Histopathological Changes in Naïve and Sensitised Goats Caused by Sarcoptes scabiei Infestation

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Kata kunci: Sarcoptes scabiei, naïve, sensitised, histopatologi, eosinofil, anafilaksis kulit, kekebalan

ABSTRACT


The purpose of this study is to compare the histopathological changes in naïve and sensitised goats caused by Sarcoptes scabiei infestation. Thirty goats were allocated evenly into 5 groups. Groups 1, 2 and 3 goats were sensitised once, twice and thrice, respectively; whereas groups 4 and 5 were left unsensitised or naïve. Sensitisation was done by infesting the animals with mite, then 7 week afterwards the animals were completely cured from the mange. After the sensitisation, all, except group 5, goats were infested on both auricles each with approximately 2000 life mites. Biopsies were collected from each group at 2 day then at weekly intervals from 1 to 7 weeks following infestation. The samples were routinely processed, paraffin blocked, and tissue sections were stained with Haematoxyllin and Eosin (H & E), Giemsa, Carbol Chromatrope, and Gram’s when indicated. Lesions in the naïve goats developed progressively characterised by thick parakeratotic crusts honeycombed with tunnels containing large number of mites. Lesions in sensitised goats, which were different qualitatively to those in naïve goats, developed rapidly characterised by copious amount of serocellular exudates in and on the surface of the epidermis, and marked oedema and cell infiltrations in the dermis. Dermal infiltration by eosinophils, which was rare in naïve goats, was apparently an important feature in the sensitised goats. Lesions developed in the sensitised goats were interpreted to be the manifestation of cutaneous anaphylaxis. Resistance or protective immunity against mite reinfection developed in the sensitised goats is supposedly attributed to this anaphylactic responses.

Key words: Sarcoptes scabiei, naïve, sensitised, histopathology, eosinophils, anaphylaxis, protective immunity

INTRODUCTION

Scabies in human or sarcoptic mange in animals is a contagious skin disease caused by the burrowing mite, Sarcoptes scabiei. The disease has been known for thousands of years and its prevalence is high in some human populations, domestic and wild animals (RONCALLI, 1987). Therefore, the economic losses caused by this mite must be very high. Despite the overwhelming economic losses and the fact that the disease has been known for a long time, no prophylactic measure is yet available to control the disease. Vaccination is considered to be one of the interesting
alternative but it remains unclear whether developing a vaccine against the disease is feasible.

Developing a vaccine against multicellular parasites is much more difficult compared to that against virus or a bacteria. This is because the interactions between the parasites and the host are more complex. Moreover, the parasite protective antigens, parasite molecule(s) that are immune protective, have to be first identified, proliferate using an appropriate cloning technology, then used as a main component in a vaccine. These array of endeavors are not only technically arduous but also costly and time consuming (TELLAM et al., 1992).

Understanding the immune mechanisms of the disease will certainly of great support to the vaccine development. Unfortunately, the mechanisms of immunity of scabies are poorly understood. Humoral immune response, as shown in an experiment in rabbits, might not play important role because no positive correlation was established between degree of immunity and the titre of antibodies (ARLIAN et al., 1994a). Cellular immune responses apparently play greater roles in the protective immunity. Studies in canine scabies suggested that neutrophils and T-lymphocytes (CD4+, CD1a+, CD3ε, CD11c and MHC class II cells) play a key role in the successful immune or inflammatory response because infiltrations by those cells occur earlier and are more intense in sensitised, immune animals than those in naïve individuals (ARLIAN et al., 1994b; STEMMER et al., 1996; ARLIAN et al., 1996). The importance of eosinophils and mast cells in the protective immunity of scabies remains unresolved. A number of studies suggest that eosinophils and mast cells play unimportant role because the population of those cells remained low at any stage of infestation (ARLIAN et al., 1994b; ARLIAN et al., 1996). Other studies, in contrast, indicate that eosinophils and mast cells are essential in the successful immunity of against mite infestation (FERNANDEZ et al., 1977; CARGILL dan DOBSON, 1979; REUNALA et al., 1984). This dispute could only be reconciled by a further, intensive, pathological study.

