

ORIGINAL ARTICLE

Comparison of cytokine gene polymorphism in drug-induced maculopapular eruption, urticaria and drug reaction with eosinophilia and systemic symptoms (DRESS)

A. Barbaud,^{1,2,3,*} J. Waton,¹ B. Herbeth,^{4,5} A.C. Bursztejn,^{1,2,3} M. Bollaert,¹ J.L. Schmutz,¹ R.M. Guéant-Rodriguez,^{2,3,4} F. Namour,^{2,3,4} J.L. Guéant,^{2,3,4} I. Aimone-Gastin^{3,4}

¹CHU Nancy, Service de Dermatologie and INGRES research Unit, pôle des Spécialités médicales, Vandoeuvre les Nancy, France

²INSERM, U954, Vandoeuvre les Nancy, France

³Université de Lorraine, Faculté de Médecine, Vandoeuvre les Nancy, France

⁴CHU Nancy, Laboratoire de Biochimie et Biologie Moléculaire, Nutrition et Métabolisme, Vandoeuvre les Nancy, France

⁵Université de Lorraine, Faculté de Pharmacie, Nancy, France

*Correspondence: A. Barbaud. E-mail: a.barbaud@chu-nancy.fr

Abstract

Background Polymorphisms of genes controlling cytokine production have not been studied in the genetic susceptibility to cutaneous adverse drug reactions (CADR).

Objectives The objective was to determine whether polymorphisms in nine cytokine genes were associated to the occurrence of drug reaction with eosinophilia and systemic symptoms (DRESS) compared to drug-induced maculopapular eruption or urticaria and to controls without drug intolerance.

Methods Results from 118 patients with a well-defined CADR were compared to 236 controls without drug intolerance living in the same area of France. We assessed nine polymorphisms: interleukin (IL)1- α -889C>T (rs 1800587), IL1- β -511C>T (rs 16944), IL1-RN intron-2-VNTR (rs2234663), IL2-330T>G (rs 2069762), IL4-33C>T (rs 2070874), IL5-745C>T (rs 2069812), IL10-592C>A (rs 1800872), IL16-295T>C (rs 4778889) and tumour necrosis factor- α -308G>A (rs 1800629).

Results Three polymorphisms exhibited a significant association with CADR ($P < 0.05$). The combination of the IL1-RN-A2 and IL1- β -511C alleles was statistically different between cases and controls ($P = 0.007$) and the A2C haplotype was associated with susceptibility to CADR, particularly in drug reaction with eosinophilia and systemic symptoms (DRESS) patients (odds ratio = 3.22; 95% confidence interval = 1.23–8.41; $P = 0.016$). The frequency of the IL10-592A allele was higher in DRESS patients than in controls (dominant model CC vs. CA + AA: $P = 0.035$). These abnormalities were not evident in maculopapular eruptions or urticaria.

Conclusions This is the first study showing that IL1-cluster polymorphisms and haplotypes and the IL10-592A allele (a low IL10 producer) are associated with DRESS. These gene variants may decrease drug tolerance and promote herpes virus reactivation.

Received: 7 September 2012; Accepted: 4 February 2013

Conflict of interest

The authors declare that they have no competing interests as defined by the Journal of the European Academy of Dermatology and Venereology, or other interests that might be perceived to influence the results and discussion reported in this article.

Funding sources

This study was sustained by regional research funds from the University Hospital of Nancy (PHRC: UF 9793).

Introduction

Hypersensitivity to drugs can induce heterogeneous cutaneous adverse drug reactions (CADR); some, such as urticaria or maculopapular eruption (MPE), occur frequently, whereas others, such as the drug reaction with eosinophilia and systemic symptoms (DRESS), are rare and severe. Peripheral blood mononuclear cells (PBMC) from subjects with drug sensitization

contained Th2 cells producing interleukin (IL) 4 in immediate reactions but Th1 cells in delayed reactions are accompanied by a drug-specific increase in the secretion of IL2, 5, 13 and interferon (IFN)- γ .¹

In DRESS, drugs could interact with different targets and thus be involved in both drug hypersensitivity and the modification of host defence against human herpes viruses (HHV).^{2–5} Thus, a

genetically predisposed deficiency in cytokine-mediated immunoregulation could be involved in delayed hypersensitivity to drugs, and anti-human herpes virus (HHV) family host defence in DRESS.

In delayed reactions, drug-specific T cells recognize drugs through their T-cell receptors. Some drugs need to bind covalently to the major histocompatibility-peptide complex (MHC) to trigger an immune response, but others may bind directly and reversibly to immune receptors (p-i concept).⁶ Studies on genetic susceptibility to drug-induced cutaneous hypersensitivity have focused mainly on the MHC, especially on the human leucocyte antigen [HLA] B genotypes that were restricted to only one drug or ethnical group.^{7–9} However, some other genes, particularly those that control cytokine production, could also modulate the intensity of this immunological response. Studies regarding cytokine gene polymorphisms showed that immediate allergic reactions to betalactam are influenced by genes of the IL13 and IL4 pathways, by TNF-alpha GG genotype and, in a subgroup of atopic women, by IL10-592A.^{10–16}

The objective of this study was to determine whether polymorphisms in nine cytokine genes were associated with the occurrence of DRESS compared to drug-induced MPE or urticaria and to controls without drug intolerance.

