

CASE REPORT

Type I allergy induced by Limulus Amoebocyte Lysate (LAL)

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Summary

During the last few years Limulus Amoebocyte Lysate (LAL) has been extensively used to detect minimal amounts of endotoxins of Gram-negative bacteria in products of the pharmaceutical industry, in food stuff, body fluids, house dust and room air. LAL is produced from cells of the haemolymph (amoebocytes) of the horseshoe crab (*Limulus polyphemus*), which respond with an extremely sensitive clotting system upon contact with endotoxins. In this study we demonstrate by typical case history, positive skin test and ELISA the occurrence of Type I allergy to LAL in a patient suffering from conjunctivitis and rhinitis at work.

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Introduction

The existence of endotoxins produced by Gram-negative bacteria is of great importance for the quality control of different products in industrial laboratories. For many years the demonstration of their presence was possible only by means of animal tests, mostly based on the pyrogenic effects of the endotoxins [1–3]. For the first time in 1956 Bang [4] described that the cells of the haemolymph—amoebocytes—of the horseshoe crab (*Limulus polyphemus*) contain an extremely sensitive clotting system which responds to endotoxins located in the cell membranes of Gram-negative bacteria.

The horseshoe crab measures up to 60 cm and inhabits the shallow waters of the North American Atlantic shelf. Its nearest relative, *Tachypleus tridentatus*, lives near the coasts of East Asia. Both are characterized by a horseshoe-like backshield and the presence of a long sting in their tail. They belong to a rare group of animals which have undergone a development independent from the other arthropods for the past 450 million years. These crabs, also referred to as 'living fossils' possess only one cell type in their haemolymph-system, the amoebocyte.

Extensive experimental studies have shown that these amoebocytes are responsible for immediate clotting of the haemolymph after contact with minimum amounts of endotoxin [4]. This reaction is initiated by discharge of enzymes stored in granules of the blood cells and subsequent formation of filamentous processes. This

action represents an important defence mechanism which allows limitation of bacterial infections.

Based on this characteristic capacity of Limulus-amoebocytes an extremely sensitive test method for the proof of endotoxins has been developed in the last few years. A lyophilized Limulus-amoebocyte extract—Limulus Amoebocyte Lysate (LAL)—makes it possible to detect endotoxins present in food stuff [5], pharmaceutical products as sera, vaccines and chemotherapeutics [6] down to concentrations of 0.05 ng/ml. In addition, it makes the examination of serum, blood, liquor and urine samples of patients with suspected infections due to Gram-negative bacteria possible [7–10] and can be used to control room air [11] and house dust in this respect [12].

In spite of the frequent use no described case of occupational sensitization to the LAL-test could be detected in the literature accessible to us. However, the following report demonstrates, that in rare instances a Type I allergy to LAL due to exposure at work can occur.

Case report

Case history

Bettina W., 23-years-old. Profession: Medical technician.

There is no known family history of atopic dermatitis or inhalant allergic symptoms. For years our patient has observed eczema after use of metal alloys. During the last 2 years she has developed rhinitis and conjunctivitis immediately after contact with cats.

In October 1987 Mrs W. began to work in a pharmaceutical laboratory. Some months later she noticed symptoms during her work similar to those previously

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only noticed after cat contact. In addition to rhinitis and conjunctivitis she observed swelling in the periorbital area. These complaints increased and remained during the following months but always stayed limited to the upper airways and face. The patient described that shortly after leaving the work place all symptoms disappeared. This fact led to the suspicion of an occupationally induced allergic reaction. In October 1989 the patient decided to consult an allergy clinic.

As a technician in the above mentioned laboratory Mrs W. was predominantly occupied with proving the existence of endotoxins using the COATEST (Kabi Vitrum Diagnostica, Copenhagen, Denmark).

The COATEST is performed as follows: the lyophilized LAL is reconstituted with sterile and endotoxin-free water, thereafter the sample to be investigated is added. Gram-negative bacterial endotoxin catalyses the activation of a proenzyme in the LAL. The enzyme formed splits off *p*-nitroaniline (*p*NA) from a chromogenic substrate S-2423 (Ac-Ile-Glu-Arg-*p*NA HCl). After stopping the reaction with acetic acid the rate at which *p*NA has been released is measured by optical density at 405 nm. The exact result is obtained by comparison with standard curves.

Methods

Skin-tests

The patient was skin-prick tested with numerous inhalant allergens such as house dust mite, animal epithelia, moulds and different pollens (reagents were obtained from ALK, Copenhagen, Denmark) and read after 20 min. Thereafter, the substances used at work—LAL and endotoxin of COATEST—were reconstituted following the manufacturer's advice and also used for prick-tests. A patch test was performed with a standard battery of common contact allergens (Brial, Greven, Germany) and read after 48 h.

