

Original article

Differential cytokine and transcription factor expression in patients with allergic reactions to drugs

Background: Allergic drug reactions (ADR) can be either immediate reaction (IR) (IgE mediated) or delayed reaction (DR) (T-cell mediated). They follow the Th1/Th2 paradigm, with DR expressing interferon- γ (IFN- γ) with down-regulation of interleukin-4 (IL-4) and IR expressing IL-4 with down-regulation of IFN- γ . We studied the extension of this polarization in DR and IR by examining the cytokine and transcription factor profile in T-cell subpopulations during the acute phase of an ADR.

Methods: Expressions of cytokines [IL-4, IFN- γ and tumor necrosis factor- α (TNF- α)] and transcription factors (c-maf, GATA-3 and T-bet) were analysed by semi-quantitative real time-polymerase chain reaction in peripheral blood mononuclear cells and in CD4 and CD8 subpopulations from ADR patients.

Results: In DR, IFN- γ , TNF- α and T-bet increased significantly in both CD4 and CD8 subpopulations, depending on the clinical severity. In IR, IL-4, c-Maf and GATA-3 were increased, but only significantly in CD4. A positive correlation existed between IFN- γ and T-bet in DR and between IL-4 and c-Maf and GATA-3 in IR. In DR, IFN- γ , TNF- α and T-bet were increased during the acute phase in CD4 and CD8. In IR, IL-4, c-Maf and GATA-3 were all increased in the acute phase, but only in CD4.

Conclusions: These results support the Th1/Th2 paradigm in ADR, confirming previous findings that include the expression in both CD4 and CD8 T cells, and extending the observation to the transcription factors involved in the polarization of the immune response. Monitoring the reactions in the cell populations implicated, could be an important tool for assessing the mechanisms involved in ADR.

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Allergic drug reactions (ADR) are unpredictable, uncommon, and usually not related to the pharmacological actions of the drug (1). Allergic drug reactions are classified, depending on the time interval between the appearance of the symptoms and the drug intake, as immediate, accelerated or delayed (2). Immediate reactions (IR) correspond to those occurring <1 h after drug intake, with anaphylaxis and urticaria being the typical manifestations. Delayed reactions (DR) appear >24 h after drug intake and have different clinical manifestations, ranging from delayed urticaria and maculopapular exanthema (MPE) to more severe reactions, such as Stevens-Johnson syndrome and toxic

epidermal necrolysis (SJS/TEN) (3). Accelerated reactions occur between 1 and 24 h after drug intake with urticaria being the typical clinical manifestation and may overlap with IR and DR.

These various clinical entities, although all mediated by effector T cells, have a number of pathological differences that are currently the objective of continued research. T cells are a key factor in all types of ADR, regulating both IgE production or effector cells, depending on their profile of cytokine production (4). Two main T-cell phenotypes have been identified, type 1 [interferon- γ (IFN- γ) secreting] and type 2 [interleukin-4 (IL-4) secreting]. In DR, different T-cell subsets have been implicated depending on the clinical entities, CD4 in MPE (5) and CD8 in SJS/TEN; these have been demonstrated in peripheral blood and skin in both blister fluids and biopsies (6–8). Monitoring the allergic response during the acute episode and the resolution period in the affected tissue and peripheral blood can provide clues concerning the mechanisms involved.

Abbreviations: ADR, allergic drug reactions; DPT, drug provocation test; DR, delayed reactions; IFN, interferon; IL, interleukin; IR, immediate reactions; MPE, maculopapular exanthema; PBGD, porphobilinogen deaminase; PBMC, peripheral blood mononuclear cells; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; TNF, tumor necrosis factor.

In a previous study, we reported that cytokine production in peripheral blood mononuclear cells (PBMC) from patients suffering an ADR followed the classical Th1/Th2 paradigm depending on the type of reaction, DR or IR (9). However, discrepancies exist between investigators (9–12). These can be explained by differences in the clinical evaluation, in the cell type analysed, or in the methodology used.

More detailed studies concerning the mechanism underlying Th1/Th2 polarization in ADR are not generally available. These responses are the result of signals through cytokine receptors that culminate in the binding of specific transcription factors to multiple regulatory elements in the promoters and subsequent activation of cytokine genes. It is increasingly apparent that different transcription factors such as GATA-3, T-bet and c-Maf, are involved in directing Th1 or Th2 immune responses (13). Thus, T-bet (T-box expressed in T-cells) is related with Th1 responses, whereas the proto-oncogenes c-Maf and GATA-3 are related with Th2 commitment. However, as far as we know, no studies have been carried out dealing with ADR.

The aim of this work was to determine Th1 and Th2 cytokine expression, as well as T-bet, c-Maf and GATA-3 expression, in PBMC and into two different T-lymphocyte subpopulations, CD4 and CD8, obtained from patients with DR and IR to drugs.

Material and methods

Subjects

Patients with a confirmed drug allergy after a clinical history, plus skin testing or drug provocation test (DPT) when required, were finally included. The clinical entities were established as described (14). Patients were classified into two groups depending on the time of occurrence and type of reaction: IR, appearing within 1 h of drug intake, including urticaria and anaphylaxis; and DR, appearing 24 h or more after drug intake, including urticaria, MPE and SJS/TEN. Accelerated reactions were not included in this study.

As controls, we selected a group of age- and sex-matched subjects to the patients with no history of ADR or any cutaneous or immunological diseases at the time of selection and good tolerance to the drugs involved in the reactions. This study was approved by the institutional review board, and informed consent for all the diagnostic procedures was obtained from patients and controls.

The procedure to establish the diagnosis was as reported elsewhere (14). Basically, in DR intradermal or patch testing was performed depending on the drug solubility. If skin testing was negative, and the reaction was not severe, a DPT was performed. In IR, prick and intradermal tests were carried out although these could only be performed with soluble drugs.

Sample collection

Blood samples were obtained from each patient, both with DR and IR, within 24 h of the appearance of the clinical symptoms (T1) and 45 days later, when the reaction had cleared (T2). Peripheral blood mononuclear cells were isolated by density gradient centrifugation (Nycomed, Oslo, Norway) from 20 ml of heparinized venous blood.

