

Evidence against HLA and immunological dependence of disease outbreak in SLE. Immunological characterisation of identical twins clinically discordant for SLE

ESA SOPPI, JUSSI ESKOLA, AND AAPO LEHTONEN

From the Departments of Medicine and Medical Microbiology, University of Turku, SF-20520 Turku 52, Finland

SUMMARY Identical female twins clinically discordant for 20 years for SLE were studied. Their HLA-haplotype was A1,28; B8w6,w35; Cw3,w7; Dr3,4. Both twins had a raised erythrocyte sedimentation rate, autoantibodies, and circulating immune complexes. The diseased sibling had a reversed OKT4/OKT8 ratio (0.43), decreased helper T cell number, defective pokeweed mitogen (PWM) induced plasma cell differentiation, and overactive hydrocortisone sensitive suppressor cells. Immunological abnormalities may be only partly HLA related (B8; Dr3), but are most probably secondary to the disease process in the sibling with SLE. Exogenous and/or endogenous factor(s) other than genetic or immunological are suggested as being operative in the predisposition to and expression of SLE.

Key words: HLA dependence, immunology.

Although the aetiology of systemic lupus erythematosus (SLE) remains unknown, genetic factors have been implicated in SLE for several reasons. Its concordance in identical twins is high (57-71%)¹ and the disease has a tendency to occur among immediate relatives.² SLE is characterised by a host of immune abnormalities. Patients often have decreased T cell function and deficiencies of normal suppressor mechanisms as well as endogenous polyclonal B cell activation.³⁻⁸ The evidence that immune responses in animals often are under control of histocompatibility antigens has prompted investigators to seek an association between SLE and HLA genetic markers.⁹⁻¹⁰ When association has been suggested, it has been linked to HLA A1,B5,B7,B8,DR2, and Dr3 antigens.⁹⁻¹⁰ Furthermore suppressor cell defect has been suggested to be a genetic marker for SLE susceptibility.⁴ It has been proposed that genetic factors predispose to autoimmunity via immunoregulatory circuits before autoantibody or clinical symptoms appear.³⁻⁸⁻¹¹ In

the present study extensive immunological analysis was carried out in identical twins discordant for SLE.

Patients and methods

PATIENTS AND CONTROLS

The salient clinical and laboratory features of the twins are shown in Table 1. A diagnosis of SLE in the diseased sibling satisfied the criteria adopted by the American Rheumatism Association.¹² Monozygosity of the twins was confirmed by an analysis of HLA antigens and nine blood group antigens (AOB, Rh, Kell, Duffy, Kidd, Lewis, MNS, P, Lutheran). There were 11 female and 4 male control subjects, mean age 36 years, SD 8 years. None of the subjects used any medication.

The controls were included in the study to provide a normal range for the assays, not a formal case control comparison.

IMMUNOLOGICAL METHODS

Autoantibodies, serum immunoglobulins, HLA, and blood group antigens were determined by

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Correspondence to Esa Soppi, MD, Department of Medicine, University of Turku, SF-20520 Turku 52, Finland.

standard techniques. T lymphocyte subsets were identified with monoclonal antibodies (OKT 3, 4 and 8) and B cells by detecting immunoglobulin positive cells with direct immunofluorescence.¹³ For lymphocyte blastogenesis whole blood and isolated lymphocytes were stimulated with three concentrations of phytohaemagglutinin (PHA M) and concanavalin A (con A).¹⁴ All counts are corrected to the reference day of ¹²⁵I-iododeoxyuridine. In addition, the effects of indomethacin (Ind), cimetidine (Cim), and D-penicillamine with copper sulphate (Pen+Cu) on lymphocyte transformation were recorded.¹⁵⁻¹⁷ B cell function was measured by a reverse plaque forming cell (PFC) assay.¹⁸ Spontaneous as well as pokeweed mitogen (PWM) induced PFC capacity and the effects of hydrocortisone and concanavalin A¹⁹ on the number of PFC induced by PWM were determined.

Results

The sibling with SLE (AS) had had symptoms for the last 20 years while her sister (BS) was symptom-

less. The diseased twin differed from the healthy one in respect of the positivity of anti-DNA antibodies and raised serum IgM and IgA concentrations. They were HLA identical (A1,28; B8,w6,w35; Cw3,w7; Dr3,4) (Table 1). Tests for complement components excluded inherited complement deficiencies.

