

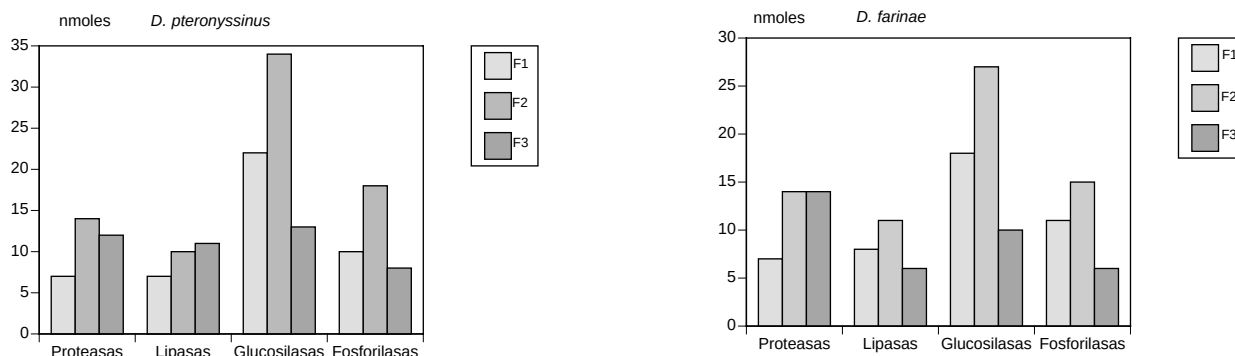


Fig. 4. Enzyme activity of the various extracts according to the phase in the growth cycle in which they were prepared.

from the 6th to the 8th week, an exponential growth phase (F2) lasting up to the 18th week for *D. pteronyssinus* and up to the 14th week for *D. farinae*, and a death phase (F3) lasting up to the 20th week.

The maximum expression of allergenic components, including that of the major allergens Der 1 and Der 2, was observed during the F2 phase (Fig. 2).

The extracts from phase F2 cultures furthermore evidenced the greatest reactivity when used as the solid phase in RAST procedures (Fig. 3)⁶ and the greatest skin reactivity in the allergic patient population included in biologic standardisation studies (Table II)⁷.

The extracts from this exponential growth phase (F2) also evidenced the greatest usefulness for in vitro cross-reactivity studies using the RAST inhibition technique⁸ and also the greatest enzymatic activity (Fig. 4)⁹. The latter reveals a greater presence of allergens, particularly considering that mite culture extracts contain a great variety of enzymes that are the mites' own and that are present both in the mite excreta and in the mite degradation products.

In conclusion, an adequate selection of the culture parameters for mite cultures crucially influences the allergenic extract finally achieved. Particular attention should be paid to the time of culture growth as this may, both by excess or by default, give rise to products with deficient allergenic presence.

References

1. Champman MD, Smith AM, Vailes LD, Arruda LK. Recombinant mite allergens. New technologies for the management of patients with asthma. *Allergy* 1997; 52: 374-379.
2. Aalberse RC. Allergens from mites: implications of cross-reactivity between invertebrate antigens. *Allergy* 1998; 53 (Suppl 48): 47-48.
3. Santos AB, Chapman MD, Aalberse RC, Vailes LD, Ferriani VP, Oliver C, et al. Cockroach allergens and asthma in Brazil: identifications of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. *J Allergy Clin Immunol* 1999; 104: 329-337.
4. Aki T, Kodama T, Fujikawa A, Miura K, Shigeta S, Wada T, et al.

Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, *D. farinae*. *J Allergy Clin Immunol* 1995; 96: 74-83.

5. Asturias JA, Arilla MC, Gómez-Bayón N, Martínez A, Martínez J, Palacios R. Sequencing and high level expression in *Escherichia coli* of the tropomyosin allergen (Der p 10) from *Dermatophagoides pteronyssinus*. *Biochim Biophys Acta* 1998; 1397: 27-30.

6. Eraso E, Guisantes JA, Martínez J, Sáenz-de-Santamaría M, Martínez A, Palacios R, et al. Kinetics of allergen expression in cultures of house dust mites, *Dermatophagoides pteronyssinus* and *D. farinae* (Acari: Pyroglyphidae). *J Med Entomol* 1997; 34: 684-689.

