Studies on the Immune Mechanisms and Immunopathology of Severe Skin and Meibomian Gland Demodicosis in Cattle

Abu-Samra M. T. and Shuaib Y. A.


DOI: 10.5455/jva.20141220110819
Studies on the Immune Mechanisms and Immunopathology of Severe Skin and Meibomian Gland Demodicosis in Cattle

*1Abu-Samra M. T. and 2,3Shuaib Y. A.

1Department of Veterinary Medicine and Surgery, College of Veterinary Medicine (CVM), Sudan University of Science and Technology (SUST), P.O. Box: 204 (Hilat Kuku), Khartoum North, the Sudan.
2Department of Preventive Veterinary Medicine, College of Veterinary Medicine (CVM), Sudan University of Science and Technology (SUST), P.O. Box: 204 (Hilat Kuku), Khartoum North, the Sudan.
3Research Center Borstel, Parkallee 18, 23845 Borstel, Germany.

Abstract

Cattle infected with severe and generalized skin and simultaneous meibomian gland lesions of demodectic mange; had higher total serum proteins, significantly higher globulin and lower albumin concentration than non-infected ones. The protein content of the soluble antigen of *Demodex* mites and associated bacteria was 70 µg /100 µl. Hypersensitivity testing of infected cattle by the intradermal injection of soluble mite and bacterial antigens produced immediate, delayed cell-mediated and Arthus immune-complex reactions, while non-infected cattle were refractory to these tests. The enzyme-linked immunoelectrotransfer blot gave positive reaction bands at 56 kilodaltons (KDD) when the resolved soluble antigen was probed with sera from infected cattle and no reaction bands were demonstrated in sera from non-infected animals. The nature of association between *Demodex* mites and bacteria in demodectic mange lesions was synergistic and of equal significance. Pathogenic and opportunistic bacteria facilitated the establishment of *Demodex* mites in the lesions produced and provided an excellent microclimate for the mites to propagate and reproduce, resulting in severe and progressive disease. Histopathological examination showed severe changes characterized by massive ‘high-turnover’ granulomatous reaction with influx of macrophages and lymphocytes proving that *Demodex* mites and associated bacteria were both persistent and immunogenic. Giant cells destroyed, engulfed and digested the bacteria and the mites, resulting in healing of the lesions as judged by the progressive proliferation of connective tissue and degeneration of the granulomas. In spite of the rapid and exaggerated humoral and cell-mediated immune responses demonstrated *in vivo* and *in vitro*, the mites and associated bacteria remained viable in chronic lesions for 2-3 years. Failure of these cattle to develop acquired immunity against repeated exposure to the mites and associated bacteria was probably due to the development of tolerance resulting from the release of large amounts of soluble and particulate antigens.

Keywords: Bovine demodicosis; hypersensitivity; immunopathology; Western blot.
STUDIES ON THE IMMUNE MECHANISMS AND IMMUNOPATHOLOGY OF ...

Introduction

The Genus Demodex belongs to the family Demodicidae, of the sub-order Trombidiiformes of the order Acarina of the class Arachnida of the phylum Arthropoda (Soulsby, 1968). Among 65 species of the genus Demodex, ten species were reported to be of medical and veterinary concern (Nutting, 1976). Most of the species were called after their hosts (Soulsby, 1968; Radostits, et al., 2007), and regarded to be host specific. However, the occurrence of more than one species of Demodex infesting the same host was reported by some workers. Accordingly, the co-existence of synhospitalic Demodex mites was reported by many workers in the different species of animals and man (Desch and Nutting, 1972; Slingenbergh, et al., 1980; Bukva, 1986; Baima and Sticherling 2002; Bikowski and Del Rosso 2009). Abu-Samra and Shauib (2014 a, b, c) isolated Demodex bovis and D. ghanensis from skin and meibomian glands, respectively, and concluded that Demodex bovis and D. ghanensis were found to exist on the one host and were regarded as being host specific to cattle, and habitat specific to skin and meibomian glands, respectively. In man the occurrence of the pilosebaceous follicle mites Demodex folliculorum and Demodex brevis which inhabit most commonly and densely certain facial skin areas with numerous sebaceous glands (forehead, meibomian glands of the eyelid, root of the eyelash, chest, armpits and pubis) were reported by many workers (Baima and Sticherling, 2002; Bikowski and Del Rosso, 2009; Karaman et al., 2010; Escudero Pastor et al., 2013). Abu-Samra and Shauib (2014 a, b, c) reported severe clinical signs, visible and extensive gross lesions and marked histopathological changes compatible with cell mediated immunity in both skin and meibomian gland demodicosis in cattle.

