HLA-DQA1, -DQB1 and DRB1 alleles in black IBBM patients and controls from Zimbabwe. *Tissue Antigens* **40**, 145–149.
12. Just J.J., King M.C., Thomson G. and Klitz W. (1997). African-American HLA allele and haplotype diversity. *Tissue Antigens* **49**, 547–555.
13. Belich M.P., Madrigal J.A., Hildebrand W.H. *et al.*(1992). Unusual HLA-B alleles in two tribes of Brazilian Indians. *Nature* **357**, 326–329.
14. Watkins D.I., McAdam S.N., Liu X., Strang C.R. *et al.* (1992). New recombinant HLA-B alleles in a tribe of South Amerindians indicate rapid evolution of MHC class I loci. *Nature* **357**, 329–333.
15. Mack S.J. and Erlich H.A. (1998). HLA polymorphism in the Ticuna of Brazil: evolutionary implications of the DRB1*0807 allele. *Tissue Antigens* **51**, 41–50.
16. Grahovac B., Sukernik R.I., O'hUigin C. *et al.* (1998). Polymorphism of the HLA loci in Siberian populations. *Hum. Genet.* **102**, 27–43.
17. Mizuki M., Ohno S., Ando H., Sato T. *et al.* (1997). Major histocompatibility complex alleles in Kazak and Han populations in the Silk Route of north-western China. *Tissue Antigens* **50**, 527–534.
18. Jenkins T. and Tobias P.V. (1977). Nomenclature of population groups in Southern Africa. *Afr. Stud.*, **36**, 50–55.
19. Bidwell J.L. (1992). Applications of the polymerase chain reaction to HLA typing. *Vox Sang.* **63**, 83–89.
20. Andersson G., Andersson L., Larhammer D., Rask L. and Sigurdardottir S. (1994). Simplifying genetic locus assignment of HLA-DRB genes. *Immunol. Today* **15**, 58–61.
21. Long J.C., Williams R.C. and Urbanek M.(1995). An E-M algorithm and testing strategy for multiple-locus haplotypes. *Am. J. Hum. Genet.* **56**, 799–10.
22. Nam J. and Gart J.J. (1987). On two tests of fit for HLA data with no double blanks. *Am. J. Hum. Genet.* **41**, 70–76.
23. Kinoshita T., Hashimoto M., Yamasaki M., Ihara H., Ichikawa Y. and Fukunishi T. (1994). Striking conservation of three extended HLA-DR13 haplotypes in the Japanese population. *Tissue Antigens* **44**, 294–299
24. Salamon H., Klitz W., Easteal S. *et. al.* (1999). Evolution of HLA molecules. Allelic and amino acid site variability across populations. Genetics **152**, 393–400.
25. Begovich A.B., Moonsamy, P.V., Mack, S.J. *et al.* (2001). Genetic variability and linkage disequilibrium within the HLA-DP region: analysis of 15 different populations. *Tissue Antigens* **57**, 429–439.
26. Sanchez-Mazas A. (2001) African diversity from the HLA point of view: influence of genetic drift, geography, linguistics and natural selection. *Human Immunology* **62**, 937–948.
27. Rousseau J., Pokorny L., Glaser J., Creemers P.C. (2003). Predisposing and protective HLA-DR and -DQ alleles for rheumatoid arthritis in South African mixed-ancestry and Xhosa populations. *S. Afr. J. Sci.* **99**, 89–91.
28 Bhettay E., Martell R., Creemers P.C.(1994). Association of HLA-DR10 with juvenile chronic arthritis in South Africans of South African mixed ancestry population. *Tissue Antigens* **43**, 47–49.
29. Wordsworth B.P., Stedeford J., Rosenberg W.M.C., Bell J.I. (1991). Limited heterogeneity of the HLA contribution to susceptibility to rheumatoid arthritis is suggested by positive association with HLA-DR4, -DR1 and Drw10. *Br. J. Rheumatol.* **30**, 178–180.
30. Rousseau J., Mathew, C.G.P., Rees J.S., Du Toit E., Botha, M.C & Harley E.H. (1983). Incidence of Hb Barts and alpha thalassaemia genotypes in a South African population. *Acta Haematologica* **73**, 159–169**.**
31. Lonjou C., Clayton J., Cambon-Thomsen A., Raffous C. (1995). HLA-A, -B, -DR haplotype frequencies in France – implications for recruitment of potential bone marrow donors. *Transplantation* **60**, 375–378.
32. Creemers P., Jacobs P., Bird, A., du Toit E.D. (1993). The South African bone marrow donor registry. *S. Afr. Med. J.* **83**, 819–821.