Table 1. Clinical data of patients with immediate and delayed allergic reaction to drugs. Results of the allergological work-up for the drug involved in the reaction

Patient	Sex	Age	Reaction type	Drug involved	Clinical symptoms	Skin test	DPT
1	M	19	IR	AX	URT	–	+
2	F	21	IR	MET	URT	+	ND
3	M	49	IR	MET	URT	+	ND
4	F	68	IR	CEF	URT	+	ND
5	M	39	IR	AX	URT	–	+
6	F	21	IR	AX	URT	+	ND
7	F	35	IR	CEF	ANAPH	+	ND
8	F	35	IR	AX	ANAPH	+	ND
9	M	57	IR	AX	ANAPH	+	ND
10	M	54	IR	AX	ANAPH	+	ND
11	M	24	IR	AX	ANAPH	+	ND
12	M	35	IR	AX	ANAPH	–	+
13	M	31	IR	CEF	ANAPH	–	+
14	F	43	IR	AX	ANAPH	–	+
15	M	26	IR	AX	ANAPH	+	ND
16	F	43	IR	AX	ANAPH	+	ND
17	F	52	DR	AX	URT	+	
18	M	25	DR	CEPH	URT	–	+
19	F	21	DR	AX	URT	AX	ND
20	F	46	DR	CEPH	URT	–	+
21	F	31	DR	AX	URT	–	+
22	F	62	DR	SPI	URT	–	+
23	F	58	DR	MET	URT	–	+
24	M	55	DR	MET	URT	–	+
25	F	38	DR	AX	MPE	–	+
26	F	52	DR	AX	MPE	+	ND
27	M	62	DR	AX	MPE	–	+
28	F	35	DR	PHE	MPE	–	+
29	F	28	DR	AX	MPE	–	+
30	F	39	DR	AX	MPE	ND	ND
31	F	59	DR	AX	MPE	+	ND
32	F	30	DR	PHE	MPE	–	+
33	M	37	DR	CAR	MPE	–	+
34	F	30	DR	AX	MPE	–	+
35	F	20	DR	PAR	MPE	–	+
36	F	77	DR	DIP	SJS/TEN	ND	ND
37	F	29	DR	ALO	SJS/TEN	ND	ND
38	F	50	DR	DIP	SJS/TEN	ND	ND
39	F	55	DR	ALO	SJS/TEN	ND	ND
40	F	52	DR	ALO	SJS/TEN	ND	ND
41	F	38	DR	DIP	SJS/TEN	ND	ND

IR, immediate reactions; DR, delayed reactions; AX, amoxicillin; CEF, cefuroxime; MET, metimazol; PAR, paracetamol; ALO, alopurinol; CEPH, cephalixin; DIP: diphenyl-dantoine; CAR, carbamazepine; PHE, phenobarbital; SPI, spiramicin; URT, urticaria; ANAPH, anaphylaxis; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; MPE, maculopapular exanthema; DPT, drug provocation test; ND, not done.

T-cell subpopulation isolation

CD4 and CD8 T-cell subpopulations were obtained from PBMC by negative selection using magnetic beads (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany), according to the manufacturer's instructions. The purity of the subpopulations was checked by flow cytometry, using CD3- and CD14-PerCP, CD16-FITC, CD4-APC and CD8- and CD19-PE monoclonal antibodies (Becton-Dickinson, San José, CA, USA) on a Facscalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) using Cell Quest software as described (15).

Semi-quantification of cytokine and transcription factor mRNA expression

Total RNA was extracted in TriPure (Roche, Indianapolis, IN, USA) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed with random hexamers and moloney murine leukaemia virus-reverse transcriptase at a final volume of 20 μ l. Specific cDNA amplification of human cytokines IL-4, IFN- γ , tumor necrosis factor- α (TNF- α) and transcription factors T-bet, c-Maf and GATA-3 was carried out with 2 μ l of cDNA by using FastStart DNA Master SYBR Green I (Roche). The specific primers for cytokines, transcription factors and the housekeeping gene [porphobilinogen deaminase (PBGD)] used were: 5'-CAGATGTA-GCGGATAATGGA-3' and 5'-ACCTTGAAACAGCATCTGAC-3' for IFN- γ ; 5'-CCCAGGCAGTCAGATCAT-3' and 5'-GATGGT-GTGGGTGAGGAG-3' for TNF- α ; 5'-TGCTGCCTCCAAGAA-CAG-3' and 5'-TCACAGGACAGGAATTCAAG-3' for IL-4; 5'-CCCATTCTGTCTATTACTG-3' and 5'-GGCTCTCCGTCG-

TTCACCTC-3' for T-bet; 5'-GGGACGCGTACAAGGAGAAA-3' and 5'-TCAGGGGTAGGTGGTTCTCC-3' for c-Maf; 5'-ACA-AAATGAACGGACAGAAC-3' and 5'-CTTTTGGATTGCT-AGACA-3' for GATA-3 and 5'-TCCAAGCGAGCCATGTCTG-3' and 5'-AGAATCTTGTCCCCTGTGGTGG-3' for PBGD. The concentration was obtained by crossing-point extrapolation into a standard curve with known cDNA concentrations by using Light Cycler system software (Roche, Mannheim, Germany) and results were expressed as relative units that represent the ratio of concentration between the specific mRNA and the housekeeping mRNA.