In the present study a relatively large number of animals with various degrees of previous exposure to the mite were used. In addition, histological samples were not terminal samples but sequential biopsies. Goats were used as the experimental animals because scientific information on immunology and pathogenesis of sarcoptic mange in these animals is meagre. More importantly, sarcoptic mange is apparently one of the most important disease affecting this animal (BROTOWIDJOYO, 1987; MANURUNG et al., 1990; DORNY et al., 1994; PARIJA et al., 1995; GABAJ et al., 1992). This study is a sequel to our previous study in which we observed that goats recovered from sarcoptic mange develop a significant degree of resistance against mite reinfestation (TARIGAN, 2003).

MATERIALS AND METHODS

Source of mite

A goat suffered from severe sarcoptic mange was purchased from a nearby farm. This goat was used to infect healthy goats by housing them together in a special, isolated pen. This colony of mangy goats was maintained as source of mite for infestation of experimental animals.

Infestation

A severe mangy goat from the colony of mangy goat was euthanised by removing all the blood by venipuncture. After clipping and shaving the hair, skin showing encrustation dermatitis was scraped deeply. The skin scrape was chopped into about 2-mm³ pieces, mixed thoroughly and kept at 4°C overnight. The number of life mites per gram of skin scraping was determined by placing the skin scraping at the edge of a Petri disc. A beam of light was directed to the centre of the Petri disc, and mites migrating toward the light were counted after 6 hours under a microscope.

A piece of cloth (6 x 4 cm²) was placed on the convex surface of a goat auricle, the bottom, left and right edges of the cloth were attached to the auricle with an adhesive tape. Skin scraping containing approximately 2000 mites was inserted under the cloth through the top edge then the top edge was attached to the auricle. After 48 hours, the cloth together with the skin scraping were removed and infestation was allowed to develop.

Sensitisation

Seven weeks after infestation, at which stage infestation is usually reflected by severe encrustation dermatitis involving almost the whole skin, the mangy goats were injected subcutaneously with 0.5-ml Ivermectin (MSD AGVET, Holland). Injections were repeated 1 and 2 weeks later and complete healing was obtained three weeks after the last injection. To acquire goats sensitised twice with the mites, the recovery animals were infested and then treated for the second time similar to that described for the first sensitisation. Thrice sensitisation was done by repeating the infestation and Ivermectin injections in animals healed from the second infestation.

Experimental goats, challenge and biopsy
Thirty young female goats were allocated into 5 groups each group contained 6 animals. Group 1, 2 and 3 goats had been sensitised with the mite once, twice and thrice, respectively. Group 4 and 5 goats were naïve or goats that previously had not been exposed to *S. scabiei*. All goats, except those of group 5 were infested with the mite on both auricles, each auricle received about 2000 mite. For the ease of biopsy collections, each group was further divided into 2 sub groups, A and B, each subgroup therefore contained 3 animals. Biopsies were collected at 2 days then at weekly intervals from 1 to 7 weeks following infestation. At 2-day sampling, biopsies were collected from the right auricles of goats in subgroup A of each group; at 1-week sampling, biopsies were taken from the right auricle of goats in subgroup B. At 2-week post infestation, biopsies were taken from the left auricles from subgroup A goats, 3-week from left auricle of subgroup B goats. At 4- and 5-week post infestations, biopsies were taken from the right auricles of subgroups A and B, respectively. At 6- and 7-weeks post infestation biopsies were collected from the left auricles of subgroups A and B, respectively. Biopsies were taken from a representative site using a sponge forceps and a number-11 scalpel blade. The biopsies were elliptical in shape with the longest and the shortest diameters of 1.5 and 1 cm, respectively. Prior to incision, a local anesthetic (Lidocain-HCl) was infiltrated, and after incision was sutured, an iodine solution (Betadine®) was applied to prevent bacterial infection.

All samples, except those taken at 2 day post infestation, were fixed in Phosphate-buffered formolsaline. Samples taken at 2 day were first fixed in Bouin’s for 24 hours then in 70% ethanol. The samples were routinely process, paraffin blocked, cut at 3 μm, then stained with Haematxillin and eosin (H & E), Giemsa, Carbol chromatotrope staining (a special staining for eosinophils), and when indicated with Gram’s.

RESULTS

No pathological change either grossly or histotologically was observed in any of the control untreated goats throughout the course of experiment.

