

Allergy to lysozyme/egg white-containing vaginal suppositories

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Seven patients who received a lysozyme, nystatin, and tetracycline containing vaginal suppository because of suspected vaginal infection, developed local or systemic allergic reactions. The coincidence of the symptoms with the repeated use of the suppository as well as skin and lymphocyte transformation tests indicated that the lysozyme in the suppository was responsible for the allergic reactions. This lysozyme preparation contained additional egg proteins, which contributed to the allergic reaction in certain patients: three patients with a previous history of egg allergy and serologic and/or skin test evidence for egg-white sensitization developed the allergic reaction after the first suppository. Four patients had urticaria or anaphylaxis after treatment for at least three days; none of these four patients developed egg allergy. Five of seven individuals had positive skin tests (prick or scratch) to ovomucoid and lysozyme, but none of the patients had lysozyme-specific IgE in the circulation.

All seven patients, with or without egg allergy, showed vigorous T cell responses to purified lysozyme and partly to other egg-white proteins in the lymphocyte transformation test, which was absent in controls. Vaginal suppositories that contain lysozyme and other contaminating egg white proteins can either elicit allergic reactions in patients with a preexisting egg white allergy or induce sensitization to lysozyme and other egg white components.

INTRODUCTION

Lysozymes are small globular proteins with molecular weights of 14,300 D. They are contained in many tissues, in cells of the hematopoietic lineage and in body secretions. The amino acid sequence and the three dimensional structure of lysozymes of various species are known.¹

Since the lysozymes of various species differ, the human immune system may mount a reaction to lysozymes of other species. The most frequently encountered lysozyme stems from hen egg. Although earlier studies suggested that hen egg lysozyme is not a major allergen in egg-allergic individuals,² more recent studies suggest that hen egg lysozyme can be a potent allergen. Both patients with food allergy who react to eggs³ and patients, who work in an egg-processing factory and develop respiratory sensitiza-

tion to inhaled egg proteins,⁴ were shown to be sensitized to egg lysozyme.

Due to its bactericidal and potentially virustatic effect,^{5,6} lysozyme is added to various medications. In Switzerland, hen egg lysozyme-containing medications are on the market in the form of tablets, lozenges, capsules, eye drops, nasal drops, and recently in vaginal suppositories. In spite of the potential immunogenicity of egg lysozyme, allergic reactions of humans to lysozyme-containing preparations have not yet been reported.

Here we present seven patients with severe allergic reactions to vaginal suppositories (Cervicaletten, Prospa) that contained lysozyme together with tetracycline and nystatin. On the basis of skin test reactivity and lymphocyte proliferation tests, the lysozyme component, which also contained other egg proteins like ovomucoid, could be identified as the allergen. This lysozyme/egg white preparation either elicited an allergic reaction in already sensitized, egg-allergic individuals or sensitized the patients with subsequent allergic reactions.

PATIENTS, MATERIAL AND METHODS

Patients

Seven women who were patients and six control persons were investigated. A summary of the patients' histories is given in Table 1. The control persons included three healthy individuals, one atopic patient who had tolerated Cervicaletten and two patients with egg-bird syndrome.⁷

Lysozyme

Hen or turkey egg lysozyme was obtained from Società Prodotti Antibiotici S.p.A., Milan, Italy. This is the factory providing the lysozyme in the incriminated suppositories (Cervicaletten) and in the lysozyme tablets of Prospa (Yverdon sur Bains, CH). Additional lysozyme preparations were obtained from Sigma (Buchs, Switzerland), as normal (grade I) or high purity (grade VI) preparations, from Galenica (Bern, Switzerland), distributor of Eyebel eyedrops (5 mg/mL lysozyme) or from Calbiochem (Luzern, CH) which also provided the turkey and human lysozyme. Livetin, ovalbumin, and ovomucoid were ob-

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Table 1. Patient Histories

Patient/age, yr	Symptoms	Previous Exp, Time Interval	Egg Allergy	Respiratory Allergy
PB, 46*	Gen. urticaria, glottic edema, bronchoconstriction, exanthem	1st course of therapy, 1st day, 12 hr	Yes	No
GC, 28*	Urticaria, angioedema, dyspnea, malaise	1st course of therapy, 1st day, min.	Yes	No
EZ, 53*	Local symptoms: pruritus, redness, burning	1st course, 1st day, 1 hr	Yes	No
AM, 31	Gen. urticaria angioedema glottic edema	1st course of therapy, 10th day (hr), 11th day (min)	No	No
EU, 33	Urticaria, angioedema, dyspnea, diarrhea, weakness, nausea	2nd course of therapy, 1st day, 2 hr, 3rd course, 1st day, 3 hr	No	No
EH, 38*	Gen. urticaria, angioedema, glottic edema, collapse	1st course, 3rd day, 8 hr, 2nd course, 2nd day	No	No
CS, 23*	Urticaria, angioedema	1st course, 8th day, 3-4 hr	No	No

* These patients have had contact with birds in previous years (budgerigar).

tained from Sigma (Buchs, Switzerland).

