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Impairment of Neutrophil Functions in a Dog with an Eosinophilic Dermatosis

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Thomsen, M. K., A. Lundorff Jensen, E. Bindseil and F. Kristensen: Impairment of neutrophil functions in a dog with an eosinophilic dermatosis. Acta vet. scand. 1991, 32, 519–526. – An eosinophilic dermatosis was diagnosed in a dog with a multifocal distribution of pruritic, pustular and erosive lesions on the trunk. Ectoparasites, fungi and bacteria were not detected in the skin of the dog. At a subsequent admission to the university clinic, bacterial conjunctivitis and superficial pyoderma had developed. At a time when the dog received no medical therapy, evaluation of phagocytosis and chemotactic migration towards a variety of chemotactic factors revealed a general suppression of patient neutrophil responsiveness, as compared to a control dog. Viability of the patient's neutrophils was normal. It was proposed that deactivation of neutrophil functions occurred following exposure to suppressive factors from mast cells, or as a consequence of surface receptor down-regulation due to prolonged cell activation by as yet unknown stimulants. The observed susceptibility to infections in the dog suffering from a primary, sterile eosinophilic dermatosis may be related to impaired host defence against opportunistic microorganisms.

eosinophil; pustular dermatosis; chemotaxis; phagocytosis.

Introduction

An intact inflammatory cell system is vital to host defence against microbial invaders. Consequently, defective chemotaxis or phagocytosis in the most important phagocyte, the neutrophil, is an important cause of increased susceptibility to opportunistic pathogens in mammalian species (*Rotrosen & Gallin* 1987). In the dog, a variety of inflammatory mediators have been found to supplement each other in the neutrophil activation process (for a review, see e.g. *Thomsen* 1991). Normal neutrophil function has been observed in dogs suffering from experimentally induced (*Gosset et al.* 1983) as well as spontaneous inflammatory disorders (*Kroese et al.* 1981), indicating that inflammatory disorders per se are not associated with an altered state of neutrophil responsiveness.

In man, certain dermatoses associated with tissue and/or blood eosinophilia have been shown to be associated with the presence of abnormal neutrophil responsiveness (*Hill et al.* 1974). The aim of the present study was to evaluate canine peripheral blood neutrophil chemotaxis and phagocytosis in a dog with a dermatosis presenting with blood and tissue eosinophilia of unknown origin.

Materials and methods

Animals

The dog, a 3 year male old Icelandic Sheepdog, was referred to the university clinic with a history of chronic pruritus caused by a pustular dermatosis, that was poorly steroid-responsive. At the time of analysis of neutrophil chemotaxis and phagocytosis, the

dog was not receiving any pharmacotherapy. A sex- and age-matched healthy Beagle dog served as control.

Bacteriology

Routine bacterial cultures were performed on blood agar in order to detect the gram-positive and -negative pathogens capable of colonizing canine skin (*Muller et al.* 1989). Pus was obtained by aspiration from pustules that were intact at the time of sampling.

Histopathology

Full-thickness 6 mm punch biopsies were obtained as previously described (*Thomsen & Thomsen* 1989). The biopsies were processed via conventional techniques and embedded in paraffin. Stepsections were cut from each block and stained with haematoxylin-eosin.

Isolation of PMN

EDTA-stabilized blood (30 ml) was obtained from the jugular vein of the dermatosis-suffering dog and the control dog. Neutrophils were isolated at room temperature according to a previously described procedure (*Thomsen & Ahnfelt-Rønne* 1989). Briefly, red blood cells were sedimented by methylcellulose for 45 min. Following this, leukocyte-rich plasma was layered on Lymphoprep® (density 1.077) (Nycomed, Norway) and centrifuged at 400 g for 30 min. Finally, the resulting cell pellet was washed twice in Hanks' balanced salt solution (Gibco, U.S.A.; HBSS) and cell density was adjusted according to the assay. The final cell suspension contained 91 % neutrophils, 7 % eosinophils and 2 % mononuclear cells. Viability in patient and control cells was 98 % and 97 %, respectively, as judged by the eosin-Y dye exclusion test (*Thomsen & Ahnfelt-Rønne* 1989). Chemotaxis and phagocytosis assays were carried out in duplicate, and mean values are presented.

