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Title: Subjects with allergic reactions to drugs show in vivo polarized patterns of cytokine expression depending on the chronology of the clinical reaction

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Abstract

Background: The mechanisms involved in adverse drug reactions with an immunologic basis (ADRIB) can be antibody dependent, mainly IgE or T cell dependent (sensitized T cells). These mechanisms are regulated by a number of cytokines, including IL-2, IL-4, IL-5, IFN-γ, and TNF-α, which follow the classical T_H1/T_H2 immunologic paradigm. Although evidence for this has been seen in ex vivo studies, the results are heterogeneous, and few in vivo studies have been carried out in subjects with ADRIB.

Objective: We studied a group of patients who experienced either immediate reactions (n = 10) or nonimmediate reactions (n = 9) to drugs to determine the cytokine pattern profile during the acute stage of the response, as well as after recovery.

Methods: PBMCs were taken at different time intervals of 24 hours or less and 7, 15, and 30 days after the onset of the reaction, and the specific cytokine transcription and production were determined by using quantitative competitive RT-PCR and ELISA, respectively.

Results: There was a transient polarized pattern corresponding to a T_H1 response with IL-2, IFN-γ, and TNF-α in nonimmediate reactions and to a T_H2 response with IL-4 in immediate reactions.

Conclusions: This is the first in vivo demonstration of these T_H1/T_H2 patterns in subjects with ADRIB and confirms that çan immunologic process is occurring related to the mechanisms involved in the pathologic manifestation. These findings are relevant to the understanding of the pathophysiologic mechanisms involved in ADRIB, suggesting that further studies in this direction are warranted.

Key words: *Allergy, drug reactions,* T_H1, T_H2, *cytokines*

Abbreviation used

ADRIB: Adverse drug reactions with an immunologic basis

Introduction

Two types of specific immunologic responses to drugs can be identified: those induced by antibodies and those caused by effector T cells (cytotoxic T ymphocytes).[1,2] In general, the clinical symptoms are pathognomonic for each response, with IgE-mediated responses producing immediate reactions, and T cell–mediated responses producing contact eczema or other types of cutaneous reactions, such as drug-induced exanthema, Steven-Johnson syndrome, or toxic epidermal necrolysis.[3-5]

IgE-specific reactions represent the best-studied model of drug allergy in human subjects, with penicillin (β-lactams) being the most frequent drug involved.3 However, there are a number of reactions induced by drugs that are nonantibody-dependent, and it is believed that T-cell involvement takes part in the immunologic response, including the effector phase.[3,5] Although the role of lymphocytes is relevant in the pathophysiology of these reactions, the precise mechanism by which they participate needs to be clarified.

Although different organs can be involved in adverse drug reactions with an immunologic basis (ADRIB), the skin is the most common target.[5] Most studies of ADRIB have been made in vitro, and therefore despite the great amount of basic evidence,[6-8] few data from in vivo studies are available.[4,9] This contrasts with the number of other studies monitoring the immunologic and allergic responses in human subjects.[10-12] Changes in lymphocyte subpopulations, increases in cytokine expression, and the presence of activation markers in T cells have been observed in different allergic diseases.[13-15]

Our group, as well as others, have recently provided information about the activation of PBMCs in ADRIB.[9] Increased activation markers are expressed in T cells at the moment of the acute reaction in both peripheral blood and the affected skin, becoming normal with time.[4,6,9,16,17] However, it can be considered that the in vitro studies are

complementary to the in vivo findings, although further research is required to determine the relation between these two models.[6,8]

ADRIB are considered to be inflammatory responses with participation of the immunologic system and can be divided into two categories according to a well-established paradigm: T_H1 and T_H2 responses.[18] T_H1 responses are characterized by the production of IL-2 and IFN-γ and induce T cell–mediated reactions, such as viral or intracellular bacterial infections. T_H2 responses are characterized by the production of IL-4 and IL-5 and are responsible for antibody production, including IgE. Although this classification has proved to be more complex,[19] it is considered acceptable for the understanding of the immunologic response.[18] Despite these considerations, because not much is known about in vivo immunologic responses to drugs, we studied the phenotype response in PBMCs from subjects with T_H1 and T_H2 patterns in ADRIB.

