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Cellular exudation induced by carrageenan in the peritoneal cavity of chicks

Exsudação celular induzida pela carragenina na cavidade peritoneal de pintos

Alda Maria Backx Noronha MADEIRA[1]; Nair Massako Katayama ITO[2]

CORRESPONDENCE TO:
Alda Maria Backx Noronha Madeira
Departamento de Patologia
Faculdade de Medicina Veterinária e Zootecnia da USP
Av. Prof. Dr. Orlando Marques de Paiva, 87 - Cidade Universitária Armando de Salles Oliveira
05508-900 - São Paulo - SP - Brasil
e-mail: albackx@usp.br

1 - Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia da USP
2 - Spave Consultoria, Estudo e Comércio em Produção e Saúde Animal Ltda.

SUMMARY

The cellular response, induced by the carrageenan injection (4.5 ml; 0.2% w/v) in Ringer-Locke solution into the peritoneal cavity of chicks (three to six-week old Isa Brown males), was evaluated by total and differential inflammatory cell counting of exudates collected 0.5 to 4 hours after stimulus. An increased leucocyte exudation, with predominance of heterophils, was observed within 1 and 4 hours post-stimulus. Macrophages were the second predominant cell type, followed by lymphocytes, eosinophils and basophils, in decreasing order. Thrombocytes were not significant in the peritoneal inflammatory exudate. Results presented in this work indicate that the peritoneal cavity can be utilized as a good model for studying the acute inflammatory response of chickens.

UNITERMS: Carrageenan; Inflammation; Exudates; Cell counting; Hens.

INTRODUCTION

Leucocyte exudation has been extensively studied in acute inflammatory processes induced in body cavities (pleura and peritoneum) of mammals. Several experimental models have been described and allowed the quantification and/or study of the inflammatory cells *in vitro* (Parente *et al.*[23], 1979) leucocyte enzymes (Sopata *et al.*[27], 1989), and evaluation of the effects of chemical mediators (Horacova *et al.*[9], 1980) or antiinflammatory drugs (Kheifets *et al.*[11], 1986).

Characterization of cellular events in chickens has been largely carried out in tissue sections and impression smears. However, the usefulness of these techniques in studying cellular exudation is limited (Chansoriya *et al.*[5], 1993). They do not allow the quantification or characterization of inflammatory cells *in vitro* (Klasing[12], 1991), the clear differentiation of heterophils from eosinophils (Maxwell[16], 1984) and of monocytes with engulfed carrageenan particles from basophils (Awadhiya *et al.*[1], 1981).

This work aimed at evaluating the cellular exudation induced by carrageenan in the peritoneal cavity of chicks. A methodology that permits total and differential cell countings of the cells is also described.

MATERIAL AND METHOD

Chicks

Three to six-week old Isa Brown male chicks were used throughout the experiment. The chicks were kept in wire mesh cages during the rearing period, with free access to water and commercial initial food designed for layers.

Induction of peritonitis

The inflammatory process was induced by the peritoneal injection of 4.5 ml of carrageenan (potassium salt, Sigma Chemical Co.), 0.2% w/v in Ringer-Locke solution. The solution of irritant was injected in the posterior third of the peritoneal cavity, nearby the cloaca. Six randomly chosen chicks were used for each time period studied. The control group was injected with the same volume of Ringer-Locke solution.

Cell suspension collection and counting

The chicks were sacrificed within periods of 0.5, 1, 1.5, 2, 2.5, 3 and 4 hours after carrageenan or control injection. In order to collect the cellular exudates, peritoneal cavities were exposed and washed with 10 ml of saline solution (NaCl 0.85% w/v in H_2O). Cell suspensions were harvested and transferred to siliconized tubes kept at 4°C.

Total leucocytes, non-inflammatory cells and thrombocytes were counted in a Neubauer chamber filled with a cell suspension diluted 1/2 to 1/40 in Natt-Herrick dye. Cell differentiation was determined as described by Natt; Herrick[20] (1952). Differential counts were carried out on cell films prepared in a Suta chamber and stained with May-Grünwald-Giemsa (Rosenfeld[26], 1947). Cell differentiation was determined according to Lucas; Jamroz[13] (1961) and Campbell[4] (1988). Small lymphocytes and thrombocytes were differentiated according to Swayne *et al.*[29] (1986).

The differential counts were performed on the circular area of the sedimented cell film, which was divided into three thirds. In each third, 100 cells, except erythrocytes, were randomly counted, totalizing 300 cells per glass slide. The cell film was examined with a Nikon microscope under oil immersion.

Statistical analysis

The results were subjected to analysis of variance followed by a Bonferroni's test (Winer[30], 1971). They were also submitted to

a test of comparison of two regression lines (Elian[6], 1988). When different variances and abnormal curves were observed in the results from the experimental group, the data were transformed into logarythmic values.

