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TH1/TH2 repolarization in induction of immune tolerance to non-steroidal anti-inflammatory drugs during the management of sickle cell disease vaso-occlusive crisis

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Abstract

Respiratory manifestations related to the intake of non-steroidal anti-inflammatory drugs (NSAIDs) during the treatment of the painful vaso-occlusive crisis of sickle cell disease are either a type I hypersensitivity mechanism of the Gell and Coombs classification, or a pharmacological mechanism of NSAIDs. The use of NSAIDs is essential in the Abidjan school because of the absence of therapeutic alternatives in the management of the inflammatory crisis of this disease. The induction of tolerance to NSAIDs initiated by the authors has had clear clinical success. The basic biological reasons for this tolerance were evaluated in this study. A group of 11 sickle cell patients aged 12 to 39 years in whom post-NSAID respiratory manifestations disappeared for at least 6 months following a short tolerance induction protocol with ibuprofen, was assayed by ELISA for TNFα, INFγ (Th1 cytokines), IL-4 (Th2 cytokine), IL-10, TGF-β (immunosuppressive cytokines) and total IgE, before induction or pre-induction (D-1) and at day one (D1), D2- 3, one month (M1), and M6 after induction. A repolarization of the Th1/Th2 balance was noted during the post induction period. The high concentration of IL-4 observed at D-1 gradually decreased in favor of the cytokines TNFα, INFγ. The decrease in cytokine IL-4 with the level of total IgE was accompanied by the increase of IL-10 and TGF-β demonstrating the regulatory role of these cytokines in the control of allergic diseases. In conclusion, the induction of immuno-tolerance to NSAIDs through a short protocol is well supported by immune regulation. The medium-term effects are real, unlike the results of allergen desensitization or specific immunotherapy. However, this protocol could be used in certain circumstances such as in the case of intolerance to trimethoprim-sulfamethoxazole, used as the treatment of choice for the prevention of opportunistic diseases in people living with human immunodeficiency virus.

Keywords: Tolerance induction, Immune-suppressive cytokines, Th1/Th2 Cytokines, NSAID.

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Introduction

Vaso-occlusive crises are one of the main manifestations of sickle cell disease. Its management in the Abidjan school necessarily involves the use of nonsteroidal anti-inflammatory drugs (NSAID), known for their mechanism of inhibition of the cyclooxygenase pathway during arachidonic acid metabolism.[1] This promotes the synthesis of leukotrienes which are broncho constrictors.[2-5] In atopic patients who suffer from sickle cell disease, 5% have shown proven respiratory signs related to taking NSAIDs.[3]

In the absence of therapeutic alternative for the management of this pain, an induction of immune tolerance to NSAIDs was carried out in 26 patients who presented intolerance to NSAIDs (Ibuprofen) and in the form of respiratory disorders. The result of this short 3-day protocol showed the complete disappearance of respiratory signs in 80% of patients.[6] Understanding the basic mechanisms of this tolerance induction led the authors to evaluate the profiles of cytokines involved in the immune response.

Subjects and Methods

Presentation of Patients

The study enrolled a total of 11 patients expressing following phenotypes of sickle cell disease (with hemoglobin phenotypes of SC, SSFA2, SFA2). They were 15 men and 7 women, aged from 12 to 39 years (age mean: 22.41 ± 7.88). They were selected from 1500 patients followed up in the Department of Immunology-Hematology and Allergology of CHU (Centre Hospitalo-Universitaire) Cocody-Abidjan. Patients were included after giving informed consent to the protocol approved (dated, February 3, 2014) by the ethical committee of the University Hospital of Cocody (n013-14/MSHPCMU/CECHU-COC-ak). None of the patients had taken antihistamine (within the last 3 days) or corticosteroid medication (within the last 14 days) before the procedure. All patients were known to have a documented history of at least one episode of adverse reactions following the intake of Ibuprofen (Table 1).

Table 1. Clinical syndromes induced by NSAID (Ibuprofen) intake before the tolerance induction procedure.