Statistical analysis

Comparisons for quantitative variables were performed by non-parametrical analysis, Mann-Whitney and Kruskal-Wallis tests for nonrelated samples. Comparisons at two different times (T1 and T2) were carried out with the Wilcoxon test for related samples. Correlation studies were performed by using Pearson's test. All reported

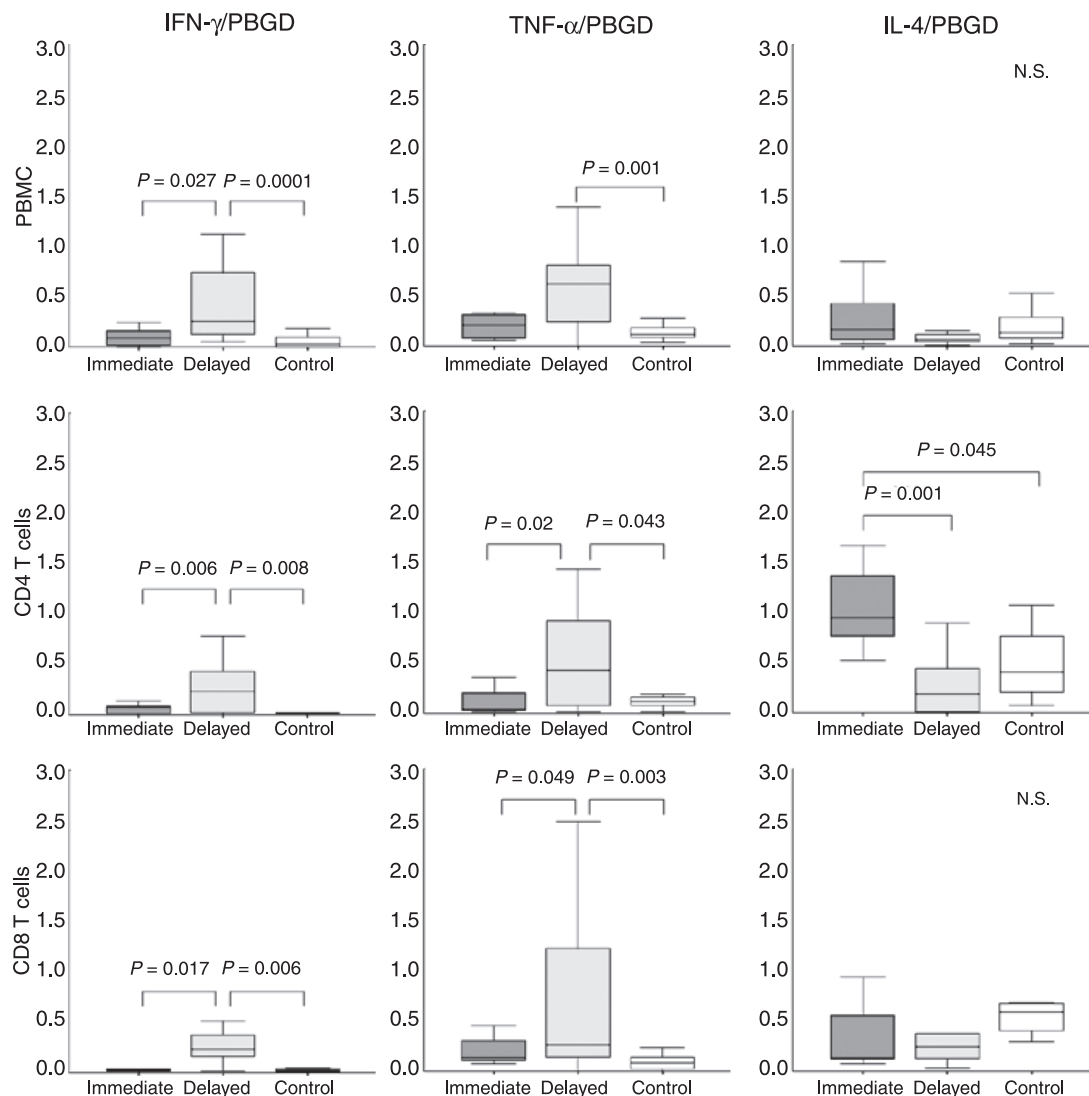


Figure 1. Box plots of the relative IFN- γ , TNF- α and IL-4 mRNA levels in patients during the acute phase of immediate (IR) and delayed reaction (DR) compared with the control group in peripheral blood mononuclear cells (PBMC), CD4 and CD8 T cells. Results were normalized to the levels of porphobilinogen deaminase (PBGD) and expressed as arbitrary units.

P-values represented two-tailed tests, with values ≤ 0.05 considered statistically significant. For the statistical analysis the SPSS program, version 11.5 was used.

Results

A total of 41 patients (mean age: 42 ± 14.76 years), 28 female and 13 male, finally diagnosed as allergic to drugs, were included (25 with DR and 16 with IR). The drugs most frequently involved in the reactions were betalactams in 12 DR cases and 14 IR cases, nonsteroidal antiinflammatory drugs in three DR and two IR, anticonvulsant in six DR cases, alopurinol in three DR cases and macrolide in one DR case. The percentage of atopy of this group of patients,

tested with a panel of inhalant and food allergens, was 20%. This was similar to that of the general population. Table 1 shows the clinical characteristics and the results of the allergological work-up (skin test and DPT) of the patients.

Cytokine expression from patients with DR and IR at the acute phase of the allergic reaction (T1)

We found a significant increase in IFN- γ expression in PBMC in DR compared with both IR ($P = 0.027$) and controls ($P = 0.0001$). With respect to TNF- α production, we found significant differences with controls ($P = 0.001$) and although the median of this was higher in DR than in IR, this was no significant (Fig. 1). For

