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دراسة المقاومة الفيبرة تخصيصًا في الكتاكيت ضد ميكروب كوليرا الطيني.

حلمي تركي، محمود السيد، محمد عقيل.

تم دراسة دور المقاومة الفيبرة تخصيصًا ضد ميكروب كوليرا الطيني.
ولقد أمكن اكتشاف وجود علاقة بين المستوى المعياري للأجسام المناعية في الدم وقوة التحكم من الميكروب بالخلايا المتخصصة (Phagocytic cells) وكان لوجود الاستثناءات في وجود الخلايا المتخصصة وزيادة نشاطها. وكانت الخلايا الالتهابية (Phagocytic cells) تتعقد اعتماداً على خلايا الدم البيضاء (Polmorphismnuclear) أو أكثر من خلايا (Monocytes) حيث أن التحليل الإحصائي أثبت ذلك.
The role of nonspecific resistance to Pasteurella multocida was studied. A correlation between levels of systemic antibodies (titres) and chemotactic and phagocytic activity of phagocytic cells was proved. The presence of specific opsonin not only result in high significant shift of phagocytic cells toward the microorganism (chemotaxis) but also it increase the phagocytic activity of these cells. The phagocytic activity depends mainly on the polymorphonuclear cells (PMN) than monocytes as $P<0.05$ in case of PMN and $P>0.05$ in case of monocytes.

INTRODUCTION

P. multocida serotypes 5a, 8a, and 9a, were found to be pathogenic for chickens by NAMIAKA and BRUNER (1963), later NAMIOKA (1970) refered to type 2aD and -2D to be associated with chronic fowl cholera. In Egypt EL-GHAWAS (1975) investigated 200 strains of P. multocida from acute fowl cholera and were serotyped 5a, 8a and 9a, while AOUAD (1978) and EYZZAT (1983) found that 5a, 8a, 9a and 2aD serotypes of P. multocida are the causative agents of chronic fowl cholera. Moreover EYZZAT (1983) reported that chickens vaccinated with bacterins of this serotypes survived challenge by (95.7%) and P. multocida was not recovered from dead birds. While the control group died from fowl cholera after 3 days post-challenge. The extraordinary high virulence exhibited by many chicken and turkey strains seems to correlate with the ability of this organisms to resist phagocytosis, once it enters the tissues (WOOLCOCK & COLLINS, 1976 and COLLINS, 1977).

Since the mechanism of acquired resistance to P. multocida infection remains poorly understood, specially the role of non specific resistance, studies were performed to measure the phagocytic activity and levels of immunity and protection to challenge. This knowledge could help in devising a more effective immunization program against fowl cholera in chickens.

MATERIAL and METHODS

Bacterial antigens:

4 virulant strains of P. multocida serotypes 5a, 8a, 9a and 2aD were used for the preparation of vaccine, challenge and measuring the phagocytic activity of leukocytes from vaccinated as well as from non vaccinated chickens. These strains were isolated, serotyped, tested for their virulence in the Department of Microbiology and Animal Hygiene Faculty of Veterinary medicine university of Alexandria.

Birds:

12 Dokki 4 of 10 weeks old broilers were obtained from a local source. They were devided into two groups (6 birds in each group) and housed in separate rooms for three weeks before vaccination. The tested group was subcutaneously vaccinated with 0.5 ml of oil-adjuvant polyvalent bacterins at 13 weeks of age and a second dose at 15 weeks as described by (EYZZAT, 1983).

Agglutination test:

An assay was carried out for measuring the humoral immunity using the ordinary tube agglutination test as recommended by NAMIOKA and BRUNER (1963).

Assay for phagocytosis:

The test was carried out essentially as described by the Department of Microbiology, Veterinary Academy, MOSCOW, (1980).

Chemotaxis studies:

A modified technique of chemotaxis assay under agarose as described by NELSON et al. (1975), was used. Where leukocyte suspension containing PMN and mononuclear cells were utilized. Chemotaxis was measured after 2 hours of incubation i.e. measuring the distance of migrating cells towards antigens, using blood leukocytes from immunized birds. Quantitation of the results was carried out by calculating the mean surface area of migration to antigen and comparison to the control tubes which contained only blood leukocytes from non immunized birds not containing any agglutinating antibodies.

Antigen for chemotaxis Assay:

P. multocida antigens were prepared as described by NAMIOKA and BRUNER (1963). They represented the packed cells obtained from 16 hours old culture, suspended in 10ml of NHCl saline (86ml conc. HCl + 9gm Nacl + 914 distilled water) and incubated in a screw capped bottles at 37°C for 16-18 hours. The suspension was centrifuged at 3000 r.p.m. for 15 min. and the sediment was twice washed with 0.3% formalinized buffer saline. The final pH of the suspension was adjusted to 7 using 10% sodium carbonate solution.

RESULTS

P. multocida serotypes 5;A, 8;A, 9;A and 2;D were not equally phagocytosed by blood leukocytes before and after vaccination, i.e. in the absence of specific opsonins, phagocytosis activity were very low and statistically was not significant (P > 0.05). After vaccination the activity became high and was significant statistically where P/ < 0.05 (Table I). However it appears that the phagocytic activity depends mainly on the PMN than monocytes as P/ < 0.05 in case of PMN and P/ > 0.05 in case of monocytes (Table II).

A correlation between levels of systemic humoral antibodies and phagocytic activity was found (Table II).

The results of chemotaxis assay showed that the migration was increased significantly post-vaccination where P/ < 0.05 (Table I).
DISCUSSION

The nature of the defence mechanism by various immunoprophylactic (vaccines) agents used against fowl cholera in chickens remains enigmatic. DUA and MAHESWARAN (1978) found a correlation between levels of systemic humoral antibodies (titres) and cell-mediated immunity responses and resistance to challenge with a virulent strain of P. multocida. However, independent studies by HEDDELESTON and WATKO (1965), BHASIN and BIBERSTEIN (1968) and ALEXANDER and SOLTYS (1973) found no correlation between serum titres and resistance. The data generated from statistical analysis of correlation between levels of antibody titres and phagocytic activity indicated that systemic immunity played a significant role $(r = 0.52-0.57)$. These results also confirm and explain earlier findings by ALEXANDER and SOLTYS (1973), BIERER and DERIEUX (1975), and HEDDELESTON et al. (1975) that turkeys which were passively immunized with antisera raised from immunized birds were protected against challenge. The extraordinary high virulence exhibited by many poultry strains are correlated with the ability of this organism to resist phagocytosis (COLLINS, 1977). These seems that vaccination cannot protect chickens against P. multocida unless the phagocytic activity of the phagocytic cells is increased. The presence of specific opsonin not only results in high significant shift of phagocytic cells towards the microorganisms (chemotaxis) but also there is an increase in the phagocytic activity. Unfortunately, it should also be pointed out here that the significant statistical difference $(P < 0.05)$ between the phagocytic activity of PMN to phagocytose the different serotypes of P. multocida can destroy the cross protection.

A commercial bacterins, only contain a few of available strains, so it cannot protect against P. multocida infection, under all field conditions and protection is often fully effective when the autologous vaccine strain is used.

REFERENCES


Table (i): Effect of immunization with bacteria of P. multocida on phylogencs of chemokines

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Table (II): Analysis of variance of phytochrome activity and correlation

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significant at P = 0.05

n = 24