

Contact Hypersensitivity to Stainless Steel Cages (Chromium Metal) in Hairless Descendants of Mexican Hairless Dogs

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Received 26 October 2006; accepted 3 December 2006

ABSTRACT: Canine allergic contact hypersensitivity is an uncommon skin disease as compared with human beings because hair coat is a good natural barrier to environmental contactants. In our colony of hairless dogs housed in stainless steel cages, we have encountered spontaneously occurring contact hypersensitivity. The author has attempted to study the toxicological effects of environmental sensitizing substances on the canine skin. The purpose of this study is to elucidate dermatological characteristics in canine species with contact hypersensitivity. This skin lesion was investigated by patch tests, macroscopic observations, and histopathological examinations. Patch tests exhibited positive reactions to potassium dichromate. Macroscopically, early lesions were macules and/or papules and they gradually progressed to severe inflammatory dermatitis over the dorsum. In the chronic phase, lichenification, hyperkeratosis, hyperpigmentation, dryness, scaliness, and fissuring were observed in the skin. Avoidance of contact with the stainless steel cages resulted in clinical improvement. Histopathologically, the epidermis apparently showed hyperkeratosis, thickening, hyperplasia, and rete ridge formation. Lichenified lesions had clumps of melanin granules in the *stratum basale* and *spinosum*. In the dermis, there was marked edema and dense mononuclear cell infiltration. Vasodilation, hemorrhage, and hyperplasia of sebaceous glands were also found. Both dermal mast cells and epidermal Langerhans cells significantly increased in the skin lesions, as compared with nonlesional sites. The present results revealed that constant contact with stainless steel cages (chromium metal) caused contact hypersensitivity in hairless dogs with very sparse hairs. © 2007 Wiley Periodicals, Inc. *Environ Toxicol* 22: 176–184, 2007.

Keywords: chromium; contact hypersensitivity; hairless dogs; histopathology; Langerhans cells; mast cells; skin; stainless steel cages

INTRODUCTION

Allergic contact hypersensitivity is an uncommon skin disease seen in dogs by a type IV or a delayed-type hypersensitivity reactions. There is a paucity of available data dealing with experimentally induced contact hypersensitivity in dogs (Nobreus et al., 1974; Krawiec and Gaafer, 1975; Shultz and Adams, 1978; Thomsen and Kristensen, 1986;

Thomsen and Thomsen, 1989; White, 1991; Kimura and Doi, 1994). It is difficult to induce contact hypersensitivity in dogs, and the obtained results are inconsistent in comparison with tests on humans and guinea pigs. Very few reports of spontaneously occurring contact hypersensitivity in dogs have been documented by exact investigations including patch tests and histopathological examination (Willemse and Vroom, 1988).

Recently, we have established a colony of hairless descendants of Mexican hairless dogs. Hairless dogs have been utilized for investigating delayed contact hypersensitivity with chemical substances such as dinitrochlorobenzene,

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Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/tox.20243

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agricultural chemicals, and hair dyes (Kimura and Doi, 1994, 1999; Kimura et al., 1998). In this colony, we have encountered spontaneously occurring contact hypersensitivity, which was resulting from constant contact with chromium metal.

The purpose of this study is to investigate dermatological characteristics in hairless dogs with spontaneously occurring contact hypersensitivity. In this article, we presented diagnostic results of patch tests and macroscopic and microscopic changes in the skin lesions of contact hypersensitivity. In addition, we demonstrated numerical data of dermal mast cells and epidermal Langerhans cells (LCs) in the skin lesions.

MATERIALS AND METHODS

Dogs

Six male hairless dogs affected with severe chronic dermatosis were used in this study. Six dogs included: two 7-year-old N₄ hairless hybrids (male N₃ hairless hybrids × female Beagles), two 3-year-old N₆ hairless hybrids (male N₅ hairless hybrids × female Beagles), and two 1.5-year-old N₇ hairless hybrids (male N₆ hairless hybrids × female Beagles).

