation between these autoantibodies ($r = 0.55$, $P < 0.0001$, by Spearman's rank test).

Overall, 1,257 of the 1,723 patients (72.9%) were positive for RF, and 1,191 (69.1%) were positive for

Table 1. Distribution of rheumatoid arthritis patients into serologic subgroups according to RF and anti-CCP antibody status*

	RF+	RF−
Anti-CCP+	1,088	103
Anti-CCP−	169	363

* There was a strong association between rheumatoid factor (RF) positivity and anti–cyclic citrullinated peptide (anti-CCP) antibody positivity ($P < 0.0001$, by 2-sided test; odds ratio 22.7 [95% confidence interval 17.3–29.8]).

anti-CCP. At least 1 SE allele was present in 1,353 patients (78.5%) in our cohort. These values were lower in the SONORA cohort (65%, 55%, and 67% positive for RF, anti-CCP, and SE, respectively) than in the NARAC collection (78%, 77%, and 85% positive, respectively). This is to be expected, since all of the SONORA patients had recent-onset disease, whereas the NARAC collection is enriched for patients with longstanding erosive disease (16). Nevertheless, the analyses described below did not differ substantially between the 2 groups, and therefore, the combined results are reported.

The various autoantibody subgroups were stratified in order to assess the influence of the SE on each serologic phenotype. Because we sought to assess the independent effects of the SE on anti-CCP and RF, the 4 subgroups shown in Table 1 (CCP+/RF+, CCP+/RF–, CCP–/RF+, and CCP–/RF–) were compared with regard to SE status. As shown in Table 2, the presence of the SE was strongly associated with the presence of anti-CCP antibodies, independently of RF status (OR 5.8 for CCP+/RF+ versus CCP–/RF+, and OR 3.1 for CCP+/RF– versus CCP–/RF–). In contrast, when we controlled for the presence of anti-CCP antibodies, the SE was not significantly associated with the presence of RF, as shown in Table 3. These results were confirmed by multiple logistic regression analyses.

To assess whether any particular SE allele was preferentially associated with anti-CCP antibodies in RA patients, we compared specific DRB1 genotype combinations, as shown in Table 4. In order to be able to make comparisons between genotypes, all analyses were performed using SE–/SE– patients as the reference group, and only the more common SE alleles (DRB1*0101, *0401, and *0404) were studied. There was no significant difference in the results for these 3 alleles, although the OR for the 0101/X genotype was lower. This is reminiscent of the generally lower ORs for the association of RA per se with the 0101 allele (5). The presence of 2 SE alleles in various combinations showed a stronger association with anti-CCP antibodies, without striking differences among them.

A further analysis of the most common HLA–DRB1 SE– allele groups was also performed to explore whether any of these alleles might influence the quantity of autoantibodies (RF or anti-CCP) in serum. These included HLA–DR2 (n = 133 alleles), DR3 (n = 318), DR5 (n = 217), and DR7 (n = 242). There was no association between these alleles and the titer of RF (data not shown). However, as shown in Table 5, HLA–DR3 was associated with markedly reduced titers of

Table 2. Association of the SE with anti-CCP antibodies, independently of RF status*

	RF+		RF−	
	SE+	SE−	SE+	SE−
Anti-CCP+	960	128	84	19
Anti-CCP−	95	74	214	149

* There were strong associations between the shared epitope (SE) and anti–cyclic citrullinated peptide (anti-CCP) antibodies in both the rheumatoid factor (RF)–positive group ($P < 0.0001$; odds ratio 5.8 [95% confidence interval 4.1–8.3) and the RF-negative group ($P < 0.0001$; odds ratio 3.1 [95% confidence interval 1.8–5.3).