The numbers of leucocytes, lymphocytes, and T Lymphocytes of the twins were within normal range. The reversed OKT4/OKT8 ratio (0.43) in the SLE patient (AS) was due to the increased number of suppressor/cytotoxic cells and decreased number of helper cells. The healthy sibling had a slightly decreased number of helper cells, resulting in an OKT4/OKT8 ratio of 1.16 (Table 2).

PHA induced lymphocyte blastogenesis of the twins did not differ from that of the controls. The same was true of con A responses in whole blood cultures. When isolated lymphocytes were used, con A responses of the twins were lower than those of the controls and the healthy twin had lower responses than the diseased one. The findings with all mitogen concentrations gave similar results. The data with

Table 1 *Clinical data of the twins*

Twin	Sex	Age	Diagnosis and clinical features			HLA haplotypes				
			IgG	IgM	Type	SDU	IgM	IgG	IgA	
AS	F	35	Symptoms at the age of 12 years; butterfly rash, severe arthralgia and arthritis, 1976 pleuritis, and pulmonary infiltrations Diagnosis: SLE			A1,28;	B8,w6,w35;			
BS	F	35	Clinically totally healthy, sedimentation rate 40-100 mm/h since 1971			Cw3,w7;	Dr3,4			
						A1,28;	B8,w6,w35;			
						Cw3,7;	Dr3,4			
Twin	ESR mm/h	Waller-Rose	Anti-nuclear antibodies			Anti-DNA antibodies	Circulating immune complexes*	Immunoglobulins (g/l)		
			IgG	IgM	Type			SDU	IgM	IgG
AS	58	<32	640	80	H(P) [†]	10	10	4.1	11.5	4.3
BS	67	128	160	80	P(S)	Neg	6	2.2	15.9	1.3

* PIPA test measures IgG complexes. Fresh platelets are used as indicators. SDU standard deviation units. Values greater than 3 SDU are regarded as positive (serum dilution 1:10)

[†] Staining pattern: H=homogeneous, P=peripheral, S=speckled.

Table 2 *Numbers of leucocytes and lymphocyte subsets*

Twin	Leucocytes	Lymphocytes	OKT3+ cells	OKT4+ cells	OKT8+ cells	OKT4/OKT8 ratio	B lymphocytes
AS	6.7	2.7	1.8	0.54	1.25	0.43	0.054
BS	6.1	2.3	1.6	0.84	0.72	1.16	0.010
Controls	7.9±3.7	3.4±1.7	2.0±1.0	1.35±0.63	0.75±0.38	1.86±0.26	0.24±0.17

The number of cells is expressed as $\times 10^9/l$. The controls provide a normal range for the number of different cells populations.

Table 3 Lymphocyte capacity for blastogenesis and immunoglobulin secretion

Lymphocyte blastogenesis (cpm)								
	PHA 125 µg/ml				Con A 25 µg/ml			
	Whole blood		Isolated lymphocytes		Whole blood		Isolated lymphocytes	
	Native per 10 ⁷ T-cells		Native per 10 ⁷ T-cells		Native per 10 ⁷ T-cells		Native per 10 ⁷ T-cells	
AS	63075	14079	34144	20693	29376	6557	10307	6244
BS	60694	16125	31538	18022	30718	7777	4128	2359
Controls	67158±	16883±	46939±	30589±	32648±	8336±	30589±	21204±
	13902	9864	9614	6859	14446	4651	6859	6136
Immunoglobulin production								
	Spontaneous PFC ^a			Polyvalent PFC in cultures stimulated by				
	IgG	IgM	IgA	RPMI	PWM	PWM+HC	PWM+con A	
AS	180	300	640	1700	1800	7400	100 (94) ^b	
BS	60	200	640	2200	10800	15600	3400 (70)	
Controls	76±	58±	264±	1721±	17129±	28803±	5671± (70±	
	110	69	465	1498	15660	19188	8630 22)	

^a Mean counts per minute (cpm)±SD are given. The controls provide a normal range for the assays.

^b PFC=plaque forming cells.

^c Suppression percentage is given in parentheses.

the optimal concentrations of PHA and con A are presented in Table 3. The augmentation indices obtained in lymphocyte cultures with Ind, Cim, and Pen+Cu from patients and controls were of the same magnitude (data not shown).

