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PRELIMINARY INVESTIGATION OF ANTIGEING PROPERTIES OF TWO KINDS OF MALLEINS

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SUMMARY:

The antigeic composition of two kinds of malleins (Old mallein and PPD mallein) were analysed by gel filtration chromatography on sephacryl S 300 column. Two major protein peaks were determined and were termed peak 1 (mol. wt > 10^{-6} daltons) and peak 2 (mol. wt < 5000 daltons). Fractions of mallein were tested for its haemagglutination inhibition activity (HI) and delayed type hypersensitivity reaction (DTH) in a P. mallei sensitized guinea pigs. Most of the HI activity of the fractions was correlated to the fractions of peak 1. Moreover peak 1 produced good DTH reaction in sensitied guinea pigs, but peak 2 failed to produce satisfactory results.

It was concluded that the antigenic activity of both Old and PPD malleins were associated with the high molecular weight protein fraction which is found in both mallein preparations.

INTRODUCTION

Mallein was first introduced by Kallning and helman in 1891, and it has been used extensively in the diagnosis of glanders in horses, ponies and donkies. However, several attempts were made to improve mallein potency and specificity (Rafyi and Mir-chamsy, 1952, Stepkows and Parnas, 1952, Huitema 1969 and

Marathe et. al. 1977) but non has been made to separate and investigate the active components of the mallein which is responsible for the DTH response produced by the intrademo-palpebral inoculation of mallein. Both Old and purified protein derivatives (PPD) of mallein preparations were proven to be valuable in the diagnosis of P. mallei infection yet, false positive results were not uncommon (stable forth, A. W. and Galloway, I. A. 1959) therefore, it become more important to analyse the antigenic component of the mallein in an attempt to identify the factors which are responsible for the specific immune response in sensitized animals.

MATERIAL AND METHODS

Mallein analysis by gel filtration, Aliquots of mallein preparations ((2 ml of PPD "iffameriux" or Old mallein (VET institute of immunology, Sofia))) were fractionated on Sephacryl S 300 Column (2.6 × 96 cm. pharmacia). The column was calibrated with a molecular weight protein standard at rate 2.5 $ml^{-2} . cm^{-2} , h^{-1}$.

The protein concentration of the fractions was monitored by 2138 UV cords (LKB) at 280 nm.

Each fraction was tested for its inhibitory activity to specific haemagglutination reaction and the regions containing detectable amounts of protein were pooled separately. Tow pools were formed in each mallein which were termed peak 1 and peak 2.

Protein concentration of malleins were determined following the method of Bradford et. al. (1976).

Skin testing for DTH; To render guinea pigs mallein sensitive, each guinea pig was subcutaneaously inoculated with 2 mg (moist weight) of heat killed P. mallei emulsified in complete freunds adjuvents (CFA).

Skin test for DTH was performed 3 weeks later by intradermal injection of 0.1 ml of various preparations of mallein or mallein fractions. Twenty four hours later, the diameters of the reactions were measured, a control group of non sensitized guinea pigs received a similar treatment.

HAI TEST : Sheep red blood cells (SRBC) : Sheep blood was collected from the jugular vein in Alsevers solution. SRBCs were sedemented by centrifugation and washed several times with phosphate buffer saline solution (PBS), pH 7.2.

Preparation of formalinized SRBCs : One volume of 3% solution of formalin was added to one volume of 8% suspension of SRBCs. After 18 hr. incubation time at 4C, the cells were sedemented and formalin was discarded. The cells were then washed several times with PBS and stored at 4C in PBS containing 1 : 10000 part of merthiolate as preservative.