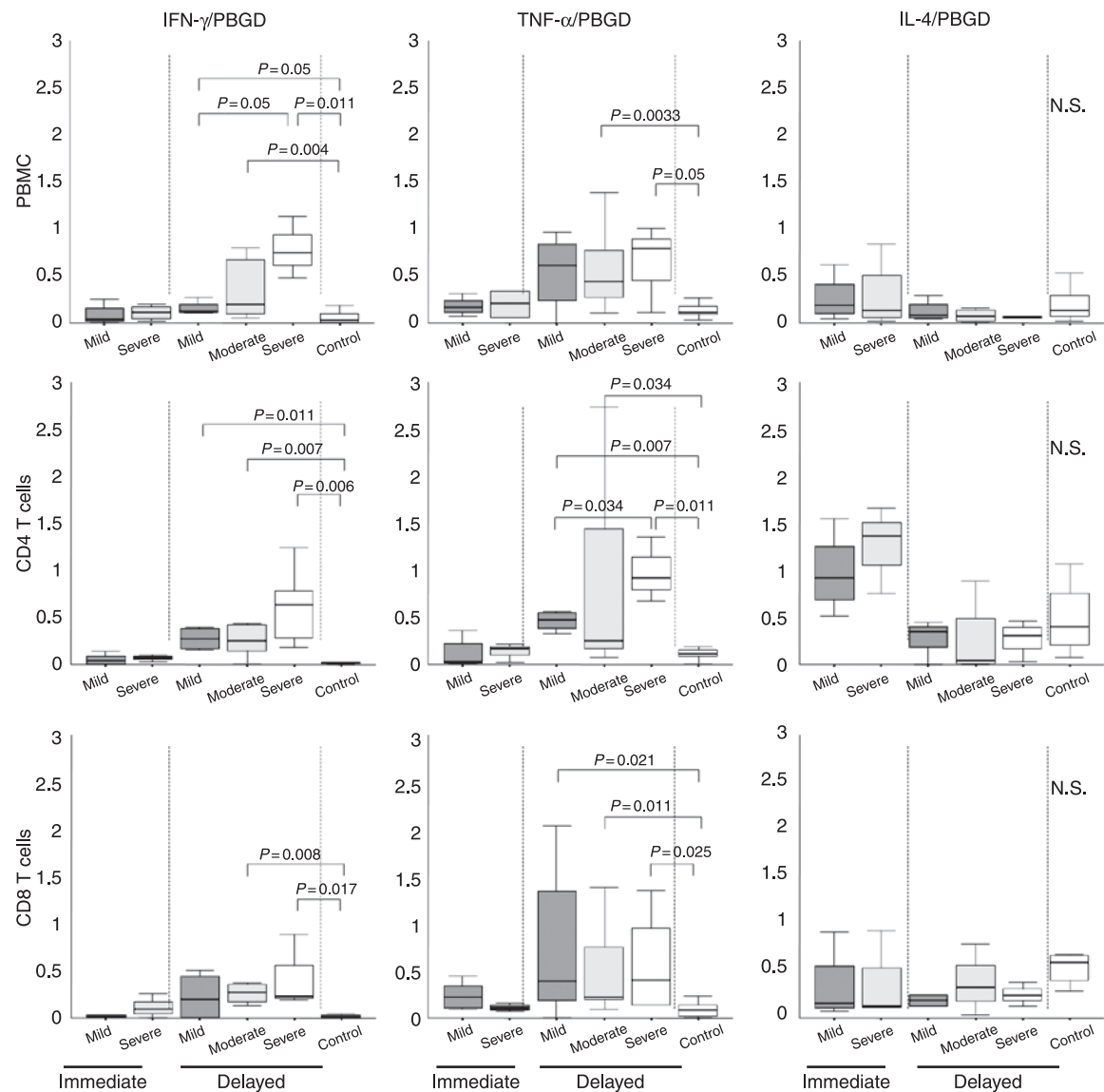


Figure 2. Box plots of the relative IFN- γ , TNF- α and IL-4 mRNA levels in patients with immediate (IR) and delayed reaction (DR) with varying degrees of severity of the clinical symptoms (mild, moderate and severe) compared with the control group. Results were normalized to the levels of porphobilinogen deaminase (PBGD) and expressed as arbitrary units.

IL-4 expression, although an increase was detected in IR compared with DR and controls, this was not statistically significant (Fig. 1).

When we analysed these comparisons into two T-cell subpopulations (CD4 and CD8), we found an increase in IFN- γ in DR compared with IR and controls in CD4 ($P = 0.006$ and $P = 0.008$ respectively) and CD8 ($P = 0.017$ and $P = 0.006$ respectively). Tumor necrosis factor- α expression had similar variations in both CD4 ($P = 0.02$ and $P = 0.043$ respectively) and CD8 ($P = 0.049$ and $P = 0.003$ respectively) (Fig. 1). In IR we found an increase in IL-4 production compared with DR and controls only in the case of the CD4 T-cells ($P = 0.001$ and $P = 0.045$ respectively).

Delayed reactions can be classified, according to the severity of the clinical symptoms, as mild (urticaria),

moderate (MPE) and severe (SJS/TEN). Comparison showed that IFN- γ was increased in all three groups compared with controls ($P = 0.05$ for mild, $P = 0.004$ for moderate and $P = 0.011$ for severe reactions). Although higher in the most severe cases, significant differences were only obtained when compared with mild reactions ($P = 0.05$). Tumor necrosis factor- α analysis showed statistical increases in moderate ($P = 0.003$) and severe reactions ($P = 0.05$) compared with the controls (Fig. 2).

When we analysed the two T-cell subpopulations we found that in the IFN- γ production for CD4 T cells ($P = 0.011$ for mild, $P = 0.007$ for moderate and $P = 0.006$ for severe reactions) whereas for CD8 the differences were only found for moderate ($P = 0.008$) and severe ($P = 0.017$) reactions compared with controls. In the case of TNF- α , we found greater differences