RESULTS

Morphology

Mononuclear leucocytes, granulocytes, erythrocytes and some thrombocytes were observed by the Natt-Herrick method in the peritoneal exudate, induced either by the injection of carrageenan or control solution. A distinct cell type was also found in both peritoneal exudates. This cell was elongated, larger than a thrombocyte, had a light violet stained cytoplasm and nucleus and was observed as a single cell, or forming clusters and sheets.

Heterophils, macrophages, monocyte-like cell, lymphocytes, eosinophils, basophils, some thrombocytes and non-inflammatory cells were observed, in both carrageenan-induced and control exudates, when stained with May-Grünwald-Giemsa dye. The non-inflammatory cells were oval, polygonal or elongated, and presented a weak basophilic cytoplasm, with a centrally positioned, round or oval nucleus. The nuclei had a coarsely granular chromatin and prominent nucleoli, and the cells were arranged on the film in clusters, sheets, alone or in irregular aggregates.

Regarding the carrageenan-specific effects, typical macrophages, as well as monocyte-like cells containing engulfed basophil granules, were observed 30 minutes to 4 hours after injury (Fig. 1 (D)). At 4 hours post-stimulus, some granulocytes, different from normal heterophils (Fig. 1 (A)) and eosinophils (Fig. 1 (C)), were either vacuolized or had non-refringent brick red granules, punctiform or round in the cytoplasm. The nucleus seldom presented visible clumped chromatin. These cells were considered as morphologically altered heterophils (Fig. 1 (B)).

Kinetics of the inflammatory process

Carrageenan injection into the peritoneal cavity of chicks induced an increase of the total leucocyte number (data not shown). The number of non-inflammatory cells observed after carrageenan injury was similar to that found after control injection ($p < 0.05$). Few thrombocytes were observed, and this cell type was not submitted to statistical analysis (data not shown).

The leucocyte exudation was characterized by a progressive response of heterophils (Fig. 2 (A)) and a less intense response of macrophages (Fig. 2 (B)). The amounts of heterophils found at 2.5 and 3 hours after carrageenan or control injections were higher than those observed at 30 minutes and 1 hour. At 4 hours post-injury, the heterophil migration, in the carrageenan-induced group, reached a maximum and was significantly higher than the control group in the same period of time ($p < 0.05$). Significant macrophage migration was only observed at 4 hours after carrageenan injury ($p < 0.05$).

Differential counts of lymphocytes (Fig. 3 (A)), eosinophils (Fig. 3 (B)) and basophils (Fig. 3 (C)) presented high variability among different samples and time periods, and did not allow to establish a reliable kinetic pattern. The absolute numbers of these cells, when comparing to the heterophil number, were two to three orders of magnitude lower.

DISCUSSION AND CONCLUSIONS

This paper describes the kinetics of cellular exudation in the peritoneal cavity of chicks after stimulus with carrageenan. The use of Suta's chambers to prepare glass slides with sedimented inflammatory cells allowed to characterize and discriminate the different cell subpopulations that migrate to the peritoneal cavity after carrageenan injection.

The Suta's chamber, an apparatus similar to the Sayk's chamber, was originally designed to study the cerebrospinal fluid (Sörnäs[28], 1967). In chickens, this chamber was utilized to evaluate the effect of antiinflammatory drugs in the carrageenan-induced peritonitis (Hara *et al.*[8],1994). These authors did not evaluate the type of cell present in the peritoneal cavity. In this work, the method

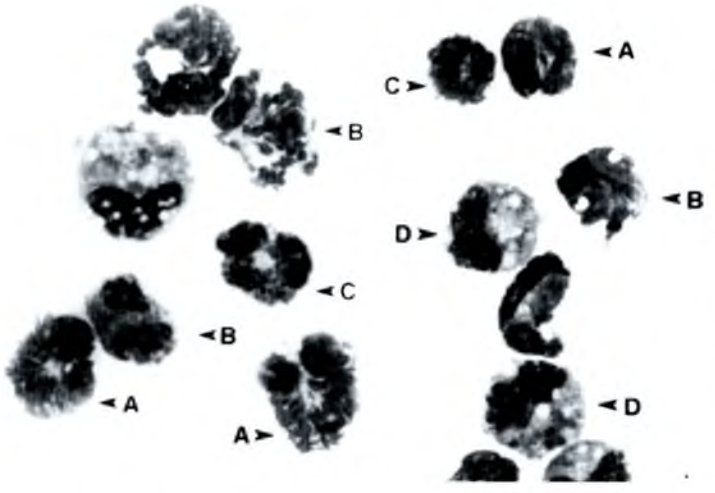

Figure 1
Photomicrographs of cell films of peritoneal exudate collected 4 hours after carrageenan injection. Normal heterophil (A), altered heterophil (B), eosinophils (C) and macrophages phagocyting carrageenan (D). May-Grünwald-Giemsa (x1000).