Sensitization of formalinized SRBCs : One millilitre of packed SRBCs were resuspended in 1 ml. PBS. To this suspension equal volume of fresh tannic acid solution (1:10000) was added and vigorously shaken and the whole content were agitated for 15 min. After which the cells were palated gently at 200 G and the supernatant discarded. Tanned cells were resuspended in 1 ml. PBS and 1 ml. of mallein PPD (containing 0.25 mg ml protein) was added to the cells and the whole mixture was gently agitated for 40 min. The cells were then washed three with PBS and resuspended in PBS containing 1% heat inactivated normal rabbit serum A non sensitized preparation of tanned SRBCs were used as a non coated control.

Heamagglutination reaction : the heamagglutination reaction was performed in microtitre plates. Various dilutions of standard anti- P. mallei sera were prepared with PBS as diluent (rabbit anti-Pseudomonus mallei Vet. Inst. Sofia). Then 0.05 ml. of 1% suspention of senitized SRBCs were added and the plate was shaken and incubated at room temperature for 2 hours. The

titre was expressed as the highest dilution of anti-sera which gave a definite agglutination reaction.

Heamagglutination inhibition reaction (HIR) : For the determination of specific antigen which produce complete inhibition of a specific agglutination several dilutions of PPD, Old mallein or their fractions were incubated with the highest dilution of anti-P. mallei antisera which produce good heamagglutination reaction. To this reaction mixture a similar volume of sensitized SRBCs was added. The whole plate were incubated at room temperature for 2 hours. Inhibition of agglutination was considered positive for the presence of specific antigen.

RESULTS

Result of fractionation of both PPD and Old malleins presented figure 1 and 2 were similar with regard to protein peaks profile. Both preparations showed an earlypeak of high molecular weight (peak 1 $>10^{-6}$ dalton) which was eluted at the viod volume and several minor low molecular weight protein peaks (peak 2, <5000 dalton). Antigenic analysis of the fractions of both malleins showed that peak 1 was associated with all humoral antigenic activity of mallein since it was the only region which produced complete inhibition of the hacmagglutination reaction (See figures 1 and 2).

Guinea pigs sensitied with P. mallei reacted typically to both ,allein types in a dose dependent manner(table 1). Furthermore, samples from peak 1 tested in pre-sensitized guinea pigs produced typical skin reactions. However, samples from peak 2 produced only a slight reaction which does not differ from that produced in the control group (table 2).

Table 1 Delayed type hypersensitivity reactions(mm) to old mallein in sensitized guinea pigs.

	Dilution of mallein µg/ml		
Type of mallein	7	30	120
Old mallein	6.5	10	13.5
PPD	3.3	6	8

Five guinea pigs were used for each group.

Table 2 Delayed type hypersensitivity reactions(mm) to mallein and mallein factions in sensitized guinea pigs.

	Diameter of reaction mm								
Type of mallein	crude			F1			F2		
	N	$\bar{X}$	SE	N	$\bar{X}$	SE	N	$\bar{X}$	SE
PPD mallein	5	16	± 3	5	14	± 4.5	5	5	0.5
Old mallein	4	10	± 1.5	4	10	± 2.6	4	0	0

N = Number.
$\bar{X}$ = Mean.
SE = Standard.

These results may indicate that cell-mediated immune response is also directed against peak 1 region of both mallein types.

A slight skin reaction to mallein was noticed in non sensitized control group.

