0014-4754/93/490553-04$1.50 + 0.20

A decrease in the estimated frequency of the extended HLA haplotype B18 CF130 DR3 DQw2 is common to non-insulin-dependent diabetes, juvenile rheumatoid arthritis, and Berger's disease

J. R. Regueiro[a], A. Arnaiz-Villena[a], J. L. Vicario[c], J. Martinez-Laso[a], A. Pacheco[a] and J. M. Rivera-Guzman[b]

[a]*Inmunología and* [b]*Medicina Preventiva, Hospital 12 de Octubre, Facultad de Medicina, Universidad Complutense, 28041 Madrid (Spain), and* [c]*Inmunología, Centro de Transfusión, Comunidad de Madrid, 28009 Madrid (Spain)*
Received 18 May 1992; accepted 3 March 1993

Abstract. Extended HLA haplotypes frequencies were estimated from the HLA, C2, Bf and C4 phenotypes of 74 patients with non-insulin-dependent diabetes (NIDD), 92 with juvenile rheumatoid arthritis (JRA), 44 with Berger's disease (BD), 83 with insulin-dependent diabetes (IDD), and 140 healthy controls. The extended HLA haplotype B18 CF130 DR3 DQw2, which is common (around 10% phenotype frequency) in healthy Spaniards and in other populations of paleo-North African origin, was found to be significantly less frequent in NIDD, JRA and BD, whereas its frequency was normal in IDD (although DR3 DQw2 haplotypes were increased in the latter disease). These data support the existence of a common HLA-linked pathogeneic mechanism in NIDD, JRA and BD, and point to a genetic difference between IDD and NIDD at the HLA level. This effect is readily detectable in our population because the uncommon BfF1 allele marks that haplotype instead of the more common BfS, which marks B8 CS01 DR3 DQw2 in other Caucasians. Our results support the hypothesis of strong selective pressures operating at the HLA level to preserve extended HLA haplotypes with advantageous gene sets from dilution by crossing-over. Imbalanced incomplete haplotypes may give rise to inappropriate T-cell repertoire selection in the thymus and/or antigen handling in the periphery, and be partly responsible for the pathogenesis of certain HLA-linked diseases (i.e. NIDD, JRA, and BD).
Key words. HLA; haplotype; disease; diabetes; arthritis; Berger's disease.

Extended HLA haplotypes, also called supratypes[1], are highly conserved *cis*-associations of certain human major histocompatibility complex (MHC) class I (HLA-A, -B, -C), class II (HLA-DR, -DQ, -DP) and class III (C2, Bf, C4A, C4B) alleles. They are present in more than 50% of healthy Caucasians[2] but the reason for their prevalence is still unknown. Several authors postulate the existence of selective advantages to explain their incidence. In some cases, they may contain synergistic *cis*-arrays of balanced 'immune response' genes united and linked to give an effective immune response[3]. In other cases, polymorphic class III components could also play a role in such a gene complementation scheme[4]. More recently, it has been proposed that certain MHC class I molecules are selected, and thus their genes carried together with a given class II haplotype, because they interact more efficiently with the T-cell receptors left after clonal deletion by that particular class II allele combination[5]. Finally, the recent description of putative peptide transporters within the class II region has led to the proposal that certain MHC haplotypes are favored when they carry compatible peptide-handling allele sets which offer advantages in responses to pathogens[6].

In contrast to the possible selective benefits enjoyed by carriers of extended MHC haplotypes, we have previously proposed that deletereous or disadvantageous consequences (i.e. disease) may follow in carriers of disrupted extended HLA haplotypes, where the intragenic balance had been broken by recombination[7]. This hypothesis can be tested by analyzing the prevalence of incomplete extended HLA haplotypes in HLA-linked diseases. In the present report, extended HLA haplotype frequencies were estimated from phenotypic data in four diseases with controversial linkage to HLA, namely insulin-dependent diabetes (IDD)[8], non-insulin-dependent diabetes (NIDD)[9], juvenile rheumatoid arthritis (JRA)[10] and Berger's disease (BD)[11], and compared to those of healthy controls. A significant decrease in extended HLA haplotype B18 CF130 DR3 DQw2, which is common in healthy Spaniards, was found in NIDD, JRA and BD, whereas its frequency was normal in IDD.

Materials and methods

Subjects. We studied 74 NIDD, 92 JRA, 44 BD and 83 IDD unrelated patients. 140 unrelated healthy individuals were used as controls. Diagnostic criteria have previously been described[12–14].

