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Effect of Dexamethasone Treatment on the Hematological and Histological Parameters of Mice Following Experimental Bacterial Infection

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Abstract: There are four groups of balb/c mice with each of it consists of 6 animals. Group 1, 2 and 3 were injected with dexamethasone daily for three days and experimentally infected with *Pasteurella multocida*, *Escherichia coli* harboring a recombinant plasmid ABA392 and *Staphylococcus aureus* respectively. Whereas, group four serve as control. At 24, 48 and 72 hours post-infection a marked increase in total leucocytes counts while differential leucocytes count revealed a marked increase in neutrophils and decrease in lymphocytes and monocytes in all dexamethasone-treated animals and was more predominant in group 1 and 2 post infection in all animals. At 72 hours post-infection, the histological examination reveals haemorrhagic (hemorrhagic septicaemia) features of lungs in dexamethasone-treated mice and infected with *P. multocida* and the recombinant plasmid ABA392 but not with the dexamethasone-treated and infected with *S. aureus* indicate that recombinant plasmid ABA392 may harbor a sequence that may code for virulent factor of *Pasteurella multocida*.

Key words: Dexamethasone, *Pasteurella multocida*, recombinant plasmid, haemorrhagic septicaemia, mice

Introduction

Pasteurella multocida infection or pasteurellosis has been encountered in many species of domestic animals including several avian species and it is an animal pathogen of considerable economic importance; for example septicemic pasteurelosis and atrophic rhinitis in pigs, fowl cholera in chickens and turkeys. The disease occurs predominantly in the tropics and in many other parts of the world such as in Asia, Africa, Southern European and near and middle East Countries (Christensen *et al*., 1998; Muhairwa *et al*., 2001; Rubies *et al*., 2002).

Mice are very susceptible to *P. multocida* infection and are often used as experimental animals to determine the virulence of stain (Collins, 1973). Dexamethasone has been used to mimic stress resulting in more severe lesion than in the absence of dexamethasone treatment (Chiang *et al*., 1990). Dexamethasone is synthetic glucocorticoids that have anti-inflammatory effect as well as immunosuprresive action (Gower, 1979). The invasion by *P. multocida* was further enhanced due to infection by *Mycoplasma hyopneumoniae*, indicating that the activity of *P. multocida* increased when the immune system of the animal has been suppressed. This means that information on the expression of virulence due to infection by *P. multocida* can be obtain through indirect studies on healthy animals subjected to stress conditions.

Corticosteroids have at least three effects on cells of the immune system: destruction, inhibition of function, and redistribution. The susceptibility to these effects depends on the animal species and cell type, and its location, physiological maturity and state of activation (Claman, 1972; Bach and Strom, 1985). A single and multiple injections of mice with the dexamethasone have contrasting effects upon the antibody production (Benner and Van Oudenaren, 1979) and background immunoglobulin production (Sabbele *et al*., 1983) in spleen and bone marrow.

The abrogation of acquired resistance to nematode infection in rodent and sheep by immunosuppressive glucocorticoids has been well documented (Mathews *et al*., 1979; Moqbel, 1980) In sheep this was associated with a reduction in the number of circulating leucocytes (Mathews *et al*., 1979) and in rats, with reduced numbers of intestinal intraepithelial mast cells and eosinophils (Moqbel, 1980). Both cortisone and corticotropin treatment reduced the number of globule leucocytes in rat mucosal tissue (Kent *et al*., 1954)

Corticosteroid drugs have been widely used in several host species to modify both natural

resistance and acquired immunity to nematode parasites. By their use it is possible to achieve maturity of parasites in otherwise unsuitable host (Cross, 1960), to increase natural susceptibility to infection (Mathies, 1962), to suppress the development of immunity during a primary infection (Coker, 1955) and to prevent the action of an established immune response upon a challenge infection (Ogilvie, 1965) .
The objective of the current study was to investigate the effect of *P. multocida* and an *E. coli* strain harboring recombinant plasmid ABA392 containing an insert cloned from *P. multocida* and *Staphylococcus aureus* infection on the hematological and histological parameters in dexamethasone-treated balb/c mice

Materials and Methods

Experimental animals: Four groups of male balb/c mice 8-12 week-old, each consist of six mice were used.

Bacterial culture, recombinant plasmid and mouse inoculation: All bacterial cultures were grown in brain heart infusion broth overnight at 37°C. All animals were given intramuscularly injection of dexamethasone (dexamethasone sodium phosphate, Intervet International B.V. Boxmeer-Holland) 0.1 ml for three days. A physiological saline containing 10^7 cfu of the *Pasteurella multocida* strain PMB202, *Escherichia coli* carrying a recombinant plasmid containing insert cloned from the chromosomal DNA fragment of a *Pasteurella multocida* strain PMB202 and *Staphylococcus aureus* ATCC25933 were inoculated subcutaneously (0.2 ml) to group 1, group 2 and group 3, respectively.

