complete sequences of 6 of the most IgE-reactive allergens from ash pollen codified for polypeptides ranging from 50 amino acids for Fra e 6 to 341 for Fra e 11 (see Fig 2, *A*). Ash allergens possessed physicochemical properties similar to those of olive pollen: acidic to mildly acidic proteins (4.3 to 6.5) except Fra e 7 with a basic pI of 8.2, low to medium MWs (5.8-37.4 kDa), and the presence of signal peptides for Fra e 6, Fra e 10, and Fra e 11. The sequence identity between ash and olive pollen allergens ranged from 84% to 97%, and sequence similarity ranged from 94% to 99%, except for Fra e 11, which presented the lowest identity and similarity (79% and 90%, respectively) with its olive counterpart (see Fig 2, *A*).

Considering the high amino acid sequence identity and similarity between ash and olive pollen allergens, we hypothesized that purified olive pollen allergens could be used to assess the significance, potency, and prevalence of ash pollen allergens. We then performed IgE-inhibition assays of the ash pollen extract with ash-sensitized patients' sera and the panel of purified olive pollen allergens as inhibitors. By densitometry of 1DE and 2DE IgE-inhibition assays, we measured a 95% reduction in the IgE binding obtained with the IgE serum pool without inhibitors (see Fig 2, *B* and *C*). The residual IgE-positive signals might be due to the recognition of specific isoforms not so cross-reactive to olive pollen allergens because of differences in their amino acid sequence or glycosylation patterns. Alternatively, the presence of other allergens different from those present in olive pollen should not be discarded. In a recent publication analyzing ash pollen components by proteomics based on 2D and mass spectrometry,[7] the authors identified Fra e 1, Fra e 2, and Fra e 3 as relevant allergens and β-galactosidase, malate dehydrogenase, and a 4-EF hand Ca-binding protein as minor ash pollen allergens, with the vast majority of major allergen spots unidentified.[7] Then, the IgE reactivity observed in the IgE-inhibition assays might be associated with these minor allergens.[7]

As IgE-inhibition assays validated the use of purified olive pollen allergens to characterize the IgE immune response of ash pollen–sensitized patients, we tested their IgE-binding ability by using ELISA. The deduced prevalence for the cross-reactive ash allergens ranged between 64% and 92% for the major allergens Fra e 1, Fra e 2, Fra e 9, and Fra e 11 and between 4% and 32% for the minor allergens Fra e 3, Fra e 6, Fra e 7, Fra e 10, and Fra e 12 (see Fig 2, *D* and *E*). These findings are in accordance with previous reports involving related and nonrelated allergenic sources, except for Fra e 10, which was previously described as a major allergen in olive pollen.[6,8]

Collectively, our findings show that the *F excelsior* allergogram responsible for allergy symptoms in ash-sensitized patients is highly similar to that obtained from *Olea europaea*, which supports the high cross-reactivity of patients to both pollens. We suggest that it is possible to use indistinctly purified allergens from ash or olive pollen for diagnosis purposes.

Additional information is available (see this article's Methods section and References in the Online Repository at www.jacionline.org).

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The expression of cannabinoid receptor 1 is significantly increased in atopic patients

To the Editor:

The human endogenous cannabinoid system (ECS) is a complex signaling network involved in a large number of physiological processes. Recently, 2 articles reported in the *Journal of Allergy and Clinical Immunology* describe that endocannabinoids and cannabinoid receptor (CB) 1 signaling may