N	Age (Years)	Sex	Weight (Kg)	Hemoglobin phenotype	NSAID (Ibuprofen)	Clinical syndrom	Within the time limite
1	21	F	52	SC	Ibuprofen	Rhinitis/pruritus/angio-oedema	Within 15 min.
2	16	M	45	SC	Ibuprofen	Rhinitis/asthma	Within 15 to 30 min.
3	19	F	52	SC	Ibuprofen	Hypotension/dyspnoea	Within 15 min.
4	13	F	41	SAFA2	Ibuprofen	Angio-oedema	Up to 6 hours
5	30	M	58	SAFA2	Ibuprofen	Angio-oedema/dyspnoea	Within 15 min.
6	19	M	51	SC	Ibuprofen	Rhinitis/asthma	Within 15 min.
7	15	F	43	SSAFA2	Ibuprofen	Hypotension /dyspnoea	Up to 1 hour
8	30	M	52	SSAFA2	Ibuprofen	Rhinitis/asthma	Within 15 min.
9	33	M	57	SAFA2	Ibuprofen	Rhinitis/pruritus/angio-oedema	Within 15 min.
10	16	M	50	SC	Ibuprofen	Hypotension/dyspnoea	Within 15 min.
11	14	M	45	SSAFA2	Ibuprofen	Rhinitis/pruritus/angio-oedema	Within 15 to 30 min.

Sex: F = female; M = male. min.=minutes

Immune tolerance induction protocol:

As previously described according to the NSAID tolerance induction protocol [3], the procedure of tolerance induction was carried out at the Allergy Unit of the department of Immunology-Hematology and Allergology of CHU Cocody-Abidjan, in the 11 patients known to have documented history of at least one episode of adverse reactions following the intake of Ibuprofen. Before and during the tolerance procedure, cardiovascular parameters, naso ocular, pulmonary, and cutaneous symptoms were monitored in all patients. In patients with a history of bronchial asthma or respiratory symptoms induced after NSAID intake, a pulmonary function test was performed before the beginning of the procedure. The oral initial dose (diluted in distilled water) was the lowest that gave a positive reaction to the prick-test carried out before the procedure (8.82 mg of Ibuprofen). We gradually increased every 60 minutes to reach in 6 hours the effective therapeutic dose. The first day (D1), this last dose was renewed 6 hours later. The second (D2) and third (D3) days, the therapeutic dose has been orally administrated with an interval of 6 hours for 12 hours as indicated in Table 2.

Table 2. The rapid tolerance induction protocol to Ibuprofen.

Days (D)	Time of administration (Hour: H)	Cumulative doses of Ibuprofen (mg)
D1	H0	8.82
	H1	17.625
	H2	35.25
	H3	75
	H4	150
	H6	400
	H12	800
D2	H0	400
	H6	800
	H12	1200
D3	H0	400
	H6	800
	H12	1200

Skin Tests and Desensitization Evidence

Skin tests were performed by prick method in order to evaluate the sensitivity of each patient to NSAIDs (principally to Ibuprofen) and to determine minimal doses inducing reactions that will be taken as the initial dose in the tolerance induction protocol. Consequently, before the procedure and one (M1), six (M6) months after the procedure, daily doses of Ibuprofen (1200 mg) geometrically diluted with distilled water were tested in each patient. The results were compared to the histamine positive control (10 mg/ml) as well as to the distilled water negative control. We have limited the induction of tolerance to NSAID to the lack of clinical syndromes during the treatment of the painful crisis since skin test is not evidence of desensitization and the oral challenge test was not performed because of the severity of the induced clinical signs.

The follow up procedure

The clinical syndromes reported during the clinical history, physical examination and skin test were monitored on day two (D2), D3, M1 and M.[3] Despite that some cases of failure might be related to the severity of symptoms or possible patho-physiological mechanism, more than 80% of patients have successfully tolerated Ibuprofen. In fact, 72 hours after the end of the procedure, hypo responsiveness was induced in

76%, 84% and 88% of the patients respectively at D1, D2-D3. No objective symptoms appeared except for two cases. The first was case number 6 (no 6), who presented the Asthma/Rhinitis couple in pre-induction, saw the persistence of his rhinitis at D3 and an appearance of eczema at M1. The other, no 10 presented the hypotension/dyspnea syndrome in pre-induction. He reacted clinically with pruritus and rhinitis, respectively at D1 and M1. Whatever, all these 11 patients tolerated ibuprofen. In order to confront this clinical evolution with the fundamental data that could support them, we explored the different cytokine profiles before induction, at D1, D2, M1 and M6 after induction.