MHC class I and class II alleles. HLA-A, -B, -C, -Bw4/Bw6, -DR and -DQ typing was performed by the microlymphocytotoxicity assay as described previously[15].

MHC class III alleles. C2, Bf, C4A and C4B typing was done by electroimmunofixation[16].

Statistical analysis

The presence of extended HLA haplotypes within phenotypes in our population was estimated as described[17]. They have previously been confirmed in families[16], and correspond to 'supratypes' in unrelated individuals[1], comprising markers from HLA-B to HLA-DQ. All the described markers were used when possible (in IDD and NIDD, but not in JRA or BD). Significant deviations of phenotype frequencies between normal and diseased individuals were detected in 2×2 contingency tables using a chi-square test with Yate's correction. Relative risks (RR) were calculated as described[18], and all p values were corrected for the number of alleles used when this was greater than 20.

Nomenclature

MHC markers were named according to their order in chromosome 6 which is, telomere to centromere, HLA-A, -C, -B, C2, Bf, C4A, C4B, HLA-DR, -DQ. Complement allotypes, alleles and haplotypes are named as proposed earlier[19] and abbreviated in complotypes in the order C2-Bf-C4A-C4B (CF130 meaning C2C BfF1 C4A3 C4BQ0).

Results

The frequency of various HLA markers or their combinations forming haplotypes were calculated from phenotypes in IDD, NIDD, JRA and BD, and compared to those of healthy controls as explained above. Relevant results are shown in tables 1 and 2.

Insulin-dependent diabetes. Table 1 summarizes our results on IDD regarding two diabetogenic haplotypes previously reported in Caucasians (1): B18 CF130 DR3 DQw2 and B8 CS01 DR3 DQw2. DR3 DQw2 haplotypes were significantly raised in IDD (RR = 6.6; p = 0.00000041) by themselves (that is, independently of HLA-B18, BfF1 and C4A3BQ0). It was not possible to evaluate the relative contribution of DQw2 (as compared to DR3) to IDD susceptibility by this type of analysis, because it is also associated with DR7, the frequency of which is significantly lowered in this disease (table 2). The second haplotype (the B8-bearing one) was not significantly more frequent in our IDD sample[20]. Further analysis of B18 CF130 DR3 DQw2 (table 2) confirmed that its diabetogenicity was mainly restricted to DR3 DQw2 (with the highest RR). The putative 'protective' haplotypes B7 CS31 DR2 DQw1 and B17 CS61 DR7 DQw2 were decreased or absent in IDD, although this seemed to be primarily due to a DR2 (RR = 0.3; p = 0.026) and DR7 (RR = 0.4; p = 0.025) decrease (table 2).

Non-insulin-dependent diabetes. When the same diabetogenic haplotypes were analyzed in NIDD (table 1), striking differences were evident compared to both IDD

Table 1. Phenotype frequencies (%) and relative risks (RR) of different HLA markers in IDD, NIDD, JRA and BD