We examined a well-characterized group of subjects with two different types of ADRIB to determine the degree of involvement of cytokines and their expression and production in PBMCs and serum. A competitive RT-PCR and ELISA were used to quantitate the specific transcription and production of several cytokines (IL-2, IL-4, IL-5, and IFN-γ) that have been reported to be relevant in the T_H1/T_H2 response and also TNF-α in inflammatory processes.

METHODS

Patient selection

A total of 19 subjects who had an ADRIB were included in the study. Ten (mean age, 45.80 ± 8.7 years) had an immediate reaction after drug intake, and 9 (mean age, 43.89 ± 12.86 years) had a nonimmediate response. The criterion for considering an immediate reaction was the appearance of urticaria or anaphylactic shock within 30 minutes of drug intake. The criterion for diagnosing a nonimmediate reaction was the development of classical cutaneous lesions 24 hours or more after drug intake. The clinical reactions involved are shown in Tables I and II. In the immediate reaction group subjects 3, 4, 6, and 7 had a reaction on two occasions; subjects 1, 8, and 9 had a positive skin test response to β-lactams; and subjects 2, 5, and 10 had a response after challenge with the drug involved in the reaction. For those patients taking corticoids, these were administered after the first blood extraction. Patients 1, 2, 5, 7, 8, and 10 were administered a tapering dose of 80 mg of prednisone over 1 week. In all patients 6 mg/d diphenhydramine was also administered for 1 week. In the nonimmediate reaction group patients 3, 7, 8, and 9 had a positive patch test response 48 hours after drug administration. Patients 1, 5, and 6 were not challenged because of the risk of inducing a similar reaction to the one reported, and patients 2 and 4 had reactions on two occasions. In patients 1, 2, 3, 5, 6, 7, and 9 corticoids were administered to control the reaction. Tapering doses of 80 mg of prednisone administered 3 times daily were given over 18 days to patient 1 and over 12 days to patients 5 and 6. Patients 2, 3, 7, and 9 received a total of 500 mg of prednisolone over 1 week. Patients 4 and 8 were not treated with corticoids, and their symptoms improved satisfactorily after drug withdrawal and administration of antihistamines.

Control subjects

To assess whether the drug itself could induce any change in cytokine expression as a result of pharmacologic or other mechanisms, a group of 9 subjects were administered the same drugs as those involved in the reactions. These drugs are the same as those mentioned in Tables I and II. Because a total of 10 different drugs were involved, in one control subject the experiment was repeated twice at different times, once with amoxicillin and once with ampicillin. None of these control subjects had a previous history of ADRIB or presented any cutaneous or immunologic diseases, including atopy, at the moment of selection.

Sample collection

In all patients and control subjects, 4 blood samples were obtained at 24 hours or earlier and 7, 15, and 30 days after the reaction. In each sample 20 mL of peripheral blood was obtained. Ten milliliters in EDTA were processed immediately for RNA extraction, complementary (c)DNA synthesis, and competitive RT-PCR analysis, and 10 mL were obtained in vacutainer tubes for ELISA analysis.

PBMC isolation, RNA isolation, and cDNA synthesis

PBMCs were isolated on Ficoll-Paque (Pharmacia Biotech Inc, Piscataway, NJ) gradients. Total RNA was isolated from mononuclear cells by the method of Chomczynsky and Sacchi.[20] Total RNA was incubated for 15 minutes at 37°C with Dnase I (Boehringer Mannheim Corp, Indianapolis, Ind). Rnase inhibitor was present during all enzymatic manipulations of RNA. Concentrations of total RNA were determined in a spectrophotometer (Ultrospec III; Pharmacia LKB, Uppsala, Sweden), and the OD260/280 ratio was between 1.8 and 2.2 in all samples. The cDNA template for RT-PCR was synthesized from RNA by using reverse transcription with a first-strand cDNA Synthesis Kit (Clontech Laboratories, Palo Alto, Calif). In all samples the quantity of total RNA that was retrotranscribed was 1 μg per reaction. For comparison of cytokine messenger RNA, cDNA concentrations were normalized to

yield equivalent β-actin products (house-keeping gene). Results with this control amplification can be used to adjust the amount of the cDNA used in the PCR to use equivalent amounts of cDNA in each reaction.