Chemotaxis assay

Cell migration was studied in a 48 well chemotaxis microchamber (Neuroprobe Inc., Bethesda, MD) using 10 μm-thick, 3 μm-pore polyvinyl pyrrolidone-free polycarbonate filters (Nucleopore Corp., Pleasanton, CA), as described previously (Thomsen et al. 1991b). Briefly, 25 μl of the lipid mediators, leukotriene B_4 (LTB_4; Ultrafine Chemicals, U.K) and platelet-activating factor (PAF; Sigma, U.S.A.), or the peptide mediators, complement C_{5a}/C_{5a} desArg (*Strøm & Thomsen* 1990) and interleukin-8 (IL-8; *Thomsen et al.* 1991b), was diluted in HBSS containing 0.5 % BSA and placed in the lower well. Fifty μl cell suspension (2×10^6 cells per ml) was placed in the upper chamber. Incubation proceeded for 45 min at 37°, 5 % CO_2 and 98 % humidity. Following this, filters were fixed and stained with Coomassie brilliant blue (Sigma). Migration was estimated by lower surface counting. The mean of the medians of 5 individual readings on each of duplicate filters was calculated and the results were expressed as chemotactic differentials.

Phagocytosis

Complement C_{3b}-mediated phagocytosis was studied by visual inspection of *Candida albicans* blastospore ingestion as previously reported (*Thomsen* 1989). The yeast: neutrophil ratio was 2 to 1 and the incubation period was 10 min after which a specific staining method utilizing a double vital dye was used to discern ingested from free blastospores. Results were expressed as the percentage of phagocytically active neutrophils (% phagocytosis) and the number of ingested yeast cells/50 neutrophils (phagocytic index).

Results

Clinical examination

At the first admission, the dog presented with a history of a chronic, pruritic dermatosis. Pustules of variable size, and erosions due to pustular rupture, with a generalized distribution were observed. Skin scrapings consistently failed to reveal the presence of ectoparasites. No extracutaneous manifestations were present. A biopsy was taken at this time. A differential cell count performed on peripheral venous blood revealed: 85 % neutrophils, 8 % eosinophils, 4 % monocytes and 3 % lymphocytes. The total leukocyte count was 21.1×10^9/l, indicating the presence of leukocytosis, absolute neutrophilia and eosinophilia, and lymphopenia (reference range from *Jain* 1986). At a subsequent hospitalization, the dog had developed bacterial conjunctivitis and superficial, staphylococcal pyoderma.

Bacteriology

At the first admission to the university clinic, bacterial culture of sampled pustular material was negative, indicating that the pustules were sterile.

Histopathology

The histopathological examination revealed a slight orthokeratotic hyperkeratosis and follicular keratosis. There were focal subcorneal granulocytic pustules dominated by eosinophils (Fig. 1a and b). No acanthocytes