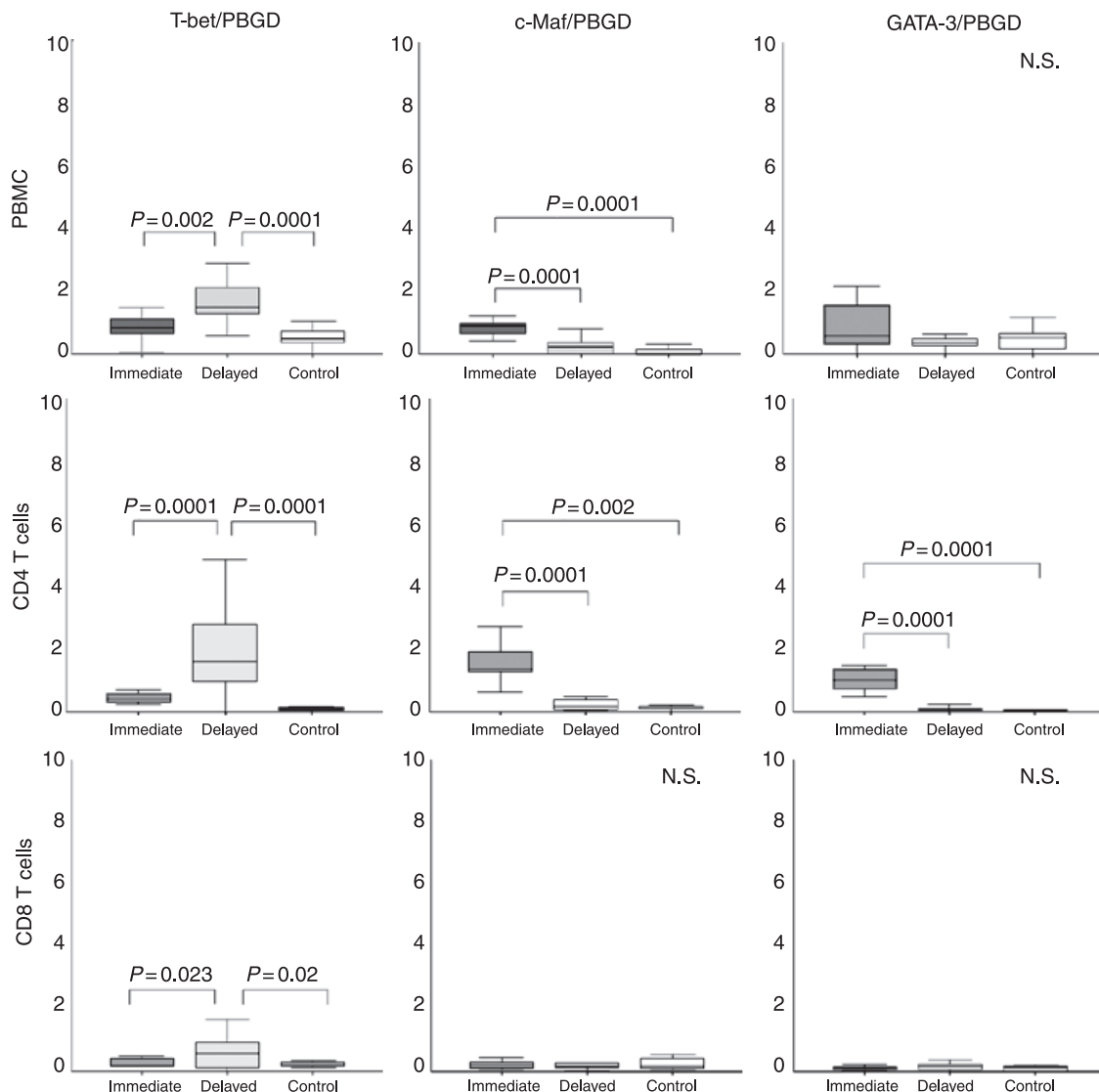


Figure 3. Box plots of the relative T-bet, c-Maf and GATA-3 mRNA levels in patients during the acute phase of immediate (IR) and delayed reactions (DR) compared with control group in peripheral blood mononuclear cells (PBMC), CD4 and CD8 T cells. Results were normalized to the levels of porphobilinogen deaminase (PBGD) and expressed as arbitrary units.

in both CD4 T cells ($P = 0.007$ for urticaria, $P = 0.034$ for MPE and $P = 0.011$ for SJS/TEN) and CD8 cells ($P = 0.021$ for urticaria, $P = 0.011$ for MPE and $P = 0.025$ for SJS/TEN) (Fig. 2).

Depending on the severity of the clinical symptoms, IR can be classified as mild (urticaria) or severe (anaphylaxis). Comparisons showed no significant differences for any of the cytokines in PBMC, CD4 and CD8 subpopulations. However, a tendency for increased IL-4 production was found in CD4 cells from patients with severe IR reactions (Fig. 2).

Transcription factor expression in patients with DR and IR at the acute phase of the allergic reaction (T1)

The determination of transcription factors showed that T-bet was increased in DR compared with IR and

controls in PBMC ($P = 0.002$ and $P = 0.0001$ respectively), CD4 ($P = 0.0001$ for both) and CD8 ($P = 0.023$ and $P = 0.02$ respectively). The c-Maf was increased in IR compared with DR and controls in PBMC ($P = 0.0001$ for both) and CD4 ($P = 0.0001$ and $P = 0.002$ respectively) but not in CD8. In GATA-3, the differences were only found in the CD4 subpopulation an increase in IR compared with DR and controls ($P = 0.0001$ for both) (Fig. 3).

When we analysed the transcription factor expression in patients suffering a DR according to the severity of the clinical symptoms, significant differences were found compared with controls in T-bet expression in PBMC ($P = 0.016$ for mild, $P = 0.0001$ for moderate and $P = 0.007$ for severe), in CD4 ($P = 0.005$ for mild, $P = 0.014$ for moderate and $P = 0.013$ for severe) and in CD8 ($P = 0.007$ for mild and $P = 0.014$ for severe). Interest-