PCR

PCR was performed with a mixture containing 10 mmol/L TRIS-HCl (ph 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.2 mmol/L deoxyribonucleoside triphosphate, 0.4 μmol/L 5′ and 3′oligonucleotide-specific primers, and 2 U of Taq polymerase (Boehringer Mannheim Corp). Aliquots were then amplified by 35 cycles in a GeneAmp 9600 thermocycler (Perkin-Elmer Corp, Irvine, Calif). Each cycle consisted of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 2 minutes. Aliquots of PCR products were electrophoresed in 1.8% agarose gels and visualized by means of ethidium bromide staining. The sequences of 5′ and 3′ cytokine-specific primer pairs were provided by Clontech Laboratories.[21-23]

Competitive PCR for cytokines

To determine the number of molecules expressing IL-2, IL-4, IL-5, IFN-γ, and TNF-α, we used competitive PCR experiments. In this method a DNA competitor containing the same primer template sequences as the target cDNA competes for primer binding and amplification. To quantitate the relative amounts of gene transcripts present in various samples, the individual PCRs were performed by coamplifying the cDNA of interest with an internal PCR control. Differences as low as 2-fold in cytokine gene expression may be detected by using this competitive template technique. The competitor DNA fragment (higher molecular weight) was derived from the *v-erb* B gene (Clontech laboratories) to which the primer templates had been added. The amplification product of each synthetic competitor differs in size from the original cDNA product. Using the competitor fragment as an internal standard in RT-PCR allows amplification of both the wild-type original DNA and the competitor fragment

DNA in the same reaction by using gene-specific primers and separating the products on the basis of size. At similar concentrations of the PCR product, comparable band intensities will occur in the ethidium bromide gel electrophoresis. The target cDNA was adjusted to equal concentrations by competitive PCR between β-actin cDNA and competitor fragment.[24] Evaluation of competitive RT-PCR experiments was performed on digitalized agarose gels by using image analysis software (TDI 1D Manager; Technology for Research and Diagnosis, Madrid, Spain). The detection limit of this method was determined by using serial dilutions of a known concentration of positive control cDNA, and after PCR optimal amplification, this was equivalent to 200 molecules of competitor fragment.

ELISA analysis of cytokines

IL-2 and IL-5 levels were determined by using a sandwich assay (Chemicon International, La Jolla, Calif), and the detection limit for both was 7.8 pg/mL. Absorbance was read at 492 nm. IL-4, IFN-γ, and TNF-α were also determined by using sandwich assays (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service). The lower limits of detection were 0.6 pg/mL for IL-4, 2.5 pg/mL for IFN-γ, and 2.5 pg/mL for TNF-α. Absorbance was read at 450 nm (Labsystems Oy, Helsinki, Finland).

Statistical studies

The values obtained with competitive PCR and ELISA of the different cytokines determined in the assay were correlated by using the Pearson correlation coefficient. The values of the different cytokines obtained by using either PCR or ELISA were also compared by means of bivariate analysis. A nonparametric test (Kruskall-Wallis) was used for the analysis of the cytokine levels at 24 hours or less and 7, 15, and 30 days. To be able to correlate the cytokine levels with the severity of the disease, we classified immediate reactions as severe (+++), intermediate (++), or mild (+) and nonimmediate

reaction as extremely severe (++++), severe (+++), intermediate (++), or mild (+). These were compared by using the Spearman rank correlation coefficient.

RESULTS

Comparison between RT-PCR and ELISA quantitation of the cytokines

Signals were detected in almost all instances of PCR analysis but in only some of the ELISA determinations. IL-4 was detected by using ELISA in 6 patients and competitive RT-PCR in all but one. The Pearson correlation coefficient was 0.532 ($P < .001$). The correlation between IL-2 levels determined by ELISA and IL-2 expression by RT-PCR was 0.717 (P < .001). The correlation for IFN-γ was 0.836 (P < .01). The result for TNF-α was 0.43 (P < .001).

Comparison between synergistic and antagonistic cytokines by RT-PCR

This relationship was examined by pooling the immediate and nonimmediate groups together at the 24 hours or less determination point. There was a very high correlation between IFN-γ and TNF-α, with a Pearson value of 0.99 ($P < .001$; Fig 1, *A*). There was a very high association between IL-2 and IFN-γ (Pearson value, 0.99; $P < .001$; Fig 1, *B*). A positive association was also found between IL-2 and TNF-α (Pearson value, 0.99; $P < .001$). The relationship between IFN-γ and IL-4 (Fig 1, *C*) showed that in the samples where IFN-γ was expressed, IL-4 was absent and vice versa. The Pearson value was –0.99, with a negative association ($P < .001$). The same polarized pattern was seen between TNF-α and IL-4 (Pearson value, –0.99; $P < .001$), but when IL-2 was related with IL-4, the association was similar to that found between IFN-γ and IL-4 (Pearson value, 0.99; $P < .001$). These results confirm that those cytokines with synergistic effects are positively associated, whereas those with opposing effects are negatively associated.