Table 3. Absence of a significant association between the SE and RF, independently of anti-CCP antibody status*

	Anti-CCP+		Anti-CCP−	
	SE+	SE−	SE+	SE−
RF+	960	128	95	74
RF−	84	19	214	149

* There were no significant associations between the shared epitope (SE) and rheumatoid factor (RF) in the anti–cyclic citrullinated peptide (anti-CCP) antibody–positive group ($P = 0.07$; odds ratio 1.7 [95% confidence interval 1.0–2.9) or the anti-CCP antibody–negative group (P not significant; odds ratio 0.9 [95% confidence interval 0.6–1.3]).

Table 4. Associations between different DRB1 genotypes and anti-CCP antibodies in RA patients as compared with SE-negative subjects*

DRB1 genotype	OR (95% CI)
0101/X	2.9 (1.7–4.8)
0401/X	6.0 (3.7–9.8)
0404/X	5.5 (2.9–10.7)
0401/0401	12.3 (3.7–40.7)
0401/0404	12.6 (5.6–28.2)
0401/0101	9.1 (4.0–20.5)

* All comparisons are for rheumatoid arthritis (RA) patients with a shared epitope (SE)–negative genotype: SE−/SE− (i.e., X/X). Anti-CCP = anti–cyclic citrullinated peptide; OR = odds ratio; 95% CI = 95% confidence interval.

Table 5. Median titers of anti-CCP antibodies in 4 common HLA–DR groups

HLA–DR allele	Median anti-CCP titer
DR2 (n = 133)	73.3
DR3 (n = 318)	23.1*
DR5 (n = 217)	31.7†
DR7 (n = 242)	63
All (n = 1,723)	76

* $P < 5.4 \times 10^{-6}$ versus anti–cyclic citrullinated peptide (anti-CCP) antibody titers in all DR3-negative subjects, excluding those with a shared epitope (SE)–positive genotype (SE+/SE+), by Mann-Whitney U test.
† $P = 0.02$ versus anti-CCP antibody titers in all DR5-negative subjects, excluding those with an SE+/SE+ genotype, by Mann-Whitney U test.

anti-CCP antibody ($P = 5.4 \times 10^{-6}$). A subgroup analysis was performed to directly control for the effect of the SE allele on the association with DR3 (Table 6). DR3 was associated with significantly reduced titers of anti-CCP antibody in the presence of an SE allele on the opposite chromosome ($P = 0.001$ for DR3+/SE+ versus DR3–/SE+, by Mann-Whitney 2-tailed U test). Although anti-CCP titers were also lower in the DR3+ group in the absence of an SE allele, the difference was not significant.

HLA–DR3 was also significantly associated with anti-CCP– disease in our study population (OR 1.6 [95% CI 1.2–2.1]). However, the effect of DR3 on lowering anti-CCP titers cannot be ascribed to higher numbers of anti-CCP– patients in the DR3+ group, since the degree of association between DR3 and lower mean titers of anti-CCP antibody was similar when only CCP+ patients were considered in the analysis (data not shown). HLA–DR5 also had a significant, but less pronounced, effect on the anti-CCP antibody titer (Table 5), but this effect did not persist when the analysis was controlled for the presence of an SE allele (data not shown).

Table 6. Association between DR3 and lower median titers of anti-CCP antibodies, by SE status

	DR3+	DR3−
SE+	41 (n = 175)*	84.5 (n = 663)
SE−	13.1 (n = 143)†	11 (n = 227)

* $P = 0.001$ versus anti–cyclic citrullinated peptide (anti-CCP) antibody titers in the shared epitope (SE)–positive/DR3-negative group, by Mann-Whitney U test.
† $P = 0.6$ versus anti-CCP titers in the SE−/DR3− group, by Mann-Whitney U test.

DISCUSSION

Anti-CCP antibodies have been clearly associated with the development of RA, although a distinct pathogenic role in the development of disease remains to be established. We have shown a strong association between the anti-CCP antibody response and the presence of the HLA–DRB1 shared epitope. A recent study has shown that citrullinated peptide antigens may bind more tightly to this group of alleles, which suggests that "determinant selection" may be the mechanism for the association (19). Further studies will be required to establish exactly which citrullinated peptide antigens are driving the anti-CCP response in RA patients and how these particular antigens interact with SE alleles.