Assessment of cytokines and total IgE concentrations

-Preparation of samples and Standards

A total of 55 sera samples were collected from the 11 study patients, followed up over 6 months (Pre-induction, D1, D3, M1, M6) as previously described.[6] These samples were stored at -80°C for 8 years (2014 to 2022), were gradually thawed overnight at ambient laboratory temperature, then they were well homogenized. They were first diluted at 1:20. The Standards were gradually diluted according to the manufacture's instruction of each cytokine.

-Assessment methods

The Th2 cytokine, IL-4, was assessed using the commercial ELISA kits (Ref: KHC0041, Invitrogen™ ELISA kit for human IL-4, Fisher Scientific, France). The Th1 cytokines were assessed using commercial ELISA kits (two different kits for assessment of TNFα and IFNγ, respectively, Ref: KHC3014, Invitrogen™ Novex for human TNFα, Fisher Scientific, France, and Ref: 10617714, Invitrogen™ ELISA kit for human IFNγ, Fisher Scientific, France). For assessment of immune suppressive cytokines, commercial ELISA kits were used (Ref: 10434383, Invitrogen™ ELISA kit for human IL-10, Fisher Scientific UK, and Ref: 10701195, Human TGF-beta 1 DuoSet ELISA, Fisher Scientific, France). Finally, for Total IgE commercial ELISA kits were used (Ref: 15540957, Invitrogen™ Kit ELISA Ready-SET-Go!™ human IgE. Fisher Scientific, France).

All kits contained pre-coated 96 well plates, standards, assay diluent concentrate, biotinylated detection antibody, streptavidin-horseradish peroxidase, wash buffer, chromogen, stop solution, and adhesive plate covers.

All ELISA tests were assayed according to the manufacturer's instructions at the Immunology Laboratory (Immunopôle) of Abidjan Medicine Faculty. Briefly, the 96 wells of the supplied microplate were pre-coated by cytokine-specific antibodies. We added diluted samples, 25 to 100µl (according to the cytokine), standards, or controls into the wells and incubated the plates to allow binding to the immobilized (capture) antibody. The sandwich was formed by the addition of the second (detector) antibody, a substrate solution was added that reacts with the enzyme-antibody-target complex to produce measurable signal. The intensity of this colored signal was directly proportional to the concentration of target (cytokines or total IgE assayed in the sample and the control) present in the original specimen. The optical density of the final color was measured using a microplate reader (BioTek Epoch2® Microplate Readers, Agilent, USA). A standard curve was plotted of the concentration of the controls related to the optical density, lead to directly read the concentrations of the cytokines and IgE by extrapolation.

Statistical analysis

The comparison of total IgE concentrations distributions at different periods of the tolerance follow-up was done by applying the Friedman test. A significant level was considered at $p<0.05\%$.

Results

Clinical changes:

The clinical syndrome reported previously during the history, physical examination and skin test was monitored on D2, D3, M1 and M6.[3] Despite some cases of failure that might be related to the severity of symptoms or

possible patho-physiological mechanism, more than 80% of patients have successfully tolerated Ibuprofen. In fact, 72 hours after the end of the procedure, hypo responsiveness was induced in 76%, 84% and 88% of the patients respectively at D1, D2-D3. No objective symptoms appeared except for two cases. The first one, n 6, who presented the Asthma/Rhinitis couple in pre-induction, saw the persistence of his rhinitis at D3 and an appearance of eczema at M1. The other, n 10 presented the hypotension/dyspnea syndrome in pre-induction. He reacted clinically with pruritus and rhinitis respectively at D1 and M1. Whatever, these 11 patients all tolerated ibuprofen (Table 3).

Table 3. Clinical evolution during the induction of immune tolerance to NSAIDs (Ibuprofen).

