Interleukin-4RA gene polymorphism is associated with oral mucous membrane pemphigoid.

UNIVERSITÀ DEGLI STUDI DI TORINO

Oral Diseases - Volume 20, Issue 3, pages 275–280, 2014 - DOI: 10.1111/odi.12106

Oral Diseases - Volume 20, Issue 3, pages 275–280, April 2014

Interleukin-4RA gene polymorphism is associated with oral mucous membrane pemphigoid

1. **M Carrozzo[1,*],**
2. **E Dametto[2],**
3. **ME Fasano[2],**
4. **R Broccoletti[3],**
5. **M Carbone[3],**
6. **S Rendine[2] and**
7. **A Amoroso[2]**

1. Department of Oral Medicine, Center for Oral Health Research, School of Dental Sciences, Newcastle University, Newcastle upon Tyne, UK
2. Transplantation Immunology Service, Departments of Genetics, Biology and Biochemistry, S. Giovanni Hospital of Turin, Turin, Italy
3. Department of Biomedical Sciences and Human Oncology, Oral Medicine Section, Lingotto Dental School, University of Turin, Turin, Italy

[*]Correspondence: Marco Carrozzo, MD, DSM (Turin 1995), Professor of Oral Medicine, School of Dental Sciences, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4BW, UK. Tel: +44 191 222 7818, Fax: +44 191 222 6137, E-mail:marco.carrozzo@ncl.ac.uk

Abstract

Objective

The aim of this study was to analyse whether the polymorphisms of several pro- and anti-inflammatory cytokines may influence the susceptibility to predominantly oral Mucous membrane pemphigoid (MMP) in a Northern Italian population.

Material and Methods

DNA was obtained from 41 MMP patients (29 with exclusively oral pemphigoid [OP]) and 140 unrelated bone marrow donors. Thirteen cytokine genes with 22 single-nucleotide polymorphisms (SNP) were studied by a sequence-specific PCR assay.

Results

There was no significant difference between the patients taken together and healthy controls for any cytokine gene polymorphism studied. However, the allele A of the IL-4 receptor A (IL-4RA) was

significantly more frequent in OP than controls ($P < 0.05$), causing an increased frequency of genotype A/A in OP patients (89.7 *vs* 67.9, odds ratio: 4.11, 95% confidence intervals 1.18–14.28, $P = 0.023$, Pc = 0.046).

Conclusion

IL-4RA-1902 A/A genotype has been associated with a reduced response to IL-4 and has been found in 90% OP patient. Giving the supposed importance of IL-4 in MMP fibrotic process, our results can partially explain the low likelihood of scarring in OP patients.

Introduction

Mucous membrane pemphigoid (MMP) is a rare subepithelial blistering disorder of late middle age, with a slight predilection for women (Scully *et al*, 1999; Chan *et al*, 2002). Most of the patients (up to 90%) have oral involvement, and the gingival is the most common intraoral site affected. Commonly the patients have exclusive oral lesions. Other mucosal tissues such as ocular, oesophageal, laryngeal and anogenital areas can be involved (Scully *et al*, 1999; Chan *et al*, 2002). Scarring is the clinical hallmark and this is why MMP is also commonly cited as cicatricial pemphigoid (Scully *et al*, 1999). This aggressive fibrotic phenomenon can have very serious consequences particularly causing sudden asphyxiation if the larynx is affected (Fleming and Korman, 2000) or requiring repeated dilatation when the oesophagus is involved (Warren *et al*, 1997). The most common and severe fibrotic sequels are however observed in the eyes where MMP can potentially leads to blindness (Scully *et al*, 1999; Chan *et al*, 2002).

Even if there is not convincing evidence to support a correlation between distinct clinical subtypes and target-specific autoantibodies (Carrozzo *et al*, 2004a,b; Calabresi *et al*, 2007), 2 very different subtypes of MMP, namely ocular pemphigoid (OCP) and oral pemphigoid (OP), are usually recognized (Chan, 2012). When lesions involve exclusively or predominantly the conjunctivas, it is referred as OCP and frequently tends to follow a progressive disease process. Recurrent conjunctivitis usually results in subepithelial fibrosis leading to fornix shortening, symblepharon and ankyloblepharon formation and then, subsequently, trichiasis and entropion. Later in the disease stage, limbal stem cell deficiency, tear deficiency and lid malposition can happen, causing a total keratinization of the whole ocular surface (Kirzhner and Jakobiec, 2011).

Conversely, OP is a MMP subtype where the disease process is limited to the oral cavity, and scarring in most of the cases does not occur, and patients tend to have a mild to moderate disease process (Chan, 2012).