Anti-CCP antibodies are a prognostic indicator for the development of more aggressive disease (20,21). The SEs encoding HLA–DRB1 alleles have also been shown to confer risk of the development of RA and have been implicated in worse outcomes, particularly increased erosions (22). By analyzing the independent effect of SE on both anti-CCP and RF, a strong association between the SE and the development of anti-CCP antibodies was confirmed in this study. The SE had no significant independent association with RF status in this large cohort of RA patients. These findings suggest that the previous associations between disease progression and the presence of RF and the SE may be indirect, reflecting a primary association of the SE with the production of anti-CCP antibodies. A recent study indicates that the presence of anti-CCP is associated with progression to erosive disease, with additional contributions by the SE (11). However, a more extended analysis suggests that the effects of the SE on erosive disease are quite modest when viewed independently of anti-CCP antibodies (Huizinga TW, et al: unpublished observations). The SONORA cohort was designed as a 5-year outcome study, including radiographic analysis, and thus, we should be able replicate these relationships and extend the genetic analysis as this cohort matures.

The more novel finding of this study is that the HLA–DRB1*03 allele was clearly associated with decreased titers of anti-CCP antibody, even in the presence of an SE allele. In addition, DR3 was associated with anti-CCP– disease. Although HLA–DRB1*03 has not previously been associated with susceptibility to RA, this allele has been associated with several other autoimmune diseases, including systemic lupus erythematosus, insulin-dependent diabetes mellitus, autoimmune hepatitis, and myasthenia gravis. Within the setting of these illnesses, the DR3 associations with autoantibody

production appear to be variable. For example, DR3 has been associated with increased production of anti-Ro/SSA and anti-La/SSB in Sjögren's syndrome (23), anti–PM-Scl in systemic sclerosis (24), multiple autoantibodies in diabetes mellitus (25,26), and anti–soluble liver antigen/liver pancreas in autoimmune hepatitis (27). In contrast, DR3 is negatively associated with anti–acetylcholine receptor (anti-AChR) antibodies in myasthenia gravis (13). In addition, the DR3 allele has been associated with a lack of antibody response in viral illnesses (28) and hepatitis B vaccination (29), as well as with immunodeficiencies, such as IgA deficiency and common variable immunodeficiency (30).

These conflicting immunologic associations with DR3 undoubtedly relate in part to the complexity of DR3 haplotypes. For example, a large proportion of the DR3 alleles in northern European populations are found on an extended haplotype designated A1;B8;DR3, which is also known as the 8.1 haplotype (31,32). It is likely that at least some of the DR3 disease associations are actually caused by genes other than DR3 on this haplotype. Indeed, we have recently shown that one or more genes located in the central portion of the 8.1 haplotype are probably associated with RA, independently of DR3 (1). A recent study of myasthenia gravis patients also implicates genes in the central MHC as accounting for disease susceptibility, while the DR3 allele itself is associated with lower titers of anti-AChR antibodies, as noted above. This may be analogous to what we observed in the current study, where the DR3 allele was associated with lower titers of anti-CCP antibodies, but genes found on a common DR3 haplotype were also associated with RA susceptibility. Clearly, it will be of interest to examine the current population for genetic variations in the central MHC as well, in order to determine whether genes that are in linkage disequilibrium with DR3 on some haplotypes might explain the DR3 effect on anti-CCP antibody titers. In addition, it will be interesting to determine whether DR3 has a protective effect on disease outcome.