N	Symptoma pre-induction	Symptoma at D1	Symptoma at D3	Symptoma at M1	Symptoma at M6
1	Rhinitis/pruritus/angio-oedema	none	none	none	none
2	Rhinitis/asthma	none	none	none	none
3	Hypotension/dyspnoea	none	none	none	none
4	Angio-oedema	none	none	none	none
5	Angio-oedema/dyspnoea	Epigastric pain	Epigastric pain	none	none
6	Asthma/rhinitis	Asthma/rhinitis	Rhinitis	Eczema	none
7	Hypotension/dyspnoea	Hypotension/dyspnoea	none	none	none
8	Rhinitis/asthma	none	none	none	none
9	Rhinitis/pruritus/angio-oedema	Angio-oedema	none	none	none
10	Hypotension/dyspnoea	Pruritus	none	Rhinitis	none
11	Rhinitis/pruritus/angio-oedema	none	none	none	none

Changes in Th1/Th2 and immune suppressive cytokines profile are shown in Figure 1: 1A, 1B. Diagrams in Figures 1 (A and B) express the simultaneous evolution (over the duration of tolerance on the abscissa), the concentration in ng/ml (on the ordinate) of Th1/Th2 cytokines (Figure 1A) and immunosuppressive cytokines (Figure 1B). The evolutionary trend of the level of Th1 cytokines (green line for INFγ and orange line for TNFα in Figure 1A) and Th2 cytokine (black line for IL-4 in Figure 1A) shows a significant drop in the secretion of the cytokine pro -Th2 (IL-4) from D1 to M6 in the 11 patients. This cytokine, strongly produced by patients, demonstrated the atopic context in which type I hypersensitivity reactions to ibuprofen occurred. On the other hand, there was a trend towards a relative increase in the level (ng/ml) of INFγ and TNFα at D1, D3, M1, M6. Concomitant with the evolution of the Th1/Th2 cytokines, that of the regulatory cytokines (blue line for IL-10 and red line for TGFβ in figure 1B) shows a trend towards increased levels (ng/ml) at D1, D3, M1 and M6.

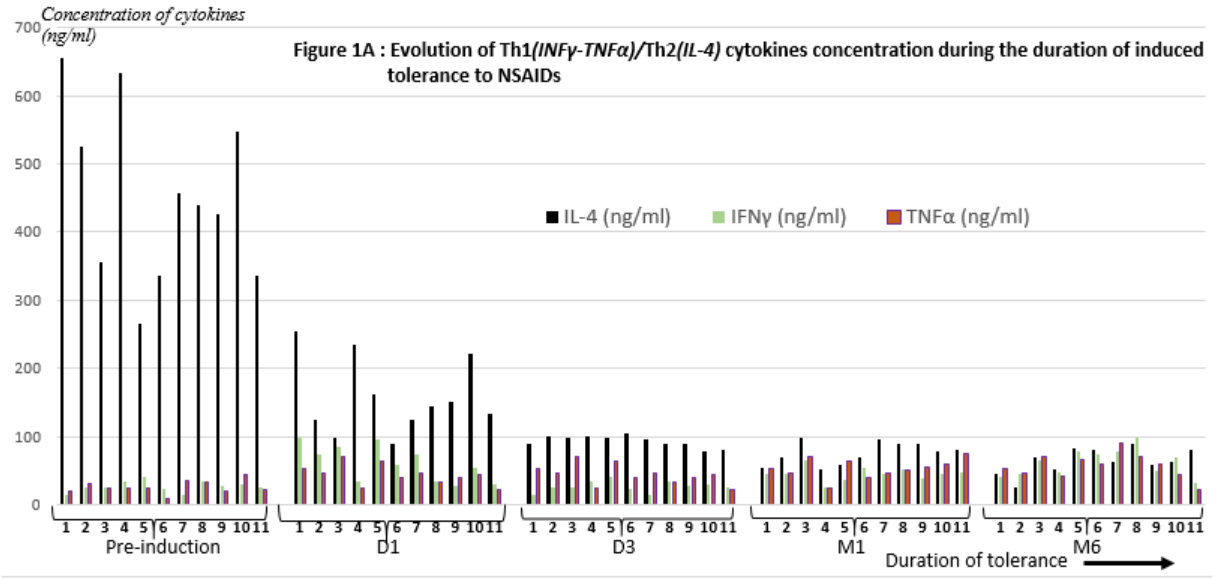

Figure 1A. Evolution of Th1/(IFNγ-TNFα)/Th2(IL-4) cytokines concentration during the duration of induced tolerance to NSAIDs.