Cytokines may have an important role in the scarring process in MMP. Increased expression of interleukin (IL)-4 and IL-4 mRNA has been seen in MMP, particularly in cicatricial pemphigoid (Caproni *et al*, 2002, 2003; Razzaque *et al*, 2003; Giomi *et al*, 2005). Increased expression of IL-4 may play an important role in the regulation of local accumulation of macrophages by inducing colony-stimulating factor and matrix accumulation by inducing heat shock protein-47 and collagen during MMP scarring (Razzaque *et al*, 2003).

Genetic factors likely play an important role in susceptibility to MMP, and its occurrence is significantly associated with mainly the HLA (human leucocyte antigen) class II allele HLA-DQB1* 03:01 (formerly known as DQB1*0301) (Marsh *et al*, 2010) apparently regardless of the clinical phenotype and detectable circulating antibasement membrane zone (BMZ) antibodies

(Ahmed *et al*, 1991; Delgado *et al*, 1996; Chan *et al*, 1997; Drouet *et al*, 1998; Carrozzo *et al*, 2001; Setterfield *et al*, 2001). A model has been proposed in which relevant portions of the four different peptides derived from BMZ involved in autoimmune response in MMP have potential sites that could be presented by an antigen presenting cell in conjunction with DQB1*03:01 to a T cell receptor to initiate the process that results in anti-BMZ antibody production (Zakka *et al*, 2011).

The production or function of cytokines is, at least partially, regulated by polymorphisms in their gene sequences (Bidwell *et al*, 1999). Numerous cytokine polymorphic variants have been associated with the development of a broad spectrum of immunologically mediated diseases, including oral and blistering diseases such as gingivitis, oral lichen planus, recurrent aphthous stomatitis and pemphigus vulgaris (PV) (Bazrafshani *et al*, 2002; Carrozzo *et al*, 2004a,b; Eberhard *et al*, 2005; Dashash *et al*, 2006; Javor *et al*, 2010) but no data are apparently available for MMP.

The aim of the present study was to analyse whether the polymorphisms of several pro- and anti-inflammatory cytokines may influence the susceptibility to MMP predominantly affecting the oral cavity in a Northern Italian population.

Material and methods

A total of 41 unrelated Caucasian patients (31 females, mean age 64.44 ± 13.80 yrs.,range: 21–90) diagnosed as having MMP were consecutively enrolled at the Oral Medicine section of the Department of Biomedical Sciences and Human Oncology of the University of Turin between 1989 and 2000. All the patients presented erosive and/or bullous oral lesions, and the blood samples for the immunogenetic investigation were always taken before the start of any treatment and in patients with active disease. The diagnosis was confirmed in all cases by histology which revealed sub-epithelial splitting and direct immunofluorescence analysis which showed linear deposits of IgG, IgA and/or C3 at the BMZ (Table 1). All patients had a complete ophthalmological and dermatological examination. A flexible nasopharyngoscope was used to visualize the nasal mucosa, epiglottis, pharynx, larynx and proximal oesophagus.

Table 1. Clinical and immunological characteristics and DQB1 alleles in patients with mucous membrane pemphigoid (MMP) and oral pemphigoid (OP)

Patients				**Direct immunofluorescence**	**DQB1* Alleles**
N	Diagnosis	Age	Sex		
1	OP	70	F	IgG	02; 03:01
2	OP	75	F	IgG,C3	03:01; 05:01
3	MMP	74	M	IgG,C3	03:01
4	OP	80	M	IgG,C3	03:01
5	MMP	81	F	IgG,IgA,C3	03:01
6	OP	60	F	IgG,IgA,C3	03:01

Table 1. Clinical and immunological characteristics and DQB1 alleles in patients with mucous membrane pemphigoid (MMP) and oral pemphigoid (OP)

Patients				Direct immunofluorescence	DQB1* Alleles
7	OP	69	F	IgG,IgA,C3	05:01;03:01
8	MMP	67	F	IgG,C3	03:01
9	OP	71	F	IgG,IgA,C3	05:01; 03:01
10	MMP	78	M	IgG,IgA,C3	03:01
11	MMP	78	F	IgG,IgA,C3	05:01; 03:01
12	OP	21	F	IgG,C3	03:01; 03:04
13	OP	69	F	IgG,IgA,C3	02; 03:01
14	MMP	55	M	IgG,IgA,C3	03:01; 05:02
15	OP	44	F	IgG,IgA,C3	06:02; 05:01
16	OP	80	M	IgG,IgA	03:01
17	MMP	62	M	IgG,IgA,C3	06:02; 03:01
18	OP	71	F	IgG,IgA,C3	02; 03:01
19	OP	29	F	IgG,C3	06:02; 03:01
20	OP	67	F	IgG	03:01
21	OP	73	F	IgG,IgA,C3	03:01
22	OP	56	F	IgG	03:01
23	OP	61	F	IgG	03:01
24	MMP	59	F	IgG,C3	03:01
25	OP	67	F	IgG,C3	03:01; 05
26	OP	73	F	IgG,IgA,C3	03:01
27	OP	41	F	IgG,C3	03:01
28	OP	77	F	IgG,C3	06