Pedersen (1999) reported that Immediate, intermediate and delayed hypersensitivity reactions can occur in the same animal. Immediate reactions are characterized by reactions that appear within 30 minutes of antigenic challenge, whereas delayed hypersensitivity reactions take 48-72 hours to reach peak intensity. The former reaction is characterized by edema, neutrophil, and eosinophil infiltrate, the latter by inflammation and mononuclear cell (Lymphocyte, macrophage, plasma cell) infiltrate. In man Baima and Sticherling (2002) reported blockage of follicles and sebaceous ducts by the mites and by reactive hyperkeratinization and epithelial hyperplasia, a vector role for bacteria, a foreign body granulomatous reaction to the mite chitinous skeleton, stimulation of the host humoral and cell-mediated immune reactions by the mite and their waste products and delayed hypersensitivity reactions (Type IV immune response) to an unknown antigen of follicular or mite origin could occur as pathogenic mechanisms of Demodex folliculorum and D. brevis infection.

The Enzyme-linked immunoelectrotransfer blot technique (Western blot) is a test that involves resolving antigens in a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferring the SDS-PAGE resolved antigen to a nitrocellulose blotting membranes by electrotransfer blot, and incubating patients’ sera and control specimens with the nitrocellulose strips containing the blotted antigen and identifying positive reactions through enzyme immunoassay. Tsang et al., (1986) reported that other simpler tests being developed might eventually supplement the Western blot technique in the diagnostic laboratory; the technique, however, will remain a powerful qualitative tool for studying the total picture of antigen-antibody systems.

Using an indirect ELISA employing as antigen a homogenate of Sarcoptes scabiei var vulpes, Bornstein et al., (1997) could successfully demonstrate antibodies to Sarcoptes scabiei in the sera of camels clinically suspected of sarcoptic mange. They confirmed the specificity of the antibodies of S. scabiei in a Western Blot analysis demonstrating similar antigens (polypeptide band pattern) as had been demonstrated for the infection in red foxes, dogs and pigs, and no specific antibodies to S. scabiei could be demonstrated in the sera from clinically healthy camels. In the available, literature only a few reports on serum protein levels, hypersensitivity or immune responses of cattle naturally infected with demodecic mange were encountered. In the current
ABU-SAMRA AND SHUAIB

investigation, these aspects are probably investigated for the first time using authentic methods and techniques. The results obtained were correlated to the histopathological changes seen in skin and meibomian gland lesions and the host-parasite interactions (Cattle/Demodex mites and associated bacteria) reported in previous investigations (Abu-Samra and Shuaib, 2014 a, b, c).