Blood parameters

Blood collection for total and differential leucocytes count: Blood was collected by sacrificing 2 mice from each group at 24, 48 and 72 hours post-infection.

Differential white blood cell counts: Thin blood smear were prepared from blood collected from sacrificing mice and also from the tail of the rest of mice. Blood was stained with Right stain and the differential while blood cell counts were determined by a standard method described by Dacie and Lewis (1975).

Total WBC count: Total leucocyte count were performed from the blood collected from sacrificing mice at 24, 48 and 72 hours post infection. Deliver 0.95 ml white cells diluting fluid (Turk's Solution) into a test tube, add 0.05 ml blood into the test tube, mix thoroughly and introduce the suspension into the counting chamber.

Histology and preparation of tissue or organ sample for analysis: Tissues such as liver, spleen and lungs of sacrificing mice were fixed immediately in 10% buffer formalin embedded in paraffin, sectioned at 4-5 µm and stained with haematoxylin and eosin (H & E).

Results

Clinical signs: All dexamethasone-treated animals were alert at 24 and 48 hours post-infection, but at 72 hours post-infection group 1 and 2 were depressed.

Blood Parameters

Total Leucocytes Counts: The results of the present study showed that the dexamethasone-treated mice and infected with bacteria exhibited leucocytosis of total white blood cells count (Table 1) and neutrophilia, lymphopenia and monocytopenia of differential leucocyte counts (Figs. 1, 2 and 3).

Bacteriology: P. multocida was isolated mostly in pure culture from the lungs of mice at 72 hours post infection in group 1 but no bacterial isolation from group 2 and 3.

Gross lesions: Group 1 and 2 dexamethasone-treated animals at day 3 post infection had lung lesions typical to pneumonic pasteurellosis with hemorrhage and were more predominant in group 1. The lung areas of group 1 were affected, mostly involving the ventral parts. None of the mice at 72 hours post infection in group 3 developed pneumonic lesion.

Table 1: Effect of Dexamethasone treatment on total leucocyte count following experimental infection with bacteria

Mice groups	Total WBC After 24 hrs	Total WBC After 48 hrs	Total WBC After 72 hrs
Dexa+Clone	5650	6450	8100
Dexa+*P. multocida*	8300		10900 14900
Dexa+*S. aureus*	5300	6100	7400
Control	3500	3550	3400

Microscopic lesions: The microscopic lesions in lungs of the group 1 mice at 72 hours post-infection had pneumonia consisted mainly of acute exudative bronchopneumonia with hemorrhage. Most inter-alveolar septa were thickened by accumulation of edematous fluid, mononuclear cells and blood congestion. The associated alveoli were filled by exudates

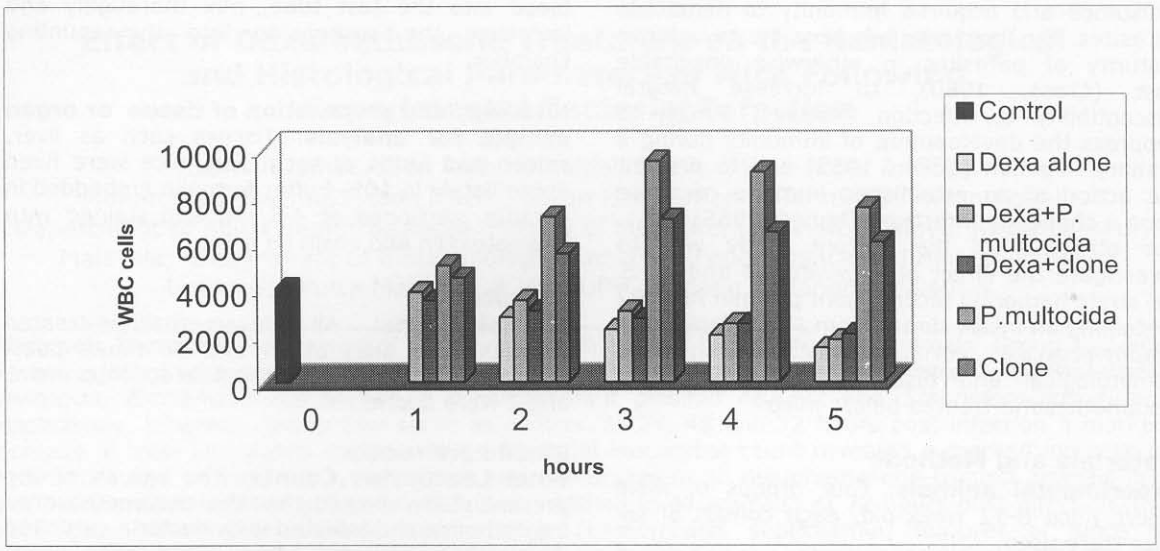

Fig. 1: Total white blood cells (WBC) count after inoculation of test organisms subcutaneously in mice with or without dexamethasone treatment