Two-day post infestation

**Naïve goats**- The auricles were mild-to-moderately swollen with thick serous exudates oozed from the primary site of infestation. Histologically, copious amount of exudates composed of neutrophils, serum and keratin fragments accumulated on the surface of the epidermis. The dermis was thickened due to severe oedema which was reflected by wide separation of collagen bundles and dilatation of veins and lymphatics. Inflammatory cells, consisted of neutrophils and mononuclear cells, were accumulated in the dermis especially around blood vessels. Special staining for eosinophils revealed that eosinophils were hardly any in the dermal or epidermal exudates. Mites were found entangled in the exudates but a significant number have successfully made their burrows adjacent to or away from the epidermal exudates. These later mites induced only mild host responses around the mites (Figure 1A).

**Sensitised goats**- Lesions developed in all sensitised goats, either sensitised once, twice or thrice, were comparable both grossly and histologically. Grossly, the changes were qualitatively similar to those in naïve group, but the lesions were much more severe. Histologically, the epidermis contained varying sizes of epidermal or sub corneal vesicles and pustules. Probably because of their large size, some pustules had burst and their contents, consisted mainly of neutrophils, precipitated on the skin surface (Figure 1B). The mites were found entangled in the exudates and none was observed successfully burrowed the skin. The dermis was severely oedematous with large number of inflammatory cells, consisting of granulocytes and mononuclear cell, scattered throughout the tissue (Figure 1C). Unlike in the naïve goats, the inflammatory cells in the dermis contained a high proportion of eosinophils. The dermal blood vessels in some animals, underwent vasculitis characterised by endothelial swelling and accumulation of inflammatory cells, especially eosinophils and mononuclear cells in and around the vessel walls (Figure 1D). The epithelial cells of hair follicles and sebaceous gland underwent mild to moderate hyperplasia.

One-week post infestation

**Naïve goats**- grossly, thin yellowish crusts were found on the site of infestation. Beyond this area, small papules could be detected by palpation of the auricles. Histologically, pathological changes caused by the mite were minimal. Mites within their burrow were found under the stratum corneum; occasionally, the mouth parts of the mites reached the dermal epidermal interface (Figure 2A). Keratin and cellular crusts were present on the epidermis, especially adjacent to the mite. The epidermis underwent moderate hyperkeratosis and acanthosis (thickening of the stratum spinosum).

**Sensitised goats**- The auricle swelling, which was very severe at 48-hour post infestation, has been waned considerably. Accumulation of dry crust or desiccated exudates was the most obvious grossly. Histologically, the crusts on the surface were consisted mostly of keratin and some cellular debris. The surface of the skin was irregularly undulating. The epidermis underwent moderate-to-severe acanthosis, hypergranulosis, acantholysis, and formation of sub corneal vesicles or...
auricles. Epidermal changes were seen on skin of both sites of the acantholysis especially adjoining the mite burrows. The hyperkeratosis and acanthosis, there were multifocal similar to those observed at 1 week. In addition to (sensitised thrice) goats exhibited a more severe lesions. epidermal cyst. Samples from the subgroup 3B hypergranulosis; and one had acantholysis and mild acanthosis, hyperkeratosis and multifocal abscesses, in addition to above described changes. In subgroup 2B (sensitised twice) goats, two also had only mild acanthosis, hyperkeratosis and multifocal hypergranulosis; and one had acantholysis and epidermal cyst. Samples from the subgroup 3B (sensitised thrice) goats exhibited a more severe lesions.

Two-week post infestation

Naïve goats. Basically, the type of lesions were similar to those observed at 1 week. In addition to hyperkeratosis and acanthosis, there were multifocal acantholysis especially adjoining the mite burrows. The epidermal changes were seen on skin of both sites of the auricles.

Sensitised goats. The epidermis was thickened due to marked hyperkeratosis, hypergranulosis and acanthosis and rete ridge formation (Figure 4A). These epidermal changes were more severe as compared to those seen at one week. However, epidermal cyts or pustules which were prominent at 1 week sampling were diminished. Epidermal micro abscesses were only marked in 2 goats of the sub group 3A. Again, neither mite nor its burrow was seen in any sensitised goats.