Background of the Study

Abu-Samra and Shuaib (2014 a, b, c) reported that bovine demodicosis was observed among emaciated animals as well as in animals in good bodily condition. In some adult cattle the disease persisted for 2-3 years. They reported that calves < 1- 1 year old and adult cattle 2-8 years old, of both sexes were equally susceptible to bovine demodicosis. Abu-Samra and Shuaib (2014 a) reported that calves had probably acquired infection from dams harboring chronic and persistent lesions of the disease during nursing in the neonatal period. Demodex bovis and D. ghanensis mites were isolated from infected purulent material extracted from skin and meibomian glands, respectively, but the mites could not be isolated from skin brushings or swabs from the eyes of non-infected cattle grazing side-by-side with infected ones. Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes (Group A) and Trueperella (Arcanobacterium) pyogenes were isolated from skin lesions (Abu-Samra and Shuaib, 2014 a, c), and Moraxella bovis and Staphyloococcus aureus were isolated from meibomian gland lesions (Abu-Samra and Shuaib, 2014 b, c). Bacillus subtilis, Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus pyogenes (Group A) were isolated from skin brushings of non-infected cattle, but no bacteria were isolated from swab cultures from the eyes of non-infected cattle (Abu-Samra and Shuaib, 2014 b, c). They reported that the nature of association between Demodex mites and bacteria in demodectic mange lesions was synergistic and of equal significance. Abu-Samra and Shuaib (2014 a, b, c) reported that pathogenic and opportunistic bacteria facilitated the establishment of Demodex mites in the lesions produced and provided an excellent microclimate for the mites to propagate and reproduce, resulting in severe and progressive disease. Histopathological examination showed severe changes characterized by massive ‘high-turnover’ granulomatous reaction with influx of macrophages and lymphocytes, proving that Demodex mites and associated bacteria were both persistent and immunogenic; producing severe and progressive disease encountered in natural field cases.

Materials and Methods

Hundred adult cattle, 2 – 8 years old with severe skin and simultaneous skin and meibomian gland demodicosis, and 30 adult non-infected cattle of the same herd; grazing side – by – side with infected ones were selected for serum protein determination and Enzyme-linked immunoelectrotransfer blot technique (PAGE). Among these animals seven infected and seven non-infected (control) cattle (4 cows and 3 bulls) in each group were selected for cutaneous hypersensitivity testing. Selection of infected cattle was based on their freedom from blood and internal parasites, and selection of non-infected animals was also based on their freedom from Demodex mites and demodectic mange lesions by clinical and laboratory examination of their skin and eyes. The animals were bled from the jugular vein and serum was separated and stored at - 20 ºC until used.

Serum Protein Determination

Absorbance of test specimen/ Absorbance of standard X Concentration of standard ( g/dl ) X 10 (g/l).

Total Serum Protein Concentration

The total serum protein concentration was determined by the Biuret method described by Weichselbaum (1946). The principle of the method was based on the reaction between the protein and copper sulphate in the presence of sodium hydroxide. Rochelle salt (Sodium potassium tartrate) found in the Biuret reagent is utilized to
keep in solution the cupric hydroxide which gives the blue colour, proportional to the protein content in the serum sample. A Pyeunicam SP 6-200 spectrophotometer at 555 nm wave length was used to measure the optical densities of the samples. A standard calibration curve was prepared by serial dilutions of a standard protein (bovine serum albumin) prepared and treated with Biuret reagent as described by Weichselbaum (1946). After a period of ten minutes, the optical densities were measured versus reagent blank (Biuret), and plotted versus concentrations of standard. Unknowns were read from the standard curve and the total serum protein concentrations of infected and non-infected cattle were calculated in g/dl and converted to SI unit – g/dl X 10.

**Serum Albumin Concentration**

Serum albumin concentration was determined by the Bromcresol Green (BCG) method described by Northman and Weddowson (1967). The principle of the method is based on the fact that the specific binding of the dye BCG with albumin results in a change in the peak of absorbance of the wave length. Serum samples were added to a buffered solution of BCG at 3.8 pH and were incubated for 10 minutes at 20 ºC. Changes in the absorbance of the spectrophotometer were recorded at 637 nm wave length against a working Blank specimen of buffered BCG. The results were calculated by the simultaneous running of a standard bovine albumin solution incubated and treated as described above. The serum albumin concentration was calculated in g/dl by the following formula:

\[
\text{Serum albumin concentration} = \frac{\text{Absorbance of test specimen}}{\text{Concentration of standard (g/dl)}} \times 10 \text{ (g/l)}.
\]

Absorbance of standard.