The dogs were individually housed in stainless steel cages (90 × 90 × 90 cm³) in an animal room controlled at 25 ± 1 °C temperature and 50 ± 10% relative humidity with 10–15 exchanges of 100% fresh air/hour and a 12-h light (6 a.m. to 6 p.m.), 12-h dark (6 p.m. to 6 a.m.) cycle. They were fed a commercial dry dog food (TC-2, Aixia Corporation, Tokyo, Japan) and water *ad libitum*.

All procedures involving animals were approved by the Animal Use Committee of National Institutes of Natural Sciences (NINS) and followed the guidelines of animal care and experiments of the NINS.

Macroscopic Examination

Firstly, we wondered if some specific allergens for canine atopic dermatitis might play a causative role in the development of the skin lesions. Intradermal skin tests and serological allergy tests showed completely negative reactions. And then, all patch tests were performed by a single investigator.

Patch testing was performed on the hairless dogs with the severe skin lesions with the International Contact Dermatitis Research Group (ICDRG) standard series and metal salts: 2% aluminum chloride, 2% chrome chloride, 2% chrome sulfate, 2% chromic acids, 2% ferri- and ferro-chloride, 0.5% potassium bichromate, 1% copper sulfate, 5% nickel sulfate, 2% zinc chloride, 2% zinc oxide, and 2% lead acetate.

All substances were applied to the nonlesional skin (dorsolumbar skin) using acrylate adhesive strips with small plates attached for test allergens (Torii Co., Tokyo, Japan). Three sets of patch test units were placed on the skin of each hairless dog. Occlusion time was 48 h and skin reactions were read 15 min after removal of the strips and 24, 48 and 72 h later.

Histopathological Examination

Two tissue specimens were obtained from both the affected and normal sites of the dorsolateral skin of the hairless dog using a 6-mm biopsy punch (Nagatoishi Co., Tokyo) under general anesthesia with medetomidine (Domitol, Meiji Seiyaku Co., Tokyo) 1 day before this study.

Half the skin specimens were rinsed in 0.1 M phosphate buffer (pH 7.4) and incubated in 0.02 M ethylenediaminetetraacetic acid (EDTA) for 2 h at 37 °C. The epidermal sheets separated from the dermis were fixed in cold 4% neutral buffered formalin for 30 min, washed twice with 0.1 M phosphate buffer (pH 7.4), and incubated for 1 h in Tris/malease buffer at pH 7.3 including adenosine triphosphate (ATP), MgSO₄ and Pb(N₂O₃). Finally, the samples were incubated in ammonium polysulphide, rinsed and mounted in glycerol.

The number of LCs in each of the ten 1 mm² fields was counted on each specimen under light microscopy, and mean number ± standard deviation (SD) was calculated.

The remaining skin specimens were fixed in 10% neutral buffered formalin, and 4-μm paraffin sections were stained with hematoxylin and eosine (HE) and toluidine blue (TB), and by van Gieson's (vG) and Weigert's (WG) staining, and by Fontana-Masson's (FM) method.

The number of mast cells in each of 10 fields was counted on the specimen stained with TB under light microscopy. Data were presented as mean number ± SD.

Statistics

Statistical significance of the differences in the number of LCs and mast cells between the lesional sites and nonlesional sites was calculated using a Student's *t* test. Differences between both sites were considered significant at *P* < 0.05.

RESULTS

Macroscopic Findings

The first signs were observed when the animals were 8–12 months old. The primary changes were erythema, edema, often macules and/or papules. Initially pruritic dermatitis was present over the thoracic and abdominal



Fig. 1. Gross appearance of the early skin lesion. Erythema, edema, often macules, and/or papules are seen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

skin (Fig. 1). The lesions gradually progressed to the dorsum, the suprascapular areas, the lateral trunk, the medial parts of extremities, and the ventral neck areas. Some portions developed secondary changes such as excoriation, exudation, and incrustation caused by self-mutilation.