Three-week post infestation

Naïve goats-Grossly, the auricles were covered with moderately thick parakeratotic crusts. Histologically, a thick parakeratotic crust honeycombed with burrows and thick serocellular exudates were seen on the epidermis. The number of mite had greatly increased. Various stage of development, embrionated eggs, larva and adults, could be seen in any microscopic field. In fact, an adult, several larvae and embrionated eggs could be seen in a cross section of a borrow (Figure 3A). Besides mites, a large number of mite’s faecal pellets and moults were found in the burrows. In some areas, there were large sub corneal abscesses, acantholysis, parakeratosis, hypergranulosis and acanthosis. Capillary congestion or even haemorrhages were seen in some area of the dermis just underneath the epidermis. Some borrows were also filled with erythrocytes and the mites’ guts engorged with spherical eosinophilic bodies or cells presumably the host erythrocytes. Sensitised goats-of the 3 biopsies from the subgroup 1B (sensitised once) goats, two had only mild acanthosis, hyperkeratosis and multifocal hypergranulosis, and slightly increased eosinophils in the dermis. One had epidermal cysts and micro abscesses, in addition to above described changes. In subgroup 2B (sensitised twice) goats, two also had only mild acanthosis, hyperkeratosis and multifocal hypergranulosis; and one had acantholysis and epidermal cyst. Samples from the subgroup 3B (sensitised thrice) goats exhibited a more severe lesions. Two had diffuse, marked hyperkeratosis, acanthosis, hypergranulosis, rete ridge formation and slightly increased number of eosinophils in the dermis. One goat had mites (2 adults and 1 larva) burrowed into the epidermis inducing severe inflammatory responses included dermal vacuolation, accumulation of mononuclear cells underneath the mites, marked hyperkeratosis, acanthosis, acantholysis, sub corneal vesicles and abscesses, accumulation of parakeratotic crust, and marked increased eosinophils and mononuclear cells in the dermis (Figure 2C).

Four-week post infestation

Naïve goats-Grossly, the area affected by the encrustation dermatitis widen. Histologically, the changes were qualitatively similar to those at 3-week but the parakeratotic crust become thicker.

Sensitised goats-biopsies taken from subgroup 1A goats exhibited marked hyperkeratosis, acanthosis, hypergranulosis and mild-to-moderate acantholysis, and increased population of eosinophils in the dermis. Subgroup 2A goats showed similar type of lesions but remarkably milder. No mite was seen in the sections of either animals of groups 1A and 2A. In group 3A goats, on the other hand, burrowing mites were seen in samples of 2 goats. Although the number of mite was small, only 2 or 3 mites per section, they induced severe lesions. Some of the mites were enmeshed in the serocellular exudates and the apparently underwent degeneration because the staining differentiation of the internal structures was lost (Figure 4B).

Five- and six-week post infestation

Naïve goats-Grossly, the encrustation dermatitis affected not only the whole infected auricle but also the opposite auricle, neck and shoulder. The hair become lusterless and the condition of animals become poorer and poorer. Histologically, the lesions remained similar to those of previous week except that diffuse sub corneal abscesses and spongiotic vesicles underneath the mites were common.

Sensitised goats-In contrast to naïve goats, sensitised goats were in good condition. Lesions could only be perceived by palpating the auricles. Histologically, the dermis showed varying degree of hyperkeratosis, acanthosis, hypergranulosis, acantholysis, spongiotic cysts, sub corneal abscesses, and accumulation of serocellular on the epidermis. At 5 weeks, small number of mites (2-4 mites/sections) were seen in one goat of subgroup 3B, and no mites were seen in subgroup 1B or 2B. By 6 week, small mites were seen in one animal in each sub groups 1A and 3A, but none in sections from groups 2A goats.
Figure 1. Two day post infestation. Naïve goat showing a mite burrowed into the epidermis inducing minimal host reactions (A). Sensitised goat showing a severe exudative dermatitis (B), dermal oedema characterised by separation of collagen bundles and dilatation oh lymphatics (arrow)(C), and eosinophilic vasculitis (D).