Overall, these data emphasize the considerable complexity of HLA associations with RA, even for the DRB1 locus alone. It is well established that the SE associations with RA are not simple. Some SE alleles, such as 0401 and 0404, confer relatively high risk, while others, such as 0101, carry lower levels of risk. Interestingly, we observed only modest differences in the strength of the associations between these alleles and anti-CCP antibodies (Table 5). Finally, in some population groups, the SE appears to have a minimal influence on disease susceptibility (33). The effect of DR3 in combination with SE alleles has not previously been examined. In addition, as noted above, consideration must also be given to the role of genes that may be in linkage disequilibrium with these alleles in the various populations. These complex relationships will become apparent only when very large population studies are performed, so that a sufficient number of the uncommon subgroups (such as CCP+ and RF– or CCP– and RF+) are available for analysis. Combining these data with those from studies of disease outcome should enable us to more fully understand the role of these genetic factors in RA.

REFERENCES

1. Jawaheer D, Li W, Graham RR, Chen W, Damle A, Xiao X, et al. Dissecting the genetic complexity of the association between human leukocyte antigens and rheumatoid arthritis. Am J Hum Genet 2002;71:585–94.
2. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis [review]. Arthritis Rheum 1987;30:1205–13.
3. Nepom GT. The role of the DR4 shared epitope in selection and commitment of autoreactive T cells in rheumatoid arthritis. Rheum Dis Clin North Am 2001;27:305–15.
4. Moxley G, Cohen HJ. Genetic studies, clinical heterogeneity, and disease outcome studies in rheumatoid arthritis. Rheum Dis Clin North Am 2002;28:39–58.
5. Hall FC, Weeks DE, Camilleri JP, Williams LA, Amos N, Darje C, et al. Influence of the HLA-DRB1 locus on susceptibility and severity of rheumatoid arthritis. QJM 1996;89:821–9.
6. Harrison B, Thomson W, Symmons D, Ollier B, Wiles N, Payton T, et al. The influence of HLA–DRB1 alleles and rheumatoid factor on disease outcome in an inception cohort of patients with early inflammatory arthritis. Arthritis Rheum 1999;42:2174–83.
7. El-Gabalawy HS, Goldbach-Mansky R, Smith D II, Arayssi T, Bale S, Gulko P, et al. Association of HLA alleles and clinical features in patients with synovitis of recent onset. Arthritis Rheum 1999;42:1696–705.
8. Del Rincon I, Battafarano D, Arroya RA, Murphy FT, Fischbach M, Escalante A. Ethnic variation in the clinical manifestations of rheumatoid arthritis: role of HLA–DRB1 alleles. Arthritis Rheum 2003;49:200–8.
9. Mattey D, Hassel AB, Dawes PT, Cheung NT, Poulton KV, Thomson W, et al. Independent association of rheumatoid factor and the HLA–DRB1 shared epitope with radiographic outcome in rheumatoid arthritis. Arthritis Rheum 2001;44:1529–33.
10. Mattey DL, Dawes PT, Clarke S, Fisher J, Brownfield A, Thomson W, et al. Relationship among the HLA–DRB1 shared epitope, smoking, and rheumatoid factor production in rheumatoid arthritis. Arthritis Rheum 2002;47:403–7.
11. Van Gaalen FA, van Aken J, Huizinga TW, Schruder GM, Breedveld FC, Zanelli E, et al. Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004;50:2113–21.
12. Berglin E, Padyukov L, Sundin U, Hallmans G, Stenlund H, van Venrooij W, et al. A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. Arthritis Res Ther 2004;6:R303–8.