Materials and methods

Study population

This unicentre, case-control study was conducted from November 2006 to December 2011. A total of 118 patients were prospectively included, based on the following eligibility criteria: (i) Age of 18 years or older; (ii) previous diagnosis with a well-defined CADR (urticaria, MPE or DRESS) as evaluated by a dermatologist; (iii) an urticaria with or without angio-oedema, a MPE (due to either only one new, recently introduced drug or positive systemic re-administration of a drug with progressively increased dosages under hospital surveillance), or a DRESS scored as plausible (class 4 or more according to the criteria of Kardaun *et al.*¹⁰ and (iv) a well-known chronology of the intake of each drug.

The study was also performed on 236 healthy controls (Table 1), including 132 subjects attending a health check-up at a local Centre for Preventive Medicine and 104 patients referred for a diagnostic coronary angiography in the context of suspected coronary artery disease but with a normal coronary angiogram.¹¹ All control subjects had no history of drug intolerance or hypersensitivity, no inflammatory and autoimmune diseases.

This study was approved by the ethic committee of Nancy (Comité de Protection des Personnes de Lorraine). All experimental procedures were conducted with written informed consent obtained from each patient, in accordance with the Ethical Principles of Helsinki's Declaration.

Drug skin tests and systemic provocation tests

The methods used are summarized in Table 2, following previously published methods.^{12,13} Briefly, all patients had drug patch tests and, when negative, prick tests. Except in patients with DRESS, intradermal tests (IDTs) followed by re-challenges were done when other tests were negative.

Assays and DNA genotyping procedures

Fasting venous blood was collected in EDTA-containing tubes, immediately centrifuged, and stored at -20°C until analysis. DNA was isolated from a lymphocyte-enriched fraction of whole blood using the NUCLEON BACC3 genomic DNA extraction kit (Amersham Pharmacia Biotech, Milan, Italy) and stored at -20°C for genetic analyses.

To investigate cytokines involved in immediate drug hypersensitization^{14–20} or delayed cutaneous hypersensitivity,²¹ nine cytokine polymorphisms were assessed: IL1-alpha-889C>T (rs 1800587), IL1-beta-511C>T (rs 16944), IL1-RN intron 2-VNTR (four repeats = allele A1; two repeats = allele A2; rs2234663), IL2-330T>G (rs 2069762), IL4-33C>T (rs 2070874), IL5-745C>T (rs 2069812), IL10-592C>A (rs 1800872), IL16-295T>C (rs 4778889) and TNF-alpha-308G>A (rs 1800629). Eight of these sequence-validated gene variants were identified by hybridization probes using LC 480 real-time polymerase chain reaction (PCR) followed by melting curve analysis (Light Cycler[®] 480, Roche Applied Science, Mannheim, Germany). The IL1-RN intron 2-VNTR polymorphism was characterized by variable tandem repeats composed most often by two or four 86-bp units. Analysis of this IL1-RN polymorphism was adapted from a previously described method²² and performed using an SYBR Green PCR restriction fragment length polymorphism. For a few subjects, data for polymorphisms were missing because of technical problems during determination. Therefore, the number of cases and controls varied for each class of polymorphisms.

Power calculation

Using a dominant model, our sample of 236 controls and 118 cases is sufficient for detecting an increase of 0.10 in the minor allelic frequency in cases with an alpha risk of 0.05 and a power of 90%, 72% and 60% when the allelic frequency of controls is 0.10, 0.20 and 0.30 respectively. Using a dominant model, our sample of 220 controls and 17 DRESS cases is sufficient for detecting a difference of 100% in the minor allelic frequency in cases with an alpha risk of 0.05 and a power of approximately 60%.

Statistical analysis

Basic statistical analyses were performed using SAS 9.2 statistical software (SAS Institute Inc., Cary, NC, USA). Allele frequencies were estimated by gene counting, and departure from Hardy-Weinberg (HW) equilibrium was tested using a chi-squared test

Table 1 Characteristics of controls and patients with cutaneous adverse drug reactions

	Controls	Patients with cutaneous adverse drug reactions			
		All cases	MPE	Urticaria and angioedema	DRESS
Number (N)	236	118	70	31	17
Age (y)*	64.5 ± 12.0	58.7 ± 14.9	61.8 ± 14.3	53.6 ± 13	55.1 ± 17.6
Sex †					
Males	163 (69.1)	45 (38.1)	35 (50.0)	4 (12.9)	6 (35.3)
Females	73 (30.9)	73 (61.9)	35 (50.0)	27 (87.1)	11 (64.7)
Blood sample taken after the CADR or OPT+		88	55	27	6
Drug responsibility determined by:					
Positive skin tests		82	53	16	13
Positive re-challenge		9	3	6	–
Only one recently introduced drug		27	14	9	4
Responsible drugs:					
Betalactam		34	20	10	4
Radiocontrast media		38	32	6	0
Pristinamycin		4	–	–	4
Vancomycin		3	–	–	3
Carbamazepine		3	–	–	3
Allopurinol		3	–	–	3
Aminoside		1	–	–	1
Minocyclin		1	–	–	1
Corticosteroid		1	–	–	1
Other classes		35	18	15	2

*Mean ± standard deviation.