Predisposing and protective HLA-DR and -DQ alleles for rheumatoid arthritis in South African mixed-ancestry and Xhosa populations

J. Rousseau*†, L. Pokorny*, J. Glaser* and P.C. Creemers*

We have investigated the distribution of the HLA-DRB1, -DQA1 and -DQB1 alleles in rheumatoid arthritis (RA) by comparing the allele frequencies in blood from 65 Cape coloured (mixed-ancestry) RA patients and 114 controls, and from 25 Xhosa RA patients and 94 controls. The strongest positive association with RA was found for the DRB1*0401 allele, followed by the DQA1*0301 and DQB1*0302 alleles, which are strongly linked with DRB1*0401. Data for both populations were statistically significant. In addition, DQB1*0501, which is in linkage disequilibrium with DR1 and DR10, showed a positive association with RA. These findings are in agreement with those for Caucasoids; they indicate that haplotypes that predispose for RA are highly conserved during evolution. Negative associations, that is, a protective effect for RA, were also found, but only for broad specificities; the associations were generally weaker. New findings were negative associations for DRB1*03, DRB1*0701, DQA1*0501 and DQB1*06. The DRB1*0301 and DQA1*0501 alleles are in linkage disequilibrium; a negative association was found in both populations. The negative association of DRB1*0701 was found only in the mixed-ancestry population and was absent in Xhosa. The effect of DQA1*06 was significant in both populations. Thus, the protective HLA-DR and DQ alleles show a greater ethnic diversity.

*Department of Clinical Laboratory Sciences, Faculty of Health Sciences Core Facility, Falmouth Building, University of Cape Town Medical School, Observatory 7925, South Africa.

†Author for correspondence. E-mail: jeanne@chempath.uct.ac.za

Introduction

The human leukocyte antigen (HLA) class II genes play an important role in the genetic predisposition to many autoimmune diseases. In the case of rheumatoid arthritis (RA), predisposing or susceptibility as well as protective genes[1] have been identified. Studies of HLA disease associations are complicated by the great extent of ethnic differences in HLA alleles and linkage disequilibrium between the DR and DQ loci.[2–4] Since HLA alleles may be targets for future therapeutic intervention, defining the HLA disease associations in every population is important. The Cape coloured population is unique in that it is of mixed origin, that is, it has Khoisan, South African Negroid, western European and southeast Asian (Malaysian, Indonesian and Indian) ancestry. South African Xhosa are Bantu-speaking African blacks in whom miscegenation with Khoisan has frequently occurred. Serologically determined frequencies of HLA-A, -B, -DR and -DQ in these populations differ considerably from those described for western Europeans.[5] The majority of previous disease association studies in the Cape coloured and southern African black populations have been performed using serological HLA typing for the HLA-DR and DQ loci; in the present investigation, high-resolution DNA class II typing was performed. DRB1*04 has been implicated as a predisposing factor for RA in many populations.[6–8] Similar results were found for a population of Zimbabwean blacks.[9,10] In Mediterranean populations, DRB*01 and DRB1*10 were predominantly found to be predisposing factor;[11] we found that DRB1*10 was a predisposing factor for juvenile RA in the mixed-ancestry population.[12]

