STUDIES ON FACTORS INFLUENCING IMMUNE RESPONSES IN CHICKEN

IV. EFFECT OF PIND-AVI ON THE PHAGOCYTIC ACTIVITIES OF CHICKEN MACROPHAGES IN-VITRO.

MONA EL-ENBAWY, R. SOLIMAN AND M. REFAI

DEPARTMENT OF MICROBIOLOGY, FACULTY OF VETERINARY MEDICINE, CAIRO UNIVERSITY.

SUMMARY: In the present work the effect of the paramunty inducer PIND-AVI on the phagocytic activities of chicken macrophages against Candida albicans in-vitro was investigated. The in-vitro sensitization of chicken macrophages with PIND-AVI for 24 hrs. was found to induce a marked activation of the process of phagocytosis, where in PIND-AVI treated cells the rate of phagocytosis reached to 80.97% and the phagocytic index was equal to 1.88, compared with phagocytosis rate of 44.25% and phagocytic index of 0.97 in the untreated macrophages.

INTRODUCTION

Macrophages are recognized as key elements in the host defence against various microbial invaders (Wasencraft, et al., 1984).

Using appropriate manipulations, macrophages can be induced to undergo profound biological and biochemical modifications which are generally referred to as activation (Adam, D., 1982). Activated macrophages manifest strong microbicidal and tumorcidal activities (Bellanti and Dayton, 1975).

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Because of the potential immunological value of the activated macrophages in disease resistance, much efforts are devoted to the study of agents which induce macrophage activation and potentiation of the phagocytic activities. These agents include glucan (Riggi, 1961), zymosan (Heller, 1960), estrogen (Nicol, et al., 1960) endotoxin (Benacerraf, 1957), BCG (Rosenthal, 1980), Corynebacterium parvum (McBride et al., 1974 and Halpern et al., 1973) and PIND-AVI from avipoxviruses and PIND-ORF from parapoxviruses (Mayr, 1978 and Mayr et al., (1979).

The effect of PIND-AVI as non specific immunostimulant on phagocytic cells has been reported by Mayr, (1980), (1981) and (1982). Also Brunecker et al., (1986) found that PIND-AVI preferentially stimulated the spontaneous macrophage activities.

In the present work the effect of PIND-AVI on the phagocytic activities of chicken macrophages against Candida albicans was investigated.

MATERIAL AND METHODS

Materials:
1. Chicken peripheral blood: Two ml of heparinized blood were collected by heart puncture from each chick. Heparin (Preservative free) was added at dilution of 20 IU/ml blood.
2. Ficoll-hypaque-sp. gr. 1.075 (Sigma, U.S.A.).
3. RPMI-1640 tissue culture medium (Flow. Lab.).
4. Candida albicans group A (ATCC-28367), the culture was kept and propagated on Sabouraud's dextrose agar.
5. PIND-AVI was obtained from Institute of Microbiology, Veterinary School Munich, Germany.
6. Cell culture and staining chamber (GCSC) with rounded cover slip (Greiner, Germany).

Methods
1. Separation of the mononuclear cells (MNC) using Ficoll-hypaque was done according to Boyum (1968).
2. Separation and cultivation of mononuclear adherent phagocytes from the MNC-suspension was carried out according to Wardley et al., (1980) as follow: The separated mononuclear leucocytes were adjusted in suspension to 10^7 cells/ml, the cells suspended in RPMI-1640 tissue culture medium (containing 15% fetal calf serum "FCS") were distributed in CCSC cups (1 ml/cup).

The cups were incubated at 37°C for 1 hr in the presence of 5-7% CO₂ tension. Then the non adherent cells were removed by 3 time washing using warm serum free RPMI-1640 medium.

The remaining adherent cells were supplied with 1 ml RPMI-1640 containing 15% FCS and kept at 37°C and 5-7% CO₂ for 24 hours before sensitization with PIND-AVI.