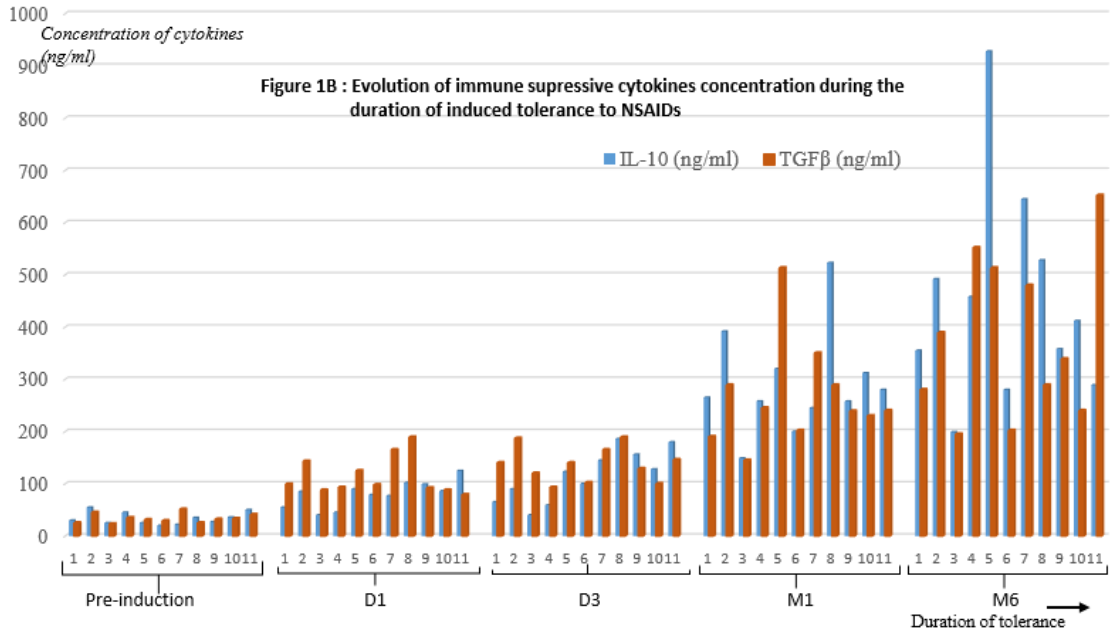

Figure 1B. Evolution of immune suppressive cytokines concentration during the duration of induced tolerance to NSAIDs.

Evolution of total IgE concentration:

The whisker diagram in Figure 2 expresses the variation in total IgE levels (in KUI/L on the ordinate) in the 11 patients at each follow-up period (Pre-induction, D1, D3, M1, M6, on the abscissa). The comparison of the means of the total IgE levels at each period showed a significant difference using the two-way analysis of variance by Friedman ranking for related samples ($p < 0.001$). There was a decrease in the level of total IgE during the tolerance period.

