

## ALLERGENIC ACTIVITY OF BOVINE DANDER EXTRACT

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### ABSTRACT

This study was conducted in Basrah at period from March through July 2000. The protein extract from Bovine dander was prepared with extraction followed by purification and fractionation by gel filtration into two peaks. Elisa inhibition with serum samples from 77 patients who were sensitive to bovine dander, revealed similar IgE, IgG binding patterns for each peak. Also, there is a cross-reaction between these two peaks. Peak II exhibited the highest IgE binding inhibition and showed a specific allergenic activity about two fold higher than that of peak I. The allergenicity of purified allergen was not lost during purification process.

### INTRODUCTION

The Elisa-inhibition is a non radioactive method based on the measurement of allergen specific IgE. It can be used in the allergen quantification when the binding of patients IgE to the solid-phase allergen is inhibited by the same allergen (1).

The amount of the allergen is calculated from the ratio of 50% inhibition points of the sample and the standard curves, it is possible to measure all allergens in crude extract or a single allergen.

Allergenic potency described the true biological activity of an allergen extract or a single allergen. It is expressed in international units (2), biological units or allergy units.

The most common method used for calculation of allergenic potency is Elisa-inhibition (3).

Since a near-perfect correlation has been found between the doses for 50% inhibition between IgE and IgG antibodies in comparative studies with a large series of allergens. The use of a less costly and more easily accessible IgE-based assay has been suggested for the potency control of allergenic extracts (4), (5).

The aim of this study was therefore determine the allergic activity of Bovine dander and purified allergen extract using the Elisa-inhibition test.

### MATERIALS AND METHODS

#### Serum Samples:

The serum samples were obtained from 77 patients seen at the center of asthma and allergic disease in Basrah during the six months from March 2000 through July 2000

**Antigen:**

Bovine *Bos domesticus* dander extract was prepared essentially as described previously (6).The protein content was determined according to Whitakavs and Granum (7).

The gel chromatography was used for the purification and fractionation of the dander extract into molecules of different molecular size using G-75 Sephadex according to the method of Leslic and Frank (8).

**Rabbit antihuman immunoglobulines conjugates:**

Rabbit antihuman Ig and horse radish had been conjugated as previously reported (8).

**Elisa-inhibition:**

For competition elisa microtiter 96 were coated with allergen extract at 100ug/ml for 1 hour at 37C (9).The plates were then blocked for 1 hour with PBS-BSA 1%.After wards 25 ul of the serum pool(diluted 1:3 PBS) and 25ul of serial dilution of allergens extract were added to the wells and incubated for 2 hours at room temperature. The plates were washed again and developed by incubating with 50ul/well of a solution of orthophenylene Diamine( OPD 0.5 mg/ml) for 30 minutes. The reaction was stopped by adding 50ul of 2M Hcl and the absorbance was measured at 492 nm .Assays were performed in duplicate.

Inhibition of specific IgE was out by incubating the pool diluted 1:5 for 3 hours with serial dilution of inhibitors ranging from 5 ug protein/ml to 0.02 ug/ml. After the mixture was transferred to the plates and further incubated for 3 hours at room temperature. Followed after washing ,by an overnight incubation with peroxidase-labeled rabbit IgG antihuman IgG.The plates were developed as above.

**RESULTS**

On fractionating Bovine dander ,two major peaks were observed (Fig 1),the allergenic activity of these two peaks (peak I and Peak II) and the crude protein extract was evaluated by means of competition Elisa as have been show in (Table 1) ,either peak I and peak II could inhibit to a high extract(peakI:76%,PeakII74%)Then suggesting that these proteins bear major allergenic determine of the source material extract. The upper vaue in each entry means the amount of protein in micrograms per well needed to reach 50% inhibition .The lower values represent the maximum inhibition reaching ( %).Futhermore ,the IgE binding capacity of peakII protein was found to be higher than that of peakI protein ,since lower concentration of peakII protein was needed to inhibit up to 50% the binding of specific IgE to the allegrosorbant phases.