3. Sensitization of the adherent phagocytic cells with PIND-AVI.

The lyophilized PIND-AVI powder was resuspended in 2ml RPMI-1640 medium. 200 µl of the PIND-AVI suspension were added to the adherent cells in each cup and incubated for 24 hrs at 37°C and 5-7% CO₂ tension. Control groups of non PIND-AVI treated cells were included.

The adherent cells were then washed twice with warm, serum free RPMI-1640 medium and new fresh RPMI-1640 medium containing 15% FCS was added.


To the prepared PIND-AVI treated or not treated adherent cells in CCSC cups, Candida albicans cell suspension in a conc. of 10^7/ml RPMI-15% FCS medium was added, then incubated for 1 hr at 37°C and 5-7% CO₂. The non adhered or phagocytosed yeast cells, were removed by 3 gentle washing using cold serum free RPMI-medium, the cover slides in the CCSC cups were stained with Giemsa stain, examined under light microscope and the phagocytic activity was evaluated as follow:
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The phagocytosis rate =

Number of phagocytes with ingested blastospores X 100
Total No. of counted phagocytes

The phagocytic index = The average No. of yeast cellsphagocytes.
Total No. of yeast cells phagocyted by macrophages
No. of counted macrophages which show phagocytic activity.

RESULTS

As shown in Table 1 and Fig 1a and 1b chicken macrophages treated with PIND-AVI in vivo manifested strong phagocytic activities compared with the untreated macrophages, where the phagocytosis rate and the phagocytic index were 80.97% and 1.88 respectively, compared with 44.25 and 0.97 in the control untreated cells.

Table (1): The effect of in-vitro sensitization of chicken macrophages with PIND-AVI on its phagocytic activity against Candida Albicans.

<table>
<thead>
<tr>
<th>The total number of</th>
<th>Activated with PIND-AVI</th>
<th>Non-activated Control.</th>
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<tbody>
<tr>
<td>No. of counted cells(A)</td>
<td>226.00</td>
<td>226.00</td>
</tr>
<tr>
<td>No. of phagocytes with ingested yeast cells(B).</td>
<td>183.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Total No. of yeast cells in all counted phagocytes (C).</td>
<td>325.00</td>
<td>97.00</td>
</tr>
<tr>
<td>Rate of phagocytosis (\frac{B}{A} \times 100)</td>
<td>80.90</td>
<td>44.25</td>
</tr>
<tr>
<td>Phagocytic index (\frac{C}{B})</td>
<td>1.88</td>
<td>00.97</td>
</tr>
</tbody>
</table>
Fig. (1) Twenty six hours old, non-activated chicken macrophages stained by Giemsa stain (1000x.)

Fig. (2) Twenty six hours-old-chicken macrophages activated in-vitro by PIMD-AVI for 24 hours. Note the strong phagocytic activity of the cells against *Candida albicans*. Giemsa stain (1000x).
DISCUSSION

In recent years, the attention of immunologists has turned to the reticuloendothelial system (RES) as a locus for initiation of immune responses. One of the important RES functions is phagocytosis which is not only involved in non-specific defence but also in initiation and regulation of specific responses. Therefore, it must be important whether the functional activity of macrophages is high or low. In this connection the stimulation of macrophages by drugs is of interest.

Clarification of paraspecific effects of pox vaccines lead to the development of the inducer PIND-AVI from avipoxviruses (Mayr, 1978). This inducer do not act primarily as immunostimulant in the sense of augmentation of specific immune responses, but it activate responses that consistently affect the antigen-non-specific part of the endogenous defence mechanisms (Brunecker, et al., 1986).

The marked increase in the phagocytic activities of chicken macrophages after in-vitro treatment with PIND-AVI as proved by the present work agree well with what recorded by Mayr, (1978), (1980) and (1982) about the preferential stimulation of macrophage activities by PIND-AVI treatment.

Also, the activation of macrophages underline the reduction of rate of tumor induction and the inhibition of murine osteosarcomas in PIND-AVI treated laboratory animals (Erfle, et al., 1982).

REFERENCES


Effect of Pind - Avi


Effect of Pind - Avi.