	Normals (n = 140)	IDD (n = 83)			NIDD (n = 74)			JRA (n = 92)			BD (n = 44)		
HLA marker	%	%	RR	p	%	RR	p	%	RR	p	%	RR	p
B8	11.3	16.9	1.6	NS	17.6	1.7	NS	-	-	-	-	-	-
B18	14.7	38.6	3.7	0.00000055	5.4	0.3	0.047	-	-	-	22.7	1.7	NS
Cw5	16.0	31.3	2.4	0.0016	4.1	0.2	0.011	-	-	-	-	-	-
DR3	27.1	70.9	6.6	0.00000041	28.4	1.1	NS	18.4	0.6	NS	-	-	-
DQw2	54.0	77.0	2.9	0.0053	51.0	0.9	NS	36.0	0.5	0.039	-	-	-
BfF1	10.0	31.2	4.1	0.00000080	9.5	0.9	NS	2.2	0.2	0.030	6.8	0.7	NS
C4BQ0 (A3)	22.9	37.7	2.0	0.031	13.5	0.5	NS	10.9	0.4	0.032	31.0	1.5	NS
C4AQ0 (B1)	18.6	22.1	1.2	NS	31.1	2.0	NS	28.3	1.7	NS	-	-	-
B18-Cw5	10.0	27.7	3.5	0.0011	0.0	0.0	0.012	-	-	-	-	-	-
B18-non Cw5	2.1	10.8	5.6	0.013	5.4	2.6	NS	-	-	-	-	-	-
B18-DR3	12.5	32.7	3.4	0.0053	4.1	0.3	NS	-	-	-	-	-	-
B18-non DR3	3.1	3.6	1.2	NS	1.4	0.4	NS	-	-	-	-	-	-
B18-BfF1	8.5	25.3	3.7	0.000069	4.1	0.5	NS	-	-	-	6.8	0.8	NS
B18-non BfF1	7.3	10.1	1.4	NS	1.4	0.2	NS	-	-	-	20.0	3.3	0.02
B18-C4BQ0	7.9	23.4	3.6	0.0026	1.4	0.2	NS	-	-	-	13.6	1.9	NS
B18-non C4BQ0	4.3	14.3	3.7	0.018	4.1	0.9	NS	-	-	-	9.1	2.2	NS
B8-DR3	6.3	16.4	2.9	NS	13.5	2.3	NS	-	-	-	-	-	-
B8-non DR3	6.3	0.0	0.0	NS	4.1	0.6	NS	-	-	-	-	-	-
B8-C4AQ0	5.0	10.4	2.2	NS	12.2	2.6	NS	-	-	-	-	-	-
B8-non C4AQ0	5.7	6.5	1.1	NS	5.4	0.9	NS	-	-	-	-	-	-
DR3-B18	12.5	32.7	3.4	0.0053	4.1	0.3	NS	-	-	-	-	-	-
DR3-non B18	14.6	38.2	3.6	0.0019	24.3	1.9	NS	-	-	-	-	-	-
DR3-BfF1	12.5	26.4	2.5	NS	8.1	0.6	NS	2.3	0.2	0.022	-	-	-
DR3-non BfF1	14.6	43.4	4.5	0.00022	20.3	1.5	NS	16.3	1.1	NS	-	-	-
DR3-C4BQ0	11.5	29.1	3.2	0.012	8.1	0.7	NS	2.3	0.2	0.034	-	-	-
DR3-non C4BQ0	15.6	41.8	3.9	0.00074	20.3	1.4	NS	16.1	1.0	NS	-	-	-
DR3-DQw2	27.1	70.9	6.6	0.00000041	28.4	1.1	NS	18.4	0.6	NS	-	-	-

NS = $p > 0.05$.
- = not done.

Table 2. Analysis of different estimated extended HLA haplotypes in IDD, NIDD and JRA Spanish individuals

Allelic combinations									IDD (n = 52)		NIDD (n = 74)		JRA (n = 86)	
HLA-A	-Cw	-B	C2	Bf	C4A	C4B	-DR	-DQw	RR	p	RR	p	RR	p
30	5	18	C	F1	3	Q0	3	2	0.3	NS	0.0	NS	-	-
	5	18	C	F1	3	Q0	3	2	1.1	NS	0.0	0.011	-	-
		18	C	F1	3	Q0	3	2	1.3	NS	0.1	0.039	-	-
			C	F1	3	Q0	3	2	1.8	NS	0.4	NS	0.0	0.0059
					3	Q0	3	2	3.2	0.018	0.6	NS	0.2	NS
				F1			3	2	2.6	0.048	0.6	NS	0.2	0.022
							3	2	6.6	0.00000057	1.1	NS	0.6	NS
								2	2.9	0.0068	0.9	NS	0.5	0.039
3		7	C	S	3	1	2	1	0.0	NS	0.8	NS	-	-
		7	C	S	3	1	2	1	0.0	NS	1.1	NS	-	-
			C	S	3	1	2	1	0.3	0.036	0.5	NS	0.5	NS
							2	1	0.3	0.026	0.7	NS	0.7	NS
								1	0.3	0.0055	1.6	NS	1.9	NS
1		17	C	S	6	1	7	2	0.0	NS	0.4	NS	-	-
		17	C	S	6	1	7	2	0.0	NS	0.3	NS	-	-
			C	S	6	1	7	2	0.0	NS	1.0	NS	0.0	NS
							7	2	0.4	0.025	1.1	NS	0.9	NS
								2	2.9	0.0068	0.9	NS	0.5	0.039

NS = $p > 0.05$.
- = not done.

patients and normals. B18 and Cw5 were significantly decreased ($p = 0.047$ and 0.011, respectively) and they were never present together in the same individual ($p = 0.012$). A closer look at B18-bearing extended haplotypes confirmed their decreased frequency in NIDD (RR = 0.0; $p = 0.011$, table 2). DR2- and DR7-bearing haplotypes frequencies were normal in NIDD, in contrast to IDD, where they were lower.

Juvenile rheumatoid arthritis. Table 1 shows DR, DQ and complement data for JRA. The most significant results were again a decrease of full extended (B18) CF130 DR3 DQw2 haplotypes ($p < 0.034$), as confirmed in table 2.

