DISEASE MARKERS, VOL. 11, 187–190 (1993)

HLA ASSOCIATIONS IN VITILIGO PATIENTS IN THE DUTCH POPULATION

GERARD T. VENNEKER*, LEO P. DE WAAL†, WIETE WESTERHOF*, JOE D'AMARO§, GEZINA M.TH. SCHREUDER§, SYED S. ASGHAR*

*Department of Dermatology, University of Amsterdam, Academisch Medisch Centrum, Amsterdam

†Department of Transplantation Immunology, Central Laboratory of the Red Cross Blood Transfusion Services, Amsterdam

§Department of Immunohaematology and Blood Bank, Leiden University Hospital, Leiden, The Netherlands

SUMMARY

This study characterizes the HLA class I and class II antigens in a group of patients with vitiligo and a control group, both of Dutch descent. Earlier reports had shown a significant positive association with DR4 and a significant negative association with DR3. We found that, after correction for the broad antigens studied, only Cw7 and DR6 were significantly associated with vitiligo. The significant positive association of DR6 with vitiligo is interesting since vitiligo has an autoimmune component in its pathogenesis and DR6 may be a marker for high immune responsiveness.

KEY WORDS Vitiligo HLA DR6 Immune responsiveness

INTRODUCTION

Vitiligo is a patchy disease with depigmentation of the skin which has a variable age of onset, equal sex distribution and is occasionally familial with an autosomal dominant mode of inheritance with variable penetrance and expression (Finco *et al*, 1991). This disease entity is commonly associated with several autoimmune diseases (McGregor *et al.*, 1972; Cunliffe *et al.*, 1969; Bor *et al.*, 1969; Lerner, 1959). The incidence of vitiligo in patients with autoimmune disease is 8% to 15% compared to 1% in the general population (Koransky, 1980). There are autoantibodies directed against melanocytes (Bystryn and Pfeffer, 1988; Norris *et al*, 1988). Recent immunophenotyping studies suggest that cellular immunity against melanocytes may also be involved in vitiligo (Abdel-Nasser *et al.*, 1991). These observations suggest that there may be an auto–immune component in its etiology and pathogenesis. There is no consensus in previous reports of HLA-A and HLA-B associations with vitiligo in different caucasoid populations (Metzker *et al.*, 1980; Retornaz *et al.*, 1976; Gunther and Richter, 1976). For that reason, we decided to carry out a search for HLA associations in patients with vitiligo in the Dutch caucasoid population. We attempted to confirm the earlier reports of a significant positive association with HLA-DR4 (Foley *et al.*, 1983) and of a sig-

Correspondence to: Syed S. Asghar, Ph. D., Academisch Medisch Centrum, K2-209, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Received 30 June, 1993

nificant negative association with DR3 (Finco *et al*, 1991), and to determine if there were any additional HLA-DR and DQ associations.

PATIENTS AND METHODS

Forty-eight unrelated Dutch caucasoid patients with generalized vitiligo without any associated disease were identified by the Department of Dermatology of the Academic Medical Centre of the University of Amsterdam. The control cases consisted of 703 healthy unrelated Dutch caucasoid blood donors provided by the Central Laboratory of the Blood Transfusion Service in Amsterdam.

HLA-A, B, C typings were performed with the standard NIH lympho-cytotoxity method (van Rood, 1979) and the HLA-DR, DQ typings with the two-colour fluorescence test (van Rood *et al.*, 1976). All typings were performed in the HLA typing laboratory of the Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands.

Haldane's modification of Woolf's method was used to calculate the Relative Risk (RR) and its significance in this study (Woolf, 1955; Haldane, 1955). When indicated, P values were corrected for multiple comparisons using the formula suggested by J. Edwards: $Pc=1-(1-Pu)^n$, where Pu is the uncorrected and Pc the corrected p value and n is the number of comparisons (Edwards, 1974). Antigenic splits were not included in the analyses because the validity of their estimated frequencies would be poor in the modest number of patients studied.

RESULTS

An overview of the borderline of clearly significantly different frequencies of the HLA-A, B, C, DR and DQ broad antigens in vitiligo patients and controls are set out in Table 1. HLA-DR3 and DR4 are included in the table because other investigators have reported significant associations for those antigens (Finco *et al.*, 1991; Foley *et al.*, 1983). In the set of 48 patients HLA-A2, B14, DR4 and DR6 were increased and HLA-B7, Cw7 and DR3 were decreased. Among these 7 antigens, only Cw7 and DR6 were still significant after correction for the 45 broad HLA antigens (8+16+7+10+4) which were examined (p values of 0.04 and 0.02 respectively). No additional significant differences were found in the remaining 38 (45-7) broad HLA antigens.

DISCUSSION

Foley *et al.* reported a significant positive association between HLA-DR4 and vitiligo (RR=2.367, Puncorr=0.0049) and Finco *et al.*, reported a negative association between HLA-DR3 and vitiligo (RR=0.4216). However, the p values in both studies were not corrected for the number of antigens examined.

In our results, HLA-Cw7 and DR6 are the only antigens which retain their significance after correction for the 45 antigens which were compared. That fact is interesting since vitiligo is commonly associated with several autoimmune diseases (McGregor *et al.*, 1972; Cunliffe *et al.*, 1969; Bor *et al.*, 1969; Lerner, 1959), where its incidence in patients with autoimmune disease is 8% to 15% compared to only 1% in the general population (Koransky, 1980), and there is evidence that DR6 may be a marker for high immune responsiveness (Hendriks *et al.*, 1983; Hendriks *et al.*, 1986; Hendriks *et al.*, 1983).