†N (%). With respect to the drug classes involved in DRESS, some patients had two responsible classes. These multi-sensitizations were demonstrated by chronological criteria but also by specific positive drug skin tests in all cases.

CADR, cutaneous adverse drug reaction; DRESS, drug reaction with eosinophilia and systemic symptoms; OPT, oral provocation test; MPE, maculo-papular eruption.

with one degree of freedom. Genotype and allele frequencies were compared between cases and controls using a chi-squared test. Odds ratios (ORs) and a 95% confidence interval (CI) were calculated using logistic regression analysis after adjustment for sex and age. Pairwise linkage disequilibria between polymorphisms of IL1- α , IL1- β and IL1-RN were estimated and expressed in terms of the D' and r^2 statistics, as implemented in THESIAS software.²³ THESIAS software was used to compare the haplotype frequency distributions of cases and controls using a likelihood ratio test and haplotype effects (95% CIs were expressed as haplotypic OR).

Results

Subject characteristics are shown in Tables 1 and 3. The control group included 73 females and 163 males (mean age = 64.5 ± 12.0 years); the patient group consisted of 73 females and 45 males (mean age = 58.7 ± 14.9 years). Seventy patients presented with MPE (mean age = 61.8 ± 14.3 years; 35 females vs. 35 males); 31 patients with an urticaria (mean age = 53.6 ± 13 years; 27 females vs. 4 males); and 17 subjects with DRESS

were enrolled (mean age 55.1 ± 17.6 years; 11 females vs. 6 males), all of them were categorized as class 4 or greater according to the criteria of Kardaun *et al.*¹⁰ PCR detected herpes virus DNA in the blood of 11 of 13 patients with DRESS, including Epstein–Barr virus (EBV) in five cases, human herpes virus 6 (HHV6) in five cases and cytomegalovirus (CMV) in one case; two cases were negative for EBV, CMV and HHV 6 and 7.

Genotypes of each of the nine polymorphisms were in Hardy–Weinberg equilibrium in cases and control subjects. For the nine gene variants, genotype and allelic frequencies were not significantly different in males and females in both cases and controls. For the eight gene variants IL1- α -889C>T, IL1- β -511C>T, IL2-330T>G, IL4-33C>T, IL5-745C>T, IL10-592C>A, IL16-295T>C and TNF- α -308G>A, there was no significant difference between cytokine polymorphisms when comparing the CADR population and controls (Table 4).

The frequency of the IL1- β -511C allele was higher in DRESS patients than in controls, with the presence of at least one IL1- β -511T allele associated with a lower risk of DRESS

Table 2 Methods used for immuno-allergological investigations (drug skin tests and provocation tests)

Undertaken at least 6 weeks after clinical symptoms of the suspected CADR were resolved Patients were denied beta-blockers, antihistamines and systemic corticosteroids for 7 days and 1 month respectively
Patch tests:
When the active compound was available it was diluted at 10% in petrolatum. The commercialized form of the drug was diluted at 30% in water, petrolatum and alcohol Readings were done at 20 min, 48 h and 96 h
Prick test
Done on the volar forearm skin with the commercial form of the drug The positive prick test control was histamine diluted at 10 mg/mL Reactions were considered positive at 20 min if the weal of the reaction had a diameter at least 3 mm greater than that obtained with negative control (0.9% saline) A prick test reading done at 24 h was considered positive if an erythematous weal was present
Intradermal tests (IDTs)
Performed only if: (i) prick tests showed negative results; (ii) in non-severe CADR and; (iii) if an injectable form of the drug was available Done using a sterile solution of the suspected drug diluted sequentially in saline The negative control was saline (0.9% NaCl). Performed on the extensor surface of the arm The injected volume (0.02 ml) was that which produced an immediate weal of 3–5 mm in diameter Considered positive on immediate reading when the diameter at 20 min was equal to or more than twice the diameter of the injection weal Delayed reactions occurring >24 h after challenge were considered positive if an infiltrated erythematous reaction was observed
Re-challenges:
Performed only when skin test results were negative and only in patients with maculopapular eruption or urticaria, not in cases of DRESS The drug was given at six dosages, starting at $1.10^{-4} \times$ the dosage, to full dosage, vs. placebo, conditioned like the other dosages Re-challenges were stopped if abnormal cutaneous or general signs appeared Re-challenge was considered positive if any clinical reaction occurred

(sex- and age-adjusted OR = 0.26; 95% CI = 0.08–0.85; $P = 0.025$, data not shown).

For the IL1-RN intron2-VNTR (rs2234663) polymorphism analysis, we used only A1A1, A1A2 and A2A2 genotypes. The comparison between 112 cases and 217 controls showed statistically significant differences both when comparing genotype frequencies ($P = 0.009$) and in the dominant model, A1A1 vs. A1A2 + A2A2 ($P = 0.013$), with a higher frequency of allele A2 in CADR patients than in controls. The presence of at least one A2 allele was associated with a higher risk of CADR without partition (sex- and age-adjusted OR = 1.80; 95% CI = 1.10–2.94; $P = 0.018$).