Table 1. Clinical and immunological characteristics and DQB1 alleles in patients with mucous membrane pemphigoid (MMP) and oral pemphigoid (OP)

Patients				Direct immunofluorescence	DQB1* Alleles
29	OP	58	F	IgG,C3	03:01
30	OP	60	F	IgG,C3	03:01; 04
31	OP	36	F	IgA	03:01
32	MMP	73	M	IgA	03:01
33	OP	60	F	IgG,C3	02; 03:01
34	OP	65	F	IgG,C3	03:01; 05
35	MMP	78	F	IgG,IgA,C3	03:01; 04
36	OP	65	F	IgG	03:01; 05
37	OP	72	M	IgG,IgA,C3	02
38	OP	54	F	IgG,IgA,C3	03:01
39	MMP	74	F	IgG,C3	03:01; 06
40	MMP	72	M	IgG,C3	03:01
41	OP	67	M	IgG,C3	03:01

All the patients were followed up for almost 2 years (median 41 months) and clinically reassessed at each follow-up visit. They were considered affected by OP if they had exclusively oral lesions during the entire follow-up period. As healthy controls, 140 unrelated bone marrow donors (81 females, mean age 54.89 ± 10.92 yrs., range 23–85) without evidence or history of MMP were studied. Patients and controls were all Italian and their geographical origin had been carefully checked to compare subjects from the same area. Patient's informed consent and approval from ethical committee of the University of Turin were obtained.

HLA–DQB1 typing

DNA of our samples was previously extracted from peripheral blood samples drawn in EDTA (ethylenediaminetetraacetic acid) anticoagulant tubes using a micro-salting-out procedure (Miller *et al*, 1988) or an automatic apparatus (Genomic DNA Extraction, Talent s.r.l.).For intermediate-resolution DQB1* typing, locus-specific amplification was performed using primer pairs designed to amplify the entire exon 2 of the locus as previously published (Carrozzo *et al*, 2001).

Cytokine genotyping

Cytokine typing was performed in patients and controls by a sequence-specific polymerase chain reaction (PCR-SSP) assay as previously published (Carrozzo *et al*, 2004a,b). The allele and genotype frequencies of the following cytokine genes have been determined: IL-1α (–889 T/C), IL1β (–511 C/T, +3962 T/C), IL12 (–1188 C/A), IFNγ (UTR 5644 A/T), TGFβ (codon 10 C/T, codon 25 G/C), TNFα (–308 G/A, –238 G/A), IL2 (–330 T/G, +166 G/T), IL4 (–1098 T/G, –590 T/C, –33 T/C), IL6 (–174 G/C, nt565 G/A), IL10 (–1082 G/A, –819 C/T, –592 C/A), IL1R (C/T pst1 1970), IL1RA (T/C mspa1 11100), IL4RA (–1902 G/A).

Statistical analysis

Because of the rarity of the disease and the lack of previous data on cytokine polymorphisms in MMP, no preliminary power calculation was performed. Allele frequencies were estimated by direct gene counting. To test the Hardy–Weinberg equilibrium, observed and expected frequencies of the various genotypes were compared using the chi-square test or Fisher's exact test, where appropriate. Odds ratios, confidence intervals and significance values were calculated using the Statistical Package for Social Sciences (SPSS) software program (version 12, Chicago, IL, USA). A P value <0.05 was considered statistically significant. Significant probability values obtained were corrected for multiple testing where appropriate (Bonferroni correction; Pc) (Chang *et al*, 2006, 2007).

Results

Exclusive oral lesions were seen in 29 patients (OP) whereas 12 patients had also extra oral lesions (MMP). All but four patients (all with exclusive oral lesions) (92.7%) were HLA-DQB1*03:01 (Table 1). Scarring lesions were seen in five patients with MMP (41.7%) (3 ocular and 2 nasals).

All allele and genotype frequencies were in Hardy–Weinberg equilibrium but TGFβ codon 25 in controls (P = 0.00025, chi-square test) and IL2-330 in OP (P = 0.034, chi-square test). There was no significant difference between the patients taken together and healthy controls for any cytokine gene polymorphism studied (Tables 2 and 3). However, when patients with OP were compared with the controls, some differences were observed. Indeed, the allele A of the IL-4 receptor A (IL-4RA) was significantly more frequent in OP than control (P < 0.05), causing an increased frequency of genotype A/A in OP patients [89.7 *vs* 67.9 odds ratio (OR) 4.18, 95% confidence intervals (CI) 1.18–14.28, P = 0.023, Pc = 0.046] (Table 4). Notably, the genotype A/A was only present in 60% of the patients with a scarring phenotype but because of the small number of patients a direct comparison with the OP was not made.