The concentrations used for skin and lymphocyte transformation tests are indicated in the results.

Lysozyme-Specific IgE

Prick (SPT), scratch, and intradermal tests were performed according to standard techniques. Circulating specific IgE was determined by means of a radioallergosorbent test (RAST) using the following antigens: hen egg lysozyme (grade VI, Sigma), hen egg lysozyme chloride (Calbiochem), turkey egg lysozyme (Sigma), lysozyme tablets (Prospa, Yverdon sur Bains, CH), and Eyebel eye drops (Galenica, Bern). Briefly, nitrocellulose (Millipore, Bedford, Mass. USA, HAWAG 04753) was washed, dried, and discs were cut. These were incubated in an allergen solution for two hours, then washed in TBS and further incubated for two hours in casein (0.15% in TBS) to block reactive sites. The following steps were identical to usual RASTs.

Results, the means of quadruplicate assays, were expressed as percentage of total radioactivity. Values more than twice that of healthy controls were considered positive.

Determination of Specific IgG

Assays of specific IgG have been performed by means of a dot-system⁸ against hen egg lysozyme and lysozyme from commercial eye drops (Eyebel). Briefly, allergen (1 μ L) was "dotted" on previously washed and dried nitrocellulose. After incubation for two hours the still reactive sites were saturated by a further 2-hours incubation with casein 0.15% in TBS. Strips were cut and incubated overnight with sera, diluted 1:200 in casein. After washing, the strips were incubated for six hours with peroxidase-labeled rabbit anti-human IgG (Medac, Hamburg, FRG) diluted 1:1000 in casein. The specific binding was visualized by addition of the substrate chloro-I-naphthol (Merck, Darmstadt, FRG).

Lymphocyte Transformation Test

Principally, the test was performed as described previously.⁹ Blood lymphocytes were isolated over Ficoll/Ronpacon, washed and cultured (1×10^6 /mL) for five days in RPMI 1640 supplemented with either 20% autologous plasma or 20% AB-Serum. The test reagents (lysozyme, nystatin, etc) were added to the cul-

tures in the concentrations indicated in the results. After five days, cultures were pulsed with 1 μ Ci of ³H-thymidin (Amersham) for 16 hours, harvested (Cell-Harvester, Skatron), and counted in a beta-counter. Results are expressed as stimulation indices (S.I.), that are the counts of sample plus antigen divided by counts of samples without antigen (= control). Values of >2 were considered positive.

RESULTS

Patient characteristics and symptoms are summarized in Table 1. One of the patients had only localized symptoms, while the other six patients developed generalized symptoms such as urticaria and angioedema of various body parts. In three patients hoarseness, difficulty in swallowing, inspiratory stridor, and fear of choking indicated that glottic edema was developing. Other patients had asthma, abdominal pain, and hypotension/collapse. All patients needed medical care and five of seven patients were immediately transferred and supervised in the emergency unit. Treatment consisted of injection of corticosteroids, antihistamines, and volume substitution. Two patients received suprenen by inhalation.

Three patients had symptoms already on the first day of treatment, minutes to hours after the first treatment (PB, GC, and EZ). These patients were most likely sensitized previously to egg white proteins. Patient PB had a clinically manifest egg allergy (nausea, vomiting, urticaria) and complained about recurrent angioedema of the lips and the tongue when she ate salads that were dressed with an egg-containing sauce. Patient GC had for 4 years a strong aversion to eggs and avoided them completely. EZ had recently developed an aversion to eggs and had developed an exanthem after egg ingestion. Two of the patients also had serologic evidence for egg white sensitization. Five patients had been exposed to budgerigars in their childhood with-

out any allergic symptoms. Skin tests to common aeroallergens were negative in all seven patients.

In three patients the relationship between drug intake and symptoms was further underlined by an unintended provocation test. Patient AM developed urticaria and angioedema on day 10, hours after introduction of the suppository. These symptoms were, however, not thought to be related to the drug. On day 11, minutes after introduction of a new suppository, the symptoms worsened and the patient developed signs of glottic edema. Patient EH's symptoms during the first treatment were not attributed to the drug. As she inserted the suppository ten months later again the symptoms reappeared. Patient EU developed symptoms two hours after inserting the first suppository of the second course. Similar symptoms reappeared 1 year later during the third treatment course.

