

of the patient's serum with pure PSA, the immunoreactive bands were absent. Furthermore, when serum from a healthy female or from a female with pollen allergy was used instead of the patient's serum, neither pure PSA nor PSA in seminal plasma was recognized (Fig 1). Together, these results strongly suggest that PSA was selectively recognized by IgE antibodies contained in the patient's serum.

A cellular allergen stimulation test (CAST; Bühlmann Laboratories, Schönenbuch, Switzerland) yielded positive results with high leukotriene release after incubation with husband's seminal plasma diluted 1:10 to 1:160, and with PSA 0.04, 0.02, and 0.01 $\mu\text{g/mL}$, whereas no reactivity was seen in 3 healthy controls. Furthermore, quantitative determination of *in vitro* basophil activation performed with the Basotest kit (Orpegen Pharma, Heidelberg, Germany) showed more than 50% basophil activation after incubation with all dilutions (1:10 to 1:160) of the husband's seminal plasma as well as PSA, whereas basophils from the normal subjects were unreactive (Fig 2). The description of both techniques has recently been published in detail.⁵

The nature of human seminal plasma allergens has been unknown so far, but results from previous studies suggested that the site of origin might be the prostate.¹ In the current case, for the first time, PSA could be identified as the causative allergen as strongly indicated by positive SPT, immunoblotting, cellular antigen stimulation, and basophil activation testing.

Prostate-specific antigen is well known as a marker for detection and management of prostate cancer⁵; it is the most abundant protease in human seminal plasma, where it functions to degrade high-molecular-weight proteins synthesized in the seminal vesicles. In prostate tissue and in seminal plasma, PSA predominantly exists in a free form as a noncomplexed 33-kd protein consisting of a single polypeptide chain of 240 amino acids, whereas in serum, PSA is present mainly in a complex form with protease inhibitors such as α_1 -antichymotrypsin.⁶

The findings in the presented case report are in line with previous studies performed before limitations caused by HIV and ethical limitations, which could demonstrate positive SPT reactivity in sensitized women against seminal plasma of different men but not other species⁷ and against expressed prostatic secretions and prostatic tissue extractions.⁸ In addition, in early chromatographic investigations, 90% of the allergenic activity of human seminal plasma was found in fractions with a molecular mass between 12 and 75 kd,⁶ and allergenicity is not modified after vasectomy.⁹

In the presented case, intravaginal desensitization using the husband's whole seminal plasma failed. Graded intravaginal challenges have been shown to be less effective as traditional subcutaneous desensitization, but have been recommended as first therapeutic approach before pursuing subcutaneous desensitization because they are less time-consuming and easier to administer.¹⁰ It might be speculated whether in the presented patient successful desensitization could be achieved by using purified PSA obtained from the husband's seminal plasma or serum.

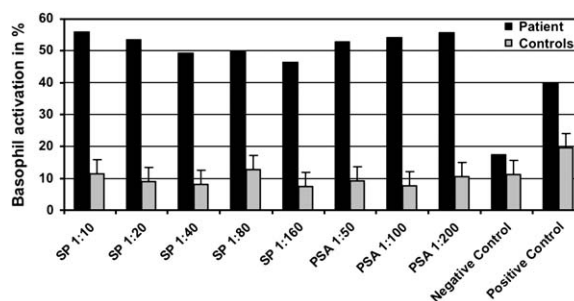


FIG 2. Basotest. Mean basophil activation (percentage of CD63⁺ basophils) and SD in the patient and 4 healthy controls after incubation with seminal plasma (SP) up to a dilution of 1:160 and PSA 0.04, 0.02, and 0.01 $\mu\text{g/mL}$.