Various theories have been proposed to explain the mode of action of HLA alleles. The 'shared epitope' hypothesis was developed to explain that different HLA-DRB1 alleles have been identified as susceptibility genes for RA: similar motifs within the third hyper variable (HV3) region of some HLA-DRB1 alleles have been found, which may affect the peptide binding characteristics of the HLA molecules. The HV3 region proteins induce stronger proliferative T-cell responses when derived from non-predisposing DRB1 chains,[13] suggesting an abnormal T-cell response. Another study found that the HV3 region of predisposing DRB1 chains constitutes a binding motif for the highly

Table 1. HLA-DRB1, -DQA1 and -DQB1 associations predisposing for rheumatoid arthritis in South African Cape Coloureds and Xhosa.

Locus/allele	Population	Frequency (%)		RR(95% CI)[a]	*P*-value[b]
		RA[c]	Controls		
DRB1*0401	CC[d]	24.6	5.7	2.3 (1.8–2.9)	<0.001
	Xhosa[e]	22.0	3.7	3.5 (2.2–5.5)	<0.001
DQA1*0301	CC	35.4	14.3	1.7 (1.4–2.2)	<0.001
	Xhosa	36.0	9.7	2.9 (1.8–4.4)	<0.001
DQB1*0302	CC	24.6	10.5	1.8 (1.3–2.3)	<0.001
	Xhosa	22.0	4.8	3.1 (1.9–5.0)	<0.001
*0501	CC	30.0	13.6	1.7 (1.3–2.2)	<0.001
	Xhosa	24.0	14.4	1.6 (0.4–1.6)	NS

[a]RR, relative risk; Taylor series 95% confidence limits; [b]*P* value was determined with the Mantel-Haenszel chi-square test with Yates' correction; [c]RA, rheumatoid arthritis; [d]CC, Cape coloureds; 65 patients and 114 controls were tested; [e]For Xhosa, 25 patients and 94 controls were tested.

conserved family of 70-kDa heat-shock proteins.[14] These findings suggest a central role for antigen presenting events in the aetiology of RA. More recently, another model was proposed in which HLA-DQ predisposes while HLA-DR protects.[13–17] In a number of DQ alleles, the p1 pockets that constitute an essential part of the antigen-binding groove are identical. These predisposing DQ alleles would bind the HV3 region of protective DRB1 alleles. This model implies that the HLA-DR-DQ combination, rather than the alleles of the individual DR and DQ loci, exert the predisposing or protective effect. DR and DQ molecules may be present in the *cis* as well as in the *trans* position.[15]

The aim of this study was twofold: to determine by DNA typing the allele specificity of the HLA-DR4 locus implicated in other studies, and whether other HLA class II specificities, or the complete haplotype, incurred a protective effect.

Patients and methods

Patients. Ninety unselected RA patients attending the Rheumatic Diseases Clinic at Groote Schuur Hospital, Cape Town, and satisfying the criteria for RA were included in the study; 25 patients were Xhosa and 65 patients were Cape coloured. Ninety-four healthy Xhosa and 114 healthy Cape coloured local factory workers served as controls. An approximately equal number of men and women were present in each group.

DNA typing. HLA-DNA typing was performed using the PCR-SSOP method. Briefly, DNA extracted from blood specimens was amplified by polymerase chain reaction (PCR) with DRB1, DQA1 and DQB1 group-specific primer pairs. Amplified samples were dot-blotted onto a nylon membrane and hybridized with sequence-specific oligonucleotide probes (SSOPs) obtained from the XIth International HLA workshop. Alleles were assigned based on the reaction pattern as determined by two independent observers.[18]

Statistical analysis. Antigen frequencies were estimated by direct counting, and compared with the frequencies of the normal controls from the corresponding ethnic group. Differences between antigen frequencies were tested for significance using the Mantel-Haenszel chi-square test and the Yates' correction, using StatCalc computer software. The relative risk (RR) and Taylor series 95% confidence intervals were also determined in this manner.