**Serum Globulin Concentration**

The serum globulin concentration was obtained by the subtraction of the value of serum albumin concentration from the value of total serum protein concentration of each serum sample.

\[
\text{Serum globulin concentration} = \text{Total serum protein} - \text{serum albumin}.
\]

**Preparation of Soluble Antigen from Demodex Mites and Associated Bacteria**

Pooled infected material was extracted from skin and meibomian gland lesions following sterile techniques. The pooled infected material was placed in a sterile 250 ml flask and was washed several times with sterile double distilled water until the supernatant fluid became clear. The infected material was examined to ensure that it was rich in mites and associated bacteria, and was then transferred to a sterile Griffith’s tube and homogenized. The homogenized material was then suspended in a sterile bottle by the addition of 100 ml of sterile physiological saline. The bottle was cooled in a jacket of water and ice while disruption of the infected material was carried out by an ultrasonic disintegrator (Mountain States Equipment Company – MSE, USA) for one hour. Complete disruption of the mites and associated bacteria was ensured by placing a drop of the disintegrated material in the middle of a clean microscope slide, covered with a coverslip and examined. The disintegrated material was left for 48 hours at 4 ºC, after which period it was centrifuged in a cooled high speed centrifuge (MSE Company) at 4500 R. P. M. for one hour. The supernatant fluid was then transferred to Visking dialysis bags, immersed in distilled water and left to dialyze for 48 hours at 4 ºC. While still in the dialysis bags, the antigen was concentrated on polyethylene glycol. The protein content of the soluble antigen, so prepared, was determined by a Gilford Stasar II spectrophotometer – Germany. The concentrated antigen was then divided into small aliquots in sterile bijou bottles and stored at – 20 ºC until used for hypersensitivity testing and the Western blot technique (PAGE).