The number of B cells secreting spontaneously IgA and IgM was increased in the twins. In the PWM-driven system the SLE patient (AS) showed a reduced PFC capacity. In the presence of hydrocortisone the PFC capacity of diseased sibling showed a four fold increase, while the increase in the healthy sibling and in controls was below 2. Con A induced suppression of PFC was similar in both the twins and the controls (Table 3).

Discussion

The twin with SLE (AS) had a reversed OKT4/OKT8 ratio. This finding is in accordance with previous data in SLE, in which it has been associated with a reduction of OKT4+ cells.^{20, 21} The number of OKT4+ lymphocytes was reduced in our patient, but in addition an increase of OKT8+ cells was recorded. The healthy sibling (BS) had lowered OKT4/OKT8 ratio due to a decreased number of OKT4+ cells. Serologically inactive patients have previously shown no abnormalities in this respect.²¹

Indomethacin and cimetidine sensitive suppressor cells and copper sulphate plus D-penicillamine sensitive helper cells were normal in the twins. In

the PFC assay the activity of con A sensitive suppressor cells was observed to be normal or slightly increased. This is in accordance with a previous report,²² although suppressor cells have also been shown to be defective in SLE^{3, 23} and the defect has been considered a genetic marker for SLE.³ The abnormality in con A responses cannot be explained via suppressor cells.

Endogenous polyclonal B cell activation together with defective B cell function and regulation has been linked to SLE.⁵⁻⁷ We found slightly enhanced spontaneous and decreased PWM induced PFC capacity in both twins; the abnormalities were more pronounced in the diseased sibling (AS) in concordance with previous studies.^{5, 6} The former finding may reflect endogenous B cell activation and the latter defective helper T cell activity.^{5, 24} The latter is also supported by the decreased number of OKT4+ cells in both twins (Table 2). Furthermore overactive hydrocortisone sensitive suppressor cells were demonstrated in the diseased sibling (AS) (Table 3).

Our twins have close similarities with the HLA-identical male twins discordant for lupus described very recently by Schroeder *et al.*²⁵ These include circulating autoantibodies, B cell function with and without PWM stimulation, and some enhanced suppressor cell activity. However, Schroeder and colleagues did not determine T cell subpopulations and reported exuberant T cell function, which was not recorded in the present investigation.

Previously, decreased T lymphocyte responses have been associated with HLA-B8 and B8 and Dr3 antigens,^{26 27} as a reflection of the action of genes predisposing to autoimmunity. In this study HLA-B8 and Dr3 antigens could be associated only with a decrease of helper T cell number and decreased con A responses.

The genetic factors may be associated with different immunological and clinical features of SLE.⁹⁻¹¹ The finding of more concordance of SLE in monozygotic than dizygotic twins provides evidence for genetic factors, whereas a documented discordance in monozygotic twins supports the importance of environmental factors.^{1 28} In twins concordant for SLE the outbreak of the disease has occurred within five years (in one pair after an interval of 16 years). The follow-up period of twins discordant for disease has been only up to five years.¹ Although our twins were monozygotic, the healthy one (BS) had been symptomless over 20 years after the outbreak of the disease in the other. Steinberg *et al.*²⁹ reported HLA-A,B,Dr genotyping in three families with SLE, and the affected persons did not share HLA haplotypes. Furthermore, it has been proposed that genetic factors predispose to autoimmunity via immunoregulatory circuits before autoantibodies or clinical symptoms appear.^{3 8 11} Both of the twins studied have autoantibodies; but the OKT4/OKT8 ratio as well defective PWM induced plasma cell differentiation were abnormal only in the SLE patient. The observations are not in accordance with the successive events resulting in autoimmunity: genetic factors – defect in immunoregulatory circuits – formation of autoantibodies – clinical symptoms.³ Our findings show that SLE susceptibility is not dependent on chromosome 6 (HLA region), as shown also in previous studies.^{11 25 28 29}

In the present study we describe female monozygotic twins discordant for over 20 years for SLE. Thus genetic factors (e.g., HLA B8, Dr3) are unlikely to be crucial in predisposing to the disease. In addition the extensive immunological analysis does not either support the notion of an inherited deficiency in immune functions²⁸; the aberrations observed are most probably secondary to the disease process. We suggest that environmental and/or endogenous factor(s) as well as genetic or immunological operate in predisposition to and expression of the illness.

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