When we compared the frequency of IL1-beta-511C and of IL1-RN-A2 between DRESS patients and other cases (MPE, urticaria and angioedema), no significant difference was shown: $P = 0.065$ and 0.671 respectively (data not shown).

The polymorphisms of IL1-beta and IL1-RN showed strong linkage disequilibria among patients and control subjects ($D' = 0.26$, $P \leq 0.001$); conversely, IL1-alpha and IL1-RN

polymorphisms were not in linkage disequilibrium ($D' = -0.07$, $P = 0.514$; data not shown). Haplotype analysis was done only on IL1-beta and IL1-RN polymorphisms.

We found an association between the haplotypes for IL1-RN intron2-VNTR and IL1-beta-511C>T and CADR. The combination of the IL1-RN-A2 and IL1-beta-511C alleles was statistically different between cases and controls (OR = 2.13; 95% CI = 1.23–3.70; $P = 0.007$) and the A2C haplotype was associated with susceptibility to DRESS (OR = 3.22; 95% CI = 1.23–8.41; $P = 0.016$; Table 5).

The frequency of the IL10-592A allele was higher in DRESS patients than in controls (dominant model CC vs. CA + AA: $P = 0.035$).

Discussion

In a very select and well-defined CADR population and in a control population living in the same area, there were no statistically significant differences between MPE patients and controls for 9 nucleotide cytokine polymorphisms. Maybe due to geographical diversity, in contrast to previous studies focused on immediate allergic reactions to betalactams^{18,19} we did not observe any difference between the IL4 polymorphism genotype (rs 2070874) or the TNF-alpha-308GG genotype (rs 1800629) in our French urticaria patients (betalactams, $n = 10$) and controls. In another French study, there was no difference between the same IL4 polymorphism genotype between patients with immediate hypersensitivity to betalactam antibiotics and controls.¹⁶

An association between immediate hypersensitivity to betalactams and some SNPs of IL4-Ralpha (on chromosome 16p12.1) has also been reported.^{14–16,20} However, we identified three cytokine gene polymorphisms, IL1-beta-511C>T, IL1-RN (intron2-VNTR) and IL10-592C>A, exhibiting significant positive associations ($P \leq 0.05$) with DRESS compared to controls.

In MPE and urticaria cytokine polymorphisms are comparable to those of controls. Phenotypes i.e. cytokine levels are probably different but it is not due to a genetically induced over or sub production of some cytokines. Moreover, even if some IL4 gene polymorphisms have been associated with urticaria due to betalactams, maybe due to geographical diversity, diversity of the responsible drugs and a limited number of patients we did not observe any difference between the IL4 polymorphism genotype in our urticaria patients.

The frequency of the IL1-beta-511C allele was higher in DRESS patients than in control subjects. In the chromosome 2q12-q21 region, the A2C haplotype was associated with susceptibility to DRESS [OR = 3.22 (1.23–8.41), $P = 0.016$; Table 4]. If the –511C allele is associated with normal production of IL1-beta, the IL1-RN-A2 allele probably is functionally significant to the regulation of IL1-Ra production.²⁴

The term 'IL1' usually designates a group of three molecules; two of these molecules, IL1-alpha and IL1-beta, are known to be involved in inflammation and host defence. The third, IL1-Ra or

Table 3 Characteristics of 17 patients with DRESS

	Sexe	Age	Skin rash	Fever	ELN	Eosinophil max	Hepatic failure	Other organ failure	DRESS score	Virus reactivation	Highest suspected drugs or classes	IL1 -beta -511C>T	IL1-RN intron 2-VNTR	IL10 -592C>A
1	M	56	+	+	+	4156	ALP $\times$ 1.5 N		6	nd	Corticosteroid*	CC	A1A1	AC
2	F	40	+	+		320 with atypical lymphopenia	T $\times$ 4 N		4	nd	Pristinamycin*	CC	A1A2	AC
3	F	51	+	+	+	800			4	nd	Glycopeptides*, and proton pump inhibitor*	CC	A2A2	AC
4	F	75	+	+		7400	T $\times$ 1.5 N	Renal dysfunction	6	–	Strontium ranelate, betalactams*	CC	A1A2	CC
5	M	55	+	–	+	1400	T $\times$ 2 N	Renal dysfunction	5	–	Pristinamycin*	CT	A1A1	AA
6	M	61	+	+	+	1310			4	EBV, HHV7	Betalactams*	CC	A1A1	AC
7	F	69	+	+		1580	T $\times$ 15N; ALP $\times$ 3N	Renal dysfunction	6	EBV, HHV6, HHV7	Allopurinol	CC	A1A2	AA
8	F	63	+	+	+	1750			5	HHV7	Aminosids* and glycopeptides*	CC	A1A2	CC
9	M	41	+	+	+	3020	T $\times$ 15 N		6	EBV	Betalactams*	CC	A1A2	CC
10	M	63	+	+	+		T $\times$ 6 N, ALP $\times$ 2 N		4	EBV, HHV7	Carbamazepine*	CC	A1A1	CC
11	F	60	+	+		3570	T $\times$ 10N; ALP $\times$ 5N		5	nd	Carbamazepine*	CT	A1A1	AA
12	F	47	+	+		742	ALP: 85 GGT: 87		4 score during the 2nd onset	IgM anti-CMV	Pristinamycin and macrolides 2nd onset of DRESS due to pristinamycin	CT	A1A2	AC
13	F	91	+	+	+	2700	ALP $\times$ 2N	Renal dysfunction	7	CMV, HHV6	Allopurinol	CC	A1A2	CC
14	F	29	+	+	+	1040	ALP $\times$ 1.7N		5	HHV7	Betalactams*	CC	A1A1	AC
15	M	18	+	+	+	2950	T $\times$ 9N; ALP $\times$ 4N	Pericarditis on echography	7	HHV6	Minocyclin	CC	A1A2	AC
16	F	71	+	–		3190	T $\times$ 8 N		4	EBV	Carbamazepine*	CC	A1A1	CC
17	F	46	+	+	+	2480	T $\times$ 4N; ALP $\times$ 3N		6	EBV, HHV7	Pristinamycin* and glycopeptides*	CT	A1A1	AC