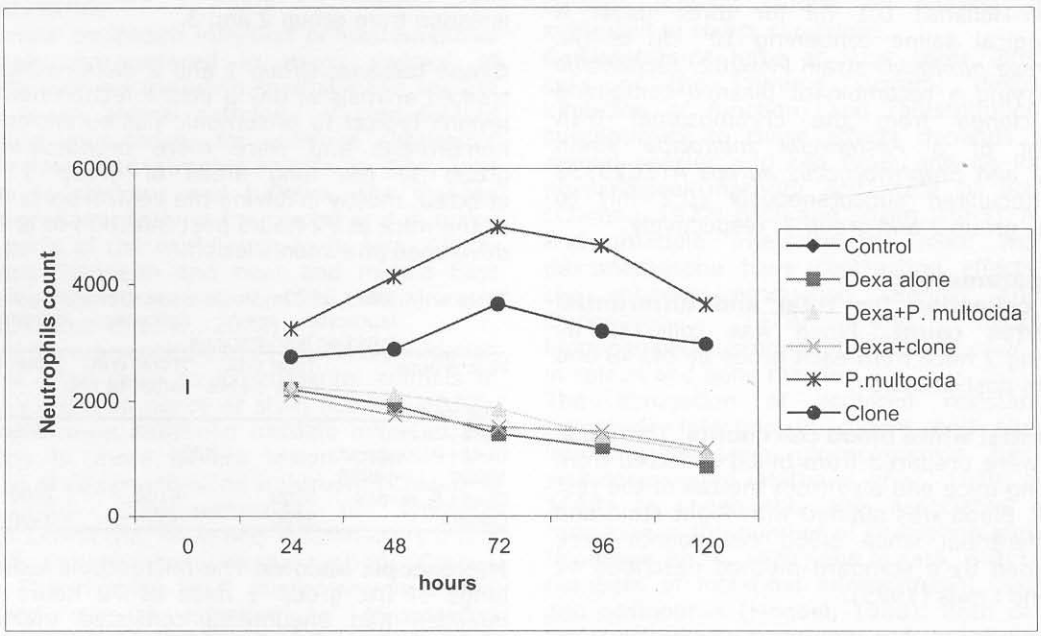

Fig. 2: Different white blood cells (neutrophils) count after inoculation of test organisms subcutaneously in mice with or without dexamethasone treatment

