SPECIFIC POLYMORPHISMS OF CYTOKINE GENES ARE ASSOCIATED WITH DIFFERENT RISKS TO DEVELOP SINGLE-SYSTEM OR MULTI-SYSTEM CHILDHOOD LANGERHANS CELL HISTIOCYTOSIS (LCH)

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ABSTRACT (word count 103)

Cytokines and chemokines determine mobilization of Langerhans cells and their dysregulation is implicated in the pathogenesis of LCH. Twenty point mutations of 12 different cytokine genes were studied in 41 Italian children, 15 with single-system and 26 with multi-system disease.

The allele and genotype distributions of IL-4 and IFNγ were significantly different in patients vs 140 controls (p=0.007, and p=0.018). Older children with single-system disease shared the "anti-inflammatory profile" determined by the intermediate producer genotype IFNγ +874A/T (p=0.029) and the high-producer genotypes IL-4 –590C/T and T/T (p=0.029).

Our findings suggest that specific cytokine gene variants affect susceptibility to LCH and its clinical heterogeneity.

INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare disorder (3.5-7 cases/million/year) affecting any age (*Aricò et al, 2003*) with dysregulated growth, activity and trafficking of Langerhans cells (LC). The clinical manifestations and course are highly variable, ranging from a solitary bone lesion to a disseminated disease with multiple-organ involvement and high mortality rate, despite aggressive treatment (*Aricò & Egeler 1998*).

Familial clustering together with the propensity to develop tumor and the association with specific immune response genes, support the hypothesis that LCH is genetically determined (*Aricò & Danesino 2001; Beverley et al, 2005*).

LC reside in the epidermis serving as sentinels of the immune system and are characterized by the CD1a-antigen (*Cresswell et al, 2005*). The ability of LC to migrate from the epidermis to regional lymph nodes is of pivotal importance for the induction of cutaneous immune response. There is increasing evidence that both cytokines and chemokines determine the mobilization of LC and that dysregulation in this pathway is implicated in the pathogenesis of LCH (*Annels et al, 2003*).

Here we addressed the issue whether functional variants of 12 different cytokine genes (20 point mutations) are implicated in the predisposition to LCH and whether the clinical heterogeneity parallels a genetic heterogeneity.

PATIENTS AND METHODS

Forty-one consecutive Italian patients, all with biopsy-proven LCH, diagnosed according to current criteria established by the Histiocyte Society (*Broadbent et al, 1989*) were studied. The pattern of disease manifestations was classified into single-system (SS) and multi-system (MS).

The patients, 25 males and 16 females, were diagnosed at a median age of 6.1 years (range, 2 months to 17 years); 15 had SS disease (median age, 7.5 years) and 26 had MS disease (median age, 5.3 years); 16 were diagnosed with LCH when younger than 2 years of age, while 25 later.

One hundred and forty healthy Italian subjects were tested as controls.

Typing was performed at the genomic level on DNA extracted by the peripheral blood samples using a salting out method .

Special kits were used, set up at the Heidelberg University (Brown Deer, WI, USA). The technique employed was the PCR-SSP as described elsewhere (*Uboldi de Capei et al, 2003*). The following biallelic single nucleotide polymorphisms (SNPs) were defined: Interleukin (IL)-1α -889C/T, IL-1β (-511C/T; +3962T/C), IL-1R pst1 1970C/T, IL-1RA mspa111100T/C, IL-4Ra +1902G/A, IL-12 -1188C/A, +874A/T, TNF-α (-308G/A; -238G/A), IL-2 (-330T/G; +160G/T), IL-4 (-1098T/G; -590T/C; -33 T/C), IL-6 (-174G/C; nt565G/A), IL-10 (-1082G/A; -819C/T;-592C/A).

Univariate statistical analysis was performed using EPI5 statistical package. Chi square analysis and Fisher's exact tests were used to compare frequencies of cytokine alleles, haplotypes and genotypes in patients versus the controls. A logistic regression model was further applied.

RESULTS

Table 1 summarizes all the significant associations found at the allelic level. The only allele with a significantly different frequency in the entire group of patients was IL-4 -590T.

Two additional alleles had a different distribution only in specific subgroups: IL-1β +3962T (exon 5), more frequent among patients > 2 years of age and the allele IL-1R +970T (5'UTR untranslated region), among patients with MS disease.

Table 2 summarizes all the significant associations found at the genotypic level. Functionally, the patients may be considered high producers of IL-4 (as per IL-4 –590C/T and T/T genotypes) and intermediate producers of interpheron γ (as per IFNγ +874A/T genotype). The first profile was more evident in the SS and > 2 years subgroups, while the latter in the SS and ≤ 2 years.