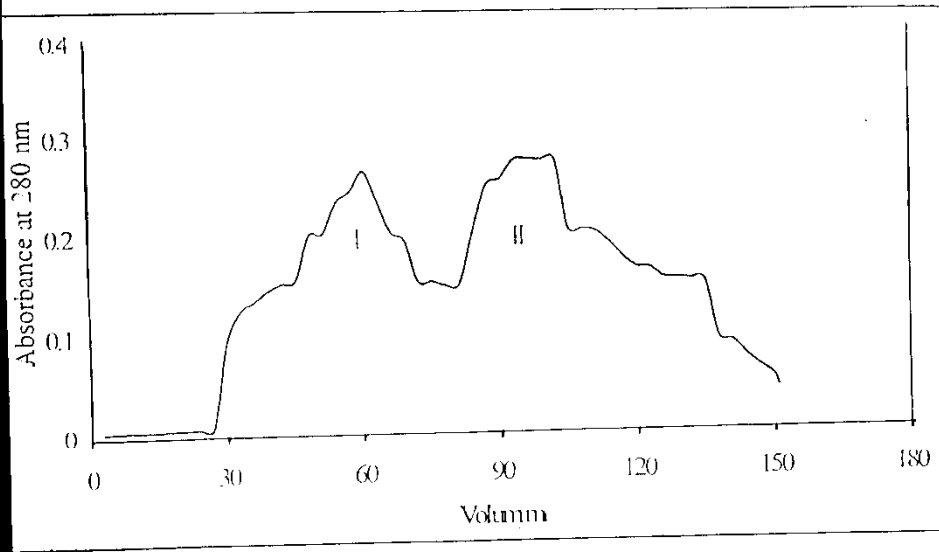


Fig. 1. Elution profile of bovine dander protein.

Table 13. Cross-inhibition of specific IgE, IgG binding among dander extract (DE), Peak I and Peak II, using ELISA inhibition.

Allergosorbent	Inhibitor		
	Dander extract	Peak I	Peak II
Dander extract	14.2 / 96	2.5 / 76	1.5 / 74
Peak I	28 / 85	1.3 / 98	0.6 / 91
Peak II	36 / 88	1.9 / 90	0.7 / 95

## DISCUSSION

As far as the allergenic activity of protein in peak I and Peak II is concerned data presented in this work clearly evidence that protein of these peak are the most clinically relevant allergens from the tested source materials.

Beside allergens of peak I and peak II independently accounted for a high percentage (about 75%) of the total allergenic activity. Demonstrating that they are the allergens from their source material and consequently other allergens might be present in the extract with a little allergenic importance in comparison with that of peak I and peak II.

On the other hand, peak I and peak II cross-reacted in IgE binding inhibition, completely inhibiting the binding of specific IgE to each other.

It seems clear that both proteins bear the same allergenic epitopes and this fact supports the idea of extremely high homology between them. This finding is in line with those previously reported (10) that cross reactions may extend to the presence of carbohydrates structures sheared by several components of the same protein extract. Nevertheless, a slight difference between these peaks was observed for the amount of protein needed to inhibit up to 50% the binding of specific IgE to each other or to whole extract, about two fold peak I to peak II amount were necessary to reach similar inhibition level.

In Conclusion: our data support the hypothesis that the allergenicity of purified allergen do not lost during the purification process.

### الفعالية المستأرجة لخلصة قشرة جلد الأبقار

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### الخلاصة

اجريت هذه الدراسة في محافظة البصرة للفترة من مايس الى اب ٢٠٠١، تم تحضير واستخلاص محلول بروتيني من قشرة جلد الأبقار ومن ثم تمت تنقيته وتجزئته بالترشيح الهلامي حيث تم فصل مستخلص قشرة جلد الأبقار الى قسمين رئيسيين. و اجراء فحص تثبيط الأليزا على مصل ٧٧ مريضا متحسنا لقشرة جلد الأبقار وجد بأن هذه القمم لا تختلف كثيرا عند ارتباطها بكل من IgG او IgA وان هناك تقاطع تصالبي بين هذه القمم وان القمة ٢ أظهرت قدرة عالية على تثبيط ارتباط IgA وكذلك فعالية مستأرجة أعلى يقدر الضعف سن هو موجود في القمة ١ وان الفعالية المستأرجة للمستأرج النقي لا تفقد أثناء عملية التنقية.

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