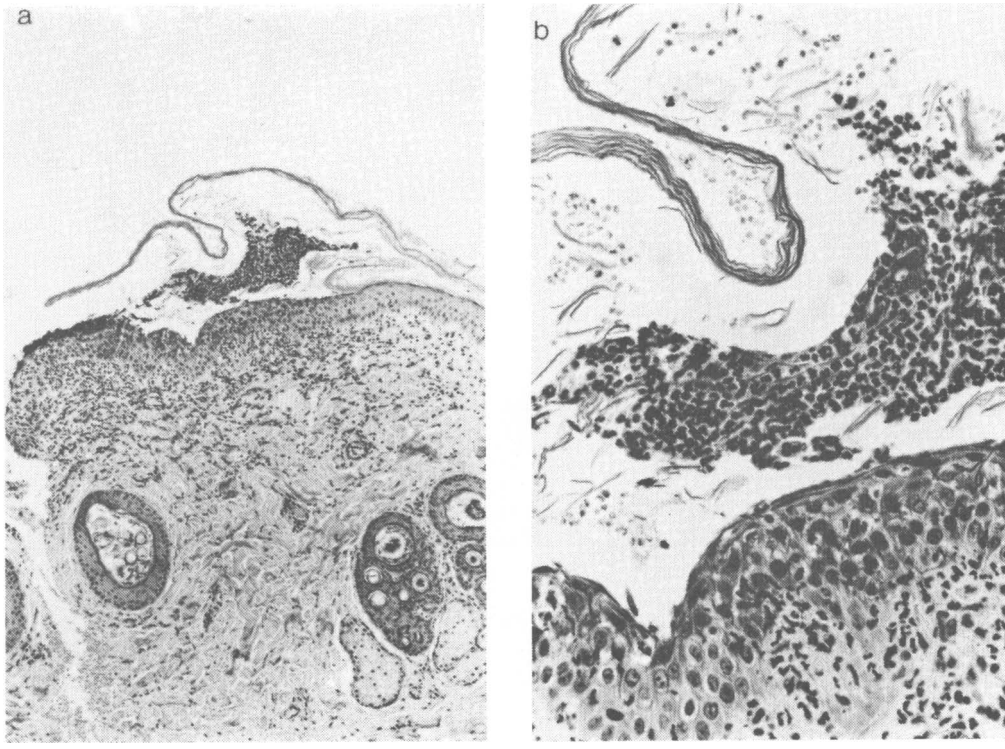

Figure 1. Low (a) and high (b) power photomicrographs showing eosinophil-dominated pustular dermatitis changes in an Icelandic Sheepdog. Haematoxylin-Eosin stain.

were seen in the pustules. Neither pustular changes, nor folliculitis was observed, and glandular structures appeared normal. A mixed cellular infiltration dominated by eosinophils, mast cells, and lymphocytes was seen in the superficial part of the dermis and particularly beneath the epidermis and perivascularly. In most subepithelial blood vessels, including the capillaries, free eosinophils were detected in the lumen. Parasites, fungi, and bacteria were not observed in the biopsy.

Chemotaxis

From previously reported dose-response relationships (*Thomsen & Jensen* 1991), concentrations of 0, 10 and 100 nM LTB_4 or PAF were chosen for assay of migration. Neutrophils from the control dog migrated towards the gradient of chemoattractants (Figs. 2 and 3). Compared to these cells, as well as a previously reported canine reference range for chemotaxis (*Thomsen & Jensen* 1991), neutrophils from the patient exhibited normal random, but a complete lack of chemoattractant-directed migration (Figs. 2 and 3). A similar lack of chemotactic migration was observed in experiments using the peptide chemoattractants IL-8 and complement C_{5a}/C_{5a}desArg (data not shown) as previously reported (*Strøm & Thomsen* 1990, *Thomsen et al.* 1991b).