7. Eraso E, Martínez J, García-Ortega P, Martínez A, Palacios R, Cisterna R, et al. Influence of mite growth culture phases on the biological standardization of allergenic extracts. *Invest Allergol Clin Immunol* 1998; 8: 201-206.

8. García-Robaina JC, Eraso E, De la Torre F, Guisantes JA, Martínez A, Palacios R, et al. Extracts from various mite species contain cross-reactive and noncross-reactive IgE epitopes. A RAST inhibition study. *Invest Allergol Clin Immunol* 1998; 8: 285-289.

9. Martínez J, Eraso E, Palacios R, Guisantes JA. Enzymatic analyses of house dust mite extracts from *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Acari: Pyroglyphidae) during different phases of culture growth. *J Med Entomol* 1999; 36: 370-375.

ADVERSE REACTIONS TO TETANUS TOXOID

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INSULIN DESENSITISATION

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The prevalence of allergic reactions to insulin has decreased substantially with the use of insulin of human

origin. However, primary allergic reactions to human insulin may still occur, requiring the application of a desensitisation protocol. The present clinical observation represents an example of that situation.

Key words: Drug allergy. Insulin. Desensitisation.

DESENSIBILIZACIÓN A INSULINA

La prevalencia de reacciones alérgicas a la insulina ha disminuido sustancialmente con el uso de insulinas de origen humano. Sin embargo, aún se pueden presentar reacciones alérgicas primarias a la insulina humana que precizarán de la práctica de un protocolo de desensibilización. La siguiente observación clínica es un ejemplo de ello.

Palabras clave: Alergia a fármacos. Insulina. Desensibilización.

Allergic reactions to insulin have dropped dramatically, initially with the use of purified mono-component insulins and then with the introduction of recombinant insulins of human origin (with the same aminoacid residue sequence as the endogenous human one)¹. However, cases of (human) insulin allergy are still reported, in patients who had been previously treated with bovine or porcine insulin - (with the possibility of cross-reactivity)² but also as primary reactions³. It has been speculated that this immunogenicity, in patients who had never been exposed to insulin of other than human origin, might be due to changes in the tertiary structure of the insulin protein molecule or, perhaps, to modifications occurring along the administration route (binding to tissue proteins, conformational changes, polymerisation...). Some cases of hypersensitivity reactions to retard insulins might be due to their protamine sulfate^{4,5} or zinc⁶ contents. One novel analogue of recombinant human insulin has been recently introduced, "lispro" insulin, with a faster onset and greater duration of action than the regular one, which might be better tolerated than other insulins⁷.

Most patients with insulin allergy report a history of intermittent administration. The reactions induced may be of two types:

(a) Local reactions: erythema, pruritus and induration at the site of injection. These reactions may be immediate, delayed or biphasic. They should not lead to discontinuation of insulin therapy, as the later reintroduction of the drug might predispose to systemic reactions. These reactions usually remit after three to four weeks of therapy.

(b) Systemic reactions: these are quite infrequent and are characterised by generalised urticaria, angioedema, bronchospasm and arterial hypotension.

Case Report

Our patient was a 36-year-old female with gestational diabetes; she had no previous history of atopy, nor had she had previous insulin therapy. In her present pregnancy, insulin therapy had been initiated on gestational week 18 (Actrapid HM and Monotard HM insulins). Two weeks later she developed increasingly severe local reactions at the site of injection.

Primary insulin allergy was suspected as the cause of the reactions, and an allergy study was performed:

(1) Skin tests: the intradermal test with human insulin (0.04 U/ml) was positive, with an 11-mm wheal, while the intradermal tests with zinc and protamine had negative results.

(2) Specific anti-insulin IgE: the specific IgE level had been 0 PRU/ml prior to insulin therapy, and had increased to 8 PRU/ml after eight weeks of insulin therapy.

Desensitisation

The aim of desensitisation is to reproduce the allergic reaction, at a smaller scale and in controlled fashion, by inducing release of mast cell and basophil mediator release to the point of exhausting the mediator contents of these

Table I. rapid insulin desensitisation protocol

Dose No.	Insulin, U ^a
1	0,00001
2	0,0001
3	0,001
4	0,005
5	0,01
6	0,05
7	0,1
8	0,2
9	0,5
10	1
11	2
12	4
13	8
14	10

^aHuman regular insulin; subcutaneous administration of successive doses at 15-minute intervals.

cells. The patient is admitted into hospital and insulin is started subcutaneously, at first at very high dilutions and then at gradually increasing dosages until therapeutic doses are reached⁸. Table I presents a proposed rapid desensitisation protocol⁹. These protocols must be flexible, with modification of the doses and of the inter-dose intervals according to the local and/or systemic reactions that may arise. If insulin therapy is suspended, its later reintroduction entails a high risk of anaphylaxis.