In the chronic phase, the affected areas revealed the development of lichenification, hyperkeratosis, hyperpigmentation, dryness, scaliness, and occasional fissuring of the skin. The eruptions were symmetrically distributed on the anterior chest, abdomen, shoulders, and dorsum. Physical examination showed erythematous papules with a coalescing tendency, and brownish pigmentation intermingled with these papules in a reticulate pattern (Fig. 2). Moderate pruritus was noted early in the course of the disease. Chronic pruritus mildly persisted at the involved sites. Avoidance of contact with the stainless steel cages resulted in clinical improvement. Indoor pens constituted of the concrete floor, concrete blocks and wooden resting areas had favorable effects on the skin lesions.

Differential diagnosis included demodicosis, sarcoptic mange, dermatophyte infections, food allergy and atopic dermatitis. Skin scrapings, fungal cultures, dietary tests,

intradermal allergy tests, and serologic allergy tests were negative.

In patch testing, positive reactions were obtained with potassium dichromate 24, 48, and 72 h after application of this agent. The intensity of erythema and edema was maximal within 48 h following application of the patch units. There was a sharp demarcation line between bright red reactive area and adjacent normal skin. The other allergens of systematic patch testing did not elicit any positive reactions. Brownish pigmentation was left at the positive patch reaction with potassium dichromate 3 weeks after the patch tests.

Histopathological Findings

Histopathological findings in the skin of six dogs are summarized in Table I. Histopathological changes in the affected skin of hairless dogs were observed in both the epidermis and dermis. The epidermis showed hyperkeratosis, marked thickening, and epidermal hyperplasia. The biopsy specimens taken from erythematous papules revealed



Fig. 2. Gross appearance of the old skin lesion. Lichenification, hyperkeratosis, hyperpigmentation, dryness, scaliness, and fissuring are seen. Brownish pigmentation is also seen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Histopathological changes of the sites of contact hypersensitivity in hairless dogs

Changes	Contact Hypersensitivity	Nonlesional Sites
Epidermis		
Hyperkeratosis	+	—
Thickening	+++	—
Hyperplasia	+++	—
Epidermal ingrowths	+++	—
Intercellular edema	++	—
Dermis		
Inflammatory cell infiltration	+	—
Mast cells	+++	±
Vasodilatation	++	—
Congestion	+	—
Hemorrhage	+	—
Sebaceous glands		
Hyperplasia	++	—

The degree of histopathological changes: — = negative; ± = slight; + = mild; ++ = moderate; +++ = marked.

severe intercellular edema of the *stratum basale* and *spinosum*. Spongiosis and disintegration could be clearly seen in the histological sections (Fig. 3).

Although notable rete ridges formation (acanthosis) was found, the dermoepidermal junction was not disrupted (Fig. 4). Lichenified lesions had deposition of melanin granules in the *stratum basale* and *spinosum* (Fig. 5).

In the dermis of the involved skin, there were marked edema and dense mononuclear cell infiltration located primarily in the upper dermal layers (Fig. 6). Intense infiltration of lymphocytes was also observed around the base of the sparse hair follicles and sebaceous glands. The vessels were dilated and filled with abundant blood. Vasodilation and hemorrhage were more prominent in the specimens of the erythematous papules. In some portions, sebaceous glands exhibited prominent hyperplasia (Fig. 7)

Histopathological examination of skin biopsy specimens from unaffected sites indicated that they were normal except for some thickening of the epidermis and slight inflammatory cell infiltration in the dermis (Fig. 8).