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REFERENCES

- Weidinger S, Ring J, Kohn FM. IgE-mediated allergy against human seminal plasma. *Chem Immunol Allergy* 2005;88:128-38.
- Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology. The diagnosis and management of anaphylaxis: an updated practice parameter. *J Allergy Clin Immunol* 2005;115(suppl 3):S483-523.
- Matloff SM. Local intravaginal desensitization to seminal fluid. *J Allergy Clin Immunol* 1993;91:1230-1.
- Frungeri MB, Weidinger S, Meineke V, Kohn FM, Mayerhofer A. Proliferative action of mast-cell tryptase is mediated by PAR2, COX2, prostaglandins, and PPARgamma: possible relevance to human fibrotic disorders. *Proc Natl Acad Sci U S A* 2002;99:15072-7.
- Eberlein-König B, Rakoski J, Behrendt H, Ring J. Use of CD63 expression as marker of *in vitro* basophil activation in identifying the culprit in insect venom allergy. *J Invest Allergol Clin Immunol* 2004;14:10-6.
- Balk SP, Ko YJ, Bubley GJ. Biology of prostate-specific antigen. *J Clin Oncol* 2003;21:383-91.
- Ohman JL Jr, Malkiel S, Lewis S, Lorusso JR. Allergy to human seminal fluid: characterization of the allergen and experience with immunotherapy. *J Allergy Clin Immunol* 1990;85:103-37.
- Siraganian RP, Schenkein I, Levine BB. Immunologic studies of a patient with seminal plasma allergy. *Clin Immunol Immunopathol* 1975;4:59-66.
- Tomitaka A, Suzuki K, Akamatsu H, Matsunaga K. Anaphylaxis to human seminal plasma. *Allergy* 2002;57:1081-2.
- Resnick DJ, Hatzis DC, Kanganis P, Liccardi FL, Lee-Wong M, Bernstein JA. The approach to conception for women with seminal plasma protein hypersensitivity. *Am J Reprod Immunol* 2004;52:42-4.

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Asthma attacks induced by low doses of celecoxib, aspirin, and acetaminophen

To the Editor:

Coxibs are a new class of potent nonsteroidal anti-inflammatory drugs that selectively inhibit the COX-2

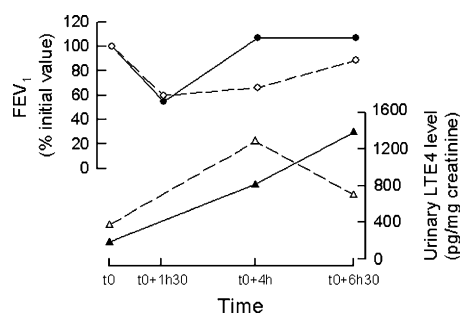


FIG 1. Evolution of FEV₁ and urinary leukotrienes during challenges with aspirin (dotted lines) and celecoxib (continuous lines).

enzyme.¹ This characteristic allows them to be administered safely to most patients with intolerance to aspirin or other conventional nonsteroidal anti-inflammatory drugs, which is thought to be precipitated by the COX-1 inhibition of these drugs.² However, anaphylaxis to celecoxib has been reported.³ In addition, urticarial reactions have been reported after ingestion of coxibs.^{4,5}

We report the case of a 45-year-old woman with a known sensitivity to aspirin who was referred to the allergy clinic for treatment of severe arthrosis. The patient was suffering from nonallergic asthma that had been diagnosed at 12 years of age when she had developed a severe bronchospasm after ingesting aspirin (1 g) for a common cold. She had also experienced difficulty with breathing after ingesting paracetamol (500 mg) for a headache. More recently, her asthma had become more difficult to control, requiring frequent courses of oral steroids in addition to high doses of inhaled corticosteroids, long-acting β_2 -agonists, and leukotriene (LT) antagonists.

Nasal polyps and chronic sinusitis had previously been surgically treated, but sinus computed tomography conducted in our clinic revealed persistent signs of sinusitis. This was treated with a course of antibiotics as well as oral and nasal steroids.

Before prescribing anything, we subjected the patient to a single-blind placebo-controlled challenge with acetylsalicylic acid (ASA; 10 mg, 30 mg, 120 mg, 300 mg) and celecoxib (15 mg, 60 mg, 130 mg), according to procedures previously described.⁶

Inhaled corticosteroids were continued, but LT antagonists and long-acting β_2 -agonists were discontinued 5 days before the challenge procedure. The use of short-acting β_2 -agonists was not allowed from 8 hours before the challenge.

No reaction was observed during the placebo procedure. Keeping the previous reaction to a relatively low dose of acetaminophen (500 mg) in mind, the ASA challenge was started using a low dose of aspirin (10 mg). Indeed, about 1 hour and a half after the ingestion of this first provocative dose, the patient did experience an asthma attack, confirming the diagnosis of aspirin-exacerbated respiratory disease (AERD; Fig 1). In addition, she also developed a severe bronchospasm when challenged 2 weeks later with celecoxib (provocative dose, 15 mg; Fig 1).