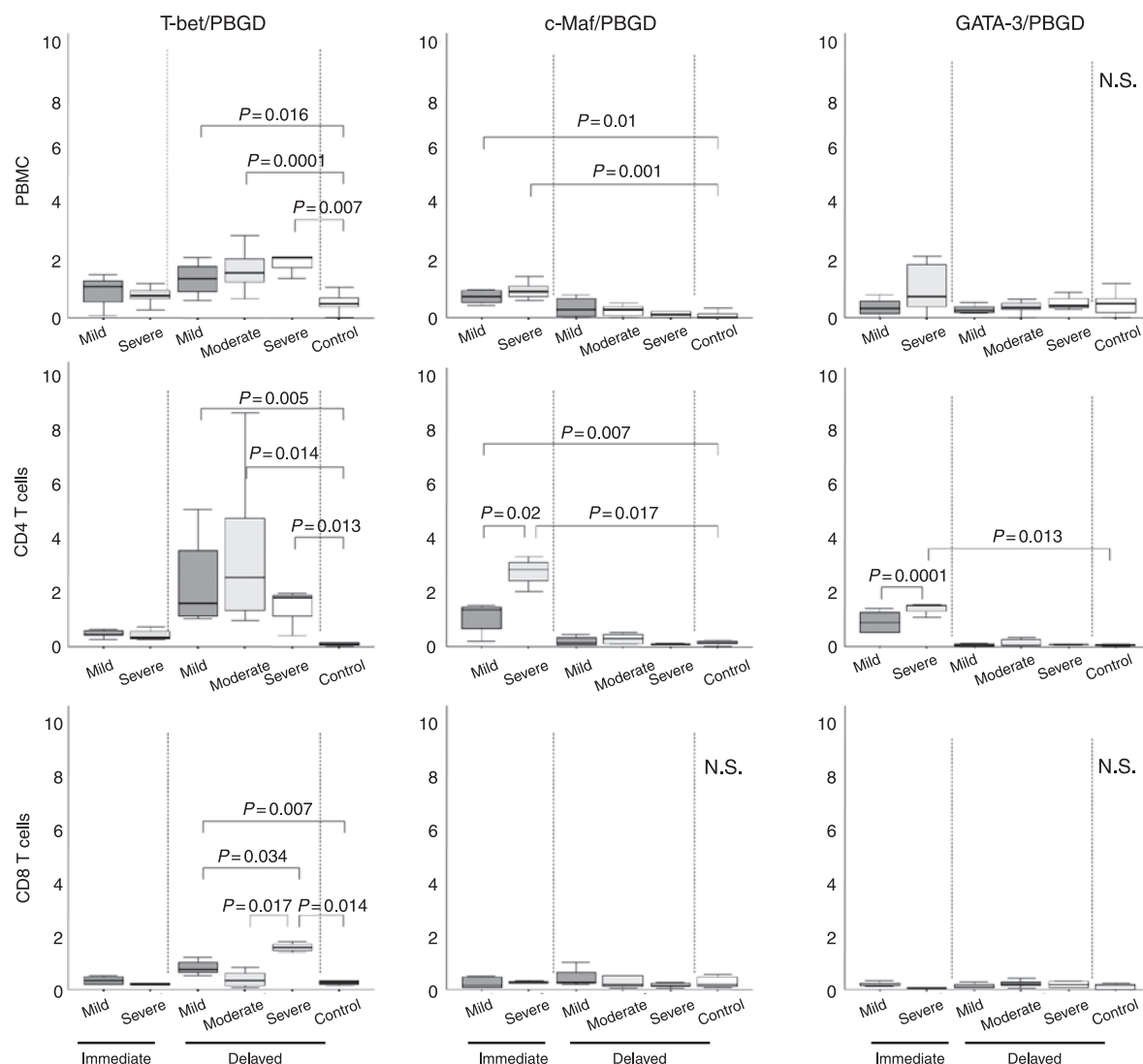


Figure 4. Box plots of the relative T-bet, c-Maf and GATA-3 mRNA levels in patients with immediate (IR) and delayed reactions (DR) with varying degrees of severity of the clinical symptoms (mild, moderate and severe) compared with the control group. Results were normalized to the levels of porphobilinogen deaminase (PBGD) and expressed as arbitrary units.

ingly, we detected an increased expression of T-bet in severe, compared with mild and moderate reactions ($P = 0.034$ and $P = 0.017$ respectively) in CD8 (Fig. 4).

The analysis of IR, according to the severity of clinical symptoms, showed differences for c-Maf in severe and mild vs controls in PBMC ($P = 0.001$ and $P = 0.01$ respectively) and CD4 ($P = 0.017$ and $P = 0.007$ respectively) but not in CD8. These differences were stronger for severe reactions with a significant increase when compared with mild in CD4 ($P = 0.02$). For GATA-3, as occurred before, differences were only observed in CD4 T-cells, with increased expression in severe reactions compared with mild reactions and controls ($P = 0.0001$ and $P = 0.013$ respectively) (Fig. 4).

Cytokine and transcription factor correlations in DR and IR at the acute phase of the allergic reaction (T1)

Statistical analysis showed that in DR, IFN- γ was associated with T-bet in both PBMC ($r^2 = 0.644$, $P = 0.018$) and CD4 ($r^2 = 0.522$, $P = 0.046$) whereas TNF- α expression correlated only in CD4 lymphocytes ($r^2 = 0.842$, $P < 0.005$). In IR, IL-4 expression, correlated with the expression of c-Maf and GATA-3 in both PBMC

($r^2 = 0.653$, $P = 0.029$; $r^2 = 0.895$, $P = 0.016$, respectively) and CD4 ($r^2 = 0.796$, $P = 0.01$; $r^2 = 0.771$, $P = 0.025$ respectively). We also found a statistical association between the two Th2 transcription factors GATA-3 and c-Maf ($r^2 = 0.818$, $P = 0.047$) (Fig. 5). No correlations were found in the case of the CD8 subpopulation for any of the transcription factors or cytokines studied in either DR or IR (data not shown in figures).

Cytokine and transcription factor expression in patients with DR and IR at the acute phase (T1) and after symptoms subsided (T2)

In DR, we observed an increase in IFN- γ expression in PBMC ($P = 0.008$), CD4 ($P = 0.05$) and CD8 ($P = 0.043$) and in TNF- α in PBMC ($P = 0.006$) and CD4 ($P = 0.001$) at T1. With respect to the transcription factors, we found that T-bet was increased in PBMC ($P = 0.05$), CD4 ($P = 0.037$) and CD8 ($P = 0.012$). Interestingly, the expression of the two Th2 transcription factors, c-Maf and GATA3, was reduced in T1 compared with T2 in those patients with DR, though only in CD4 lymphocytes ($P = 0.028$ and $P = 0.012$, respectively) (Table 2).