Sequential follow-up of cytokines in immediate reactions

The values of IL-4 in immediate reactions are presented in Fig 2, *A*. There was an initial increase in IL-4 levels that tended to normalize at 30 days, by which time levels were similar to those found in the control group. TNF-α levels were not detected at the 24 hours or less determination point or at 7 or 15 days, but they were detected at 30 days, being similar to those found in the control group (Fig 2, *B*). IFN-γ, IL-2, and IL-5 levels were not detected in any samples.

Sequential follow-up of cytokines in nonimmediate reactions

Fig 3, *A*, presents the sequential IFN-γ expression at the 4 determination points. There was a tendency to decrease with nondetection at day 30. The TNF-α expression showed a similar initial pattern (Fig 3, *B*), although it was still detected at day 30, being similar to that found in the control group. The expression of IL-2, which was only detected at the first determination point, is shown in Fig 3, *C*. IL-4 was not detected at 24 hours or less or at 7 or 15 days, but it was expressed in all patients at 30 days. The values for the control group at 24 hours or less were as follows: IFN-γ, 766.67 ± 444.41 molecules; IL-4, 1066.67 ± 132.29 molecules; and TNF-α, 1166.67 ± 427.20 molecules. IL-2 and IL-5 were not detected.

Comparison between cytokine levels and severity of the disease

The comparison between levels of IL-4 expression and disease severity in immediate reactions showed a nonsignificant association ($r_s = -0.2444$, $P < .903$), and the comparison between TNF-α expression levels and the severity of the disease in nonimmediate reactions was also not significant ($r_s = 0.169$, $P < .662$). The comparison of the other cytokines expressed in nonimmediate reactions and severity of the disease was also not significant.

DISCUSSION

Identification of the mechanisms involved in ADRIB is often difficult, with a number of questions remaining unanswered.[1,2,25] Unlike classical allergens, drugs are low-molecular-weight structures that require a protein or peptide for interaction with the immunologic system, although direct interaction of the drug with class I or class II molecules has also been reported.[26] Drugs can therefore be processed by antigen-presenting cells or interact directly with the molecules involved in the recognition processes.[27] In addition, covalent and noncovalent hapten protein binding may exist before the immunologic response occurs.[7]

Important progress has been made in recent years in understanding drug processing and recognition by T cells.[8] The development of T-cell lines and T-cell clones has shown that hapten recognition is specific and that a heterogeneous pattern of cytokine production can be observed in ADRIB. For example, in T-cell responses to β-lactams, specific clones for penicillin G were CD4+ and CD8+, whereas clones recognizing penicillin conjugated to human serum albumin were only CD8+, there being a heterogeneous cytokine pattern with IL-2, IFN-γ, and TNF-α and variable levels of IL-4 and IL-5.[7,8] In individuals allergic to sulfonamides, phenytoin, and carbamazepine, CD4 and CD8 cells expressed high levels of CD25 and HLA-DR molecules after drug stimulation in vitro and secreted high amounts of IL-5 and normal or low levels of IL-2, IFN-γ, IL-4, and TNF-α.[6]

Although a number of in vitro findings have been reported, their responses do not always mimic the in vivo situation, and discrepancies exist.[28] Therefore as part of an ongoing strategy for a better understanding of ADRIB, we assessed the cytokine expression in PBMCs from subjects with immediate and nonimmediate reactions to drugs. The approach consisted of quantitating by both RT-PCR and ELISA the expression and production of a number of relevant cytokines with an important role

in the regulation and induction of the immune response. Although there are a number of limitations with the sensitivity of ELISA,[28,29] the use of both methodologies was a more complete approach.