Berger's disease. Although only a limited sample was studied (n = 44), an increase in incomplete B18-bearing extended haplotypes was observed (RR = 3.3; $p = 0.02$, table 1), whereas no single marker was altered by itself.

Discussion

The primary association of IDD with DR3 DQw2 and not with the whole haplotype (B18 CF130 DR3 DQw2) confirms other findings[21], but is in contrast to some groups for whom the DR3 (DQw2) increase was secondary to an increase in two extended HLA haplotypes (B18- and B8-bearing ones)[1,22]. The Spanish IDD population did not have an increased B8: thus the increase of DR3 DQw2 was shared by B18 and many other non-extended haplotypes, suggesting that DR3 DQw2 is primarily associated with the putative diabetogenic factor. The low frequency or absence of the 'protective' haplotypes B7 CS31 DR2 DQw1 and B17 CS61 DR7 DQw2 found by us has been reported by others[22–24]; they may thus carry 'protective' genes mainly associated with the DR/DQ region. In summary, DR/DQ genes or closely associated factors, rather than extended HLA haplotypes, would be involved in IDD susceptibility.

Although family studies are needed to confirm the results with NIDD, JRA and BD, out data support previous data on the prevalence of incomplete HLA haplotypes in certain diseases of unknown etiology[7]. Extended HLA haplotypes could share selective advantages because they may carry balanced arrays of genes for an adequate immune response. These positive effects might take place at the level of class II-induced T-cell repertoire selection in the thymus[5], or in later stages of peripheral peptide presentation by class I and/or class II molecules[6]. Such advantages could be lost by relatively rare recombinational events, leading to inappropriate immune responses and ultimately to disease. In addition, some degree of gene complementation may exist between the recognition (HLA antigens and peptide transporters) and effector (C2, Bf, C4, heat-shock proteins, cytokines such as tumor necrosis factor) molecules controlled by the MHC region. This condition was more readily testable in our population because an uncommon Bf allele (F1, instead of the more common S allele of other Caucasians) marks the most common DR3-bearing haplotype[9].

The subdivision of diabetes into NID and ID subsets has sometimes been considered artificial, especially in some non-Caucasian groups[25]. Also, the recognition of NID as an autoimmune disease is controversial, with data for[26] and against[27] the concept. However, weak associations with HLA have been found for NIDD in populations other than strictly North European or

American Caucasians: Spanish Caucasians[20], Xhosa South African Blacks[28], Pima North American Indians[29], and Fiji and Nauru (Micronesian) populations[30,31]. Thus, it seems that in the genetics hyperglycemic syndrome, common susceptibility factors may exist within the HLA system that are only expressed within certain environments and/or HLA haplotype arrangements. This may be due to the existence of a small subset of pre-ID patients within the NID population, or to a certain degree of autoimmune activity in NID[26]. To the best of our knowledge, our present and previous studies are the first to use the common and diabetogenic halplotype A30 Cw5 B18 CF130 DR3 DQw2 (found in Spanish, Basque, Sardinian and paleo-North African populations) and to relate it to non-insulin-dependent individuals[12,20]. However, according to our present data, there could be a genetic distinction at the HLA level between insulin-dependent and non-insulin-dependent diabetes mellitus, even if these two diseases differ only quantitatively in glucose metabolism.

Acknowledgments. This work was supported in part by FIS grants. We thank J. Gomez-Reino and J. Ortuño for their collaboration.