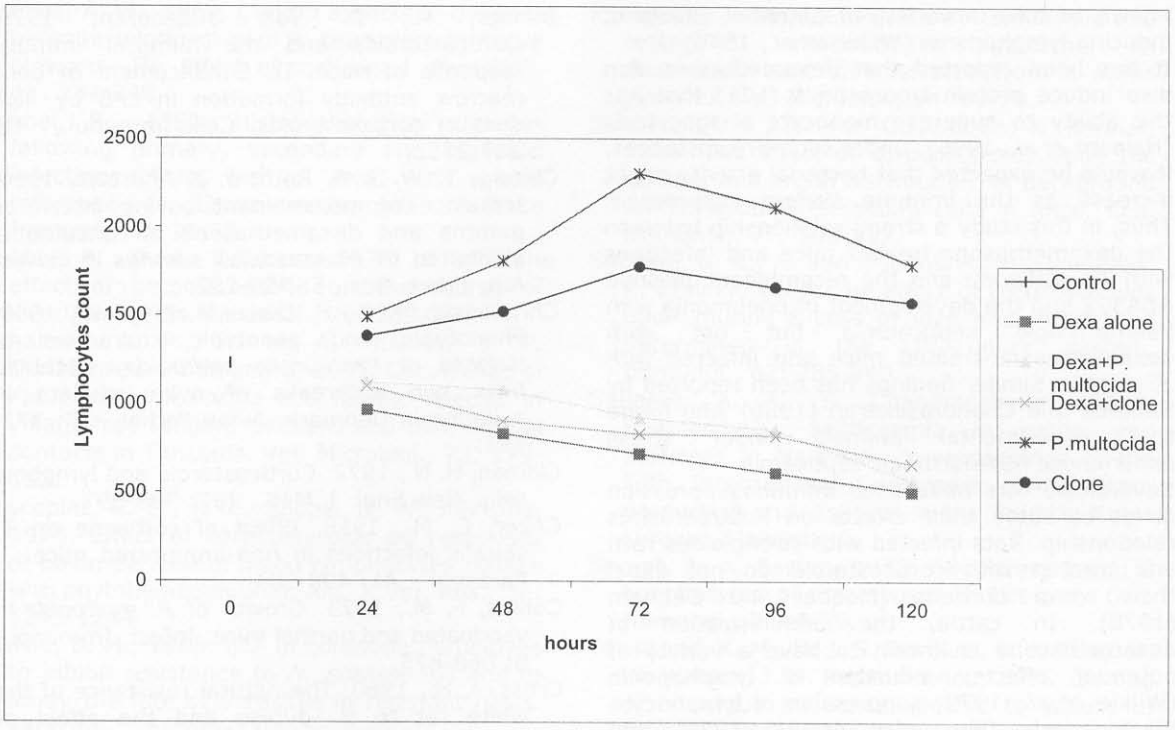

Fig. 3: Differential white blood cells (lymphocytes) count after inoculation of test organisms subcutaneously in mice with or withour dexamethasone

consisting of a mixture of numerous erythrocytes, neutrophils, lymphocytes and macrophages. Similar lesions were observed in group 2 mice at 72 hours post-infection but in less severity.

Discussion

The purpose of the current study was to investigate the effects of *P. multocida* strain and a recombinant plasmid containing chromosomal DNA insert cloned from a *P. multocida* isolate on hematological and histological parameters in dexamethasone-treated mice. The results demonstrate that dexamethasone at a dosage of 0.1 ml/animal per day exhibited immunosuppressive action in the treated mice, by the manifestation of a marked leucocytosis, lymphopenia and monocytopenia. Leucocyosis was primarily due increase in circulating neutrophil numbers (neutrophilia). Dexamethasone is a known anti-inflammatory agent, decreasing the neutrophil margination pool in the blood vessels. The reduced margination of neutrophils is probably responsible for the marked neutrophilia that is caused by dexamethasone (Roth *et al.*, 1982). The Lymphopenia and monocytopenia were probably due to two factors. Firstly, to the severe decrease of the number of pre-cursor lymphocytes and monocytes cells in bone marrow by dexamethasone treatment (Ku and Whitte, 1986) which cause a decrease production of lymphocytes and monocytes. Secondly, to the rapid turnover of the maturity of lymphocytes and monocytes in the mouse (Sprent and Basten, 1973). According to a study by Erika *et al.* (1996), dexamethasone inhibits the proliferation of human pheripheral lympocyte by down-regulating IL-2 receptor-mediated signal. This could be use as the basis to explain for the reduction in the lymphocytes counts in the dexamethasone-treaed mice in this study. Similarly, Sabbele *et al.* (1987) observed that treatment of mice with single or multiple injection of dexamethasone could greatly reduce the number of B-lymphocytes in the spleen. With the consistence of the results of the present study, the administration of the dexamethasone is

known to have a variety of potential effects of inducing lymphopenia (Wilkie *et al.*, 1979)

It has been reported that dexamethasone can also induce protein Lipocortin 1 (LC1) that has the ability to suppress monocyte phagocytosis (Halpern *et al.*, 1996). Under such circumstances, it would be expected that bacterial activity might increase as the immune system suppressed. Thus, in this study a strong relationship between the dexamethasone-treated mice and infections with *P. multocida* and the recombinant plasmid ABA392 and the development of pneumonia with haemorrhagic septicaemia but not with dexamethasone-treated mice and infected with *S. aureus.* Similar findings has been reported by Saharee and Chandrasekaran (1986) who found that experimental animals under stress experienced haemorrhagic septicemia.

Several workers have used immunosuppression drugs to study their effects on host/parasites relationship. Rats infected with strongloides ratti and treated with corticosteroid do not expel their worm burdens (Moqbel and Denham (1978). In cattle, the administration of dexamethasone is known to have a variety of potential effects; induction of lymphopenia (Wilkie *et al.*, 1979) suppression of lymphocyte blastogenesis (Muscoplat *et al.*, 1975) and impairment of the function of neutrophils (Roth and Kaeberle, 1982).

It is interesting to highlight the finding in the present study in which a recombinant plasmid ABA392 containing a chromosomal DNA insert cloned from a *P. multocida* serotype B:2 strain was able to induce haemorrhagic septicemia in the mice tested. Elsewhere, Townsend *et al.* (1996) reported on the cloning of a unique sequence specific to isolates of serotype B:2 *P. multocida* related to the pathogenesis of haemorrhagic septicaemia. Although the observation on the ability of the recombinant plasmid ABA392 to induce bleeding feature is at a preliminary stage, our results suggests that this recombinant plasmid may contain unique sequence, and further study into the characteristics of the cloned sequence is needed.

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