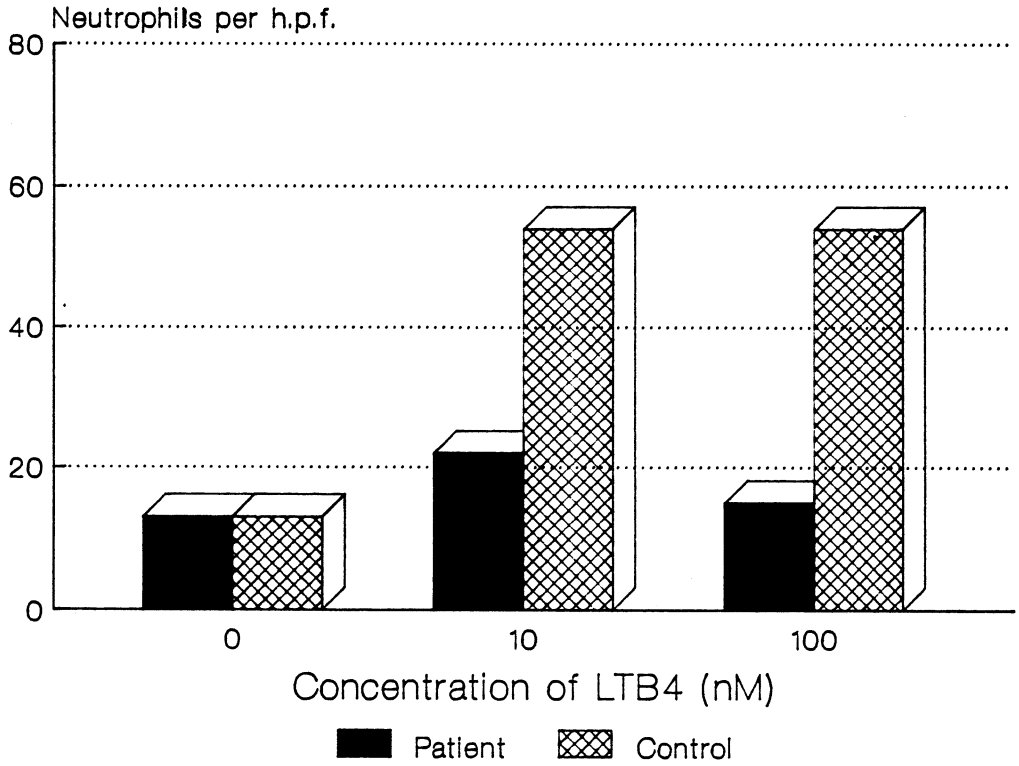

Figure 2. Migration of patient and control neutrophils towards leukotriene B_4 (LTB_4). Migration was quantified by the lower surface count technique, and expressed as the number of neutrophils per high power field.

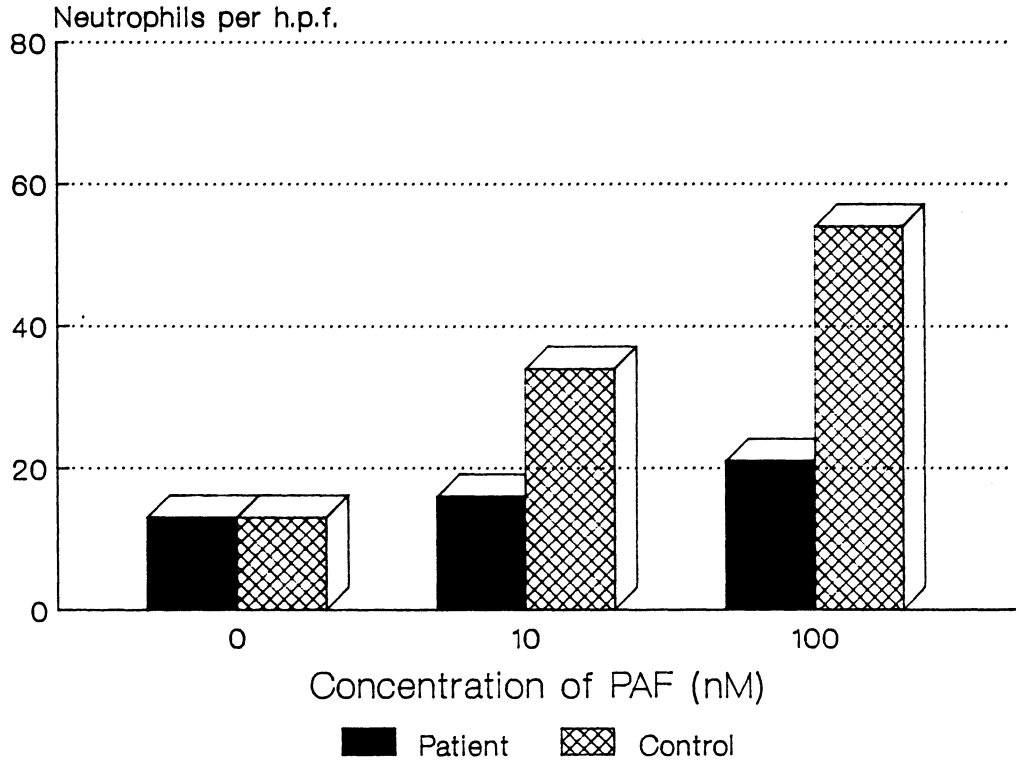

Figure 3. Migration of patient and control neutrophils towards platelet-activating factor (PAF). Migration was quantified as described in Fig. 2.