Table 2. IL-1 cluster, IL-12, IFN-c, TGF-b and TNF-a polymorphisms in mucous membrane pemphigoid patients and controls

Genotype	Patients *n* = 41 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals

1. All the comparisons resulted are not significant.

IL1α-889

Table 2. IL-1 cluster, IL-12, IFN-c, TGF-b and TNF-a polymorphisms in mucous membrane pemphigoid patients and controls

Genotype	Patients *n* = 41 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals
C/C	18 (43.9)	73 (52.1)	0.72	0.36–1.45
T/C	18 (43.9)	54 (38.6)	1.25	0.62–2.52
T/T	5 (12.2)	13 (9.3)	1.36	0.45–4.06
IL1β-511				
C/C	18 (43.9)	70 (50)	0.78	0.39–1.58
C/T	21 (51.2)	58 (42.4)	1.48	0.74–2.99
T/T	2 (4.9)	12 (8.6)	0.55	0.12–2.55
IL1β+3962				
C/C	25 (61)	76 (54.3)	1.32	0.65–2.68
C/T	12 (29.3)	56 (40)	0.62	0.29–1.32
T/T	4 (9.7)	8 (5.7)	1.78	0.51–6.25
IL1R pst1 1970				
C/C	15 (36.6)	60 (42.9)	0.77	0.38–1.58
C/T	20 (48.8)	67 (47.9)	1.04	0.52–2.08
T/T	6 (14.6)	13 (9.2)	1.67	0.59–4.72
IL1RA mspa1 11100				
C/C	2 (4.9)	4 (2.9)	1.74	0.31–9.88
T/C	13 (31.7)	57 (40.7)	0.68	0,32–1.42
T/T	26 (63.4)	79 (56.4)	1,34	0.65–2.74
IL12-1188				
A/A	23 (56.1)	73 (52.1)	1.17	0.58–2.36
C/A	14 (34.1)	57 (40.7)	0.76	0.36–1.56

Table 2. IL-1 cluster, IL-12, IFN-c, TGF-b and TNF-a polymorphisms in mucous membrane pemphigoid patients and controls

Genotype	Patients *n* = 41 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals
C/C	4 (9.8)	10 (7.2)	1.41	0.42–4.74
IFNγ UTR 5644				
A/A	11 (26.8)	42 (30)	0.86	0.39–1.87
A/T	17 (41.5)	66 (47.1)	0.79	0.39–1.61
T/T	13 (31.7)	32 (22.9)	1.57	0.73–3.37
TGFβ1 cdn 10				
C/C	6 (14.6)	32 (22.9)	0.58	0.22–1.50
C/T	23 (56.1)	63 (45)	1.56	0.77–3.15
T/T	12 (29.3)	45 (32.1)	0.87	0.41–1.87
TGFβ1 cdn 25				
C/C	0 (0)	4 (2.9)	–	–
C/G	4 (9.8)	14 (10)	0.97	0.30–3.14
G/G	37 (90.2)	122 (87.1)	1.36	0.43–4.28
TNFα-308				
A/A	1 (2.4)	3 (2.1)	1.14	0.12–11.28
G/A	11 (26.8)	20 (14.3)	2.20	0.95–5.08
G/G	29 (70.8)	117 (83.6)	0.48	0.21–1.07
TNFα-238				
A/A	0 (0)	1 (0.7)	–	–
G/A	1 (2.4)	19 (13.6)	0.16	0.02–1.23
G/G	40 (97.6)	120 (85.7)	6.67	0.87–51.26

Table 2. IL-1 cluster, IL-12, IFN-c, TGF-b and TNF-a polymorphisms in mucous membrane pemphigoid patients and controls

Genotype	Patients *n* = 41 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals

Table 3. IL-2, IL-4 cluster, IL-6 and IL-10 polymorphisms in mucous membrane pemphigoid patients and controls

Genotype	Patients *n* = 41 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals

1. All the comparisons resulted are not significant.

IL2-330				
G/G	6 (14.6)	17 (12.1)	1.24	0.45–3.38
T/G	22 (53.7)	61 (43.6)	1.50	0.75–3.02
T/T	13 (31.7)	62 (44.3)	0.58	0.28–1.22
IL2+166				
G/G	24 (58.5)	86 (61.4)	0.89	0.44–1.80
G/T	17 (41.5)	45 (32.1)	1.50	0.73–3.06
T/T	0 (0)	9 (6.5)	–	–
IL4-1098				
G/G	0 (0)	1 (0.7)	–	–
T/G	2 (4.9)	23 (16.4)	0.26	0.06–1.16
T/T	39 (95.1)	116 (82.9)	4.03	0.91–17.85
IL4-590				
C/C	35 (85.4)	110 (78.6)	1.59	0.38–1.58
C/T	6 (14.6)	28 (20)	0.69	0.52–2.08
T/T	0 (0)	2 (1.4)	–	–
IL4-33				