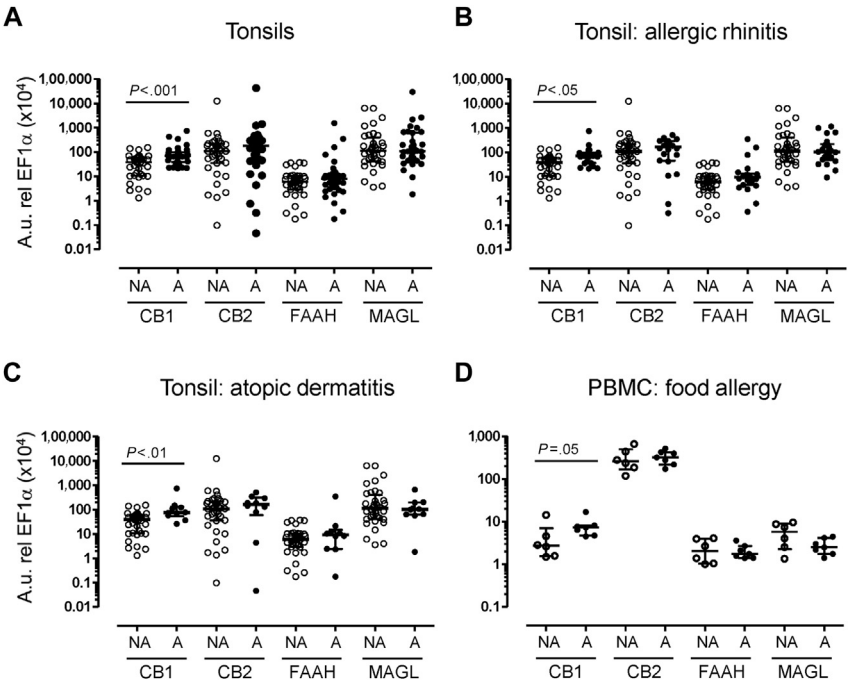

FIG 1. Increased CB1 mRNA expression in tonsils and PBMCs from atopic patients. mRNA expression levels of CB1, CB2, FAAH, and MAGL in tonsils from 35 atopic *(A)* patients **(A)**, in tonsils from 22 atopic *(A)* individuals with allergic rhinitis **(B)**, and in tonsils from 10 atopic *(A)* individuals with atopic dermatitis **(C)** compared with tonsils from 35 nonatopic *(NA)* individuals, and PBMCs from 6 healthy *(NA)* controls compared with 7 highly peanut-allergic *(A)* individuals **(D)**. Arbitrary units *(A.u.)* are $2^{-\Delta CT}$ values multiplied by 10^4, with ΔCT defined as the difference between the cycle threshold value for the gene of interest and EF1α. Data represent median with interquartile range. Mann-Whitney *U t* test. *EF1α*, Elongation factor 1 α.

play a potent inhibitory role in human mast cell (MC) degranulation and activation in the airway mucosa and skin, suggesting that targeting the ECS in these tissues might well represent a novel strategy for the treatment of allergy.[1,2] In these studies, the pharmacological blockage or gene silencing of CB1 significantly stimulated the degranulation and maturation of MCs from resident progenitors by mechanisms partially depending on the upregulation of stem cell factor production, which was counteracted by the activation of CB1 with specific agonists. Although it is plausible that the ECS may contribute to the regulation of allergic diseases, studies reporting human data are still scarce. Accordingly, we explored the potential effect of the ECS in human allergic diseases by quantifying and comparing the *in vivo* mRNA expression levels of the main components of the ECS (the receptors CB1 and CB2 and the enzymes fatty acid amide hydrolase [FAAH] and monoacylglycerol lipase [MAGL] that are involved in the hydrolysis and inactivation of endocannabinoids) in tonsils and PBMCs of atopic and healthy subjects.

This study has been reviewed and approved by the ethical committee of Satakunta Central Hospital, Pori, Finland, and the ethical committee of the Medical University of Vienna, Vienna, Austria. Detailed methodology is fully described in this article's Methods section in the Online Repository at www.jacionline.org. We initially explored the *in vivo* mRNA expression levels of main components of the ECS in palatine tonsils directly excised from 35 atopic patients suffering from allergic rhinitis, atopic dermatitis, and/or asthma and 35 nonatopic controls. The main reason for tonsillectomy was tonsillar hypertrophy or recurrent tonsillectomy without significant differences between both groups (nonatopic vs atopic). Tonsils that showed any sign of infection or microabscess were discarded. As shown in Fig 1, *A*, the mRNA expression levels of CB1 were significantly higher in tonsils ($P = .0002$) from atopic patients than in tonsils from nonatopic subjects. There was no significant difference in the mRNA expression levels of CB2, FAAH, and MAGL. We extended the analysis by subgrouping atopic patients according to allergic diseases. As shown in Fig 1, *B*, the mRNA expression levels of CB1 were significantly higher in tonsils from atopic patients with allergic rhinitis than in nonatopic subjects without significant differences in the other compared genes. We also compared tonsils from 10 atopic patients suffering from atopic dermatitis with tonsils from 35 nonatopic subjects, and significant upregulation of the mRNA levels of CB1 was also observed in patients with atopic dermatitis than in nonatopic subjects without changes for the other analyzed genes (Fig 1, *C*). These data demonstrated that CB1, CB2, FAAH, and MAGL are expressed in human tonsils and that the atopic patients displayed a significant upregulation of CB1 at the mRNA level. To strengthen our findings in a different population of patients and to determine whether changes in the mRNA expression levels of components of the ECS could also be detected in peripheral blood, we investigated patients who showed clinical anaphylaxis to peanut and an age-matched control group with no allergy. We quantified and compared the mRNA expression levels of the same ECS components in PBMCs from 7 food-allergic and 6 healthy individuals. As shown in Fig 1, *D*, the mRNA levels of CB1 were also significantly higher in peanut-allergic individuals than in healthy controls without significant differences in the

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