The indications for insulin desensitisation include systemic reactions and severe local ones.

Desensitisation methodology

The less reactive insulin is used (skin tests), in the form of regular insulin; the initial dose is 1/100 of the one inducing a positive test. The administration route may be subcutaneous or intradermal. The blood glucose levels must be monitored throughout, and the desensitisation proceeds in dynamic fashion. Once the therapeutic dose is reached, desensitisation continues with retard insulin, under monitoring of the specific IgE and IgG levels.

In the case here reported, the desensitisation protocol suggested in Table I was used. The procedure achieved a reduction of the local reactions, and the specific serum IgE levels dropped to 1.3 PRU/ml.

Comments

Human insulin is less allergenic than non-human ones, but it may still induce IgE antibodies and cause allergic reactions. In such a case, desensitisation is the only possible therapy.

A number of alternatives have been proposed for patients in whom the desensitisation procedure has not achieved insulin tolerance: continuous subcutaneous insulin infusion¹⁰, subcutaneous administration of insulin, diphenhydramine and a corticosteroid in the same injection¹¹, and oral administration of insulin and acetylsalicylic acid¹².

References

1. Leonart R. Insulin allergy. Epidemiology and management. Clin Immunother 1994; 2: 7-12.
2. García-Ortega P, Knobel H, Mirada A. Sensitization to human insulin (letter). Br Med J 1984; 288: 1271.
3. Balsells M, Corcoy R, Leonart R, Pujol R, Mauricio D, García A,

et al. Primary allergy to human insulin in patient with gestational diabetes (letter). Diabetes Care 1991; 14: 423-424.

4. Kim R. Anaphylaxis to protamine masquerading as an insulin allergy. Del Med Jrl 1993; 65: 17-23.

5. Blanco C, Castillo R, Quirarte J, García I, de Pablos P, Carrillo T. Anaphylaxis to subcutaneous neutral protamine Hagedorn insulin with simultaneous sensitization to protamine and insulin. Allergy 1996; 51: 421-424.

6. Gin H, Aubertin J. Generalized allergy due to zinc and protamine in insulin preparation treated with insulin pump (letter). Diabetes Care 1987; 10: 789-790.

7. Kumar D. Lispro analog for treatment of generalized allergy to human insulin. Diabetes Care 1997; 20: 1357-1359.

8. Liñana JJ, Montoro FJ, Hernández MD, Basomba A. Reacciones adversas a la insulina. An Med Interna 1997; 14: 369-372.

9. Leonart R, Balsells M, Corcoy R. Primary hypersensitivity to human insulin (abstract). Clin Exp Allergy 1990; 20 Suppl.1: 208A.

10. Nagai T, Nagai Y, Tomizawa T, Mori M. Immediate-type human insulin allergy successfully treated by continuous subcutaneous insulin infusion. Intern Med 1997; 36: 575-578.

11. Loeb JA, Herold KC, Barton KP, et al. Systematic approach to diagnosis and management of biphasic insulin allergy with local anti-inflammatory agents. Diabetes Care 1989; 12: 421-423.

12. Haldoway IM, Wilson JD. Cutaneous insulin allergy responsive to oral desensitisation and aspirin. BMJ 1984; 289: 1565-1566.

RE-EXPOSURE TO β -LACTAM ANTIBIOTICS

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The diagnosis of hypersensitivity to β -lactam antibiotics is based on a sequence of steps comprising anamnesis, skin tests and/or specific IgE quantitation, and controlled exposure-challenge testing. The diagnosis of β -lactam hypersensitivity may be established at any of these steps, but most usually through the skin tests and/or the assessment of the specific IgE levels. However, a number of studies have reported that both the skin tests and the specific IgE may become negative over time^{1,2}. Other studies³⁻⁵ have shown that repetition of the tests and/or controlled exposure/challenge, ten to thirty days later, may establish the diagnosis in a number of patients who would have otherwise remained undetected. Considering this, a prospective study was initiated in order to establish the