Results and discussion

HLA antigen frequencies in the study populations that were significantly or conspicuously present in a higher frequency in the RA patients than in the control groups are summarized in Table 1. The strongest predisposing association for both Cape coloureds and Xhosa was found for the DRB1*0401 allele. Relative risk was 2.3 and 3.5, respectively. The other alleles of DRB1*04 that were tested (DRB1*04, *0402, *0403, *0404, *0405 and *0408) combined did not show any significant result; RR was 1.2 and 3.0 for mixed-ancestry and Xhosa patients, respectively. Since these alleles were present in only small numbers, an effect of these alleles cannot be ruled out. These results are in agreement with other studies implicating the DRB1*0401 allele as a risk factor for the development of RA.[6–10]

The DQA1*0301 and DQB1*0302 alleles were also present in the RA patients in a significantly higher number (Table 1); this result was also highly statistically significant. For Cape coloureds and Xhosa, RR for DQA1*0301 was 1.7 and 2.9, respectively; for DQB1*0302, 1.8 and 3.1, respectively. These alleles are in strong linkage disequilibrium with the DRB1*0401 allele. Since the positive association with RA for DQA1*0301 and DQB1*0302 was less strong than the DRB1*0401 association, this may explain these results. All three alleles were present in 17/65 Cape Coloured and in 8/25 Xhosa patients. This result is similar to those found in Caucasoids, which implicate the DRB1*0401, DQA1*0301, DQB1*0302 haplotype as a risk factor for the development of RA.[6–8] Results in Zimbabwean blacks were similar;[9,10] these findings indicate that this haplotype predisposing for RA is highly conserved throughout evolution.

Another DQB1 allele which showed a positive association with RA was DQB1*0501; the effect was weak (RR for Cape coloureds, 1.7; for Xhosa, 1.6) and only the results for the Cape coloureds are statistically significant. The DQB1*0501 allele was strongly linked with DRB1*01 and DRB1*10. In a previous study, DRB1*10 was found to be strongly associated with juvenile chronic arthritis (JCA) in the mixed-ancestry population.[12] While every effort was made to exclude from the RA study group individuals who had previously presented with JCA, it is possible that a few may have been included. These findings are similar to those found for Mediterranean Caucasoids;[11] this may also be a highly conserved predisposing haplotype.

Like previous investigators,[15,16] we found a number of HLA alleles that appeared to be protective against the development of RA; the results are summarized in Table 2. Generally, the effect was associated with a broad specificity rather than with a specific allele; the associations were less strong than those for the susceptibility genes. The DRB1*0301 and *0302 alleles, when taken together, showed a significant protective effect for RA in both Cape coloureds (RR = 0.2) and Xhosa (RR = 0.4). The RR values for these alleles were comparable (Cape coloureds, DRB1*0301: RR = 0.2, DRB1*0302: RR = 0.4; Xhosa, DRB1*0301: RR = 0.3, DRB1*0302: RR = 0.4). A protective effect for DRB1*03 has not been described previously and may be specific for our population.

Table 2. HLA-DRB1, -DQA1 and -DQB1 associations protective for rheumatoid arthritis in South African Cape coloureds and Xhosa.

Locus/allele	Population	Frequency (%)		RR(95% CI)[a]	*P* value[b]
		RA[c]	Controls		
DRB1*0301,*0302	CC[d]	2.3	9.7	0.2 (0.1–0.6)	<0.001
	Xhosa[e]	8.0	22.9	0.4 (0.1–0.9)	0.028
*0701	CC	3.9	14.4	0.3 (0.2–0.8)	0.003
DQA1*0501	CC	9.2	27.3	0.4 (0.3–0.7)	<0.001
	Xhosa	10.0	16.7	0.6 (0.3–1.5)	NS
DQB1*06 (*0601–	CC	12.3	24.1	0.4 (0.6–0.9)	0.011
*0605; *0609)	Xhosa	20.0	38.3	0.5 (0.3–0.9)	0.024

[a]RR, relative risk; CI, Taylor series 95% confidence limits. [b]*P* value was determined by the Mantel-Haenszel chi-square test with Yates' correction. [c]RA, rheumatoid arthritis. [d]CC, Cape coloureds; 65 patients and 114 controls were tested. [e]For Xhosa, 25 patients and 94 controls were tested.