The symptoms pruritus, urticaria, angioedema, asthma, abdominal pain, and collapse are compatible with an IgE-mediated reaction. None of the patients had a positive scratch test to tetracycline or nystatin; only patient EZ had a weakly positive patch test after 24 and 48 hours with the antibiotic and antimycotic.

Skin prick tests (SPT) were positive with ovomucoid only (Table 2). The same concentrations of lysozyme, grade I and grade VI, did not provoke a positive SPT, but the scratch (not shown) or intradermal tests were strongly positive to purified lysozyme in five of the seven patients. One patient (GC) refused SPT, and patient EZ had a late reaction only.

The determinations of lysozyme specific IgE antibodies were performed with different lysozyme preparations (Table 3). Although some of the patients had circulating IgE antibodies to various egg white components, none had lysozyme specific IgE. The positive reactions with certain lysozyme preparations

Table 2. Skin Tests to Egg Components (SPT and intradermal)*

	Egg White	Livetin			Ovalbumin		Ovomucoid		Lysozyme (grade VI)	
		10	1	0.1	1	0.1	1	0.1	1	0.1/ i.d.
		$\mu\text{g/mL}$								
PB	+	-	-	-	-	+	++	-	-	+++
AM	+++	-	-	-	-	++	+++	-	-	+++
EU	-	-	-	-	-	+	+++	-	-	+++
EH	-	-	-	-	-	-/+++†	-	-	-	+++
CS	-	-	-	-	-	-/-†	-/-	-	-	+++/-§

* GC, 1960, refused skin testing; EZ, skin prick tests were negative to various lysozyme preparations; however, she developed an infiltrative skin reaction to lysozyme 24 hr after scratch testing; EZ was also the only patient with a delayed skin reaction to nystatin and tetracycline (patch tests). The skin tests (scratch and patch) of all other patients were negative to nystatin or tetracycline.

† Intradermal tests.

‡ Test 4 weeks after allergic reaction.

§ Test performed 30 months after allergic reaction.

Table 3. Determination of Specific IgE*

	RAST, % of Total Radioactivity				Time Interval, mo
	Eyebel	Egg White	Lysozyme (Prospa)	Lysozyme gr. VI	
PB	36.5†	48.8	5.2	1.4	2
GC	3.1	8.7	0.7	0.5	4
EZ	0.6	0.6	0.6	0.6	9
AM	0.7	1.4	0.6	0.5	34
EU	0.8	2.5	0.7	0.6	7
EH	nt	1.5	nt	nt	18
CS	nt	0.6	0.5	0.5	1
Controls (6)	<0.6	<0.6	<0.6	<0.6	

* Determination of lysozyme specific IgG negative (1 control person positive).

† Complete RAST inhibition by ovomucoid (3 $\mu\text{g/mL}$) and partly conalbumin (300 $\mu\text{g/mL}$).

were most likely due to impurities in these preparations, as ie, the RAST reactivity of the most reactive serum (PB) to "Eyebel" could be completely blocked by ovomucoid (99% inhibition with only 0.3 $\mu\text{g/mL}$ ovomucoid) and partly by conalbumin (80% inhibition of 0.3 $\mu\text{g/mL}$, 97% inhibition with 300 $\mu\text{g/mL}$ conalbumin). Ovalbumin had no blocking capacity. The determination of lysozyme specific IgG was negative in all patients, but positive in one of six control persons (without any egg related symptoms).

Since lysozyme is a well described T cell antigen in mice¹⁰ and as allergen-specific T cells regulate the IgE production by secreting IL4¹¹ we also performed a lymphocyte prolif-

eration test to lysozyme (Table 4). In all seven patients a strong proliferative response to relatively low concentrations of lysozyme was obtained. Some patients showed also a reaction to other egg components, namely ovalbumin (4/7) and ovomucoid (2/5). No proliferative response was seen to human lysozyme (evaluated in two patients), to livetin or to various concentrations of nystatin or tetracycline. In patients AM and GC the T cell immune response was only observed with lysozyme, but no other egg proteins, suggesting that lysozyme might be the relevant T cell antigen. Quite interestingly, one patient, who lost her skin test reactivity to lysozyme rather quickly (CS), had repeatedly