The score has been calculated according to Kardaun *et al.* criterias (10), value of transaminases or alkaline phosphatases are given compared to normal ranges.

Virus reactivations have been studies by polymerase chain reaction on total blood for human herpes virus (H-HV) 6 and 7, cytomegalovirus (CMV), Epstein–Barr Virus (EBV).

*Positive results on drug patch tests. Biological abnormalities were observed at least twice in all case but one (case #12). In case 12, biological abnormalities were transient because it was a 2nd onset of DRESS, the 1st onset occurred 8 years before.

ELN, enlarged lymph nodes; eosinophil max, highest eosinophil count, eosinophil count done at least three times; T, transaminases; ALP, alkaline phosphatases; nd, not done; –, done with negative results.

Table 4 Association of cytokine gene polymorphisms with cutaneous adverse drug reactions*

IL1Alpha (-889C>T) rs1800587 (Chr 2q14)	Controls (N = 234)	All cases (N = 118)	MPE (N = 70)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
CC	50.4	51.7	48.6	67.7	35.3
CT	39.8	35.7	40.0	22.6	41.2
TT	9.8	12.6	11.4	9.7	23.5
Test for difference (cases vs. controls)†					
Categorical model: CC vs. CT vs. TT	–	0.612	0.917	–	–‡
Dominant model CC vs. CT + TT	–	0.822	0.785	0.069	0.228
IL1-beta (-511C>T) rs16944 (Chr 2q14)	Controls (N = 234)	All cases (N = 118)	MPE (N = 70)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
CC	47.0	55.9	52.9	51.6	76.5
CT	43.2	35.6	37.1	38.7	23.5
TT	9.8	8.5	10.0	9.7	0.0
Test for difference (cases vs. controls)					
Categorical model: CC vs. CT vs. TT	–	0.285	0.635	–	–
Dominant model: CC vs. CT + TT	–	0.105	0.372	0.612	0.018
IL1-RN (intron 2-VNTR)§ rs2234663 (Chr 2q13–14)	Controls (N = 217)	All cases (N = 112)	MPE (N = 66)	Urticaria + angioedema (N = 29)	DRESS (N = 17)
A1A1	65.9	51.8	54.5	48.3	47.1
A1A2	27.2	43.7	40.9	48.3	47.1
A2A2	6.9	4.5	4.6	3.4	5.8
Test for difference (cases vs. controls)					
Categorical model: A1A1 vs. A1A2 vs. A2A2	–	0.009	–	–	–
Dominant model: A1A1 vs. A1A2 + A2A2	–	0.013	0.094	0.063	0.117
IL2 (-330T>G) rs2069762 (Chr 4q26–27)	Controls (N = 228)	All cases (N = 118)	MPE (N = 70)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
TT	49.1	51.7	51.4	48.4	58.8
TG	40.8	43.2	44.3	45.2	35.3
GG	10.1	5.1	4.3	6.4	5.9
Test for difference (cases vs. controls)					
Categorical model: TT vs. TG vs. GG	–	0.281	0.316	–	–
Dominant model: TT vs. TG + GG	–	0.650	0.711	0.957	0.429
IL4 (-33C>T) rs2070874 (Chr 5q31.1)	Controls (N = 229)	All cases (N = 118)	MPE (N = 70)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
CC	74.2	77.9	77.1	87.1	64.7
CT	23.6	15.3	15.7	6.4	29.4
TT	2.2	6.8	7.2	6.5	5.9
Test for difference (cases vs. controls)					
Categorical model: CC vs. CT vs. TT	–	–	–	–	–
Dominant model: CC vs. CT + TT	–	0.431	0.610	0.114	0.396
IL5 (-745C>T) rs2069812 (Chr 5q31.1)	Controls (N = 212)	All cases (N = 118)	MPE (N = 70)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
CC	48.6	51.7	48.6	48.4	70.6
CT	42.0	35.6	38.6	38.7	17.6
TT	9.4	12.7	12.8	12.9	11.8
Test for difference (cases vs. controls)					
Categorical model: CC vs. CT vs. TT	–	0.428	0.688	–	–
Dominant model: CC vs. CT + TT	–	0.559	0.973	0.996	0.077