**Enzyme-Linked Immunoelectrotransfer Blot Technique (Western Blot)**

Bio-Rad Model 360 mini-vertical slab casting chamber was used for casting the gels. The separating gel (10 ml Acrylamide stock, 11.2 ml Tris pH 8.8, 8.7 ml double distilled water) were thoroughly mixed and degassed for 15 minutes and 0.3 ml of 10 % of SDS, 100 µl of 10 % freshly prepared ammonium persulphate and 10 µl TEMED were added. The separating gel was poured leaving
4 cm at the top for the stacking gel and the wells. The gel was immediately overlaid with water saturated butanol to give a smooth interface after polymerization and was left to polymerize for 45-60 minutes. After polymerization the butanol was poured off and the gel surface was rinsed with double distilled water. The stacking gel (1.0 ml Acrylamide, 1.25 ml Tris pH 6.8 and 7.7 ml double-distilled water) was thoroughly mixed and degassed for 15 minutes and 0.1 ml SDS, 50 µl ammonium persulphate and 10 µl TEMED were added. The stacking gel monomer was poured to about 1 cm from the top of the plates. The comb was pushed down, avoiding the trapping of air bubbles below the comb teeth and the gel was left to polymerize for 30-45 minutes. After polymerization the comb was carefully removed and the wells were rinsed three times with double distilled water and the assembly was turned up-side-down to drain any liquid present. The wells were filled with running buffer (3.03 gm Tris base of 0.025 M, 14.42 gm glycine of 0.192 M and 1.0 gm SDS/1 liter of double distilled water) at pH 8.3, and loaded with bovine demodectic mange antigen prepared by adding 0.65 ml of the sample buffer (1.88 ml Tris pH 6.8, 6.0 ml of 10 % SDS, 3.0 ml glycerol and 2.12 ml double distilled water) to 0.1 ml of 1.5 M dithiothreitol (Sigma) in 0.1 % w/v bromophenol blue (BDH). Equal volumes of the sample buffer and the soluble antigen were mixed and heated at 100 °C for 3-5 minutes in closed tubes and were left to cool before loading the gel. One well was filled with a Pharmacia low molecular weight standard (Pharmacia LMW marker) for the purpose of knowing the relative molecular weights of the resolved protein bands on the transferred blot. After loading the antigen and the standard protein, the top unit of the chamber was snapped to fit it over the electrode terminals and the leads were connected to the power supply. The samples were then subjected to electrophoresis until all of the tracking dye reached the bottom of the glass plates and just began to run out of the gel sandwich. Electrophoresis took 45-60 minutes, and the power supply was turned off. The top unit of the chamber was removed and the buffer was poured out and discarded. The chamber was disassembled and the gel sandwich was removed. When the gel was still on glass, small notches were made with the scalpel to indicate different lanes as well as orientation marks. The gel was incubated twice, each for 30 minutes in the transfer buffer (20 Mm Tris base and 100 mM glycine) prepared by dissolving 9.68 gm Tris base and 30 gm glycine in 4 liters of double distilled water to eliminate swelling. During the second washing period, the gel dimensions was measured and the Nitrocellulose Paper (NCP) was cut slightly larger than the gel. When dry the NCP paper was labelled with pencil marks (strip number and orientation marks). The NCP was rolled onto double-distilled water to avoid bubbles underneath. When completely moist, it was submerged for 5 minutes in water. The wet NCP was equilibrated in transfer buffer for 10-20 minutes. Two pieces of whatman paper of the same size as the gel were wetted in the transfer buffer. One piece was used to pick up the gel, making sure to remove any air bubbles between the paper and gel. The paper – gel unit was laid down on scotch – brite pad with paper next to pad. The wet and equilibrated NCP was laid over the gel avoiding to trap air bubbles. The sandwich was completed by laying the second piece of wet whatman paper over the NCP followed by the second scotch – brite pad. The gel supporting frame was closed and placed into blotting unit prefilled with transfer buffer. The orientation of the sandwich was ensured to have the gel cathode to the NCP. Electrophoresis was conducted and transfer was performed for 90 minutes at 60 V, 0.22 Amp setting. After electrophoretic transfer was completed (SDS-PAGE), the power was shut off and the sandwich was disassembled carefully to avoid tearing the NCP or gel. The NCP was placed in the quench buffer (5 gm of BSA dissolved in 250 ml [2 %] phosphate buffered saline [PBS]) for one hour at room temperature on a rocker platform. The NCP was washed twice for 30 minutes each in the washing buffer (20 gm BSA dissolved in 4 liters of PBS to which 8 ml non-ionic detergent – Triton X-100 [0.2 %] was added). In each batch, four sera from infected cattle and one from non-infected cattle were blotted. Each serum specimen was diluted with the washing buffer in the ratio of 1:100. The blot NCP was placed on a large glass plate and
was cut into 0.5 cm strips with a strip cutter. Each 0.5 strip was placed into a separate slot of the incubation tray. Each diluted serum was put on each strip and were allowed to remain in contact for 90 minutes at room temperature with continuous agitation on a rocker. The strips were then washed four times for 20 minutes each with the washing buffer, and were then incubated with continuous gentle agitation on a rocker for 1 hour in anti-bovine peroxidase (conjugate), diluted with the washing buffer in the ratio of 1:1000. After incubation, the strips were washed four times for 20 minutes each with the washing buffer and were placed into peroxidase substrate: (5mg [3,3-Diaminobenzidine tetrahydrochloride dihydrate [DAB, Sigma] and 5 µl H2O2 per 1ml of MES buffer [2-[N-Morpholino] ethane sulfonic acid [50 mM MES, Sigma, pH 6.0] ) and were left to react for 5-10 minutes. Finally the strips were assembled and the bands were photographed.

Hypersensitivity Testing

The skin over the neck of the seven infected and non-infected cattle (4 cows and 3 bulls) was carefully inspected and palpated. On both sides of the neck of each animal, areas of 15 cm in length and 4 cm in width, that were free of demodectic mange lesions, were chosen for performing the test. The designated areas in the seven infected and the seven non-infected (control) cattle were washed, carefully shaved, disinfected and dried. Following the method described by Rook and Wilkinson (1968), the animals were skin-tested by the intradermal injection technique. Four equally spaced sites were marked. The 1st site was injected with 0.1 ml sterile normal saline and the 2nd to 4th sites were similarly injected with 1:100 dilution of the soluble antigen in sterile normal saline, using 1 ml sterile hypodermic syringes. The skin test-sites were closely inspected over a period of 4 days. Hyperaemia on non-pigmented areas, wheal formation, necrosis or indurated swellings were noted. Skin biopsy specimens were taken half an hour and one hour after testing and, then at 3 hours’ intervals when required, depending on the gross appearance of the test sites. The biopsy specimens were fixed in 10 per cent formal saline, processed, embedded in paraffin wax, cut at 5µm, stained with Haematoxylin and Eosin and examined.