Table 2. IL-1 cluster, IL-12, IFN-c, TGF-b and TNF-a polymorphisms in mucous membrane pemphigoid patients and controls

Genotype	Patients *n* = 41 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals
C/C	36 (87.8)	105 (75)	2.40	0.87–6.59
C/T	5 (12.2)	32 (22.9)	0.47	0.17–1.29
T/T	0 (0)	3 (2.1)	–	–
IL4RA+1902				
A/A	35 (85.4)	95 (67.9)	2.76	1.08–7.04
G/A	5 (12.2)	38 (27.1)	0.37	0.14–1.02
G/G	1 (2.4)	7 (5)	0.48	0.06–3.98
IL6-174				
C/C	4 (9.7)	13 (9.3)	1.06	0.32–3.43
C/G	15 (36.6)	70 (50)	0.58	0,28–1.18
G/G	22 (53.7)	57 (40.7)	1.69	0.84–3.40
IL6 nt 565				
A/A	5 (12.2)	13 (9.3)	1.36	0.45–4.06
G/A	13 (31.7)	67 (47.8)	0.51	0.24–1.06
G/G	23 (56.1)	60 (42.9)	1.70	0.84–3.44
IL10-1082				
A/A	17 (41.5)	47 (33.6)	1.40	0.69–2.86
G/A	20 (48.8)	76 (54.3)	0.80	0.40–1.61
G/G	4 (9.7)	17 (12.1)	0.78	0.25–2.47
IL10-819				
C/C	22 (53.7)	70 (50)	1.16	0.58–2.33
C/T	16 (39)	62 (44.3)	0.81	0.40–1.64

Table 2. IL-1 cluster, IL-12, IFN-c, TGF-b and TNF-a polymorphisms in mucous membrane pemphigoid patients and controls

Genotype	Patients *n* = 41 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals
T/T	3 (7.3)	8 (5.7)	1.30	0.33–5.15
IL 10-592				
C/C	22 (53.7)	70 (50)	1.16	0.58–2.33
C/A	16 (39)	62 (44.3)	0.81	0.40–1.64
A/A	3 (7.3)	8 (5.7)	1.30	0.33–5.15

Table 4. TNFα,IL-2 and IL-4RA polymorphisms in oral pemphigoid patients and controls

Genotype Patients *n* = 29 (%) Controls *n* = 140 (%) Odds ratios 95% confidence intervals

1. [a]

   $P = 0.044$, Pc = 0.176.

2. [b]

   $P = 0.018$, Pc = 0.072.

3. [c]

   $P = 0.032$, Pc = 0.128.

4. [d]

   $P = 0.023$, Pc = 0.046.

TNFα-308				
A/A	1 (3.5)	3 (2.1)	1.63	0.16–16.26
G/Aa	10 (34.5)	20 (14.3)	3.16	1.28–7.77
G/G	18 (62)	117 (83.6)	0.32	0.13–0.77
TNFα-238				
A/A	0 (0)	1 (0.7)	–	–
G/A	0 (0)	19 (13.6)	–	–

Table 4. TNFα,IL-2 and IL-4RA polymorphisms in oral pemphigoid patients and controls

Genotype	Patients *n* = 29 (%)	Controls *n* = 140 (%)	Odds ratios	95% confidence intervals
G/G[b]	29 (100)	120 (85.7)	–	–
IL2-330				
G/G	3 (10.3)	17 (12.1)	0.83	0.23–3.06
T/G[c]	20 (69)	61 (43.6)	2.88	1.22–6.77
T/T	6 (20.7)	62 (44.3)	0.33	0.13–0.86
IL4RA+1902				
A/A[d]	26 (89.7)	95 (67.9)	4.11	1.18–14.28
G/A	3 (10.3)	38 (27.1)	0.31	0.09–1.08
G/G	0 (0)	7 (5)	–	–

Moreover, in OP patients, the frequency of the –308A TNF-α allele was elevated compared to the controls (21.6 *vs* 9.3; OR 2.69, 95% CI 1.41–5.15, $P = 0.05$). This caused a significantly increased frequency of the genotype G/A in OP compared to controls that was lost when the P value was adjusted by Bonferroni correction (34.5 *vs* 14.3%, $P = 0.044$, Pc = 0.176) (Table 4). Similarly, also the frequency of genotypes TNFα-238 G/G and IL2-330 T/G was increased in OP but without any statistical significance when the P values were corrected (Table 4).

Discussion

This is apparently the first study on cytokine polymorphism in MMP, and only another study in a Chinese population has been conducted on the closely related bullous pemphigoid (BP) and found IL-1β (−511) and (−31) polymorphisms significantly associated with BP in women (Chang *et al*, 2006). The frequency of exon 1 A-G genotype of the human cytotoxic T-lymphocyte antigen 4 was also investigated in a small sample of MMP patients without any significant finding (Drouet *et al*, 2000).