Phagocytosis

Using an assay which depends on the presence of complement components as opsonins (*Thomsen* 1989), control neutrophils possessed normal phagocytic capacity, irrespective of the opsonin concentration present (Fig. 4; reference range: *Thomsen et al.* 1991a). In contrast, 40–50 % less neutrophils from the patient were phagocytically active and the number of ingested *C. albicans*/neutrophil was also 40–50 % lower.

Discussion

In man, a rare disease referred to as sterile eosinophilic pustulosis has been recognized (*Orfanos & Sterry* 1978). A few years later, *Scott* (1984) reported the existence of a canine sterile, pustular dermatitis with eosinophils predominating. This condition is considered to be analogous to the human dermatosis (*Muller et al.* 1989). In both species, the pathophysiological background of the disease is unknown (*Muller et al.* 1989) and due the low incidence of the dermatosis, functional studies on inflammatory cell activation have not been undertaken. The keystone in the diagnosis of the disease is the presence of blood and tissue eosinophilia (and in some cases neutrophilia) in a patient with a generalized, sterile, pustular dermatitis (*Scott* 1984). The Icelandic Sheepdog in the present study fulfills these criteria. With

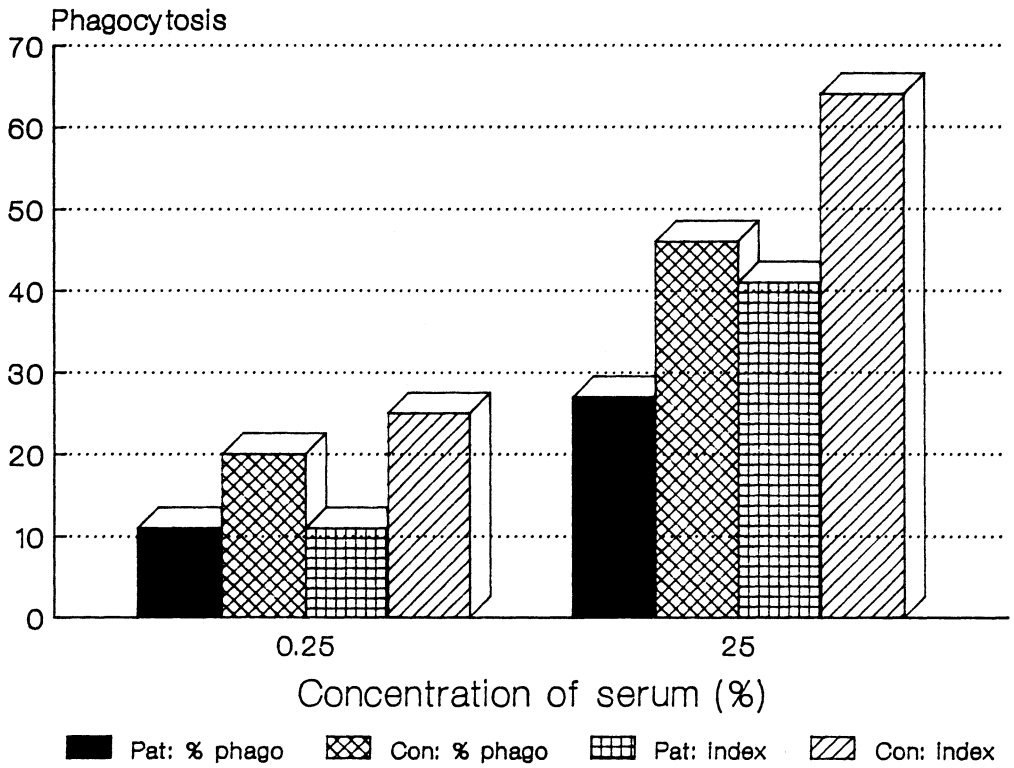

Figure 4. Phagocytosis of *Candida albicans* blastospores by patient (Pat) and control (Con) neutrophils in the presence of 0.25 or 25 per cent pooled homologous serum. Phagocytosis was estimated as the per cent phagocytosis (% phago): Per cent phagocytically active neutrophils, and as the phagocytic index (index): Number of ingested yeast cells per 50 neutrophils.

respect to the bacterial culture, attempts at detecting mykoplasms and fungi were not made. These organisms are not, however, associated with pustular dermatitis changes in the dog (*Muller et al.* 1989). We did not find a partially follicular distribution of the dermatitis, as observed by *Scott* (1984) in the 3 cases described. It nonetheless appears probable that the biological reaction patterns in the dermatoses described by *Scott* (1984) and in the present study are the same and that the diseases belong to the same entity.