Results

Serum Protein Concentrations

Infected cattle had higher total serum protein, significantly higher globulin and lower albumin concentration than non-infected cattle (Table 1). The values obtained for total serum protein, albumin and globulin concentration of non-infected cattle were within the normal range recorded for this species (Coles, 1967; Blood and Studdert, 1999).

<table>
<thead>
<tr>
<th>Serum Protein (g/l)</th>
<th>Infected cattle</th>
<th>Non – infected cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum protein concentration</td>
<td>103 – 105</td>
<td>72 – 85</td>
</tr>
<tr>
<td>Albumin concentration</td>
<td>30 – 32</td>
<td>35 – 40</td>
</tr>
<tr>
<td>Globulin concentration</td>
<td>71 – 74</td>
<td>40 – 42</td>
</tr>
</tbody>
</table>

The Soluble Antigen

The protein content of the soluble antigen of Demodex mites and associated bacteria was 70 µg /100 µl.

Enzyme-Linked Immunoelectrotransfer Blot (Western Blot)

The enzyme-linked immunoelectrotransfer blot (Western Blot) gave positive reaction bands in the lanes probed with sera from infected cattle, while no reaction bands were seen in the lanes probed with sera from non-infected cattle. The reaction bands were seen at 56 Kilo-daltons (KDD) of the resolved antigen (Fig. 1).
Hypersensitivity Testing

Immediate hypersensitivity reaction was demonstrated at the site of injection with swelling, redness, wheal and flare within 15-30 minutes after the intradermal injection of the soluble antigen. These swellings disappeared within 4 – 6 hours. Arthus reaction appeared within 1-2 hours with localized swelling, erythema and edema.

Fig. 1: Enzyme-linked immunoelectrotransfer blot (Western blot) showing positive reaction bands at 56 KDD (arrow) of the resolved soluble antigen in the lanes probed with sera from infected cattle (D₁ – D₄) and no reaction band in the lane probed with serum from a non-infected animal (C₁). BPB (Bromophenol blue), MW (Molecular weight marker) and TSG (Top of separating gel).

These areas became haemorrhagic and showed central necrosis within 8 – 12 hours after the intradermal injection of the soluble antigen. The reaction resolved within 30 – 36 hours. Delayed hypersensitivity showed first appearance within 6 – 12 hours after the intradermal injection of the soluble antigen. These areas showed erythematous non-edematous indurated swellings, reaching maximum intensity within 24 – 72 hours and regressed slowly. Control sites in infected animals that were injected with normal saline and all sites in control non-infected cattle showed non-erythematous swellings which regressed and disappeared within a short time. Biopsy specimens, taken 0.5 – 1.5 hours after skin testing of infected cattle, showed marked edema and dilatation of the blood vessels, and infiltration by mast cells and few basophils. Biopsies taken from sites showing central necrosis, 12 – 18 hours after the intradermal injection of the soluble antigen, revealed dermal degeneration, thrombosis, vascular necrosis and extravascular infiltration by numerous neutrophils, few lymphocytes, macrophages and plasma cells. Biopsies taken 18 – 72 hours. After injection showed dilatation of the blood vessels and marked infiltration by lymphocytes, macrophages and a few
neutrophils and eosinophils. Biopsies from control sites and control animals were normal and showed insignificant and transient changes which disappeared within a short time after the intradermal injection of the soluble antigen.