DQA1*0501 had a protective effect in both populations; only the results for the Cape coloureds (RR = 0.4) are significant. This allele is strongly associated with DRB1*0301 and *0302, which may explain this effect. Since the effect is stronger for the DRB1 allele, only the latter may be implicated.

The DRB1*0701 allele showed a protective effect with an RR of 0.3, but only in Cape coloureds. Interestingly, this DR allele did not exert any influence on RA in the Xhosa population: RR was 1.0. This protective effect has been described in several studies.[1,16,17] DRB1*07 in blacks is found in association with DQB1*02, whereas in Caucasoids and Cape coloureds it is mainly found in association with DQB1*03 (unpubl. obs.). This may account for the absence of protection for RA in the Xhosa population.

The DQB1*06 broad specificity (DQB1*0601, *0602, *0603, *0604, *0605, *0609) appeared to have a protective effect in both population groups. The RR value for mixed-ancestry was 0.4; for Xhosa 0.5. Except for the DQB1*0601 allele, all alleles showed an RR value of less than 1. This effect appeared to be the strongest for the DQB1*0602 allele; the *P* value for this allele for Cape coloureds was highly significant ($P = 0.006$; RR = 0.3) The DQB1*06 specificity does not show a strong association with any particular DRB1 specificity. A protective effect of DQB1*06 has to our knowledge also not been reported previously.

In summary, there is a remarkable similarity between Caucasoids, blacks and Cape coloureds in the HLA-DRB1, -DQA1 and -DQB1 alleles, which predispose for RA. However, a greater ethnic diversity exists for the factors that are protective for RA.

The Provincial Administration of the Western Cape provided financial support. The Rheumatology Department of Groote Schuur Hospital is thanked for supplying the patients' samples.

Received 1 November 2001. Accepted 21 October 2002.