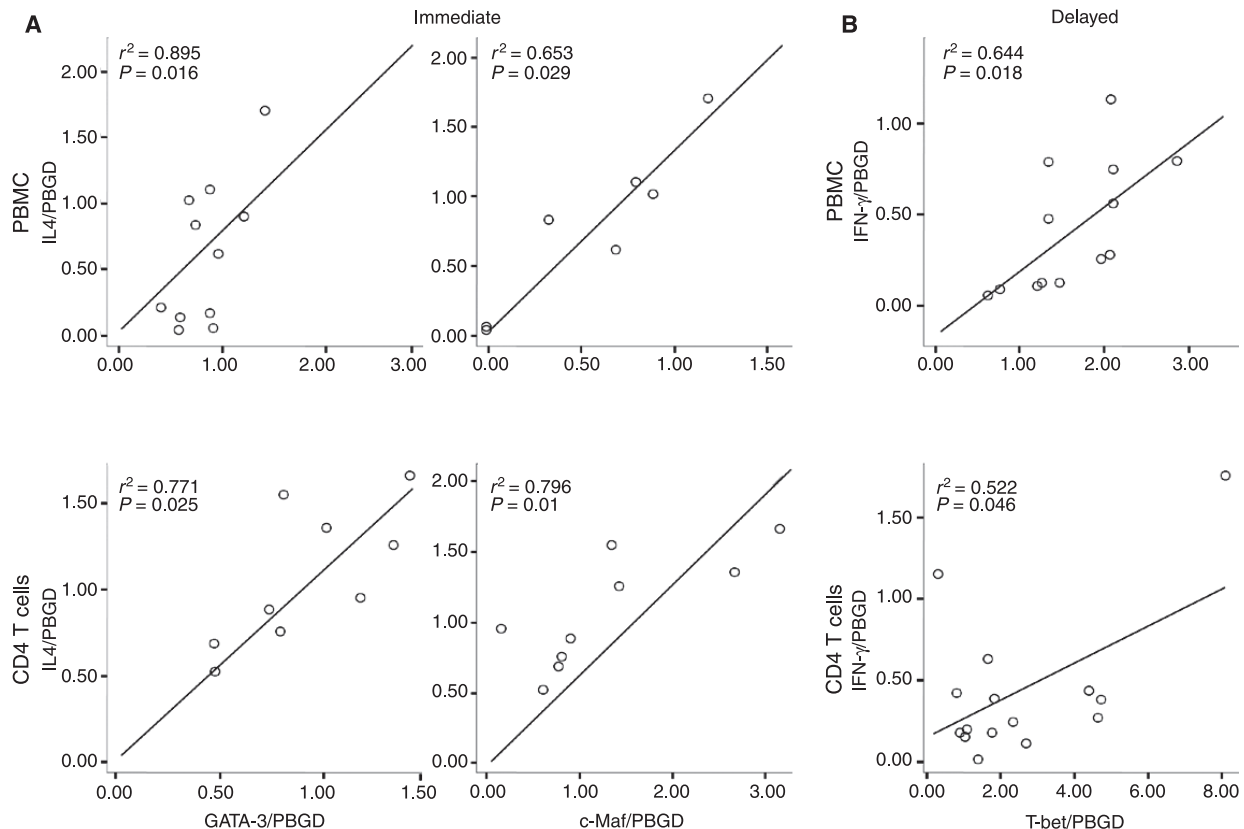


Figure 5. (A) Correlation scatter plots of IL-4 with c-Maf and GATA-3 expression in both peripheral blood mononuclear cells (PBMC) and CD4 in immediate reaction (IR). (B) Correlation scatter plots of IFN- γ with T-bet in both PBMC and CD4 in delayed reaction (DR).

Table 2. Cytokine and transcription factor expression in PBMC, CD4 and CD8 T cells from patients with IR and DR at T1 and T2

	Immediate						Delayed					
	PBMC			CD4			CD8			PBMC		
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
IFN- γ	0.11 (0.025–0.2)	0.084 (0.04–0.12)	0.073 (0.008–0.097)	0.015 (0.014–0.03)	0.02 (0.08–0.06)	0.04 (0.027–0.072)	0.28 (0.12–0.77)	0.11 (0.04–0.29)	0.28 (0.17–0.53)	0.025 (0.04–0.18)	0.23 (0.13–0.39)	0.0001 (0.00001–0.1)
TNF- α	0.21 (0.067–0.33)	0.163 (0.08–0.24)	0.045 (0.017–0.21)	0.037 (0.018–0.06)	0.14 (0.1–0.32)	0.09 (0.07–0.22)	0.48 (0.18–0.88)	0.22 (0.13–0.44)	0.48 (0.23–1.03)	0.09 (0.18–0.12)	0.31 (0.14–1.37)	0.037 (0.01–0.23)
IL-4	0.15 (0.06–0.52)	0.068 (0.006–0.1)	0.95 (0.72–1.45)	0.17 (0.14–0.36)	0.124 (0.11–0.73)	0.125 (0.11–0.53)	0.073 (0.05–0.14)	0.082 (0.06–0.12)	0.19 (0.01–0.45)	0.002 (0.001–0.009)	0.24 (0.13–0.37)	0.25 (0.18–1.06)
T-bet	0.78 (0.66–1.07)	0.48 (0.34–0.95)	0.42 (0.28–0.58)	0.33 (0.19–0.52)	0.195 (0.19–0.43)	0.177 (0.17–0.21)	1.65 (1.26–2.14)	0.59 (0.14–0.65)	1.79 (1.11–4.1)	0.15 (0.02–1.5)	0.68 (0.3–1.18)	0.064 (0.11–0.16)
c-Maf	0.9 (0.6–1.23)	0.25 (0.001–0.68)	1.45 (0.97–2.94)	0.02 (0.013–0.04)	0.21 (0.09–0.35)	0.26 (0.22–0.29)	0.22 (0.001–0.45)	0.19 (0.002–0.49)	0.15 (0.062–0.42)	0.3 (0.22–0.54)	0.16 (0.1–0.49)	0.73 (0.05–0.21)
GATA-3	0.74 (0.33–0.19)	0.49 (0.36–1.1)	1.02 (0.62–1.39)	0.092 (0.01–0.2)	0.097 (0.05–0.25)	0.154 (0.06–0.27)	0.32 (0.26–0.5)	0.92 (0.21–3.7)	0.055 (0.02–0.16)	0.24 (0.09–0.53)	0.168 (0.07–0.26)	0.083 (0.028–0.36)

All data are represented as median and interquartile range (25–75). PBMC, peripheral blood mononuclear cells; IR, immediate reaction; DR, delayed reactions; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin. Significant differences ($P < 0.05$) by Wilcoxon test are shown in bold.