Table 4. Lymphocyte Transformation Test to Egg Proteins*

	Lysozyme gr. I		Lysozyme gr. VI		Lysozyme Turkey		Ovalbumin		Ovomucoid	
	10	100	10	100	10 μ g/mL	100	10	100	10	100
PB	30.8	40.6	71.3	21.5	40.4	57.7	27.0	23.7	40.8	4.8
GC	1.9	2.0	4.4	13.0	9.8	1.9	1.8	1.6	nt	nt
EZ†	nt	nt	16.6	11.2	nt	nt	1.3	11.3	1.4	1.0
AM‡	nt	nt	2.4	6.9	nt	nt	1.0	1.2	1.8	1.6
EU	27.3§	28.0§	nt	nt	nt	nt	nt	nt	nt	nt
EH	1.5	3.6	5.0	2.9	1.6	2.1	3.8	6.2	4.3	4.3
CS	1.9	3.2	13.2	20.8	8.9	9.5	8.4	28.3	0.9	0.7
Controls (6)	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2

* Stimulation indices; the LTT to nystatin (1, 10, 100 μ g/mL) or tetracycline (1, 10, 100 μ g/mL) was negative (<2.0) in all patients.

† LTT to human lysozyme: 10 μ g/mL 0.6; 100 μ g/mL 0.9.

‡ LTT to human lysozyme: 10 μ g/mL 0.8; 100 μ g/mL 2.2.

§ LTT performed with lysozyme Prospa.

|| Controls: three healthy individuals, one atopic patient who tolerated Cervicalteten, and two patients with bird/egg syndrome.

a strong proliferative response to various egg components. In contrast to the transitory positive skin test and the negative RAST, the lymphocyte transformation test to lysozyme was found to be most consistently positive. Two patients with egg/bird syndrome and one atopic patient who tolerated Cervicalteten well and three healthy individuals without egg/lysozyme related symptoms were negative in the lymphocyte transformation tests to lysozyme, ovomucoid, or ovalbumin.

DISCUSSION

Seven patients who received a lysozyme, tetracycline and nystatin-containing vaginal suppository because of suspected vaginal infection, developed anaphylactic reactions or local allergic symptoms. Due to spontaneous provocation tests (repeated exposure), skin test reactivity and positive lymphocyte transformation tests, the egg lysozyme contained in the vaginal suppository could be identified as the provoking allergen. Only in one patient could a possible sensitization to nystatin or tetracycline (patch tests positive) be demonstrated.

These cases are interesting for various reasons. First, symptoms were quite severe and five of seven patients needed emergency room

treatment. Glottic edema in three patients was potentially life threatening. Second, two possible mechanisms of "lysozyme" allergy could be elaborated, namely precipitation of an allergic reaction in patients with a preexisting egg white allergy and sensitization of lysozyme and possibly to other egg white components by the vaginal suppository and subsequent development of an allergic reaction. This sensitization did not lead to an egg allergy.

In patients with a preexisting egg white allergy (PB, GC, and EZ), local and systemic symptoms arose minutes or hours after application of the first suppository. Patients PB and GC had serologic evidence for egg white allergy (circulating egg white specific IgE). Their symptoms were most likely provoked by contamination of lysozyme with ovomucoid, which was found to elicit a positive SPT reaction in patient PB (GC refused SPT). Indeed, the lysozyme used in the medications distributed by Prospa or Galenica seem to be rather heavily contaminated with other egg white constituents since ovomucoid completely abrogated the reactivity with "lysozyme" in a RAST inhibition assay using the serum of PB. Patient EZ had only a T cell-mediated immune response to egg white proteins, and no

indication for an IgE-mediated mechanism was found.

In contrast to these three patients with preexisting egg allergy, four patients without egg allergy developed the sensitization to lysozyme and possibly to other egg white proteins via the vaginal mucosa. Their allergic symptoms started after prolonged treatment or after re-exposure to the lysozyme/egg protein-containing suppository. It is possible that the vaginal inflammation, which was the reason for the bactericidal and mycostatic treatment, made the mucosa more permeable for the protein antigens, leading to sensitization and precipitation of the allergic reaction. These four patients continued to tolerate eating eggs or egg-containing food.

It is controversial, whether hen egg lysozyme itself is a potential immunogenic substance in humans.² The failure to demonstrate lysozyme-specific, circulating IgE as well as the negative result in the rather specific SPT argues against the allergenicity of egg lysozyme. On the other hand, we observed skin test reactivity (scratch) to purified lysozyme preparations as well as strong T cell reactivity to highly purified lysozyme preparations in all patients (but not in the controls), which would support the concept that egg lysozyme is immunogenic. The negative IgG-antibody dot blot tests probably rule out an IgG-immune complex induced anaphylactic reaction in these patients. The T cell-mediated immune reaction in the lymphocyte transformation tests was detectable using relatively small amounts of purified lysozyme (10 μ g/mL). Further, some patients had a positive lymphocyte transformation test to highly purified lysozyme only, but not to other egg white or egg yolk constituents (ovomucoid, ovalbumin, and livetin). This suggests that lysozyme is the antigen in those patients.