1. Zanelli E., Gonzalez-Gay M.A. and David C.S. (1995). Could HLA-DRB1 be the protective locus in rheumatoid arthritis? *Immunol. Today* **16,** 274–278.
2. Carcassi C., Passiu G., Lai S. *et al.* (1999). HLA-DRB1*01 and DRB1*04 alleles in Sardinian rheumatoid arthritis patients. *Tissue Antigens* **53**, 97–100.
3. De Juan M.D., Belmonte I., Barado J. *et al.* (1994). Differential associations of HLA-DR antigens with rheumatoid arthiritis (RA) in Basques: high frequency of DR 1 and DR10 and lack of association with DR4 or any of its subtypes. *Tissue Antigens* **43**, 320–323.
4. Huang H.S., Peng J.T., She J.Y. *et al.* (1995). HLA-encoded susceptibility to insulin-dependent diabetes mellitus is determined by DR and DQ genes as well as their linkage disequilibria in a Chinese population. *Hum. Immunol.* **44**, 210–219.
5. du Toit E.D., MacGregor K.J., Taljaard D.G. and Oudshoorn M. (1988). HLA-A, B, C, DR and DQ polymorphism in the South African population groups: South African Negroes, Cape coloureds and South African Caucasoids. *Tissue Antigens* **31,** 109–125.
6. Grennan D.M., Dyer P.A., Clague R., Dodds W., Smeaton I. and Harris R. (1983). Family studies in RA: the importance of HLA-DR4 and of genes for autoimmune thyroid disease. *J. Rheumatol.* **10,** 584–590.
7. Lanchbury J.S.S., Sakkas L.I., Marsh S.G.E., Bodmer J.G., Welsh K.I. and Panayi G.S. (1989). HLA-DQB 3.1 allele is a determinant of susceptibility to DR4-associated rheumatoid arthritis. *Hum. Immunol.* **26**, 59–63.
8. Wordsworth B.P., Stedeford J., Rosenberg W.M.C. and Bell J. (1991). I. Limited heterogeneity of the HLA-class II contribution to susceptibility to rheumatoid arthritis is suggested by positive association with HLA-DR4 -DR1 and Drw10. *Br. J. Rheumatol.* **30**, 178–180.
9. Martell R.W., Stein M., Davis P., West G., Emmanuel J. and du Toit E.D. (1990). The association between HLA and rheumatoid arthritis in Zimbabwean blacks. *Tissue Antigens* **36**, 125–126.
10. Cutbush A., Chikanza I.C., Biro P.A. *et al.* (1993). Sequence-specific oligonucleotide typing in Shona patients with rheumatoid arthritis and healthy controls from Zimbabwe. *Tissue Antigens* **41**, 169–172.
11. de Vries N., Ronningen K.S., Tilanus M.G.J. *et al.* (1993). HLA-DR1 and rheumatoid arthritis in Israeli Jews: sequencing reveals that DRB1*0102 is the predominant HLA-DR1 subtype. *Tissue Antigens* **41**, 26–30.
12. Bhetthay E., Martell R. and Creemers P.C. (1994). Association of HLA-DR10 with juvenile chronic arthritis in South Africans of mixed ancestry. *Tissue Antigens* **43**, 47–49.
13. Zanelli E., Krco C.J. and David C.S. (1997). Critical residues on HLA-DRB1*0402 HV3 peptide for HLA-DQ8-restricted immunogenecity. *J. Immunol.* **158**, 3545–3551.
14. Auger I., Toussirot E. and Roudier J. (1998). HLA-DRB1 motifs and heat shock proteins in rheumatoid arthritis. *Int. Rev. Immunol.* **17**, 263–271.
15. Deighton C.M., Cavanagh G., Rigby A.S. and Lloyd H.L. (1993). Walker D.J. Both inherited HLA-haploytypes are important in the predisposition to rheumatoid arthritis. *Br. J. Rheumatol.* **32**, 893–898.
16. Van der Horst-Bruinsma I.E., Visser H., Hazes J.M. *et. al.* (1999). HLA-DQ associated predisposition to and dominant HLA-DR associated protection against rheumatoid arthritis. *Hum. Immunol.* **60**, 152–158.
17. Ollier W.E.R., Stephens C., Awad J., Carthy D., Gupta A., Perry D., Jawad A. and Festenstein H. (1991). Is rheumatoid arthritis in Indians associated with HLA antigens sharing a DRB1 epitope? *Ann. Rheum. Dis.* **50,** 295–297.
18. Bidwell J.L. (1992). Applications of the polymerase chain reaction to HLA class II typing. *Vox Sang.* **63**, 83–89.

✎ New Books

Biological Sciences

The End of Stress As We Know It. By Bruce McEwen. Pp. 256. Dana Press. £20.

Does Stress Damage the Brain? Understanding trauma-related disorders from a mind-body perspective . By J. Douglas Bremner. Pp. 311. W.W. Norton. $30.

Genes for Africa: Genetically modified crops in the developing world. By Jennifer A. Thomson. Pp. 192. University of Cape Town Press. R120.

Physical Sciences

On the Shoulders of Giants: The great works of physics and astronomy. Edited by Stephen Hawking. Pp. 1266. Running Press. £20. The book features English translations of texts by five great cosmologists, Copernicus, Galileo, Kepler, Newton and Einstein. In spite of this impressive source material and a distinguished editor, one reviewer found the work rather disappointing.

The Mystery of the Moon Illusion. By Helen Ross and Cornelis Plug. Pp. 277. Oxford University Press. £29.95. The illusion of the title is the apparent change in the distance of the Moon as viewed at different elevations above the horizon, the explanation of which remains unknown.