Table 4 Continued

IL10 (-592C>A) rs1800872 (Chr 1q31–32)	Controls (N = 221)	All cases (N = 117)	MPE (N = 69)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
CC	61.1	55.6	62.3	51.6	35.3
CA	34.8	34.2	27.6	41.9	47.1
AA	4.1	10.2	10.1	6.5	17.6
Test for difference (cases vs. controls)					
Categorical model: CC vs. CA vs. AA	–	0.077	–	–	–
Dominant model: CC vs. CA + AA	–	0.301	0.886	0.299	0.035
IL16 (-295T>C) rs4778889 (Chr 15q26.3)	Controls (N = 211)	All cases (N = 118)	MPE (N = 70)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
TT	65.4	66.9	68.6	67.7	58.8
TC	32.2	32.2	30.0	32.3	41.2
CC	2.4	0.9	1.4	0	0
Test for difference (cases vs. controls)					
Categorical model: TT vs. TC vs. CC	–	–	–	–	–
Dominant model: TT vs. TC + CC	–	0.753	0.610	0.784	0.594
TNF Alpha (-308G>A) rs1800629 (Chr 6q21.3)	Controls (N = 234)	All cases (N = 118)	MPE (N = 70)	Urticaria + angioedema (N = 31)	DRESS (N = 17)
GG	77.4	75.4	75.7	74.2	76.5
GA	20.9	20.4	18.6	22.6	23.5
AA	1.7	4.2	5.7	3.2	0
Test for difference (cases vs. controls)					
Categorical model: GG vs. GA vs. AA	–	–	–	–	–
Dominant model: GG vs. GA + AA	–	0.686	0.775	0.694	0.933

*Frequency

†chi-squared test

‡only dominant models were tested for rare alleles and when expected cell sizes were ≤ 5

§only A1 and A2 alleles were taken into account. DRESS: drug reaction with eosinophilia and systemic symptoms, IL: interleukin, MPE: maculopapular eruption.

RN, is a receptor antagonist that moderates the effects of the main secreted form of IL1, that is, IL1-beta.²¹ Interleukin1-RN-A2 allele may be associated with decreased IL1-Ra levels, and lower IL1-Ra inhibitory activity.²⁴ Our data correspond to these previous reports describing a synergistic effect between IL1-beta-511 and IL1-RN polymorphisms.

Interleukin1 may be involved in pathogenesis of atopic dermatitis, asthma,²⁵ delayed-type hypersensitivity and/or contact sensitization.²⁶ The highly active pro-inflammatory cytokine IL1-beta is essential in antiviral host defence.²⁷ Over-production of IL1 can enhance viral pathogenesis by triggering a 'cytokine storm' that drives a massive and toxic inflammatory response.²⁸ These polymorphic variants in the IL1 gene cluster may affect the responses in viral infections caused by CMV, EBV.²⁹ Mechanisms related to the IL1-RN-A2 allele may be responsible for an overall increase of IL1 production in DRESS patients.

For the first time, we report a significantly higher frequency of the IL10-592A allele in DRESS patients compared to controls

($P = 0.035$). In our controls, the allele percentages were close to those observed by others.³⁰ IL10 ubiquitously expressed among innate and adaptive immune cells inhibits both Th1 and Th2 production, which limits the ability of antigen-presenting cells to promote the activation of CD4 + T cells, inhibits antigen-specific cytotoxic T cells and makes T cells irreversibly tolerant.^{17,31}

The IL10 gene, located on chromosome 1q31–q32, is regulated primarily by transcription, 75% of the variation in IL10 production is genetically determined.³¹ Three gene variants in the IL10 promoter located at positions –1082 G/A, –819 C/T and –592 C/A are reported to be correlated with IL10 production.³¹ Due to complete linkage disequilibrium with the –592 C/A polymorphism, these alleles combine to form three possible haplotypes: GCC, ACC and ATA. The presence of these haplotypes can be determined by analysing the –592 C/A polymorphism, the ATA haplotype is associated with lower IL10 levels both *in vitro* and *in vivo*.³² Penicillin allergy was not associated with the IL10 promoter genes rs1800871 C/T or with rs1800872 G/T polymorphisms in American patients.¹⁹ In 44 French

Table 5 Association of haplotypes for IL1-RN VNTR and IL1-beta-511C>T with cutaneous adverse drug reactions