The clinical data and the laboratory findings confirmed that the reactions were IgE dependent (Table I) or mediated by T lymphocytes (Table II). The pattern of expression in nonimmediate reactions showed an overexpression of IL-2, IFN-γ, and TNF-α, with no expression of IL-4, paralleling the clinical course of the disease. The kinetic expression showed that IL-2 normalized early in the immunologic response, whereas the other cytokines were still elevated after 15 days. This supports a T_H1 phenotype response. When we looked at immediate reactions, there was an elevated expression and production of IL-4 at the first determination within 24 hours, decreasing over time to become similar to that found in the control group by day 30, whereas the other cytokines studied were neither produced nor expressed. This suggests a T_H2 phenotype response. Comparison between cytokine levels and disease severity in both immediate and nonimmediate reactions showed no significant association.

Because the sample size is small, these data cannot be extrapolated further. In this study we used two nonidentical but complementary methods for estimating cytokine production, ELISA, and RT-PCR.[6,7] Different cells comprising T cells, natural killer cells, and monocytes may contribute to the cytokine expression in PBMCs.[29]

Cytokine quantitation by ELISA in sera reflects the number of molecules sent to circulation by different cells located in activated tissue or peripheral blood cells, and this comparison may be useful, although ELISA has limitations in sensitivity.[6,7] In our study there was good agreement and correlation between competitive RT-PCR and ELISA, although the pattern was detected better by the former. These data differ from those of other studies in which T-cell lines and T-cell clones from subjects with immediate and nonimmediate reactions have shown different patterns of cytokine

production not related with the T_H1/T_H2 phenotype.[6,7,30] Our observations in PBMCs represent the expression of the most predominant tendency to which different clones contribute interdependently.[18] However, these in vivo data may have some limitations because we do not know which T-cell subset or other cell is involved, although we assume that CD3+ and CD45RO+ memory cells are the most probable candidates, as suggested by others.[18,31,32] An aspect not included in the design of this study was whether the T_H1/T_H2 response was limited to CD4, the equivalent subsets at the CD8 subpopulation identified as T_c1/T_c2 according to the cytokines they secrete,[33] or both, although these studies are now being considered.[18] We have recently undertaken a different type of study concerning the T-cell response in toxic epidermal necrolysis showing that both CD4 and CD8 are activated and participate in this skin inflammatory response.[4]

It is relevant to mention the role of TNF-α in nonimmediate reactions. This cytokine has been reported to bind to different receptors in the extracellular matrix and to induce adhesion and activation of T cells and monocytes.[34,35] This could explain the activity of this cytokine in the skin of the patients reported herein.

Although eosinophilia and IL-5 have been reported in allergic drug reactions,[6] we observed no expression of IL-5 in our study, either in immediate or nonimmediate reactions. Because eosinophilia may be influenced by the administration of glucocorticoids,[36] all first determinations in our patients were taken before the initiation of glucocorticoid therapy when necessary, thus indicating that neither eosinophilia nor elevation of IL-5 was present in our patients. These results contrast with those of others indicating that eosinophilia and production of IL-5 is a distinctive marker.[6] However, because these data mainly come from in vitro studies, they are not necessarily comparable with or applicable to in vivo findings.

The role of glucocorticoids can influence the kinetics of cytokine expression during the follow-up period.[37,38] The data provided in this study, although limited in number, indicate that irrespective of whether patients were treated with corticoids, the expression pattern was similar. This suggests that a possible transitory resistance to the action of corticoids may exist, as has been described in other allergic pathologies, including ADRIB.[4,17,39] In vivo data have shown that administration of glucocorticoids in allergen-challenged asthmatic subjects does not influence the expression of IL-4, IL-2, or IFN-γ.[40-42]

As far as we know, this is the first evidence supporting the functional T_H1/T_H2 dichotomy expression in ADRIB, indicating that the clinical and laboratory findings are in good agreement with the pathophysiologic mechanism involved in the induction of these clinical entities. Further studies looking at lymphocyte subpopulations and other cytokines need to be carried out in addition to the parallel monitoring of the response in the skin lesion to understand better the pathophysiologic mechanism involved. This work is currently in progress in our laboratory.

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TABLE I. Clinical and immunologic characteristics in subjects with immediate reactions.