Some controversial data are available for another blistering disease frequently affecting the oral cavity, namely PV (Eberhard *et al*, 2005; Javor *et al*, 2010).

Although skin and multiple mucous membranes can be affected in MMP, there are apparently two distinct subsets of patients where the lesions are restricted to the ocular or oral mucosae (Chan *et al*, 1993; Mobini *et al*, 1998; Rashid *et al*, 2006). In ocular mucosa scarring is a clinical hallmark of OCP (Saw *et al*, 2009). Although the mechanisms have not been clearly demonstrated, OCP manifests as chronic recurrent cicatricial conjunctivitis potentially leading to severe conjunctival fibrosis, causing blindness in 30% of cases (Saw *et al*, 2009). Contrarily, OP patients have an excellent prognosis, they tend to have a mild to moderate disease process, and scarring usually does not occur (Chan *et al*, 2002).

Fibrosis, the hyperaccumulation of scar tissue, is characterized by the overproduction and deposition of type I and II collagen by fibroblasts and when associated with repetitive injury in chronic inflammatory disease is strongly linked with CD4+ Th2 responses involving IL-4, IL-5, IL-13 and IL-21 (Wynn, 2004). In MMP, both IL-4 and IL-13 are thought to be involved in cicatricial scarring process (Razzaque *et al*, 2003; Bhogal *et al*, 2005 Giomi *et al*, 2005).

IL-4 is a pleiotropic cytokine that is produced mostly by Th2 T cells, NK1 cells and mast cells (Bhogal *et al*, 2005). IL-4 plays a crucial role in fibrosis operating through the IL-4 receptor (IL-4R) and inducing STAT6 activation. IL-4R consists of two subunits, the α chain (IL-4Rα or IL-4RA) and the γ chain (γc) (Bhogal *et al*, 2005). Interestingly, receptors for both IL-4 and IL-13 share the IL-4Rα chain and support STAT6 activation (Izuhara and Shirakawa, 1999).

The IL4RA gene is located on chromosome 16p (16p12.1). Several single-nucleotide polymorphisms (SNPs) have been identified in the coding region of the IL4RA gene (Shirakawa *et al*, 2000). One of these polymorphisms consists of an A-to-G transition at nucleotide 1902, causing a change from glutamine to arginine at codon 576 (Q576R) in the cytoplasmic domain of the IL-4RA. This polymorphism is associated with an up regulated receptor response to IL4. The genotype IL-4RA-1902 A/A is present in 90% of our OP patients, and it has been associated with a reduced response to IL-4 (Hershey *et al*, 1997). Of note, in our small cohort of MMP with cicatricial phenotype, only 60% had genotype IL-4RA-1902 A/A.

Increased expression of IL-4 and IL-4 mRNA has been seen in MMP, including patients with exclusive oral lesions (Caproni *et al*, 2003; Razzaque *et al*, 2003) but scarring formation is very unusual in OP. Giving the supposed importance of IL-4 in MMP fibrotic process, the present results may at least partially explain the low likelihood of scarring in OP patients.

Interestingly, patients with IL-4 RA (+1902) AA homozygosis have a stable non-progressing phenotype of pulmonary idiopathic fibrosis, which is a serious disease characterized by uncontrolled fibro-production as a sequelae of alveolar injury which devastates the lung architecture (Vasakova *et al*, 2007).

There are some limitations to our study particularly related to the sample size and enrolment. Although the epidemiologic data are not very well established, MMP is considered a rare disease, with a calculated incidence in dermatological/ophthalmological cohorts ranging from 0.67 up to 2 of new cases/million/year (Bernard *et al*, 1995; Zillikens *et al*, 1995; Rauz *et al*, 2005; Bertram *et al*, 2009). Even if dental cohorts suggest a higher incidence (Scully *et al*, 1999), it is difficult to enrol a consistent number of patients from a single centre. However, the relative small cohort size in our study could have lead rather to underestimate some possible association. For example, increased serum level of TNF-α in MMP has been reported, and anti-TNF-α medication can control the disease (Lee *et al*, 1993; Canizares *et al*, 2006). According to our results, an increased production TNF-α could be genetically induced in patients with exclusive oral lesions and influence OP susceptibility, but a larger cohort is warranted to confirm our preliminary observation and increase the strength of the findings.

Moreover, the heterogenic clinical phenotype might represent a potential source of bias. Indeed, our study suggests that OP may have a different genetic background and pathogenesis than other MMP subsets. Thus, our finding could not extend to other MMP variants.

In conclusion, our data suggest that Italian MMP patients with exclusive oral lesions are associated with the genotype IL-4RA-1902 A/A that can cause a reduced response to IL-4. This can partially explain the low likelihood of scarring in OP patients.

Acknowledgements

This work has been supported by M.U.R.S.T. (ex quota 60%) and the Department of Biomedical Sciences and Human Oncology, University of Turin.

Conflict of interest

None declared.