All cases IL1-RN rs2234663 (intron 2-VNTR)	IL1-beta rs16944 (-511C>T)	Controls N = 216	All cases N = 112	OR (95% CI)*	P value
A1	C	0.584	0.581	1.00†	–
A1	T	0.200	0.181	0.95 (0.60–1.52)	0.852
A2	C	0.087	0.176	2.13 (1.23–3.70)	0.007
A2	T	0.119	0.086	0.74 (0.37–1.46)	0.396
MPE IL1-RN rs2234663 (intron 2-VNTR)	IL1-beta rs16944 (-511C>T)	Controls N = 216	MPE N = 66	OR (95% CI)	P value
A1	C	0.594	0.566	1.00	–
A1	T	0.200	0.183	0.95 (0.56–1.61)	0.855
A2	C	0.089	0.145	1.71 (0.86–3.39)	0.120
A2	T	0.119	0.104	0.87 (0.41–1.87)	0.727
Urticaria + angioedema IL1-RN rs2234663 (intron 2-VNTR)	IL1-beta rs16944 (-511C>T)	Controls N = 216	Urticaria + angioedema N = 29	OR (95% CI)	P value
A1	C	0.594	0.522	1.00	–
A1	T	0.200	0.201	1.11 (0.48–2.60)	0.795
A2	C	0.087	0.167	2.02 (0.81–5.00)	0.127
A2	T	0.119	0.109	0.95 (0.30–2.91)	0.928
DRESS IL1-RN rs2234663 (intron 2-VNTR)	IL1-beta rs16944 (-511C>T)	Controls N = 216	DRESS N = 17	OR (95% CI)	P value
A1	C	0.591	0.602	1.00	–
A1	T	0.202	0.104	0.55 (0.13–2.29)	0.411
A2	C	0.089	0.280	3.22 (1.23–8.41)	0.016
A2	T	0.117	0.014	0.48 (0.01–24.06)	0.715

*OR (95% confidence interval).

†Reference.

DRESS, drug reaction with eosinophilia and systemic symptoms; IL, interleukin; MPE, maculopapular eruption.

patients allergic to betalactam, matched for sex and atopy to 44 patients with bad tolerance to betalactam but without positive skin or provocation tests, the IL10 –819 CT/TT and IL10 –592 CA/AA genotypes were associated with immediate betalactam allergy only in a limited subgroup of atopic women.¹⁶

IL10 suppresses the ability of effector T cells to prolong inflammatory responses; moreover, it influences viral disease progression.³¹ The ATA haplotype has been associated with the seroconversion, severity or the re-activation of EBV infections in children.³³ IL10 –592 C/C carriers can more easily clear EBV than can IL10 –592A allele carriers.³⁴ As some viruses such as CMV or EBV express a gene that encodes a viral IL10 and produce an IL10 analogue, this study has some limitations.^{35,36} Our patients had a different sex ratio than did controls. However, because the genes studied are autosomal, sex differences would not be expected.

In conclusion, we hypothesize that in DRESS, a genetically induced defect in the production of IL10 and a special pattern of

IL1-beta production could facilitate hypogammaglobulinemia, the failure of drug tolerance and low antiviral host-defence capacity against HHV family reactivation. Moreover, a disturbance in IL1 control could trigger a cytokine storm that drives a massive and toxic inflammatory response.²⁸ These abnormalities have not been elucidated in patients with drug-induced MPE.

Acknowledgements

The authors wish to thank D. Forest Tramoy, T. Josse, C. Masson and A. Collet for their excellent technical assistance. The biological assays and genotypes used in this publication were processed on samples kept in the Biobank of the Biochemistry and Molecular Biology Laboratory of the University Hospital of Nancy (54 500, Vandoeuvre les Nancy, France).

Contributions

Recruitment and selection of patients (AB, JW, ACB, BM, JFC, JLS), genetic methods and analyses (IA-G, RG-R, FN, JLG),

recruitment of controls (BH, RG-R, JLG), Statistical analyses (BH, IA-G), writing of the manuscript (AB, IA-G, BH, JLG), corrections of the manuscript (AB, JW, BH, ACB, MB, JFC, JLS, RG-R, FN, JLG, IA-G).