Among the cytokine genotypes, logistic regression defined a hierarchy of risk to the disease: IL-4 –33 T/T resulted the most implicated (OR=5.63, p=0.035, 95% CI 1.13-28.2), followed by the IFNγ +874A/T (OR=3.00, p=0.006, 95% CI 1.38-6.58).

DISCUSSION

The aim of this study was to explore the hypothesis that specific polymorphisms of genes involved in the production of cytokines may be significantly associated with LCH or with its clinical variants.

Our findings support the concept that peculiar cytokine genes polymorphisms contribute to an intrinsic genetic propensity to develop LCH and its manifestations. Although the number of patients investigated remains limited (LCH is a very rare disease) our main result is that patients with MS or SS have significantly different genetic characteristics. This finding may be considered by the clinicians in keeping with the observation that SS and MS disease are really different on the clinical ground. In fact, while SS is a benign disease, with no attaint of survival, MS LCH patients have 20% risk of mortality (Aricò, 1998, Beverley 2005, *Jubran et al, 2005*).

Thus, the concept that different patterns of disease expression may reside primarily on the patient's intrinsic characteristics lends further support to genetic investigations. Two recent studies have investigated the HLA system in patients with LCH. McClain and coworkers showed that

patients presenting with single bone disease had an especially high frequency of the HLA-DR4 allele, and in this group every Caucasian patient had either HLA-Cw7 or DR4 allele *(McClain et al, 2003)*. In another independent study of Nordic patients, those with single-system disease more often had the allele HLA-DRB1*03 (*Bernstrand et al, 2003*). Altogether these data suggest an immunogenetic heterogeneity.

In synthesis, two are the major findings in this work: a) the predominance of IL-4 high producer genotypes (IL-4 -590T/C and IL-4-33T/T) and IFNγ +874A/T intermediate secretor genotype and b) the prominent association between these anti-inflammatory profiles and LCH with lower clinical aggressiveness and later onset.

IL-4 downregulates IFNγ production from Th1 lymphocytes and, together with IL-10, exerts a protective role in stress conditions. In patients with rheumatoid arthritis for instance, the phenotype high producer of IL-4 seems to protect from severe joint destruction; among patients undergoing heart transplant, those who received the graft from a donor high-producer of IL-4 have a lower incidence of episodes of rejection (*Bjilsma et al, 2002*). Our results of a positive association of the genotypes resulting in high IL-4 gene transcription (namely IL-4 -590T/C and IL-4 -33T/T) with the mild form of LCH are in keeping with these observations. A possible explanation is that the IL-4 high producer genotype could favor a Th2 mediated antibody response to pathogenic stimulation able to circumscribe the disease thus avoiding a rapid spreading.

In conclusion, this study substantiates the hypothesis that cytokine genetic variants, identified by individual SNPs or specific genotypes of IL-4 and IFNγ regulatory pathways, may confer susceptibility to – or protection from – multi-system LCH, the most severe form of a disease which may be absolutely benign while in this clinical subset is associated with a 20% fatality rate.

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REFERENCES

– Annels NE, Da Costa CE, Prins FA, Willemze A, Hogendoorn PC, Egeler RM. (2003) Aberrant chemokine receptor expression and chemokine production by Langerhans cells underlies the pathogenesis of Langerhans cell histiocytosis. *Journal of Experimental Medicine,*197(10):1385-90

– Aricò M, Egeler RM (1998) Clinical aspects of Langerhans cell histiocytosis. *Haematology/Oncology Clinics of North America,*12: 247-258

– Aricò M, Danesino C (2001) Langerhans' cell histiocytosis: is there a role for genetics? *Haematologica,*86:1009-14

– Aricò M, Girschikofsky M, Genereau T, Klersy C, McClain K, Grois N, Emile JF, Lukina E, De Juli E, Danesino C (2003) Langerhans cell histiocytosis in adults. Report from the international Registry of the Histiocyte Society. *European Journal of Cancer,*39: 2341-8

– Bernstrand C, Carstensen H, Jakobsen B, Svejgaard A, Henter JI, Olerup O (2003) Immunogenetic heterogeneity in single-system and multi-system Langerhans cell histiocytosis. *Pediatric Research,*54(1):30-36

– Bijlsma FJ, vanKuik J, Tilanus MG, deJonge N, Rozemuller EH, van den Tweel JG, Gmelig-Meyling FH, deWeger RA. (2002) Donor interleukin-4 promoter gene polymorphism influences allograft rejection after heart transplantation. *Journal of Heart and Lung Transplantation,*21(3):340-6.

– Beverley PC, Egeler RM, Arceci RJ, Pritchard J. (2005) The Nikolas Symposia and histiocytosis. *Nat Rev Cancer*. 5:488-94.