Throughout a series of more than 10,000 consecutive migration analyses performed on healthy and ill dogs, the complete lack of chemotactic responsiveness of canine neutrophils found in the present dermatological patient has never been observed (*Thomsen & Jensen* 1991). Besides chemotactic unresponsiveness, the patient's neutrophils also possessed a decreased phagocytic response, suggesting the presence of a more generalized neutrophil dysfunction. This could explain the susceptibility to secondary infection noted in this patient (*Thomsen & Strøm* 1989).

The combined presence of eosinophilia, eczematous dermatitis, susceptibility to infection and defective chemotaxis of neutrophils

is well documented in the human dermatosis termed Job's syndrome (*Hill et al.* 1974). In this disorder, defective chemotaxis is caused in part by granulocyte-suppressive products from degranulated mast cells (*Klein* 1990). Suppression of granulocyte functions in the present dog may be mast cell-mediated since mast cell infiltration was indeed observed in the skin of this patient. Eosinophil-derived mediators could also be causative since large numbers of eosinophils were present in the cell suspension used in the functional analysis. In this respect, the eosinophil is a major producer of leukotriene D_4 (LTD_4) which has recently been shown specifically to suppress LTB_4-directed migratory responses in canine neutrophils (*Thomsen & Ahnfelt-Rønne* 1989). However, LTD_4 neither affects migratory responses to PAF (*Thomsen & Ahnfelt-Rønne* 1989), nor does it influence phagocytosis (*Thomsen* 1989). Another possibility is that disease-related prolonged exposure of neutrophils to high concentrations of inflammatory mediators has caused generalized deactivation of all cell functions due to receptor down-regulation, as previously reported (*Donabedian & Gallin* 1981). Finally, the adrenal stress response which is often encountered in inflammatory disorders (*Thomsen* 1990) does not offer an explanation for the observed neutrophil dysfunction since pathophysiologically attainable circulating levels of glucocorticosteroids are non-suppressive with respect to canine neutrophil functions (*Guelfi et al.* 1985, *Thomsen* 1991).

In conclusion, deactivation of neutrophil functions has been demonstrated in a dog suffering from a sterile, eosinophilic dermatosis. Evaluation of whether neutrophil dysfunction was a primary or secondary phenomenon with respect to the disease process was not possible. However, the presence of secondary infections may be related to the neutrophil hyporesponsiveness and corrective immuno-stimulatory thrapy may consequently prove to be of value, as in man (*Rebora et al.* 1980), without treating the underlying disease process.

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Sammendrag

Hæmmet granulocytfunktion hos en hund med en eosinofil dermatose.

En eosinofil dermatose blev diagnosticeret hos en hund med multifokale, kløende, pustulære og erosive læsioner på truncus. Der kunne ikke påvises ektoprasitter, svampe eller bakterier på/i patientens hud. Ved en senere hospitalsindlæggelse diagnosticeredes bakteriel konjunktivitis og superficiel pyodermi. På et tidspunkt, hvor hunden ikke var under medicinsk behandling påvistes, i forhold til en kontrolhund, et generelt nedsat fagocytrespons, bedømt ved måling af granulocyt-kemotaxi og fagocytose. Deaktivering af nævnte cellefunktioner foreslåes sat i forbindelse, enten med mastcelle-frigørelse af suppressive faktorer, eller med receptornedregulering som følge af prolongeret celleaktivering med ukendte, inflammationsassocierede stimuli. Konklusivt kan den observerede prædisponering for infektion hos en hund med en primær, steril eosinofil dermatose muligvis sættes i relation til et nedsat forsvarsberedskab overfor opportunistiske patogener.

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