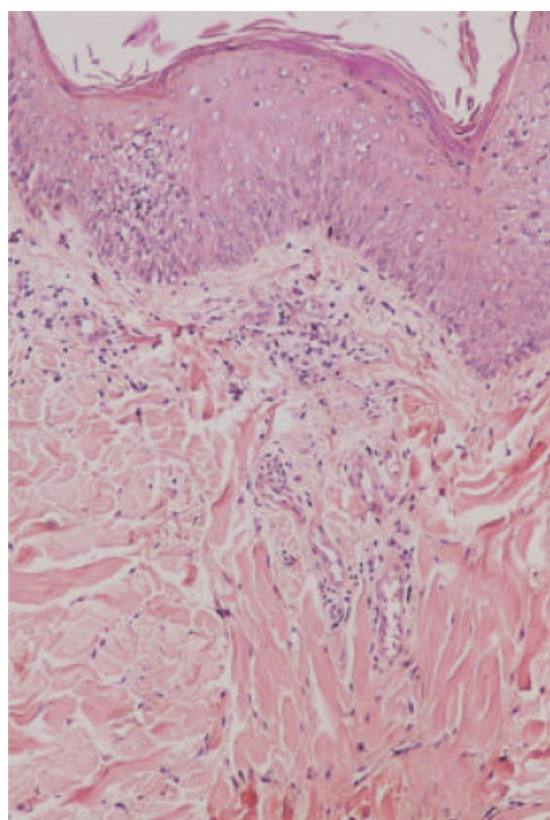


Fig. 3. Microscopic photograph of the skin lesion. Hyperkeratosis, marked thickening, and epidermal hyperplasia are seen. Severe intercellular edema of the *stratum basale* and *spinosum* is also seen. $\times 100$, H & E. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

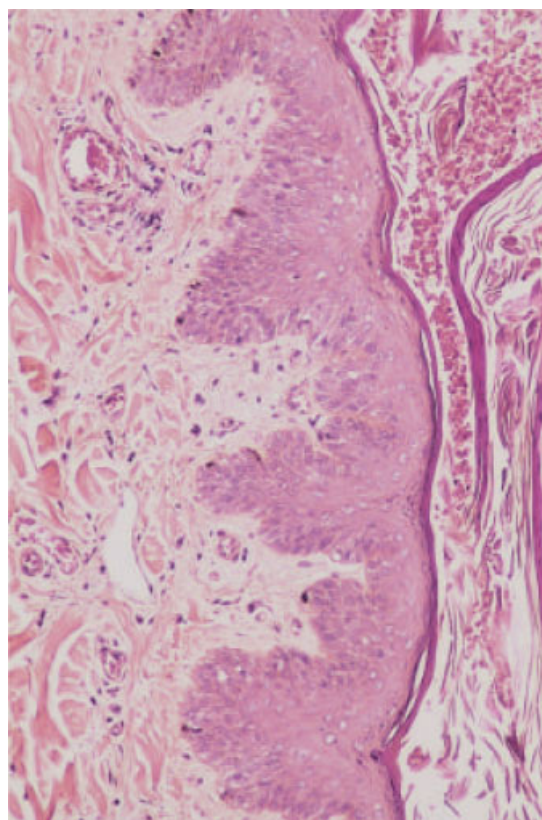


Fig. 4. Microscopic photograph of the skin lesion. Notable rete ridges are seen. $\times 100$, H & E. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

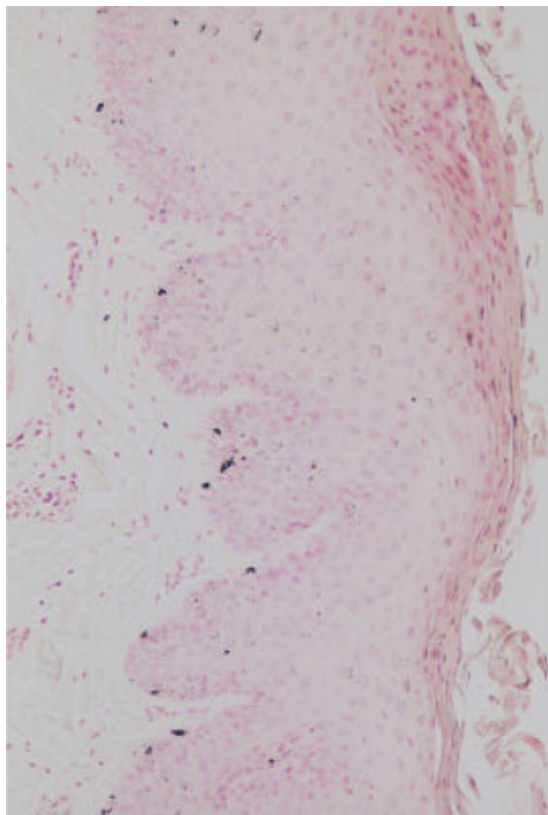


Fig. 5. Microscopic photograph of the skin lesion. Lichenified lesions have deposition of melanin granules in the *stratum basale* and *spinosum*. $\times 100$, FM method. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The Number of Mast Cells