Author contributions

Carrozzo M and Amoroso A designed the study. Carrozzo M, Broccoletti R and Carbone M recruited the patients. Fasano E and Dametto E performed the genetic analyses. Rendine S and Carrozzo M carried out the statistical analysis. All the Authors drafted the manuscript.

References

- *Ahmed AR, Foster S, Zaltas M et al (1991). Association of DQw7 (DQB1*0301) with ocular cicatricial pemphigoid. Proc Natl Acad Sci USA 88: 11579–11582.*
- *Bazrafshani MR, Hajeer AH, Ollier WE, Thornhill MH (2002). IL-1B and IL-6 gene polymorphisms encode significant risk for the development of recurrent aphthous stomatitis (RAS). Genes Immun 3: 302–305.*
- *Bernard P, Vaillant L, Labeille B et al (1995). Incidence and distribution of sub-epidermal autoimmune bullous skin diseases in three French regions. Arch Dermatol 131: 48–52.*
- *Bertram F, Bröcker EB, Zillikens D, Schmidt E (2009). Prospective analysis of the incidence of autoimmune bullous disorders in Lower Franconia, Germany. J Dtsch Dermatol Ges 7: 434–440.*
- *Bhogal RK, Stoica CM, McGaha TL, Bona CA (2005). Molecular aspects of regulation of collagen gene expression in fibrosis. J Clin Immunol 25: 592–603.*
- *Bidwell J, Keen L, Gallagher G et al (1999). Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 1: 3–19.*
- *Calabresi V, Carrozzo M, Cozzani E et al (2007). Oral pemphigoid autoantibodies preferentially target BP180 ectodomain. Clin Immunol 122: 207–213.*
- *Canizares MJ, Smith DI, Conners MS, Maverick KJ, Heffernan MP (2006). Successful treatment of mucous membrane pemphigoid with etanercept in 3 patients. Arch Dermatol 142: 1457–1461.*
- *Caproni M, Calzolari A, Giomi B, Santucci M, Ficarra G, Fabbri P (2002). IL-4, IL-5, TGF-beta1 and IFN-gamma mRNAs detected by a new in situ amplification system in cicatricial pemphigoid. Exp Dermatol 11: 421–427.*
- *Caproni M, Calzolari A, Salvatore E et al (2003). Cytokine profile and supposed contribution to scarring in cicatricial pemphigoid. J Oral Pathol Med 32: 34–40.*
- *Carrozzo M, Fasano ME, Broccoletti R et al (2001). HLA-DQB1 alleles in Italian patients with mucous membrane pemphigoid predominantly affecting the oral cavity. Br J Dermatol 145: 805–808.*
- *Carrozzo M, Cozzani E, Broccoletti R et al (2004a). Analysis of antigens targeted by circulating IgG and IgA antibodies in patients with mucous membrane pemphigoid predominantly affecting the oral cavity. J Periodontol 75: 1302–1308.*
- *Carrozzo M, Uboldi de Capei F, Dametto E et al (2004b). Tumor necrosis factor-α and interferon-γ polymorphisms contribute to susceptibility to oral lichen planus. J Invest Dermatol 122: 87–94.*
- *Chan LS (2012). Ocular and oral mucous membrane pemphigoid (cicatricial pemphigoid). Clin Dermatol 30: 34–37.*
- *Chan LS, Yancey KB, Hammerberg C et al (1993). Immune-mediated subepithelial blistering diseases of mucous membranes Pure ocular cicatricial pemphigoid is a unique clinical and immunopathological entity distinct from bullous pemphigoid and other subsets identified by antigenic specificity of autoantibodies. Arch Dermatol 129: 448–455.*