Figure 2. One week post infestation. Naïve goat showing a mite with mouth parts reaching the dermal-epidermal interface inducing mild host reactions (A). Sensitised goats showing moderate-to-severe acanthosis, hypergranolosis, acantholysis (B) and sub corneal vesico pustules (C).
Seven-week post infestation

Grossly, the naïve goats were in poor condition, and encrustation dermatitis affected almost the whole skin. Histologically, the lesions were similar to those of previous week. A large number of bacterial colonies which on Gram staining appeared to be Gram-positive cocci arranged in chains or grapelike clusters were seen (Figure 3B). The sensitised goats, in contrast, were all in good condition. Grossly and histologically, lesions were similar to those of previous week. Mites were seen in histological section of all subgroup 1B goats, in one goat in each subgroup 2B and 3B.

DISCUSSION

The present study showed that infestations of *Sarcoptes scabiei* in sensitised goats induced lesions which were different qualitatively from those in naïve goats. In naïve goats, the mites burrowed the epidermis and reproduced within the burrows inflicting minimal host responses. In sensitised goats, in contrast, mite attempt to burrow the epidermis confronted with rapid, excessive host responses that hinder mite infestation. The most important host responses exerted to fight the mites included outpouring the mites with serocellular exudates. These sort of host responses, to a great extent, were successful because at the end of the experiment, seven week post mite challenge, the goats were in good clinical condition and histological examination of the skin around the primary infestation site revealed no or only few mites. At the same time, the naïve goats were all in poor condition and the skin all over the body suffered from severe encrustation dermatitis and contained very large number of mites.

The most prominent changes in sensitised goats by 2-day post mite challenge included extravasation of fluids and neutrophils into and through epidermis, capillary and lymphatic dilatation, oedema and accumulation of eosinophils in the dermis. Those changes were indicative of local, cutaneous anaphylactic or hypersensitivity type 1 (YAGER and SCOTT, 1985; STROMBERG and FISHER, 1986). All of
the changes described above, except infiltration of dermis by eosinophils were also displayed by the naïve goats although to a lesser degree. Because dermal infiltrates in the naïve goats were devoid of eosinophils, and since infiltration by these cells is considered to be the characteristic of cutaneous anaphylactic reaction, lesions in the naïve goats were probably not the manifestation of cutaneous anaphylactic reaction.

The observation that a significant number of mites were successfully burrowed the epidermis by 2-day post mite infestation; laying many eggs, many of which had hatched into larvae, by 3 weeks; and then the population of mites rapidly increased after 3 weeks indicate that the growth and reproduction of mite in naïve goats seemingly face insignificant hindrances. This is further supported by the histological evidence that host reactions around the mite burrows were minimal. In sensitised goats, in contrast, the mite apparently have a great difficulty in infesting these goats. This is supported by the observation that at 48 hour examination, all mites were flooded by copious serocellular exudates and no mite was observed to have successfully burrowed the skin. These host responses apparently eliminated almost all the inoculated mites since no mite were seen in tissue sections at 1 to 4 week examination in any of the 18 sensitised goats. By 4-week, however, a few mites were seen in tissue sections in some animals and the proportion of sensitised goats having mite in their tissue section were increased. This indicates that the immunity possessed by sensitised animals could not completely protect the animals against the mites.

The reaction of sensitised goats against the mite was initiated by acantholysis which often developed into sub corneal vesicles and subsequent vesicopustules or micro abscesses. The abscesses were often filled with such a large number of inflammatory cells that burst and their content condensed and dried on the skin surface. The dermis contained a large number of eosinophils and monocellular cells. These epidermal and dermal changes, which were compatible with spongiosis psoriasiform which is a feature of an allergic dermatitis (STROMBERG and FISHER, 1986), were also seen in all other sensitised animals even though the mite was not seen in the tissue sections. This may indicate that mites actually existed in all of the sensitised animals but because of their small number they were frequently invisible in the tissue sections.

The formation of the sub corneal abscesses is apparently the mechanism of the sensitised hosts to destroy the mite or inhibit their burrowing the skin. This contention is supported by the observation in this study that the mites were often seen flooded by serocellular contents of the abscesses and the mites often seen degenerated. This milieu of serocellular exudates eminently unsuitable for the survival of the parasites. In addition, specific antibodies and other serum components, and toxic component produced by the cellular infiltrates such as proteases and oxygen-metabolite radicals may intoxicate the mites (STROMBERG and FISHER, 1986).

REFERENCES