Patient No.	Sex	Age (y)	Clinical entity	Drug involved	Skin test response	RAST	Challenge	No. of episodes	Severity
1	F	47	Anaphylactic shock	Amoxicillin*	+	+	ND	1	+++
2	F	50	Anaphylactic shock	Diclofenac*	-	ND	+	1	+++
3	M	40	Urticaria/angioedema	Dipyrone	+	ND	ND	2	++
4	F	59	Anaphylactic shock	Amiodarone	ND	ND	ND	2	+++
5	F	36	Anaphylactic shock	Dipyrone*	-	-	+	1	+++
6	M	42	Urticaria/angioedema	Dipyrone	-	-	-	2	++
7	F	36	Urticaria	Ibuprofen*	ND	ND	ND	2	+
8	M	52	Anaphylactic shock	Amoxicillin*	+	+	ND	1	++
9	M	38	Urticaria/angioedema	Ampicillin	+	-	ND	1	++
10	F	58	Urticaria	Benzylpenicillin*	-	-	+	1	+

ND, Not determined.
*Subjects who took corticoids during the occurrence of the allergic episode.

TABLE II. Clinical and immunologic characteristics in subjects with nonimmediate reactions

Patient No.	Sex	Age (y)	Clinical entity	Drug involved	Skin test response	Severity	Challenge	No. of episodes
1	F	19	Lyell syndrome	Carbamazepine*	-	++++	ND	1
2	M	49	Desquamative exanthema	Hidantoin*	-	++	+	1
3	M	30	Exanthema	Amoxicillin*	+	+	+	2
4	M	49	Desquamative exanthema	Amiodarone	-	++	+	2
5	M	55	Steven-Johnson syndrome	Hidantoin*	-	+++	+	1
6	M	61	Steven-Johnson syndrome	Hidantoin*	-	+++	ND	1
7	F	45	Exanthema	Ceftriaxone*	+	+	+	2
8	F	48	Exanthema	Amoxicillin	+	+	+	3
9	M	39	Desquamative exanthema	Ampicillin*	+	++	+	1

ND, Not determined.
*Subjects who took corticoids during the occurrence of the allergic episode.

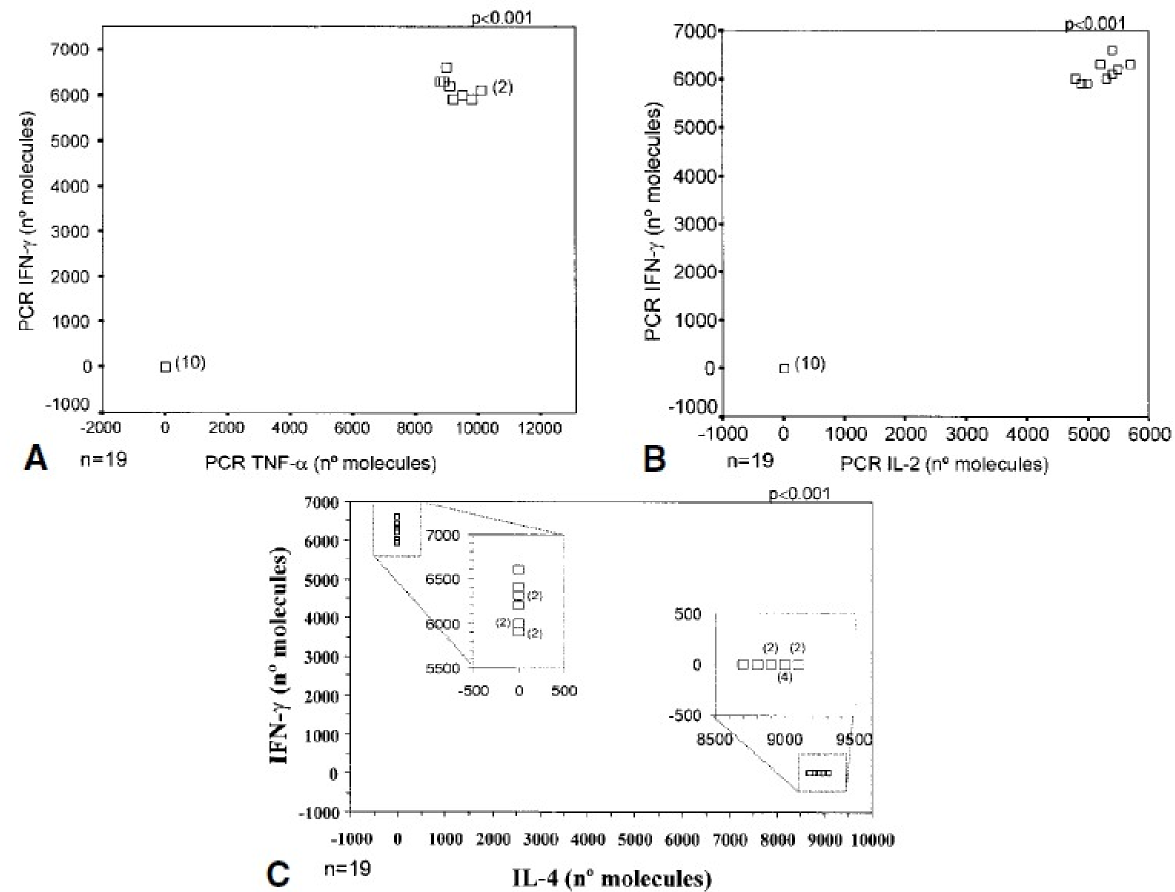
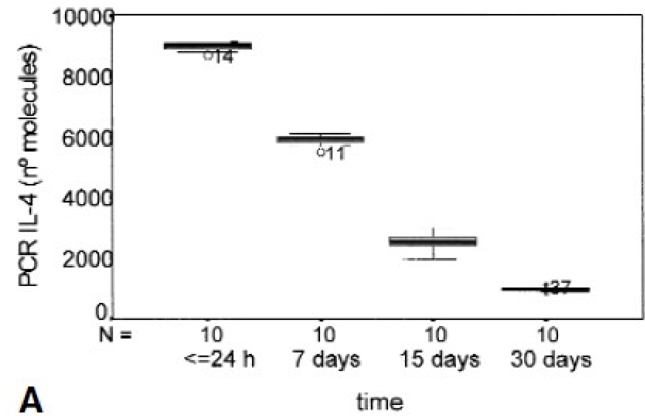