Table 1. HLA antigen frequencies in vitiligo patients and their controls in the Dutch caucasoid population.

	Vitiligo patients*	Controls*		Puncorr*
Antigen	%	%	RR*	
A2	67	51	1.90	0.050
B7	13	27	0.41	0.017
B14	10	3	4.22	0.016
Cw7	22	46	0.35	0.001#
DR3**	6	24	0.24	0.003
DR4**	34	25	1.58	n.s.
DR6	54	29	2.90	0.0004#
Total cases	48	703		

n.s.: not significant

** HLA-DR3 and DR4 are included in Table 1 because other investigators have reported significant associations for those antigens (Finco *et al.*, 1991; Foley *et al.*, 1983).

* The RR and Puncorr values are for the comparison of the Vitiligo patient versus the Controls.

\# The p values are still significant after correction for 45 comparisons, 0.04 and 0.02 for HLA-Cw7 and DR6 respectively.

REFERENCES

Abdel-Nasser, M.B., Kruger, S., Krasagalus, K., Garbe, C., Gollnick, H., Orfanus, C.E. (1991). Evidence for involvement of both cell mediated and humoral immunity in generalized vitiligo. *J. Invest. Dermatol.*, **96**, 1024a.

Bor, S., Feiwell, M., Chanarin, L. (1969). Vitiligo and its aethiological relationship to organ-specific autoimmune diseases. *Br. J. Dermatol.*, **81**, 83–88.

Bystryn, J.C., Pfeffer, S. (1988). Vitiligo and antibodies to melanocytes. *Progress Clin. Biol. Res.*, **256**, 195–206.

Cunliffe, W.J., Hall, R., Newell, D.J., Stevenson, C.J. (1969). Vitiligo, thyroid disease and autoimmunity. *Br. J. Dermatol.*, **180**, 135–139.

Edwards, J. (1974). HLA and disease. The detection of associations. *J. Immunogenetics*, **1**, 249.

Finco, O., Cuccia, M., Martinetti, M., Ruberto, G., Orecchia, G., Rabbiosi, G. (1991). Age of onset in vitiligo: relationship with HLA supratypes. *Clinical Genetics*, **39**, 48–54.

Foley, L.M., Lowe, N.J., Misheloff, E., Tiwari, J.L. (1983). Association of HLA-DR4 with vitiligo. *J. Am. Acad. Dermatol.*, **8**, 39–40.

Gunther, V.W., Richter, K.V. (1975). Häufigkeitsverteilung von Histokompatibilitätsantigenen (HLA) bei dermatologischen Erkrankungen. *Dermatol. Monatsschr.*, **161**, 402–404.

Haldane, J.B.S. (1955). The estimation and significance of the logarithm of a ratio of frequencies. *Ann. Hum. Genet.*, **20**, 309–311.

Hendriks, G.F.J., Claas, F.H.J., Persijn, G.G. *et al.* (1983). HLA-DRw6 positive recipients are high responders in renal transplantation. *Transplant Proc.*, **15**, 1136–1138.

Hendriks, G.F.J., Schreuder, G.M.T., D'Amaro, J. (1986). The regulatory role of HLA-DRw6 in renal transplantation. *Tissue Antigens*, **27**, 121–130.

Hendriks, G.F.J., D'Amaro, J., Persijn, G.G. *et al.* (1983). Excellent outcome after transplantation of renal allografts from HLA-DRw6-positive donors even in HLA-DR mismatches. *Lancet*, **2**, 187–189.

Koransky, J. (1980). Vitiligo. *Dermatology*, **3**, 30–41.

Lerner, A.B. (1959). Vitiligo. *J. Invest. Dermatol.*, **32**, 285–310.

Metzker, A., Zamir, R., Gazit, E., David, M., Feuerman, E.J. (1980). Vitiligo and the HLA system. *Dermatologica*, **160**, 100–105.

McGregor B.C., Karz, H.I., Doe, R.P. (1972). Vitiligo and multiple glandular insufficiencies. *JAMA*, **219**, 724–725.

Norris, D.A., Kissinger, R.M., Naughton, G.M., Bystryn, J.C. (1988). Evidence for immunologic mechanisms in human Vitiligo: Patients' sera induce damage to human melanocytes *in vitro* by complement-mediated damage and antibody-dependent cellular cytotoxicity. *J. Invest. Dermatol.*, **90**, 783-789.

Retornaz, G., Betuel, H., Ortonne, J.P., Thivolet, J. (1976). HLA antigens and vitiligo. *Br. J. Dermatol.*, **95**, 173–175.

Van Rood, J.J. (1979). Microlymphocytoxicity method. In: Ray, J.G. (Ed.), *Manual of Tissue Typing Techniques*, National Institutes of Health, Bethesda, 1979, pp. 104–105.

Van Rood, J.J., Van Leeuwen, A., Ploem, J.S. (1976). Simultaneous detection of two cell populations by two colour fluorescence and application to the recognition of B-cell determinants. *Nature*, **262**, 795–797.

Woolf, B. (1955). On estimating the relation between blood groups and disease. *Ann. Hum. Genet.*, **19**, 251–253.