References

- Lochmatter P, Beeler A, Kawabata TT *et al.* Drug-specific *in vitro* release of IL-2, IL-5, IL-13, and IFN-gamma in patients with delayed-type drug hypersensitivity. *Allergy* 2009; **64**: 1269–1278.
- Hausmann O, Schnyder B, Pichler WJ. Drug hypersensitivity reactions involving skin. *Handb Exp Pharmacol* 2010; **196**: 29–55.
- Picard D, Janela B, Descamps V *et al.* Drug reaction with eosinophilia and systemic symptoms (DRESS): a multiorgan antiviral T cell response. *Sci Transl Med* 2010; **2**: 46ra62.
- Kano Y, Inaoka M, Shiohara T. Association between anticonvulsant hypersensitivity syndrome and human herpesvirus 6 reactivation and hypogammaglobulinemia. *Arch Dermatol* 2004; **140**: 183–188.
- Mardivirin L, Valeyrie-Allanore L, Branlant-Redon E *et al.* Amoxicillin-induced flare in patients with DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms): report of seven cases and demonstration of a direct effect of amoxicillin on human herpesvirus 6 replication *in vitro*. *Eur J Dermatol* 2010; **20**: 68–73.
- Pichler WJ, Beeler A, Keller M *et al.* Pharmacological interaction of drugs with immune receptors: the p-i concept. *Allergol Int* 2006; **55**: 17–25.
- Kaniwa N, Saito Y, Aihara M *et al.* HLA-B*1511 is a risk factor for carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients. *Epilepsia* 2010; **51**: 2461–2465.
- Lonjou C, Borot N, Sekula P *et al.* A European study of HLA-B in Stevens–Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 2008; **18**: 99–107.
- McCormack M, Alfirevic A, Bourgeois S *et al.* HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. *N Engl J Med* 2011; **364**: 1134–1143.
- Kardaun SH, Sidoroff A, Valeyrie-Allanore L *et al.* Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? *Br J Dermatol* 2007; **156**: 609–611.
- Guéant-Rodriguez RM, Juillière Y, Battaglia-Hsu SF *et al.* Association of IL1B polymorphism with left ventricular systolic dysfunction: a relation with the release of interleukin-1 β in stress condition. *Pharmacogenet Genomics* 2011; **21**: 579–586.
- Barbaud A. Skin testing in delayed reactions to drugs. *Immunol Allergy Clin North Am* 2009; **29**: 517–535.
- Brockow K, Romano A, Blanca M *et al.* General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002; **57**: 45–51.
- Guéant JL, Guéant-Rodriguez RM, Cornejo-García JA *et al.* Gene variants of IL13, IL4, and IL4RA are predictors of beta-lactam allergy. *J Allergy Clin Immunol* 2008; **122**: 152–158.
- Cornejo-García JA, Fernandez TD, Torres MJ *et al.* Differential cytokine and transcription factor expression in patients with allergic reactions to drugs. *Allergy* 2007; **62**: 1429–1438.
- Guglielmi L, Fontaine C, Gougat C *et al.* IL-10 promoter and IL4-Ralpha gene SNPs are associated with immediate beta-lactam allergy in atopic women. *Allergy* 2006; **61**: 921–927.
- Qiao HL, Wen Q, Gao N *et al.* Association of IL-10 level and IL-10 promoter SNPs with specific antibodies in penicillin-allergic patients. *Eur J Clin Pharmacol* 2007; **63**: 263–269.
- Guéant-Rodriguez RM, Guéant JL, Viola M *et al.* Association of tumor necrosis factor-alpha -308G>A polymorphism with IgE-mediated allergy to betalactams in an Italian population. *Pharmacogenomics J* 2008; **8**: 162–168.
- Apter AJ, Schelleman H, Walker A *et al.* Clinical and genetic risk factors of self-reported penicillin allergy. *J Allergy Clin Immunol* 2008; **122**: 152–158.
- Huang CZ, Yang J, Qiao HL, Jia LJ. Polymorphisms and haplotype analysis of IL-4Ralpha Q576R and I75V in patients with penicillin allergy. *Eur J Clin Pharmacol* 2009; **65**: 895–902.
- Schnuch A, Westphal G, Mössner R, Uter W, Reich K. Genetic factors in contact allergy—review and future goals. *Contact Dermatitis* 2011; **6**: 2–23.
- Tarlow JK, Blakemore AI, Lennard A *et al.* Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Hum Genet* 1993; **91**: 403–404.
- Tregouet DA, Garelle V. A new JAVA interface implementation of THE-SIAS: testing haplotype effects in association studies. *Bioinformatics* 2007; **23**: 1038–1039.
- Dinareello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009; **27**: 519–550.
- Zeyrek D, Demir E, Alpman A *et al.* Association of interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms in Turkish children with atopic asthma. *Allergy Asthma Proc* 2008; **29**: 468–474.
- Nambu A, Nakae S. IL-1 and allergy. *Allergol Int* 2010; **59**: 125–135.
- Sergerie Y, Rivest S, Boivin G. Tumor necrosis factor-alpha and interleukin-1 beta play a critical role in the resistance against lethal herpes simplex virus encephalitis. *J Infect Dis* 2007; **196**: 853–860.
- Poeck H, Ruland J. From virus to inflammation: mechanisms of RIG-I-induced IL-1 β production. *Eur J Cell Biol* 2012; **91**: 59–64.
- Hurme M, Helminen M. Resistance to human cytomegalovirus infection may be influenced by genetic polymorphisms of the tumor necrosis factor-alpha and interleukin-1 receptor antagonist genes. *Scand J Infect Dis* 1998; **30**: 447–449.
- de Jongh CM, John SM, Bruynzeel DP *et al.* Cytokine gene polymorphisms and susceptibility to chronic irritant contact dermatitis. *Contact Dermatitis* 2008; **58**: 269–277.
- Mosser DM, Zhang X. Interleukin-10: new perspectives on an old cytokine. *Immunol Rev* 2008; **226**: 205–218.
- Eder T, Mayer R, Langsenlehner U *et al.* Interleukin-10 [ATA] promoter haplotype and prostate cancer risk: a population-based study. *Eur J Cancer* 2007; **43**: 472–475.
- Hurme M, Haanpää M, Nurmikko T *et al.* IL-10 gene polymorphism and herpesvirus infections. *J Med Virol* 2003; **70**: S48–S50.
- Cheong JY, Cho SW, Hwang IL *et al.* Association between chronic hepatitis B virus infection and interleukin-10, tumor necrosis factor-alpha gene promoter polymorphisms. *J Gastroenterol Hepatol* 2006; **21**: 1163–1169.
- Spencer JV, Lockridge KM, Barry PA *et al.* Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. *J Virol* 2002; **76**: 1285–1292.
- Chao Y, Jing Y, Jia Y *et al.* Conservation and mutation of viral interleukin-10 gene in gastric carcinomas and nasopharyngeal carcinomas. *J Med Virol* 2011; **83**: 644–650.