Fig 1 shows that the urinary levels of LTE₄—determined by an ELISA (CAST-200; Buhlman, Allschwill,

Switzerland)—increased from 368 pg/mg creatinine and 180 pg/mg creatinine to 1283 pg/mg creatinine and 1318 pg/mg creatinine when the patient experienced bronchospasms after ingestion of ASA and celecoxib, respectively. No change in urinary LTE₄ levels was observed during placebo challenge.

As far as we are aware, this is the first documented report of an asthma attack precipitated by celecoxib through its pharmacological mode of action, even though a double-blind challenge would have been more appropriate to establish the reaction firmly. Indeed, an increase in leukotriene production—thought to play a key role in the onset of bronchospasm induced by ASA in patients with AERD—was also observed when the patient was challenged with celecoxib. Whether this reaction could be expected is unclear. Indeed, even though the increase of urinary LTE₄ ($\times \sim 3.49$) observed during the ASA challenge was within the range usually observed when ASA patients undergo such procedures, the provocative dose of ASA was strikingly low (10 mg), clearly below the usual threshold doses (30–150 mg).⁷ This, together with the reaction previously noted in this patient after the ingestion of a relatively low dose of paracetamol (which has only a limited inhibitory effect on COX-1 activity at usual dosage, with the provocative dose averaging 1000–1500 mg²), suggested an exquisite sensitivity of CysLT production to COX-1 inhibition. Alternatively, considering that celecoxib only inhibits COX-1 at high concentration, some unknown mechanism unrelated to COX-1 inhibition could induce synthesis of leukotrienes by direct stimulation of mast cells (a new mast cell receptor?) with low doses of ASA, acetaminophen, and coxibs.

As mentioned, occasional reactions caused by intolerance to celecoxib (mainly skin reactions, but also rare cases of true anaphylactic reactions) have already been reported.^{3–5} Similarly, a few cases of urticaria and 1 attack of asthma have been reported with the use of rofecoxib,^{8,9}—an even more selective COX-2 inhibitor. These reactions are quite rare⁶ and do not challenge the view that COX-2 inhibitors provide a safe alternative for treatment of inflammatory states in patients with AERD. However, the severity of some reactions^{5,7} also suggests that the first dose ingestion should be in a medical doctor's office.

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REFERENCES

- Hawkey CJ. COX-2 inhibitors. *Lancet* 1999;353:307–14.
- Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 2003;111:913–21.
- Levy MB, Fink JN. Anaphylaxis to celecoxib. *Ann Allergy Asthma Immunol* 2001;87:72–3.

4. Kelkar PS, Butterfield JH, Teaford HG. Urticaria and angioedema from cyclooxygenase-2 inhibitors. *J Rheumatol* 2001;28:2553-4.
5. Murr D, Bocquet H, Lelouet H, Fischer RM, Revuz J, Cosnes A. Adverse cutaneous reaction to celecoxib: 6 cases. *Ann Dermatol Venerol* 2003;130:519-21.
6. Woessener KM, Simon RA, Stevenson DD. Safety of high-dose rofecoxib in patients with aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol* 2004;93:339-44.
7. Szczeklik A, Nizankowska E, Bochenek G, Nagraba K, Mejza F, Swierczynska M. Safety of a specific COX-2 inhibitor in aspirin-induced asthma. *Clin Exp Allergy* 2001;31:219-25.
8. Passero M. Cyclooxygenase-2 inhibitors in aspirin sensitive asthma. *Chest* 2003;123:2155-6.
9. Bavbek S, Celik G, Ozer F, Mungan D, Misirligil Z. Safety of selective COX-2 inhibitors in aspirin/nonsteroidal anti-inflammatory drug-intolerant patients: comparison of nimesulide, meloxicam, and rofecoxib. *J Asthma* 2004;41:67-75.

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Enhanced frequency of immunoregulatory invariant natural killer T cells in the airways of children with asthma

To the Editor:

Asthma is a disease characterized by eosinophil-rich airway inflammation, bronchospasm, and airway hyper-reactivity, which has recently increased dramatically in prevalence in the industrialized world.¹ In the past few decades, it has become clear that both pathogenesis and severity of asthma are mediated through T_H2 lymphocytes.² Recently, we and others have demonstrated that a new population of regulatory T cell, the Cluster of Differentiation 1d (CD1d)-restricted invariant natural killer T (iNKT) cell, plays a major role in the severity of experimental allergic asthma by enhancing harmful T_H2 immune responses.^{3,4} iNKT cells constitute a distinctive T-lymphocyte population that recognizes lipids presented by the nonpolymorphic MHC class I-like molecule, CD1d.^{5,6} They coexpress a highly restricted T-cell receptor (TCR) repertoire, composed of a single invariant V α 24J α 18 chain in humans, preferentially paired with a limited TCR V β chain repertoire.^{5,6} Another major feature of this T-cell population is its capacity to generate promptly a large range of immunomodulatory cytokines such as IL-4, IL-13, and IFN- γ .^{5,6} The influence of iNKT cells in the severity of experimental allergic asthma is presently well documented.^{3,4} However, it is still unclear whether iNKT cells influence the pathology of the human disease. One of the problems encountered in human studies comes from the difficulty to study iNKT cells locally at the site of the immune reaction rather than in peripheral blood, which is frequently the only material available. Yet, in atopic asthma, it is of foremost importance that the regulatory functions of these cells should be analyzed in the airways, where the symptoms do actually occur. Knowing that several key events that contribute to the development of allergic asthma take place in the early childhood,⁷ we analyzed here the frequency of iNKT cells in the airways of children suffering from T_H2 cell-mediated atopic asthma.

We examined 15 children with severe asthma, 12 children with airway infections (bronchiectasis), and 10

children with no obvious endobronchial inflammation or atopy (these controls are subjects with suspicion of foreign body in the airways). The children were recruited from the Necker Enfants Malades Hospital in Paris, France. Subject characteristics are outlined as follows: mean age of 4, 7, and 4 years (ranging from 6 months to 12 years) respectively, for asthma, infection, and control groups made up of female and male subjects. Severe asthma was treated according to the Global Initiative for Asthma/American Thoracic Society criteria, and all patients underwent computed tomography scan and bronchoscopy as part of their clinical work-up. The local ethical committee approved the study, and the parents gave written informed consent.

Bronchoscopy was performed under general anesthesia as described previously.⁷ Bronchoalveolar lavage fluid (BALF) was filtered on 100- μ m cell strainers to exclude debris. One aliquot was used for microbiologic evaluation. The number of total cells in the BALF was $100 \times 10^3 \pm 50 \times 10^3$, $980 \times 10^3 \pm 157 \times 10^3$, and $227 \times 10^3 \pm 41 \times 10^3$ per mL from asthma, infection, and control groups, respectively. To identify iNKT lymphocytes among the cells from BALF, we incubated them with α -galactosylceramide (α -GalCer) loaded CD1d/ β 2m tetramer-allophycocyanin (which specifically binds to the invariant V α 24J α 18 TCR of iNKT cells) or control (CD1d-unloaded or mock tetramers), as described.³ After washing, cells were further incubated with appropriate dilutions of anti-CD4-phycoerythrin, anti-TCR $\alpha\beta$ fluorescein isothiocyanate, and anti-CD8-phycoerythrin (from Immunotools, Germany). The cells were washed and analyzed on a FACScalibur (Becton Dickinson, Le Pont de Claix, France) using CellQuest software (Becton Dickinson). The percentage of the different T-cell subsets in BALF was evaluated in the lymphocyte gate according to forward-scatter/side-scatter characteristics. This lymphocyte gate represents $32\% \pm 3\%$, $22\% \pm 2\%$, and $29\% \pm 3\%$ of total BALF cells from asthma, infection, and control groups, respectively. Data are given as mean values $\pm$ SEM. The Mann-Whitney *U* test was used to establish statistical significance between patient groups.

Analysis of T-cell populations in BALF revealed a similar incidence of TCR $\alpha\beta$ ⁺ cells among children with asthma ($38\% \pm 9.9\%$) and controls ($43.2\% \pm 13.7\%$), whereas these cells were decreased in children with infections ($18.8\% \pm 3.6\%$). These findings suggest that the inflammation occurring in both pathologies could be mediated through distinct mechanisms resulting in the different recruitment of T cells to the airways.

We found a similar percentage of CD4⁺ cells among gated BALF TCR $\alpha\beta$ ⁺ lymphocytes, whether they were recovered from patients of the asthma ($35\% \pm 3\%$), the infected ($39\% \pm 4\%$), or the control group ($34\% \pm 3.5\%$). CD8⁺ cells were the predominant T-cell population in the BALF of patients with asthma ($59\% \pm 5\%$) as well as in the other 2 groups ($52\% \pm 5\%$ and $63\% \pm 4\%$ for infected and control group, respectively).

This is the first evidence for the presence of iNKT cells in BALF of children with asthma (Fig 1) using specific detection by the CD1d/ α -GalCer tetramers. Their