13. Giraud M, Beaurain G, Yamamoto AM, Eymard B, Tranchant C, Gajdos P, et al. Linkage of HLA to myasthenia gravis and genetic heterogeneity depending on anti-titin antibodies. Neurology 2001; 57:1555–60.
14. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
15. Bombardier C, Deaton RL, Gregersen P, Massarotti E, Formica C, Weisman MH. Pattern of DMARD use in a North American cohort of patients with early rheumatoid arthritis (RA) (SONORA) [abstract]. Arthritis Rheum 2002;46 Suppl 9:S344.
16. Jawaheer D, Lum RF, Amos C, Gregersen PK, Criswell LA. Clustering disease features within 512 multicase rheumatoid arthritis families. Arthritis Rheum 2004;50:736–41.
17. Wener MH. Rheumatoid factors. In: Rose NR, Hamilton RG, Detrick B, editors. Manual of clinical laboratory immunology. 6th ed. Washington, DC: ASM Press; 2002. p. 961–72.
18. Erlich HA, Bugawan T, Begovich AB, Scharf S, Griffith R, Saiki R, et al. HLA-DR, DQ and DP typing using PCR amplification and immobilized probes. Eur J Immunogenet 1991;18:33–55.
19. Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. J Immunol 2003;17:538–41.
20. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van 't Hof M, et al. The prognostic value of anti–cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831–5.
21. Forslind K, Ahlmen M, Eberhardt K, Hafstrom I, Svensson B. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 2004;63:1090–5.
22. Gorman JD, Lum RF, Chen JJ, Suarez-Almazor ME, Thomson G, Criswell LA. Impact of shared epitope genotype and ethnicity on erosive disease: a meta-analysis of 3,240 rheumatoid arthritis patients. Arthritis Rheum 2004;50:400–12.
23. Gottenberg JE, Busson M, Loiseau P, Cohen-Solal J, Lepage V, Charron D, et al. In primary Sjögren's syndrome, HLA class II is associated exclusively with autoantibody production and spreading of the immune response. Arthritis Rheum 2003;48:2240–5.
24. Falkner D, Wilson J, Fertig N, Clawson K, Medsger TA Jr, Morel PA. Studies of HLA-DR and DQ alleles in systemic sclerosis patients with autoantibodies to RNA polymerases and U3-RNP (fibrillarin). J Rheumatol 2000;27:1196–202.
25. Savola K, Laara E, Vahasalo P, Kulmala P, Akerblom HA, Knip M. Dynamic pattern of disease-associated autoantibodies in siblings of children with diabetes. Diabetes 2001;50:2625–32.
26. Kulmala P, Savola K, Reijonen H, Veijola R, Vahasalo P, Karjalainen J, et al. Genetic markers, humoral autoimmunity and prediction of type 1 diabetes in affected children. Diabetes 2000; 49:48–58.
27. Czaja AJ, Donaldson PT, Lohse AW. Antibodies to soluble liver antigen/liver pancreas and HLA risk factors for type I autoimmune hepatitis. Am J Gastroenterol 2002;97:413–9.
28. Caruso C, Candore G, Colucci AT, Cigna D, Sammartano F, Ammatuna P. HLA-B8,DR3 phenotype and the antibody response against Epstein-Barr virus. Immunol Invest 1993;22:41–51.
29. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ, et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989;321:708–12.
30. Schaffer FM, Palermos J, Zhu ZB, Barger BO, Cooper MD, Volanakis JE. Individuals with IgA deficiency and common variable immunodeficiency share polymorphisms of major histocompatibility complex class III genes. Proc Natl Acad Sci U S A 1989;86:8015–9.
31. Price P, Witt C, Allcock R, Sayer D, Garlepp M, Kok CC, et al. The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases. Immunol Rev 1999;167:257–74.
32. Mickelson EM, Nisperos B, Layrisse Z, Kim SJ, Thomas ED, Hansen JA. Analysis of the HLA-DRw8 haplotype: recognition by HTC typing of three distinct antigen complexes in Caucasians, Native Americans, and Orientals. Immunogenetics 1983;17: 399–410.
33. Teller K, Budhai L, Zhang M, Haramati N, Keiser HD, Davidson A. HLA-DRB1 and DQB typing of Hispanic American patients with rheumatoid arthritis: the "shared epitope" hypothesis may not apply. J Rheumatol 1996;23:1363–8.