**Discussion**

Infected cattle had higher total serum protein, significantly higher globulin and lower albumin concentration than non-infected cattle. The increase in serum proteins was solely attributed to the severe and generalized demodectic mange infection. This was because the values obtained for serum proteins of cattle with concurrent infection (blood or endoparasites) were excluded from this study. Coles (1967) reported that the most significant change occurring in disease is a decrease in the albumin fraction due to an increase in the concentration of globulins. Infections accompanied by invasion of the body by foreign material, whether it be of bacterial, viral, protozoal or parasitic origin, usually result in an increase in the concentration of γ-globulins (Coles, 1967). Circulating antibodies were demonstrated in the sera of infected cattle by the enzyme-linked immunoelectrotransfer blot (Western blot). Positive reaction bands were obtained when the resolved soluble antigen was probed with sera from infected cattle and no reaction bands were obtained when the resolved antigen was probed with sera from non-infected animals. In this respect, the Western blot proved to be quite reliable for studying the total antigen-antibody systems. This finding agree with Tsang et al. (1986) who reported that the technique remained a powerful qualitative tool for studying the total antigen-antibody systems. Bornstein et al. (1997) found that the technique was quite useful for confirming the specificity of antibodies to Sarcoptes scabiei in camels clinically suspected of sarcoptic mange, demonstrating similar antigen (polypeptide band pattern) as had been demonstrated for the infection in red foxes, dogs and pigs infected with the mite. The reaction bands were seen at the molecular weight of 56 KDD of the resolved antigen. This proved that Demodex mites and associated bacteria were highly immunogenic and evolved antibodies and cell-mediated immunity. This finding agree with Jackson (1978) who enlisted “foreignness” among the properties of effective immunogens. He reported that effective immunogens had molecular weights greater than 10 KDD, and had the capacity to evoke specific immune responses by elaboration of antibodies and development of cell-mediated immunity or both. Hypersensitivity testing of the infected cattle by the intradermal injection of the soluble antigen produced immediate, delayed-cell mediated and Arthus immune-complex reactions. These reactions were identical in all respects to what was previously described (Parish, 1968; Coomb’s et al., 1975; Henson, 1978; Thomson, 1978; Dick and Kirkwood, 1983). The reactions produced were also in agreement with Cypess (1978) who reported that immediate and delayed hypersensitivity were commonly associated with metazoan infections in animals and man, and attributed this to the nature of the antigens and the chronicity of infection, permitting persistence of antigens.

Thompson (1978) reported that the various types of immune mechanisms of injury do not always occur independently or separately, and in some instances, all four types may occur in the same lesion by response to different antigens or by multiple response to the same antigen. The demonstration of high γ-globulins, positive reaction bands in the lanes probed with sera from infected cattle by the Western-blot technique and hypersensitivity reactions in cattle with generalized demodicosis proved that the immune mechanisms of those animals functioned normally and were even activated and boosted by the repeated exposure to the infective agents.

The histopathological changes reported in natural field cases evolved cell-mediated immunity and were characterized by a massive granulomatous reaction with influx of macrophages and lymphocytes proving that Demodex mites and associated bacteria were both persistent and immunogenic, producing the severe, progressive and generalized lesions (Abu-Samra and Shuaib, 2014 a, b, c). These findings agree with Thomson (1978) and Dick et al., (1983) who reported that when the inflammatory agent was both persistent
ABU-SAMRA AND SHUAIB