- *Chan LS, Hammerberg C, Cooper KD (1997). Significantly increased occurrence of HLA-DQB1*0301 allele in patients with ocular cicatricial pemphigoid. J Invest Dermatol 108: 129–132.*
- *Chan LS, Ahmed AR, Anhalt GJ et al (2002). The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic indicators. Arch Dermatol 138: 370–379.*
- *Chang YT, Liu HN, Yu CW et al (2006). Cytokine gene polymorphisms in bullous pemphigoid in a Chinese population. Br J Dermatol 154: 79–84.*
- *Chang YT, Chou CT, Yu CW et al (2007). Cytokine gene polymorphisms in Chinese patients with psoriasis. Br J Dermatol 156: 899–905.*
- *Dashash M, Drucker DB, Blinkhorn AS (2006). Interleukin-10 haplotype frequencies in children with gingivitis. J Periodontol 77: 1503–1509.*
- *Delgado JC, Turbay D, Yunis EJ et al (1996). Common major histocompatibility complex class II allele HLA-DQB1* 0301 is present in clinical variants of pemphigoid. Proc Natl Acad Sci USA 93: 8569–8571.*
- *Drouet M, Delpuget-Bertin N, Vaillant L et al (1998). HLA-DRB1 and HLA-DQB1 genes in susceptibility and resistance to cicatricial pemphigoid in French Caucasians. Eur J Dermatol 8: 330–333.*
- *Drouet M, Aupetit C, Delpuget-Bertin N, Bedane C, Bonnetblanc JM, Cogné M (2000). CTLA4 exon 1 dimorphism in bullous and cicatricial pemphigoid. Eur J Immunogenet 27: 77–79.*
- *Eberhard Y, Burgos E, Gagliardi J et al (2005). Cytokine polymorphisms in patients with pemphigus. Arch Dermatol Res 296: 309–313.*
- *Fleming TE, Korman NJ (2000). Cicatricial pemphigoid. J Am Acad Dermatol 43: 571–591.*
- *Giomi B, Caproni M, Fabbri P (2005). L-4 and cellular adhesion molecule (CAM) pathway are involved in cicatricial pemphigoid scarring process. J Dermatol Sci 38: 57–59.*
- *Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA (1997). The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Engl J Med 337: 1720–1725.*
- *Izuhara K, Shirakawa T (1999). Signal transduction via the interleukin-4 receptor and its correlation with atopy. Int J Mol Med 3: 3–10.*
- *Javor J, Chmurova N, Parnicka Z et al (2010). TNF-alpha and IL-10 gene polymorphisms show a weak association with pemphigus vulgaris in the Slovak population. J Eur Acad Dermatol Venereol 24: 65–68.*
- *Kirzhner M, Jakobiec FA (2011). Ocular cicatricial pemphigoid: a review of clinical features, immunopathology, differential diagnosis, and current management. Semin Ophthalmol 26: 270–277.*
- *Lee SJ, Li Z, Sherman B, Foster CS (1993). Serum levels of tumor necrosis factor-alpha and interleukin-6 in ocular cicatricial pemphigoid. Invest Ophthalmol Vis Sci 34: 3522–3525.*
- *Marsh SG, Albert ED, Bodmer WF et al (2010). Nomenclature for factors of the HLA system, 2010. Tissue Antigens 75: 291–455.*
- *Miller S A, Dykes D D, Polesky H F (1988). A simple salting-out procedure for extraction of DNA from human nucleated cells. Nucleic Acids Res 16: 221–228.*
- *Mobini N, Nagarwalla N, Ahmed AR (1998). Oral pemphigoid. Subset of cicatricial pemphigoid?. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85: 37–43.*
- *Rashid KA, Gurcan HM, Ahmed AR (2006). Antigen specificity in subsets of mucous membrane pemphigoid. J Invest Dermatol 126: 2631–2636.*
- *Rauz S, Maddison PG, Dart JKG (2005). Evaluation of mucous membrane pemphigoid with ocular involvement in young patients. Ophthalmology 112: 1268–1274.*
- *Razzaque MS, Ahmed BS, Foster CS, Ahmed AR (2003). Effects of IL-4 on conjunctival fibroblasts: possible role in ocular cicatricial pemphigoid. Invest Ophthalmol Vis Sci 44: 3417–3423.*
- *Saw VP, Offiah I, Dart RJ et al (2009). Conjunctival interleukin-13 expression in mucous membrane pemphigoid and functional effects of interleukin-13 on conjunctival fibroblasts in vitro. Am J Pathol 175: 2406–2415.*

- *Scully C, Carrozzo M, Gandolfo S, Puiatti P, Monteil R (1999). Update on mucous membrane pemphigoid A heterogeneous immune-mediated subepithelial blistering entity. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 88: 56–68.*
- *Setterfield J, Theron J, Vaughan RW et al (2001). Mucous membrane pemphigoid: HLA-DQB1*0301 is associated with all clinical sites of involvement and may be linked to antibasement membrane IgG production. Br J Dermatol 145: 406–414.*
- *Shirakawa I, Deichmann KA, Izuhara I, Mao I, Adra CN, Hopkin JM (2000). Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. Immunol Today 21: 60–64.*
- *Vasakova M, Striz I, Dutka J et al (2007). Cytokine gene polymorphisms and high-resolution-computed tomography score in idiopathic pulmonary fibrosis. Respir Med 101: 944–950.*
- *Warren LJ, Wojnarowska F, Wilkinson JD (1997). Oesophageal involvement in cicatricial pemphigoid. Australas J Dermatol 38: 148–151.*
- *Wynn TA (2004). Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 4: 583–594.*
- *Zakka LR, Reche P, Ahmed AR (2011). Role of MHC Class II genes in the pathogenesis of pemphigoid. Autoimmun Rev 11: 40–47.*
- *Zillikens D, Wever S, Roth A, Weidenthaler- Barth B, Hashimoto T, Bröcker EB (1995). Incidence of autoimmune subepidermal blistering dermatoses in a region in central Germany. Arch Dermatol 131: 957–958.*