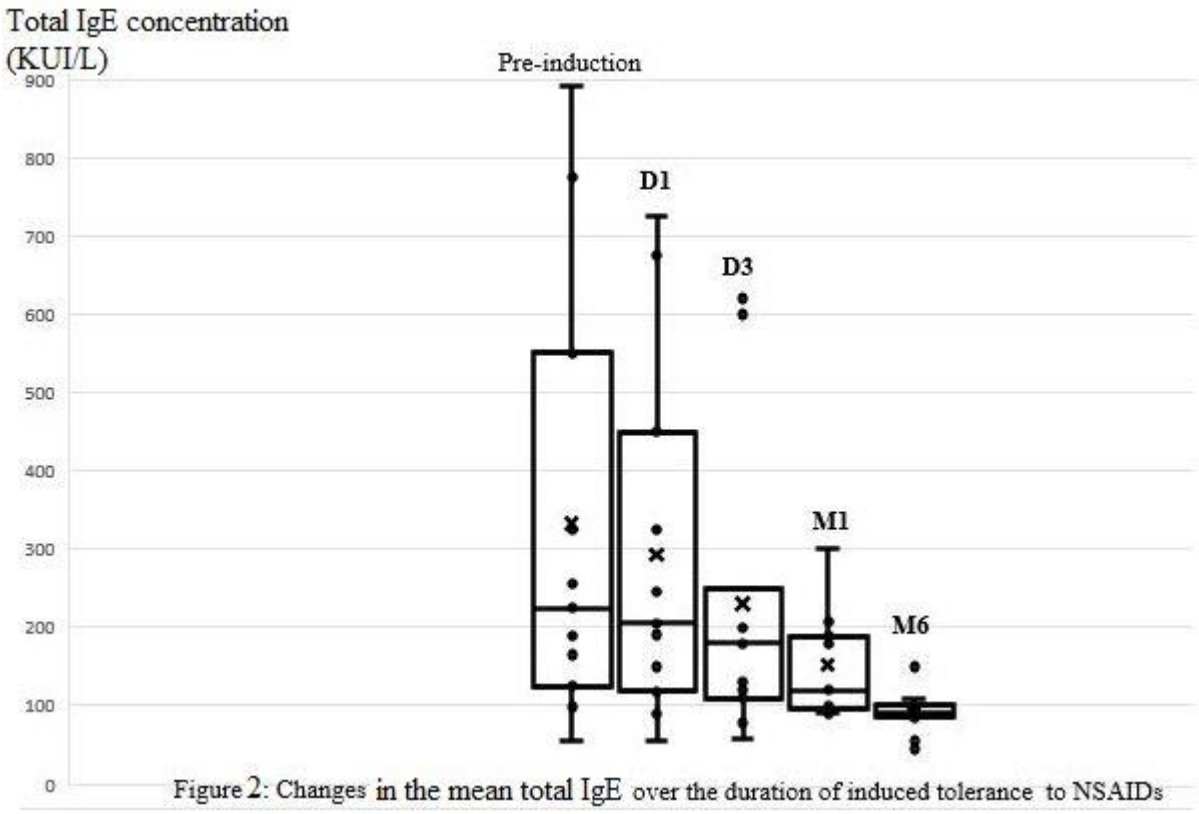

Figure 2. Changes in the mean total IgE over the duration of induced tolerance to NSAIDs.

Discussion

We have previously reported in an analytical study concerning sickle cell patients that 5% of patients showed respiratory signs after taking NSAIDs. These manifestations appeared in 80% of cases, less than 30 minutes and in 60% of cases, they yielded only under corticosteroid therapy and antihistamine drugs.[3] These results associated with data from other literature,[6;7] made us evoke a mechanism of type I hypersensitivity even if it is also known that the use of corticosteroid therapy in the consequences of inhibition of cyclooxygenases is justified.[8] NSAID-intolerant asthma frequently begins after the age of 30[9,10] and reactions to NSAIDs are not always immunological.[11]

The symptomatology found takes on a clinical form of anaphylaxis[6] to the point that it was dangerous to continue. Indeed, the involvement of inflammation in the painful vaso-occlusive crisis justifies the use of NSAIDs in the management of sickle cell disease.[12] The absence of a known alternative therapeutic that could be effective led us to set up a short three-day protocol to induce immune tolerance to NSAIDs such as Ibuprofen.[6]

During the clinical follow-up, the complete disappearance of the initial signs of intolerance was noted, as shown in Table III comparatively to Table I. Despite two cases of failure that we observed through the occurrence of rhinitis and eczema at M1, the protocol noted at M6, a success with a complete disappearance of the signs during the reintroduction of NSAIDs.

These data were confirmed by the negativity of the Ibuprofen rapid-reading cutaneous tests and the total IgE level which dropped considerably (Figure 2). These arguments demonstrate the drop in allergenic sensitization to Ibuprofen and is much more reminiscent of the type 1 hypersensitivity mechanism according to Gell and Coombs classification.[9] The absence of clinical signs after reintroduction of Ibuprofen reinforces the involvement of such a mechanism that supports allergenic

desensitization (immune tolerance) and cytokines Th1/Th2 balance.

Of the Th cell subsets, Th2 cells are established as the main Th cell subset that drives allergic tissue inflammation. The signature cytokines (IL-4, IL-5, and IL-13) that are produced during type 2 immune responses, are critical for protective immunity against infections of extracellular parasites and are responsible for asthma and many other allergic inflammatory diseases, but also it is responsible to instruct B cells to produce IgG1 and IgE.[13-16] These studies have identified a specific subset of Th2 cells that is intricately linked to allergic pathology.