The comparison of the different tests revealed that intradermal or scratch tests with the lysozyme prep-

arations and the lymphocyte transformation tests were most consistently positive. In one case (CS) the lymphocyte transformation test was performed 3 years after the anaphylactic event and was still positive, while skin tests had become negative. Thus, although this test discovers the T-cell immunity only, which cannot explain the anaphylactic symptoms, it might be useful in detecting sensitization.

Patients who got sensitized during the therapy with the lysozyme/egg white-containing suppositories still tolerated eating eggs or egg containing products. This means that lysozyme is either rapidly degraded in the GI tract and is, therefore, no major allergen in egg-allergic individuals, or that the sensitization via the vagina was not strong enough to break the tolerance to orally ingested egg proteins. Our observation that only patients with clinically manifest egg allergy had circulating IgE antibodies to egg proteins supports the latter hypothesis. In contrast the patients who presumably got sensitized via the vaginal mucosa had no circulating IgE to egg proteins.

Recently an bird/egg syndrome has been described,⁷ whereby previous exposure to birds resulted in sensitization and egg allergy. Although five of the seven patients were previously exposed to birds, this mode of sensitization appears to be rather unlikely, as sensitization in the bird/egg syndrome is mainly directed to egg yolk proteins (livetins) and no such sensitization was found in our patients (SPT, stimulation tests). Interestingly enough, atopy was also no risk factor, as none of our patients had clinical symptoms of a respiratory allergy and all were negative to skin tests to usual environmental allergens.

Our data do not permit an estimate of the frequency of egg lysozyme allergy elicited by lysozyme containing drugs. In addition to the cases presented here the Swiss drug monitoring center had been informed of at least three additional patients, who developed anaphylaxis provoked by the same vaginal suppository; therefore lysozyme has been removed from the suppository. Lysozyme is used far more frequently in eye drops or nasal sprays than in vaginal suppositories, but reports about allergic side reactions to these topical medications are rare. We heard, however, of an egg-allergic patient, who developed an anaphylactic reaction to a lysozyme containing nasal spray (B. Wüthrich, personal communication). While sensitization to egg lysozyme via the nasal mucosa appears to be very rare, already sensitized individuals may develop allergic symptoms to lysozyme containing nasal sprays. In view of these data, one should be cautious in prescribing lysozyme containing medicaments to egg-allergic people, specifically because the lysozyme preparations are often impure and contain other egg white proteins as well.

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REFERENCES

1. Atassi MZ, Lee CL. The precise and entire antigenic structure of native lysozyme. *Biochem J* 1978;171:429-34.
2. Langeland T. A clinical and immunological study of allergy to hen's egg white. *Allergy* 1982;37:521-30.
3. Anet J, Back JF, Baker RS, et al. Allergens in the white and yolk of hen's egg—A study of IgE binding by egg proteins. *Int Arch Allergy*

4. Bernstein DI, Smith AB, Moller DR, et al. Clinical and immunologic studies among egg-processing workers with occupational asthma. *J Allergy Clin Immunol* 1987;80:791-7.
5. Roitt I, Brostoff J, Male D. *Immunology*. London: Churchill-Livingston Publ. London, 1989.
6. Hanson LA, Ahlstedt St, Andersson B, et al. Protective factors in milk and the development of the immune system. *Feeding the normal infant*. *Pediatrics* 1985;75(Suppl):172-6.
7. Mandallaz MM, De Weck AL, Dahinden CA. Bird-egg syndrome—Cross-reactivity between bird antigens and egg-yolk livetins in IgE-mediated hypersensitivity. *Int Arch Allergy Appl Immunol* 1987;87:143-50.
8. Pichler WJ, Schindler L, Stäubli M, et al. Anti-amiodarone antibodies: detection and relationship to the development of side effects. *Am J Med* 1988;85:197-202.
9. Koponen M, Pichler WJ, De Weck AL. T cell reactivity to penicillin: phenotypic analysis of in vitro activated cell subsets. *J All Clin Immunol* 1986;78:645-52.
10. Sette A, Colizzi V, Appella E, et al. Analysis of lysozyme-specific immune responses by synthetic peptides. I. Characterization of antibody and T cell-mediated responses to the N-terminal peptide of hen egg-white lysozyme. *Eur J Immunol* 1986;16:1-6.
11. Parronchi P, Macchia D, Piccinni M-P, et al. Allergen and bacterial antigen specific T cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci* 1991;88:4538-42.

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