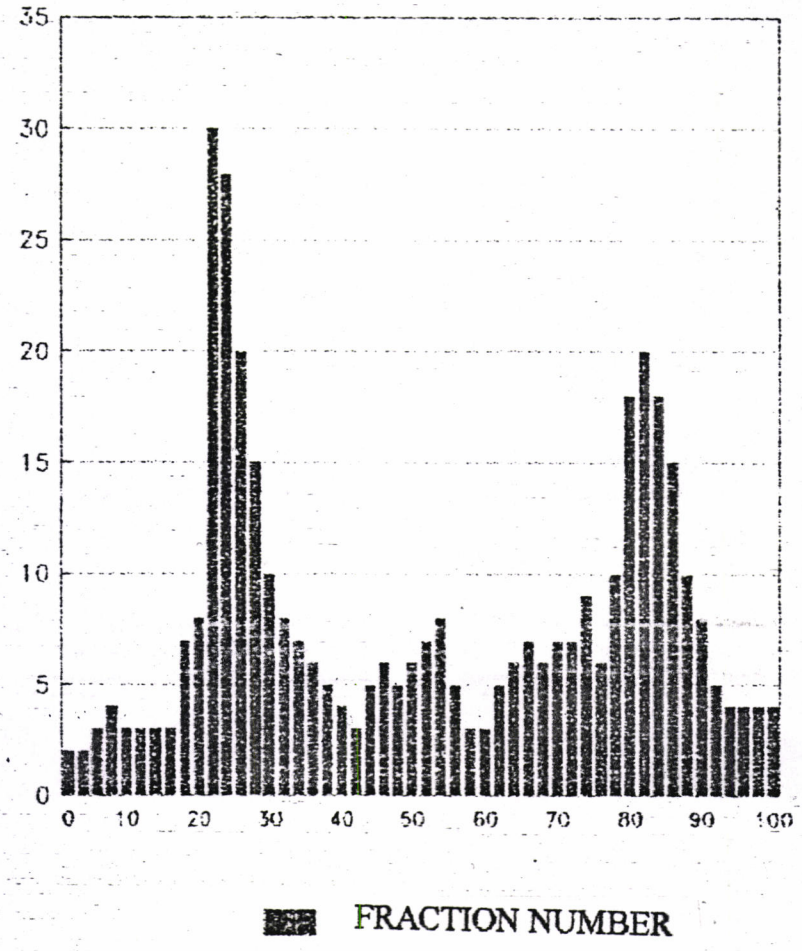

Figure 1 : Fraction of PPD mallein on sephacryl S 300 Column

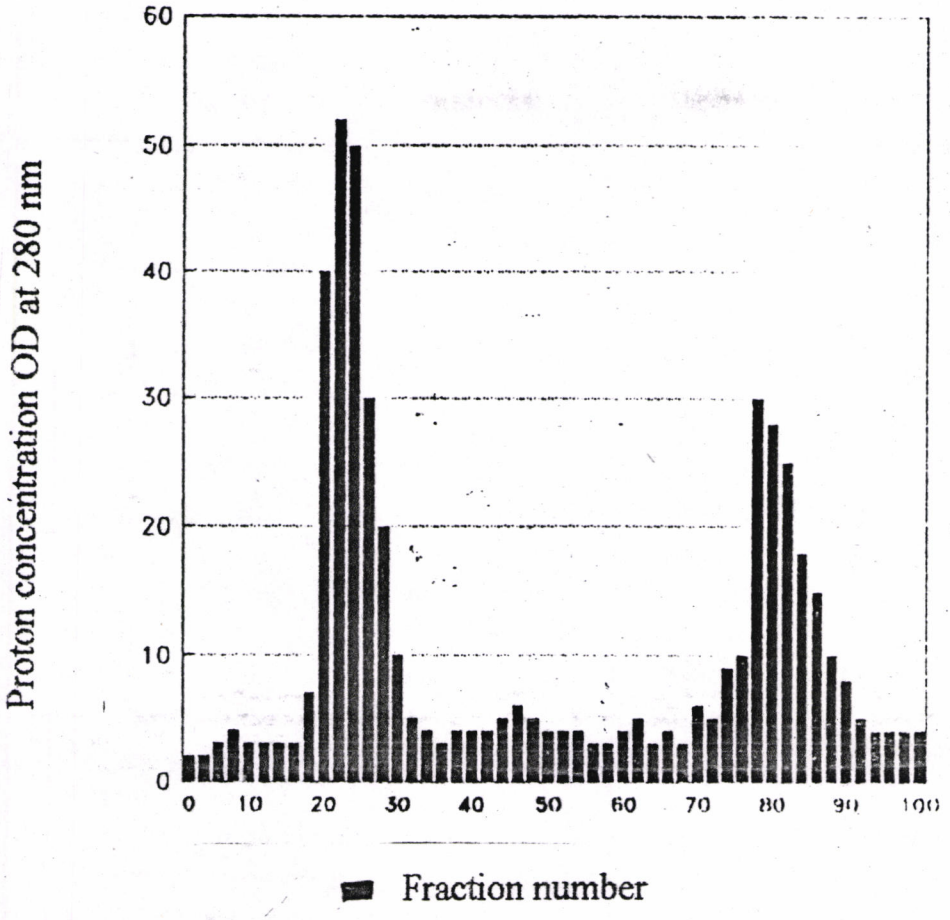

Figure 2: Fractionation of old mallein on sephacryl S 300 column

DISCUSSION:

Several authors have demonstrated the utility of haemagglutination inhibition and delayed type hypersensitivity reaction as methods for standerdization of mallein (Sen, et al 1967 and Huitema, 1969). In this paper both tests have been used to evaluate the antigenic properties of various mallein fractions in an attempt to separate the active component of mallein antigens which are responsible for the specific immunological responses in P. mallei sensitized animals recent study by AL-Bassam 1993 has shown that mallein prepared from local isolates of P. malei (Old mallein, PPD) fractionated by gel filtration on a column of (sepharose 6 B) yielded three major protein components, most of the HI & DTH activity were detected in the first fraction of high molecular weight (AL-Bassam 1993).

Fractionation of both PPD and Old malleins by gel filtration, revealed the presence of a high mol. wt. component which contained most of the biological activity of mallein, as judged by the HI activity of the fractions and DTH reactions produced by peak 1 in guinea pigs. These results may suggest that both cell-mediated and humoral immunity in P. malei. sensitized animals were confined to peak 1 in both PPD and Old mallein.

Marathe et. al. 1977 analyzed the active component in both PPD and mallein and he concluded that the biological activity in both malleins were associated with an identical high mol. wt. glycoprotein.