– Broadbent V, Gadner H, Komp DM, & Ladisch S, Clinical Writting Group of The Histiocyte Society (1989) Histiocytosis syndromes in children: II. Approach to the clinical and

laboratory evaluation of children with Langerhans cell histiocytosis. *Medical and Pediatric Oncology*,17:492-95

– Cresswell P, Ackerman AL, Giodini A, Peaper DR, Wearsch PA. (2005) Mechanisms of MHC class I-restricted antigen processing and cross-presentation. *Immunol Rev.*, 207:145-57.

– Jubran RF, Marachelian A, Dorey F, Malogolowkin M. (2005) Predictors of outcome in children with Langerhans cell histiocytosis. *Pediatrics Blood and Cancer,*45(1):37-42

– McClain KL, Laud P, Wu WS, Pollack MS (2003) Langerhans cell histiocytosis patients have HLA Cw7 and DR4 types associated with specific clinical presentations and no increased frequency in polymorphisms of the tumor necrosis factor alpha promoter. *Medical and Pediatric Oncology,*41:502-507

– Uboldi de Capei MU, Dametto E, Fasano ME, Rendine S, Curtoni ES. (2003) Genotyping for cytokine polymorphisms: allele frequencies in the Italian population. *European Journal of Immunogenetic,*30(1):5-10

Table 1: Cytokine alleles with statistically significant deviations from the controls

Cytokine	Allele	Controls (N=280)		LCH (N=82)		SS (N=32)		MS (N=50)		Age ≤ 2 years (N=32)		Age> 2 years (N=50)	
		N	%	N	%	N	%	N	%	N	%	N	%
IL-4 -590	C	248	88.6	62	75.6	24	75.0	38	76.0	25	78.1	37	74.0
	T	32	11.4	20	24.4	8	25.0	12	24.0	7	21.9	13	26.0
					p= 0.003		**p= 0.029**		**p= 0.016**		p=ns		**p= 0.056**
IL-4 -33	C	242	86.4	66	80.5	23	71.9	43	86.0	27	84.4	39	78.0
	T	38	13.6	16	19.5	9	28.1	7	14.0	5	15.6	11	22.0
					p=ns		**p= 0.029**		p=ns		p=ns		p=ns
IL-1β +3962	C	208	74.3	56	68.3	19	59.4	37	74.0	26	81.3	30	60.0
	T	72	25.7	26	31.7	13	40.6	13	26.0	6	18.7	20	40.0
					p=ns		p=ns		p=ns		p=ns		**p= 0.037**
IL-1R +970	C	187	66.8	47	57.3	22	68.8	25	50.0	20	62.5	27	54.0
	T	93	33.2	35	42.7	10	31.2	25	50.0	12	37.5	23	46.0
					p=ns		p=ns		**p= 0.022**		p=ns		p=ns

Table 2: Cytokine genotypes with statistically significant deviations from the controls

Cytokine	Genotype	Controls (140)		LCH (41)		SS (16)		MS (25)		Age ≤ 2 years (16)		Age > 2 years (25)	
		N	%	N	%	N	%	N	%	N	%	N	%
	CC	110	78.6	22	53.7	8	50.0	14	56.0	9	56.3	13	52.0
IL-4 -590	CT	28	20.0	18	43.9	8	50.0	10	40.0	7	43.7	11	44.0
	TT	2	1.4	1	2.4	0	0	1	4.0	0	0	1	4.0
					p= 0.007		**p= 0.025**		p=ns		p=ns		**p= 0.019**
	AA	42	30.0	6	15.0	0	0	6	25.0	2	12.5	4	17.0
IFNγ +874	AT	66	47.1	29	72.5	12	75.0	17	70.8	13	81.3	16	66.0
	TT	32	2.9	5	12.5	4	25.0	1	4.2	1	6.2	4	17.0
					p= 0.018		**p= 0.029**		**p= 0.049**		**p= 0.035**		p=ns
	CC	76	54.3	21	51.2	6	37.5	15	60.0	11	68.7	10	40.0
IL-1β +3962	CT	56	40.0	14	34.2	7	43.8	7	28.0	4	25.1	10	40.0
	TT	8	5.7	6	14.6	3	18.7	3	12.0	1	6.2	5	20.0
					p=ns		p=ns		p=ns		p=ns		**p= 0.042**
	CC	60	42.9	12	29.2	8	50.0	4	16.0	5	31.3	7	28.0
IL-IR +970	CT	67	47.8	23	56.2	6	37.5	17	68.0	10	62.5	13	52.0
	TT	13	9.3	6	14.6	2	12.5	4	16.0	1	6.2	5	20.0
					p=ns		p=ns		**p= 0.038**		p=ns		p=ns