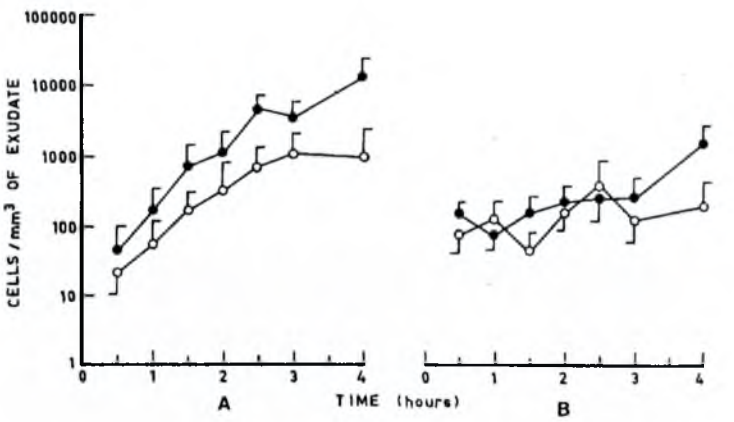

Figure 2
Absolute numbers of heterophils (A) and macrophages (B) in exudates from chick peritoneal cavities after carrageenan (•) or control (○) injections. Y axis is represented in logarythmic scale.

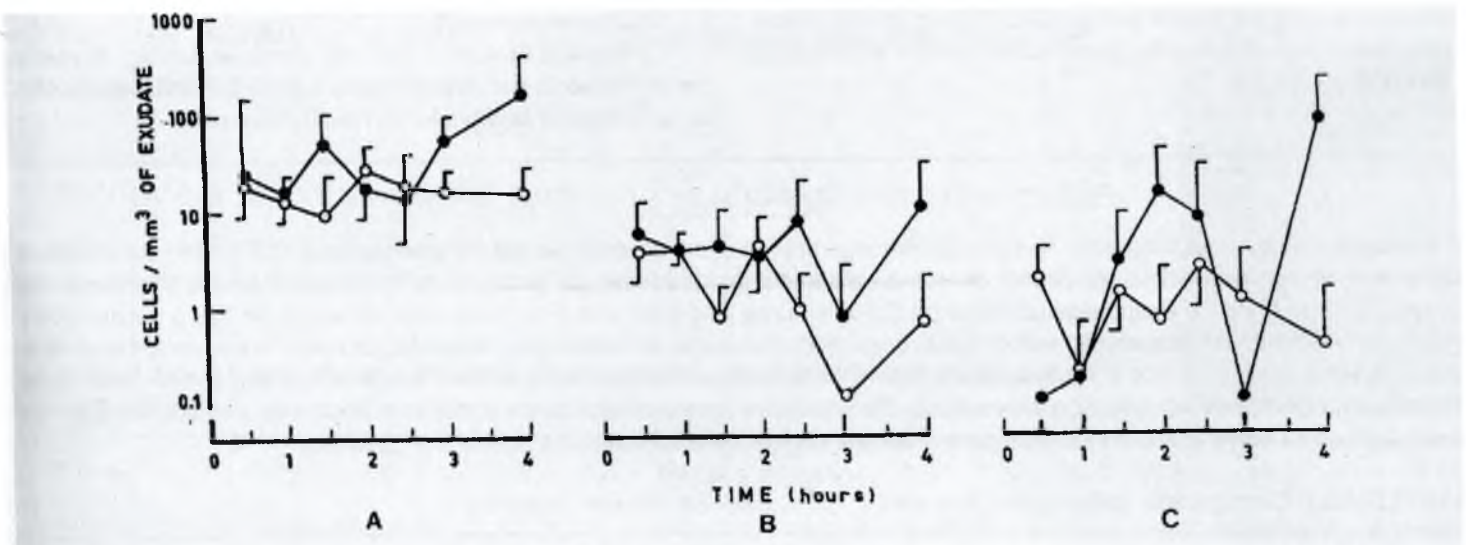

Figure 3
Absolute numbers of lymphocytes (A), basophils (B) and eosinophils (C) in exudates from chick peritoneal cavities after carrageenan (•) or control (o) injections. Y axis is represented in logarythmic scale.

utilized permitted to establish a reliable kinetic pattern of migration of heterophils and macrophages, the main cells involved in the inflammatory process of chickens (Awadhiya *et al.*[1], 1981; Klasing[12], 1991), but not of lymphocytes, eosinophils and basophils. More sensitive methods, like flow cytometry should be chosen, if a more accurate picture of the cell population were required.