The removal of non specific protein contaminants from mallein preparations might improve the efficiency of the intradermo-palpebral test for diagnosis of glanders. A large scale study to test the efficiency of peak 1 preparation from mallein in animals naturally infected with glanders is required.

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دراسة اولية على الخواص الاستضدادية لنوعين من الملينات

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الخـــلاصة

تـم تحليـل المكونـات الاسـتضدادية لنوعيـن مـن الملينـات المسـتوردة (Old Mallein)* و (PPD Mallein) ** بأستخدام تقنية كروماتوغرافياً الفصل الجزيئي على هلام السفكريل S 300. تم الحصول على ذروتين اساسيتين مـن الـبروتين لكـلا النوعيـن مـن الملين وهـي الـذروة رقـم 1 (الـوزن الجزيئي > 10^{6} دالتون) والذروة رقم 2 (الوزن الجزيئي < 5000 دالتون).

فحصت اجزاء الكلين بواسطة قدرتها على تثبيط تفاعل التلازن الدموي المنفعل واحداث تفاعل فحص التحسس الجلدي المتأخر في خنازير غينيا المحسسة ببكتريا p. mallei.

دلت النتائج على ان اغلب الفعالية الاستضدادية المثبطة لتفاعل التلازن الدموي المنفعل كانت في الذروة رقم 1 لكلا الملينين بالاضافة الى انها كانت الذروة الوحيدة التي اعطت تفاعلاً ايجابياً في فحص التحسس الجلدي المتأخر في الخنازير المحسسة بالمقارنة مع الذروة رقم 2 ، لذا فقد تم استنتاج ان الفعالية الاستضدادية لكلا الملينين كانت موجودة في الذروة رقم (1).

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* وهو عبارة عن الملين الخام والمستورد من المعهد البيطري في صوفيا (vet institute , Immunology , Bulgaris)

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