and antigenic, ‘high-turnover’ granulomas evolved with influx of macrophages and lymphocytes. Moreover, the mites and associated bacteria had triggered multiple antigen determinants which stimulated both antibody production and proliferation of specific T – cells. The response of the sensitized animals to repeated exposure by the mite and associated bacteria was by the release of mediators of the delayed hypersensitivity. Such a reaction mimics Type IV allergic reaction, the classic example of which is tuberculosis as was described by Coombs et al., (1975), and Thomson (1978) who reported that delayed hypersensitivity (Type IV) is best known as the basis of the tuberculin reaction. It was also reported that cutaneous hypersensitivity reactions of the delayed type mediated by cellular responses of the classical tuberculin type might be encountered in microbial hypersensitivity and contact dermatitis (Walton, 1968; Arbuthnott et al., 1983), and that many granulomas were generated and maintained by the delayed type of hypersensitivity when the antigen persisted (Thomson, 1978; Henson, 1978; Dick and Kirkwood, 1983; Dick et al., 1983). The mites and associated bacteria were engulfed and digested by multinucleated giant cells, resulting in complete healing of the lesions (Abu-Samra and Shuaib, 2014 a, b, c). The cellular elements of the granulomas had gradually disappeared and the connective tissue increased till the infected areas or colonies of demodectic mange became covered by concentric bundles of connective tissue (Abu-Samra and Shuaib, 2014 a, b, c). These findings agree with Baima and Sticherling (2002) who reported blockage of follicles and sebaceous ducts by the mites and by reactive hyperkeratinization and epithelial hyperplasia, a vector role for bacteria, a foreign body granulomatous reaction to the mite chitinous skeleton, stimulation of the host humoral and cell-mediated immune reactions by the mite and their waste products and delayed hypersensitivity reactions (Type IV immune response) to an unknown antigen of follicular or mite origin could occur as pathogenic mechanisms of Demodex folliculorum and D. brevis infection in man. The demonstration of hypersensitivity reactions in infected cattle implied tissue damage by the mechanisms of immunologic injury. This was probably due to the persistence of the infective agents in chronic lesions, continuous release of the mite and bacterial antigens, destruction and alteration of the host tissues by the mites and associated bacteria and difficulty of some cattle harboring chronic lesions to eliminate infection. These findings agree with Cypess (1978) who reported that parasitic infections represented an excellent example for conditions of tissue damage by any of the four mechanisms of tissue injury, and attributed this to the release and persistence of large amounts of antigens, alteration and destruction of the host tissue and chronicity of infection. In spite of the rapid and exaggerated humoral and cell-mediated immune responses that were demonstrated in vivo and in vitro studies, the mites and associated bacteria remained viable in chronic lesions for 2-3 years (Abu-Samra and Shuaib, 2014 a, c). Natural field conditions; failure of some cattle to develop acquired immunity against repeated exposure to the mites and associated bacteria was probably due to the development of tolerance resulting from the release of large amounts of soluble and particulate antigens. This finding was in agreement with Cypess (1978) who also reported that many parasites remained viable within the host for extended periods of time in spite of the often rapid and exaggerated humoral and cell-mediated immune responses. He attributed this to the development of tolerance resulting from the release of large amounts of soluble and particulate antigens.

Conclusion

Rapid and exaggerated humoral and cell-mediated immune responses were demonstrated in cattle, and previous investigations (Abu-Samra and shuaib 2014 a,b,c) demonstrated severe histopathological changes characterized by massive ‘high-turnover’ granulomatous reaction with influx of macrophages and lymphocytes in skin and meibomian gland lesions of these cattle, proving that Demodex mites and associated bacteria were both persistent and immunogenic. The mites and associated bacteria were destroyed, engulfed and digested by giant cells, resulting in healing of the
lesions as judged by the progressive proliferation of connective tissue and degeneration of the granulomas. However, in some cattle the mites and associated bacteria remained viable in chronic lesions for 2-3 years in spite of the rapid and exaggerated humoral and cell-mediated immune responses demonstrated in vivo and in vitro. Failure of these cattle to develop acquired immunity against repeated exposure to the mites and associated bacteria was probably due to the development of tolerance resulting from the release of large amounts of soluble and particulate antigens. Tolerance is brought about and maintained by the active production of blocking factors, thought to be antigen-antibody complexes which might interfere with cell mediated response against infection. In this respect further researches are warranted to investigate these complexes in animals harboring chronic lesions. If this problem is resolved then bovine demodicosis can be prevented since transmission of Demodex mite to calves was most probably acquired from dams harboring chronic and persistent lesions of the disease by direct contact during nursing in the neonatal period.

Acknowledgements

We are indebted to the Agricultural Research Council, National Council for Research, Khartoum, Sudan, for the generous support of this work. Dr. Beesley WN, formerly Head/Department of Veterinary Parasitology, Liverpool School of Tropical Medicine, UK, is gratefully acknowledged for his interest in this work and for the confirmation of the identification of Demodex bovis. The confirmation of the identification of D. bovis and D. ghanensis mites by the late Professor W. B. Nutting, Department of Zoology, University of Massachusetts, USA, is highly appreciated and acknowledged with thanks.

References