Th1 and Th2 clones can be distinguished either by the profile cytokine produced, or through the expression of different patterns of cell surface molecules. On one hand, with regard to cytokine expression, Th1 cells make IFNγ and TNFα as their signature. On the other hand, Th2 cells fail to produce IFNγ or lymphotoxin and their signature cytokines IL-4, IL-5, and IL-13.[17]

Immune tolerance is the state of quiescence to self or to foreign antigens by multiple mechanisms. Dysregulated immune tolerance can lead among other disorders, to allergic diseases. Unresponsiveness to otherwise harmless, ubiquitous allergens is provided by peripheral tolerance mechanisms. From the allergy point of view, tolerance defines the induction and maintenance of the long-term unresponsiveness to allergens, which can be induced either by natural allergen exposure or by in vivo challenges. A suppressive, non-proliferative and non-inflammatory reaction should be established and sustained.[18]

In this study, the fundamental bases of induced clinical tolerance were demonstrated by the evolution of Th1/Th2 profile cytokines and immunosuppressive cytokines (IL-10 and TGFβ). Indeed, concomitantly with the appearance of clinical tolerance, Figure 1A shows at the start of tolerance induction, a high concentration of IL-4 (250-650 ng/ml) with a low level of cytokines Th1 (INFγ, TNFα) almost undetectable (less than 20 ng/ml). This clearly shows the involvement of the type 1 hypersensitivity mechanism.

At 24-72 hours after D1 and D3 the end of the tolerance induction protocol, there was a gradual drop in IL-4 levels (250 to 80 ng/ml at D1-D3). At the same time, the levels of Th1 cytokines (INFγ, TNFα) rose slightly between 50 and 100 ng/ml without really reaching the levels of IL-4. From M1 to M6, there was a tendency for the three cytokines to overlap (their concentrations vary between 20 and 100 ng/ml).

This evolution of the Th1/Th2 cytokines is well materialized in Figure 1A. The high concentrations of IL-4 (Black line) in the pre-induction phase decreased gradually in favor of the relative increase of INFγ and TNFα concentrations (green and orange lines, respectively) from D1 to M6.

In the pre-induction phase, the two cytokines have low levels (less than 50ng/ml). These concentrations began to increase rapidly from 100 ng/ml at D1 to reach levels of about 500 ng/ml at M1 and even higher (700 ng/ml) at M6. The modulation of pro-Th2 cytokines towards the allergen tolerance effect (NSAIDs) was dependent on immunosuppressive cytokines (IL-10 and TGF-β).

The evolution of immunosuppressive cytokine levels (IL-10 Treg cell cytokine) (Figures 1B) seems to confirm the published data about the identification of biomarkers to predict the clinical outcome of allergen-specific immunotherapy.[19] Biomarkers such as IgE, IgG4, detection of IL-4 during the course of allergen-specific immunotherapy and increased IL-10 mRNA expression levels following sublingual immunotherapy could be predictive of a better clinical response and could be considered as a potential novel biomarker. Treg cells are also best known with their TGF-β and IL-10 productions where IL-10 is the major cytokine that suppresses IgE production through Treg Cell-B cell interaction.[19-22]

The decline in the IgE level could be explained by the effect of IL-10 (Figure 2). Generally, successful allergen-specific immunotherapy is associated with a marked reduction in symptoms related to prolonged exposure to allergens.[18,19]

This effect persisted for at least several years after discontinuation of immunotherapy and

the basis of clinical tolerance is immunological. The tolerance induction protocol is quite long and evaluated in years.[13] In our study, the induction protocol was rapid (Table 2) as applied elsewhere[15] and exposure to the allergen was not prolonged. This is why the tolerance effect did not last in terms of years.

In conclusion, the clinical results observed by the induction of immuno-tolerance to NSAIDs through a short protocol are well supported by immune regulation. The medium-term effects are real, unlike the results of allergen desensitization or specific immunotherapy. However, this protocol could be used in certain circumstances such as in the case of intolerance to trimethoprim-sulfamethoxazole (TMP-SMX) used as the treatment of choice for the prevention of opportunistic diseases in people living with HIV.

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Author Contributions

DSR conceptualized and designed the study. DSR, SKL and NKi drafted the manuscript. DSR, SKL, YOR, AAH critically reviewed it and contributed to its design. All authors participated in revising the different draft versions and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was approved (dated, February 3, 2014) by the ethical committee of the University Hospital of Cocody (n013-14/MSHPCMU/CECHU-COC-ak).

Informed consent

A signed consent form was obtained from each study participant.

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