In vitro tests

Total IgE was measured by PRIST (Pharmacia, Uppsala, Sweden) and RAST (Pharmacia, Uppsala, Sweden) was performed with following allergens: cat, house dust mite, crab, shrimp, chironomids and lobster, respectively, according to the manufacturer's advice.

An ELISA system was used to investigate IgE antibodies with specificity for LAL, endotoxin and the chromogenic substrate of the COATEST: microtitre plates (M129A, Dynatech MT Zug, Switzerland) were coated with different concentrations (1:2, 1:10, 1:50) of LAL, endotoxin, S2324 and a mixture of LAL and S2324 diluted in carbonate-buffer (1.8 mmol/l) for 12 hr at 4°C.

After washing three times with phosphate buffered saline (PBS)/0.05% Tween 20, the plates were saturated with PBS/0.05% Tween 20/1% HSA at room temperature (RT) for 3 hr. Thereafter, 100 µl of the patients' serum and as controls a human serum pool (from 22 healthy donors), and five individual human sera, diluted 1:2 in PBS/0.05% Tween 20/0.5% HSA, were filled into the wells and remained for 4 hr at RT. After washing three times with PBS/0.05% Tween 20, 100 µl of a horse anti-human-IgE conjugated with alkaline phosphatase (Kallestad, Chaska, U.S.A.) diluted 1:2 in the above mentioned buffer, was filled into the test-wells as detecting antibody and remained overnight at RT. After washing again the substrate polynitrophenylphosphate (PNPP) was applied in a concentration of 1 mg/ml in diethanolamine buffer, pH 9.8. The reaction was stopped after a 2 hr incubation period at 37°C with 1 M tribasic potassium phosphate. Subsequently, the optical density was measured at 405–550 nm.

As a negative control for antigen coating the carbonate buffer solution without diluting any material in it was used. As a positive control for the conjugate-substrate reaction a test with a birch pollen extract (100 µg/ml) and a serum with IgE antibodies from a birch pollen allergic patient was performed.

Results

Skin-prick tests with numerous inhalative allergens such as house dust mite, animal epithelia, moulds and different pollen showed a histamine-equivalent weal reaction to cat extract solely. Applying the substances used at work—LAL and endotoxin of COATEST—a distinct weal and flare reaction with LAL could be seen after 20 min, whereas the prick-test with endotoxin remained negative. Control tests with both substances in five volunteers showed no detectable skin-reactions.

A patch test performed with a standard battery of common contact allergens confirmed the presence of Type IV allergy to nickel and cobalt.

Using the PRIST-method, a marked rise in the total IgE level—2160 kU/l—could be found. RAST showed raised specific IgE for cat allergen. Different additional RAST-investigations with crab, shrimp, chironomids and lobster allergens remained negative, proving no Type I allergy against crustaceans or cross-reacting allergen sources in general.

Applying the ELISA-technique, IgE antibodies directed against LAL but not against the endotoxin sample or the chromogenic substrate S2324 could be detected. Control tests with a normal human serum pool (from 22 healthy volunteers) and five individual human sera showed negative results.

Discussion

The present study indicates that Type I allergy can occur during occupational exposure to lyophilized *Limulus* amoebocyte material. In this context it has to be considered that our patient showed a prior sensitization to cat allergen so that an atopic constitution can be assumed. A cross-reactivity between horseshoe crab amoebocytes and other related arthropod allergens like crab, lobster etc and allergens cross-reacting with these sources could be excluded by our RAST-results. Looking at the literature we found that Hansen and Gleich [13] immunized atopic and normal volunteers with haemocyanin from the horseshoe crab by subcutaneous and nasal routes. The authors observed the formation of specific IgM and IgA already after one week, while the presence of IgG antibodies was demonstrable in the third week. After the second intradermal application of haemocyanin 8 weeks after the start of the experiments in 2/11 cases immunized subcutaneously a weal and flare reaction could be seen and in 7/11 individuals a late reaction after 24 and 48 hr was observed. Clear differences between atopic and non-atopic persons could not be found. These and our results give evidence that blood components of arthropods are potent antigens and are even able to induce an IgE-response in atopic persons, which can lead to a typical Type I allergy on frequent exposure as we observed in our patient.

After the discussion of our test results with Mrs W. she decided to continue her work in the same laboratory but had the possibility to delegate the performance of the COATEST. A control in June 1990 showed that the patient had no complaints under these conditions. A control-measurement of the total IgE level at this time showed a significant decrease to 150 kU/l compared to 2160 kU/l in October 1989.

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