1 Dawkins, R. L., Christiansen, F. T., Kay, P. H., Garlepp, M., McCluskey, J., Hollingsworth, P. N., and Zilko, P. J., Immunol. Rev. *70* (1983) 11.
2 Awdeh, Z. L., Raum, D., Yunis, E. J., and Alper, C. A., Proc. natl. Acad. Sci. USA *80* (1983) 259.
3 Uhr, J. W., Capra, J. D., Vitetta, E. S., and Cook, R. G., Science *206* (1979) 292.
4 Porter, R. R., Mol. biol. Med. *1* (1983) 161.
5 Reich, E. P., Sherwin, R. S., Kanagawa, C. A., and Janeway, C. A., Nature *341* (1989) 326.
6 Parham, P., Nature *348* (1990) 674.
7 Regueiro, J. R., Martin-Villa, M., and Arnaiz-Villena, A., Tissue Antigens *36* (1990) 138.
8 Khalil, I., d'Auriol, L., Gobet, M., Morin, L., Lepage, V., Deschamps, I., Park, M. S., Degos, L., Galibert, F., and Hors, J., J. clin. Invest. *85* (1990) 1315.
9 Arnaiz-Villena, A., Rodriguez-Cordoba, S., Dujovne, I. L., Regueiro, J. R., Bootello, A., and Serrano-Rios, M., New Engl. J. Med. *18* (1980) 1065.
10 Vicario, J. L., Martinez-Laso, J., Corell, A., Regueiro, J. R., and Arnaiz-Villena, A., Clin. Immunol. Immunopathol. *56* (1990) 22.
11 Moore, R. H., Hitman, G. A., Lucas, E. Y., Richards, N. T., Venning, M. C., Papiha, S., Goodship, T. H., Fiedler, A., Awad, J., and Festenstein, H., Kidney Int. *37* (1990) 991.
12 Serrano-Rios, M., Regueiro, J. R., Severino, R., Lopez-Larrea, C., and Arnaiz-Villena, A., Diabetologia *25* (1983) 71.
13 Arnaiz-Villena, A., Gomez-Reino, J. J., Gamir, M. L., Regueiro, J. R., Vicario, J. L., Gomez-Reino, F. J., Alonso, A., Fernandez-Dapica, M. P., Irigoyen, M. V., Mateo, I., and Zea, A., Arthritis Rheum. *27* (1984) 1281.
14 Arnaiz-Villena, A., Gonzalo, A., Regueiro, J. R., Vicario, J. L., and Ortuño, J., Clin. Nephrol. *22* (1984) 320.
15 Arnaiz-Villena, A., Rodriguez-Cordoba, S., Vela, F., Pascual, J. C., Cervero, J., and Bootello, A., Hum. Genet. *58* (1981) 344.
16 Regueiro, J. R., and Arnaiz-Villena, A., Tissue Antigens, *31* (1988) 14.
17 Kelly, H., McCann, V. J., Kay, P. H., and Dawkins, R. L., Immunogenetics *22* (1985) 643.
18 Svejgaard, A., Jersild, C., Staub-Nielsen, L., and Bodmer, W. F., Tissue Antigens *4* (1974) 95.
19 Mauff, G., Alper, C. A., Awdeh, Z., Batchelor, J. R., Bertrams, J., Bruun-Petersen, G., Dawkins, R. L., Demant, P., Edwards, J., Grosse-Wilde, H., Hauptmann, G., O'Neill, G., Rittner, C., Roos, M. H., Skanes, V., Teisgerg, P., and Wells, L., Immunobiol. *164* (1983) 184.
20 Arnaiz-Villena, A., Regueiro, J. R., Martinez-Laso, J., Vicario, J. L., Martin-Villa, J. M., Nieto-Cuartero, J. A., Ordonez, A., and Serrano-Rios, M., Inmunologia *8* (1989) 56.
21 Todd, J. A., Bell, J. I., and McDevitt, H. O., Nature *329* (1987) 599.
22 Raum, D., Awdeh, Z. L., Yunis, E. J., Alper, C. A., and Gabbay, K. H., J. clin. Invest. *74* (1984) 449.
23 Cambon, A., Ohayon, E., Hauptmann, G., Sevin, A., Abbal, M., Sommer, E., Vergnes, H., and Ducos, J., Tissue Antigens *19* (1982) 366.
24 Wolf, E., Spencer, K. M., and Cudworth, A. G., Diabetologia *24* (1983) 224.
25 Keen, H., Diabetic Medicine *3* (1986) 11.
26 Niskanen, L., Karjalainen, J., Sarlund, H., Siitonen, O., and Uusitupa, M., Diabetologia *34* (1991) 402.
27 Chapel, H., and Haeney, M., Endocrinology: pancreatic disease, in: Essentials of Clinical Immunology, p. 371. Blackwell Sci. Pub., 2nd ed., London 1988.
28 Briggs, B. R., Jackson, W. P., Du Toit, E. D., and Botha, M. C., Diabetes *29* (1980) 68.
29 Williams, R. C., Knowler, W. C., Butler, W. J., Pettitt, D. J., Lisse, J. R., Bennett, P. H., Mann, D. L., Johnson, A. H., and Terasaki, P. I., Diabetologia *21* (1981) 460.
30 Serjeantson, S. W., Ryan, D. P., Ram, P., and Zimmet, P. Med. J. Australia *1* (1980) 462.
31 Serjeantson, S. W., Owerbach, D., Zimmet, P., Nerup, J., and Thoma, K., Diabetologia *25* (1983) 13.