

Table 1. Patients with EBV-associated drug rash to amoxicillin: synopsis of the allergy diagnostic test results

Patient no.	SPT	Patch test AMX	Patch test penicillin	Patch test cefotaxim	Challenge AMX	Challenge penicillin	Challenge cephalosporin	Challenge refused
1	Negative	Positive	Negative	Negative	–	Negative	–	
2	Negative	Positive	Negative	Negative	Positive	–	Negative	
3	Negative	Positive	Positive	Negative	–	–	–	yes
4	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	–	<i>Negative</i>	
5	AMX at 48 h +	Positive	Negative	Negative	–	–	–	yes
6	Negative	Positive	Positive	Negative	–	–	–	yes
7	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	–	–	–	yes
8	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	<i>Negative</i>	–	–	

Results written in italics describe those individuals who most probably only have a transient EBV-associated drug intolerance. EBV, Epstein–Barr virus; SPT, skin prick test; AMX, amoxicillin; –, not done.

anti-histamines and topically applied glucocorticosteroids without the necessity for hospitalization. After resolution, the oral challenge test with a cephalosporin was well tolerated.

Another amoxicillin patch test positive patient tolerated the oral challenge with penicillin, thereby revealing an isolated allergy to amoxicillin. As a challenge test would involve the risk for the patients to experience the same signs and symptoms of the previous drug reaction, the decision of four out of eight patients not to perform an oral challenge test was respected. Two of three patients, who were completely negative in the allergy diagnostic tests, agreed to an oral challenge with verum and revealed tolerance to the drug. It can be hypothesized that the third patch test negative subject may also have tolerated amoxicillin challenge. These three individuals may belong to the population, which only has a transient decrease in drug tolerance during EBV infection. Two amoxicillin patch test positive patients could be examined in a follow-up visit 2.2 and 1.5 years, respectively, after the first patch testing. Those patients revealed a patch test reaction similar in strength to the preliminary test result, which is indicative for a persistent delayed-type reaction to amoxicillin.

In conclusion, this study does not only provide additional evidence that true delayed-type allergic reactions to aminopenicillins may develop during a florid viral infection, but that they may be definitely more prevalent than previously assumed and published (5, 6). Additionally, follow-up investigations on two

patients revealed the persistence of the delayed-type reactions to amoxicillin, an approach, which has to the best of our knowledge not been published before. These observations should encourage the investigation of patients with aminopenicillin-induced exanthema for both, EBV-infection and true drug allergy. Particularly young women should be recommended an oral challenge with cephalosporins and maybe penicillins because the latter are antibiotics which may be given safely during pregnancy.

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Oral reactions to sublingual immunotherapy: a bioptic study

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Key words: biopsies; eosinophils; local reactions; mast cells; sublingual immunotherapy.

Sublingual immunotherapy (SLIT) is currently considered a feasible option to traditional subcutaneous immunotherapy (SCIT) for treating allergic rhinitis caused by sensitization to seasonal or perennial allergens, with a significant advantage in terms of safety (1, 2). However, local reactions to the allergen extract following

Mast cells and eosinophils are not the elicitors of local reactions to SLIT.