In IR, there was a significant increase of IL-4 in PBMC and CD4 T cells ($P = 0.003$ and $P = 0.028$, respectively) at T1. The c-Maf expression was increased in PBMC ($P = 0.05$) and in CD4 lymphocytes ($P = 0.028$), whereas GATA-3 expression was only significantly increased in CD4 cells ($P = 0.028$) (Table 2).

Discussion

The induction of allergic diseases depends on the coordinated regulation of many genes, whose products determine, among other processes, cytokine synthesis. The expression of gene products is regulated at multiple levels, with transcription factors being a key element in the tightly regulated mechanisms governing Th1 and Th2 cell differentiation and the maintenance of the corresponding immune response (16). T-bet plays a key role for the development of Th1 cells producing IFN- γ (17) and represses the production of Th2 cytokines, IL-4 and IL-5 (13). The proto-oncogene c-Maf is mainly expressed by Th2 cells, but, although c-Maf is specific for IL-4, it is not sufficient (18). A more important transcription factor for Th2 differentiation is GATA-3, which inhibits the production of IFN- γ , increases the transactivation of the IL-4 promoter, and directly regulates IL-5 and IL-13 expression (13, 19).

An imbalance between the Th1-specific T-bet and the Th2-specific GATA-3 and c-Maf transcription factors can result in several immunological diseases (20–25). T-bet is increased in some Th1 diseases such as celiac, Crohn’s disease and multiple sclerosis (20–22). In Th2 allergic diseases, GATA-3 is increased in patients with allergic rhinitis (25), and both GATA-3 and c-Maf in asthma (23). However, although in ADR both Th1 and Th2 responses can be involved, we are unaware of any study analysing the relationships between these transcription factors and cytokines with the exception of contact dermatitis where T-bet plays an important role (26).

The results of the present study on cytokine expression in PBMC showed a Th1 pattern with a high expression of IFN- γ and TNF- α in DR. However, the Th2 pattern was not so well defined in IL-4 expression in IR. Additionally, analysis of different T-cell subsets showed a Th1 pattern in both CD4 and CD8 cells for DR but a Th2 pattern, which did not appear in PBMCs, in just CD4 T cells in IR. Previous studies developed not only with mRNA gene expression (9, 27) but also with cytokine protein determination by flow cytometry (8) and by enzyme-linked immunosorbent assay (9), showed a similar pattern, although with lower sensitivity. This work supports those data, with additional information about the T-cell subpopulations involved. These cytokine profiles have not been so clearly found before because studies have used T cells without specifically examining subpopulations, and this study shows that this can be a limitation in the final analysis of the results (11, 12). On the other hand,

in vitro cell stimulation conditions, ionomycin, phorbol 12-myristate 13-acetate and the time of incubation can alter cytokine production, even if the analysis is performed in different T-cell subsets (10, 11). The approach followed in our study was to observe the different T-cell subpopulations involved by obtaining lymphocytes from peripheral blood, with no other additional intervention, to verify in detail the nature of the ongoing process.

Our results confirm the Th1/Th2 paradigm with the involvement of the corresponding transcription factors. In DR, a Th1 pattern with T-bet in PBMC and in both CD4 and CD8 T cells was observed, and in IR, a Th2 pattern with c-Maf in PBMC and CD4 T cells. For GATA-3, differences were only detected in the CD4 T-cells, although studies examining PBMC have shown the importance of GATA-3 in the induction of Th2 responses (19, 24, 25). This could be explained by compensation in PBMC levels with a decrease in CD8 or because the expression of GATA-3 is earlier than the induction of c-Maf (28) and our samples were collected after GATA-3 peaked.

This study also confirms previous results showing that the production of different cytokines is correlated with the severity of ADR (9). However, in the more severe reactions we also found a higher expression of T-bet (DR) and of both c-Maf and GATA-3 (IR). Subpopulation analysis in IR only showed differences in the CD4 T cells, whereas in DR these differences depended on the clinical manifestation, with higher levels of T-bet in CD4 for moderate reactions and in CD8 T-cells for severe reactions. Examination of the relationship between the severity of the reaction (MPE vs SJS/TEN) showed that CD4 is mainly involved in MPE (5) and CD8 in SJS/TEN (7). These differences are more marked for T-bet. Although T-bet is mainly produced in CD4 T-cells, this transcription factor is also required for the differentiation of naïve CD8 T lymphocytes into cytotoxic effector cells (29).

We also found significant positive correlations between cytokines and transcription factors, as reported by others (13, 30, 31). In IR, we observed a correlation between

IL-4 and c-Maf and GATA-3 expression, and between GATA-3 and c-Maf, and in DR between both IFN- γ and TNF- α and T-bet. A correlation has previously been shown between IFN- γ and T-bet in PBMC from patients with rheumatoid arthritis (30) and in CD4 T cells from allergic patients (31). We are unaware of the explanation for the positive correlation between TNF- α and T-bet. It may be as a result of two coexisting pathological phenomena: one related to the severity (TNF- α) and the other to the degree of involvement of the Th1 immunological response (T-bet).

Although drugs can affect several parameters of the immune response, including cytokine levels (32, 33), in the absence of immunological manifestations, no such effect was observed in our control group. Nevertheless, the results were not epiphenomena as cytokines and transcription factors all returned to levels detected in controls during the resolution phase of the disease in both DR and IR.

This is the first study to demonstrate the potential involvement of various transcription factors in ADR and their association with key Th1 and Th2 cytokines. We also show the relevance of monitoring the reactions as a tool for assessing the mechanisms involved. These data emphasize that, as suspected, IR show a homogeneous pathophysiological mechanism whereas in DR each clinical entity is a consequence of different mechanisms with the involvement of a cell subpopulation. This raises the need to analyse the different T-cell markers in the particular T-cell populations (CD4 or CD8). Understanding the cytokine and the transcription factors involved will enable development of immunological intervention to control the ongoing processes.

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