FIGURE 1. Scatter diagram showing the bivariate analysis between IFN-γ and TNF-α **(A)**, IFN-γ and IL-2 **(B)**, and IFN-γ and IL-4 **(C)** at 24 hours or less. The number of patients studied for each panel is 19. Points representing more than one patient are indicated with an adjacent numeral. In **A** and **B** there was a highly significant positive association ($P < .001$) between IFN-γ and TNF-α and between IFN-γ and IL-2, showing that these were agonist cytokines. In **C** there was a significant negative association between IFN-γ and IL-4 ($P < .001$).



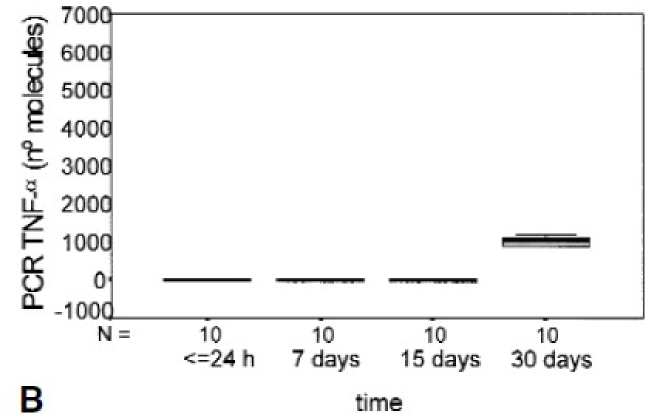


FIGURE 2. Sequential follow-up analysis of IL-4 **(A)** and TNF-α **(B)** in immediate reactions, as determined by quantitative competitive PCR. IL-4 expression decreased markedly from 24 hours or less to 30 days. At 30 days, expression was the same as in the control group. TNF-α was not detected at 24 hours or less or 7 or 15 days but was detected at 30 days, being similar to that found in the control group. *Boxes* enclose medians and interquartile ranges.