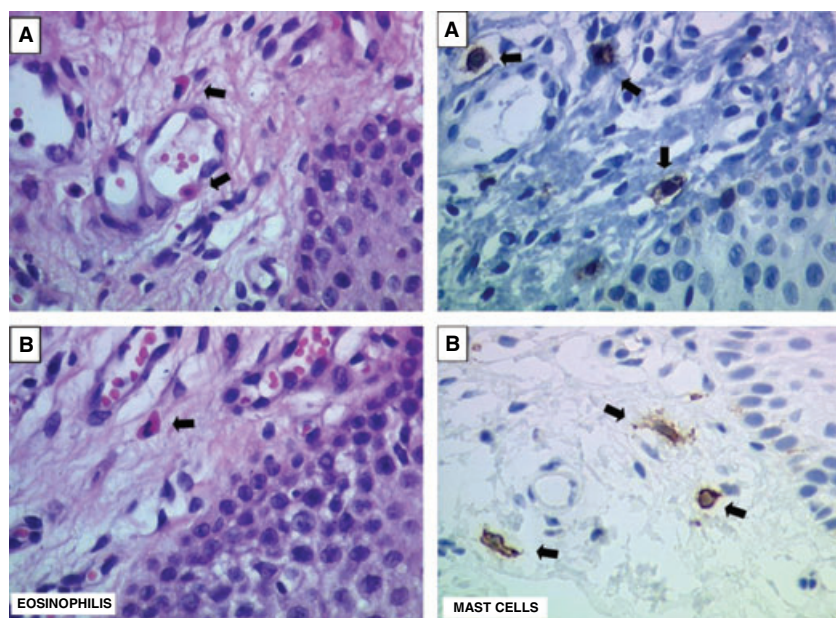


Figure 1. Eosinophils and mast cells in biopsies prior to (A) and after (B) the local reaction to sublingual immunotherapy.

its contact with the oral mucosa are quite common: in an analysis of all published studies – including 1440 patients – the prevalence of such reactions was 43% (3). Most of them are minor and short-lasting but sometimes the itching and burning of the mouth and the edema of the lips hamper the subsequent administration of the treatment and require modifications of the schedule or dosage. Concerning local reactions to SCIT, a biopptic study detected infiltration of T lymphocytes, macrophages and eosinophils in the site of injection of the allergen extract (4), while the mechanism of local reactions to SLIT is not known. In particular, the involvement of mast cells and eosinophils, which usually has a pivotal role in allergic reactions, was made unlikely by the observation that (differently from findings in nasal mucosa) in the oral mucosa of patients treated with SLIT could not be detected tryptase and eosinophil cationic protein (ECP), the markers of mast cell and eosinophils activation (5). We conducted the present investigation to directly ascertain on biopsies if mast cells and eosinophils are present in the oral mucosa of a subject developing a local reaction to SLIT. The patient was a 31-year-old male with persistent rhinoconjunctivitis to grass pollen from the age of 23 years, with skin prick tests and *in vitro* IgE tests positive

for grass pollen, who started in 2005 SLIT with a grass pollen extract (Staloral 300®, Stallergenes, Antony, France) by a preseasonal schedule, showing intense local reactions requiring several repetitions of dose. He did not suffer from any oral pathology, was not under treatment with any drug and was not a smoker. Before the second year of treatment, we planned to investigate the reactions by biopsies. The patient gave his informed consent and the local ethical Committee of the University of Perugia approved the study. Biopsies were performed before starting SLIT and during maintenance treatment, in presence of repeated local reactions as observed in the first year. They were performed by cutting with a lancet a small piece (of about 3 mm of diameter) of the oral mucosa under the tongue. A local anesthesia by infiltration of lidocaine was performed. Biopsies were fixed in 10% buffered formalin and embedded in paraffin wax. Semi-serial 5-µ sections were used for hematoxylin and eosin staining or for immunohistochemical study using CD117 or IgE as a primary antibody.

Briefly, paraffin sections, after rehydration, were immersed in citrate buffer at pH 7.6. Sections were then left to cool to room temperature, washed in phosphate buffer saline (PBS), and then incubated with primary rabbit

polyclonal antibody CD117 (Dako, Milan, Italy) diluted 1 : 100, and primary rabbit polyclonal antibody IgE (Dako), diluted 1 : 2000, followed by chromogen diaminobenzidine tetrahydrochloride (DBA, Dako) and hydrogen peroxide in PBS. Sections were then counterstained with hematoxylin. Appropriate positive and negative controls were performed.

Figure 1 shows that a low number of mast cells was found in subepidermal level in perivascular sites, some with surface IgE. The other cells present in biopsies, besides normal squamous epithelial cells of the oral mucosa, were eosinophils, they too in perivascular sites. No clear differences were noted in biopsies before (part A) and after the administration (part B) of the allergen extract. The absence of tryptase positive cells and eosinophils in the epithelium and their paucity in perivascular site in subepithelial area should lead to conclude that mast cells and eosinophils are not the elicitors of local reactions to allergen extracts administered by SLIT. Therefore, studies are needed to identify the mechanisms underlying such local reactions. A useful approach for future investigations should consist in detecting in the oral mucosa mediators, cytokines or any other cell product able to indicate their possible source and then the kind of mechanism causing local reactions to SLIT.

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