The inflammatory cells, sedimented on the slides using Suta's chamber, were morphologically similar to the circulating leucocytes found in the blood of chickens (Lucas; Jamroz[13], 1961). Non-inflammatory mononucleated cells were observed and easily differentiated from macrophages, lymphocytes and thrombocytes. These cells shared a similar morphology with mesothelial cells from the peritoneal wall (Campbell[4],1988).

From 30 minutes to 3 hours after carrageenan or Ringer-Locke injection, the exudated heterophils were morphologically similar to those described by Campbell[4] (1988) in chicken blood. However, heterophils harvested from the inflammed site 4 hours post-carrageenan injection were morphologically altered. Natt; Herrick[21] (1954), Lucas; Jamroz[13] (1961), Robertson; Maxwell[25] (1990) described the presence of these altered cells in chicken blood and proposed that they could be considered as a technical artifact. In our opinion, these vacuolated heterophils, present only at 4 hours post-injury, could be degranulated or disintegrated heterophils, commonly observed in avian inflammatory processes (Nair[18], 1973). Large amounts of heterophils were visualized 4 hours after carrageenan injection. At this time, the edema and the increase in vascular permeability were no longer observed (Ito *et al.*[10], 1989; Noronha[22], 1990). Brune; Glatt[3] (1974) suggested that these cells could have an anti-inflammatory activity. We intend to investigate in the future wheter heterophils could be negatively modulating the response to carrageenan.

Macrophages and monocyte-like cells, that could also be called macrophages (Nair[18], 1973; Nair[19], 1989), were able to engulf carrageenan particles (Awadhiya *et al.*[1], 1981) and, according to the data presented here, phagocytosis could be seen as early as 30 minutes after carrageenan injection.

Differentiation of eosinophils from heterophils in tissue sections is a difficult task, since both cells present acidophilic granules and their shapes are very similar when observed under light microscope (Lucas; Jamroz[13], 1961). Some methods have been used to discriminate eosinophil and heterophil granules such as electron microscopy (Maxwell; Trejo[14], 1970) and peroxidase marker enzyme (Maxwell[16], 1984). Our results demonstrate that eosinophils can be easily identified and differentiated from heterophils. A low number of eosinophils was observed with this technique, but the finding of this cell in the exudate is remarkable, since the methods previously standardized in mammals have failed to demonstrate the presence of eosinophils in the inflammatory response of birds. These cells were not detected at the site of the inflammatory reaction induced by non-immunologic stimuli (Nair[18], 1973) nor in immediate hipersensivity reactions (Pillai *et al.*[24], 1988), but were observed in idiopathic dermatitis (Maxwell *et al.*[15], 1979), in phytohaemagglutinin-induced reaction (McCorckle *et al.*[17], 1980) and in delayed-type hypersensivity (Awadhiya *et al.*[2], 1982). In fowls, the precise function of this leucocyte in these processes is not defined yet.

A basophil response has been commonly reported in the early stages of the acute inflammatory reaction of chickens (Nair[18], 1973; Awadhiya *et al.*[1], 1981). The present study showed that the number of basophils and the rate of increase of these cells were low when compared to other inflammatory cells. A similar finding was described in the dinitrochlorobenzene skin hypersensitivity reaction of chickens (Awadhiya *et al.*[2], 1982).

Some authors have reported the appearance of thrombocyte in the inflammatory process of chickens (Brune; Glatt[3], 1974; Grecchi *et al.*[7], 1980), even though their presence has not been described by others studies (Awadhiya *et al.*[1], 1981; Klasing[12], 1991; Hara *et al.*[8],1994). In the present work, thrombocytes were not observed in the peritoneal inflammatory reaction induced by carrageenan.

Finally, in face of the results presented in this work, and of the advantages in using peritoneal cavity exudates over tissue

sections, we conclude that the peritoneal cavity can be utilized as a good model for studying the acute inflammatory response of chickens.

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RESUMO

A resposta celular, induzida pela injeção de carragenina (4,5 ml de solução de Ringer-Locke a 0,2% w/v) na cavidade peritoneal de pintos (machos Isa Brown de três a seis semanas de idade), foi avaliada pela contagem total e diferencial das células inflamatórias de exsudatos colhidos de 0,5 a 4 horas pós-estímulo. Foi observado ao longo de 1 a 4 horas após a injúria um aumento da exsudação leucocitária, com predominância de heterófilos. Macrófagos foram o segundo tipo celular predominante, seguidos por linfócitos, eosinófilos e basófilos, em ordem decrescente. Trombócitos não foram observados em número significante no exsudato inflamatório. Os resultados apresentados neste trabalho indicam que a cavidade peritoneal pode ser usada como um bom modelo para o estudo da resposta inflamatória aguda em galinhas.

UNITERMOS: Carragenina; Inflamação; Exsudatos; Contagem de células; Galinhas.

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