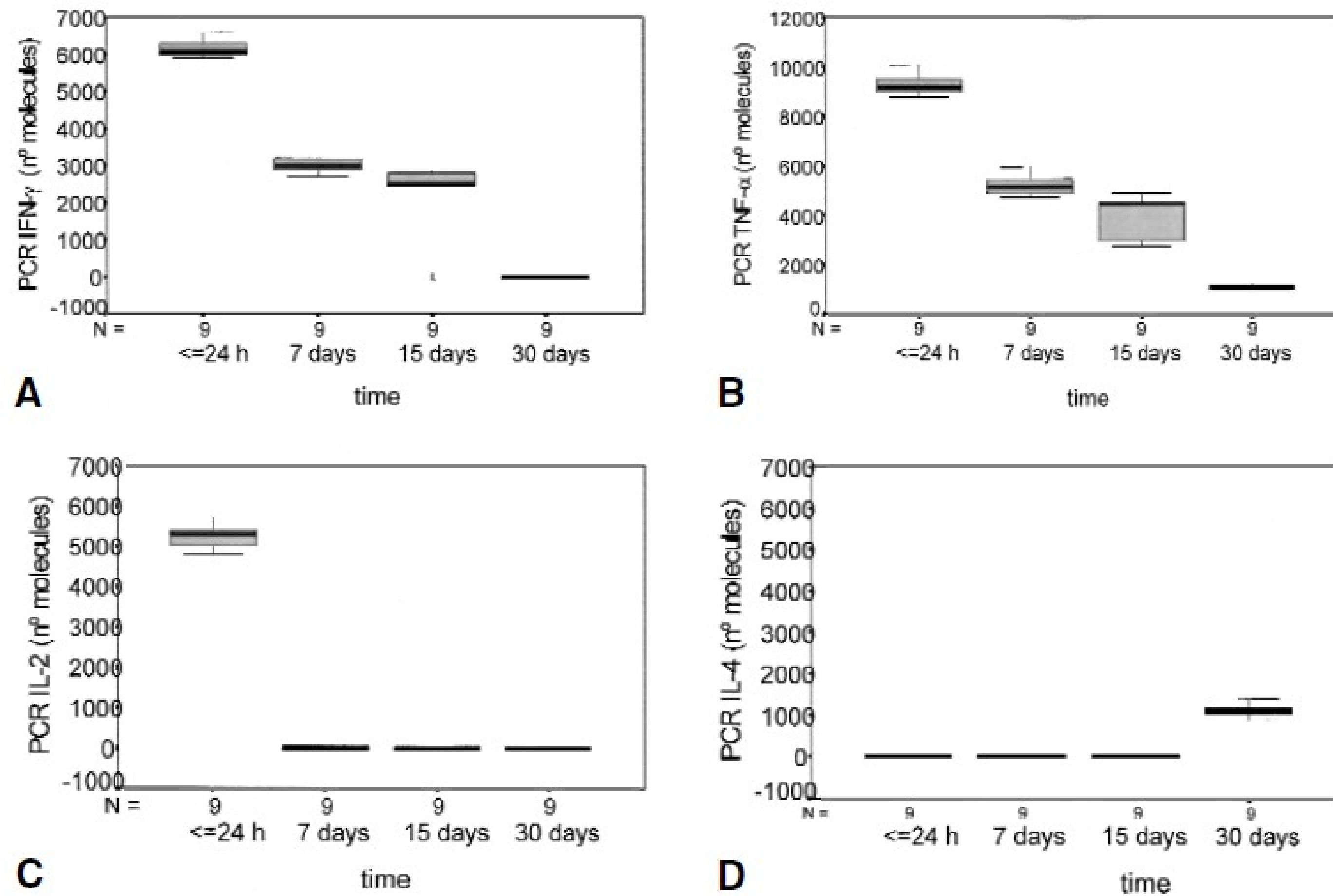


FIGURE 3. Sequential follow-up analysis of IFN-γ (A), TNF-α (B), IL-2 (C), and IL-4 (D) in nonimmediate reactions, as determined by quantitative competitive PCR. IFN-γ (A) was detected until day 15 and TNF-α (B) until day 30, at which time expression was similar to that found in the control subjects. IL-2 (C) was only detected at 24 hours or less and IL-4 (D) only at day 30, at which time IL-4 expression was similar to the control group. *Boxes* enclose medians and interquartile ranges.

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Abbreviations used

TEN:	Toxic Epidermal Necrolysis
CLA:	Cutaneous lymphocyte-associated antigen
IFN γ:	interferon gamma
TNF α:	tumor necrosis factor alpha
IL:	interleukin
MoAb:	Monoclonal antibodies
PBMC:	Peripheral blood mononuclear cells
VCAM-1:	vascular cell adhesion molecule-1
ICAM-1:	intercellular adhesion molecule-1
mRNA:	messenger ribonucleic acid
RT-PCR:	reverse transcription polymerase chain reaction
Lc:	Langerhans cells
GC:	glucocorticoids
GR:	glucocorticoid receptor
SBAP	steptavidin-biotin-alkaline phosphatase