The number of mast cells is illustrated in Figure 9. There were a few distributions of dermal mast cells in the nonlesional sites. The mean number of mast cells per square millimeter of the dermis increased from 14 ± 11 in nonlesional sites to 139 ± 24 in lesional sites. Dermal mast cells in the lesional sites significantly increased to levels about 10 times higher than those in nonlesional sites ($P < 0.001$). In the lesional sites, a number of mast cells were found throughout the upper dermis and around the microvessels (Fig. 10).

The Number of LCs

The number of LCs is illustrated in Figure 11. The mean number of LCs per square millimeter of a split epidermal sheet increased from 113 ± 45 in nonlesional sites to 355 ± 77 in lesional sites. Epidermal LC counts were significantly higher in lesional than in nonlesional sites ($P < 0.001$). In the lesional sites, LCs showed dendritic processes stretching from the cell bodies (Fig. 12).

DISCUSSION

Contact hypersensitivity to chromium in the skin of hairless dogs was clinically and histopathologically investigated.

Although the macroscopic appearance of the skin lesions in hairless dogs was not specifically characteristic, early lesions consisted of various combinations of erythema, macules, papules, and cutaneous edema. Chronic lesions were extensive plaques that were lichenified and hyperpigmented. There were no severe vesicles seen in human beings (Paustebach et al., 1992).

Experimentally, contact hypersensitivity in dogs can be induced 3–5 weeks after sensitization (Nobreus et al., 1974; Krawiec and Gaafer, 1975; Shultz and Adams, 1978; Kimura and Doi, 1994). However, the present study showed that spontaneously occurring contact dermatitis in dogs required long-term sensitization period, more than 8–12 months.

Canine contact dermatitis is usually limited to the thin-haired regions of the body or between the foot pads. The canine breed used in this study has systemically hairless skin with very sparse hairs. Stainless steel cages including

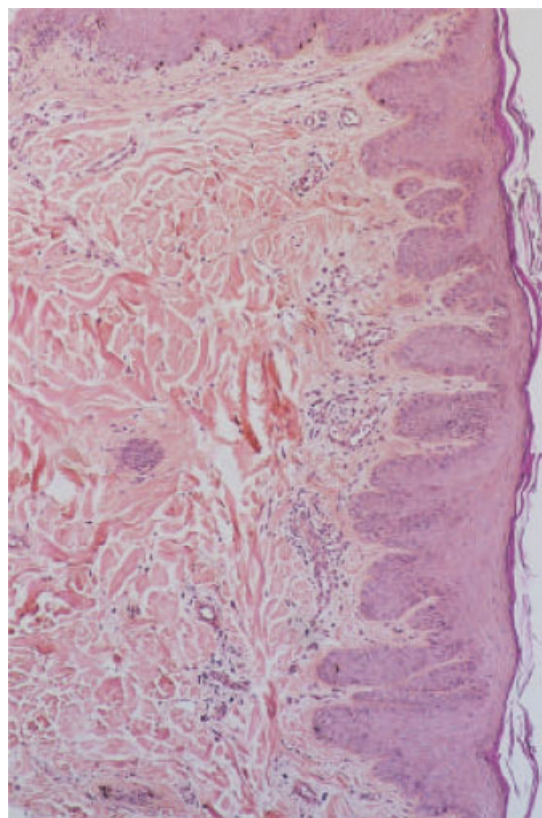


Fig. 6. Microscopic photograph of the skin lesion. Marked edema and dense mononuclear cell infiltration are seen in the upper dermal layers. $\times 40$, H & E. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

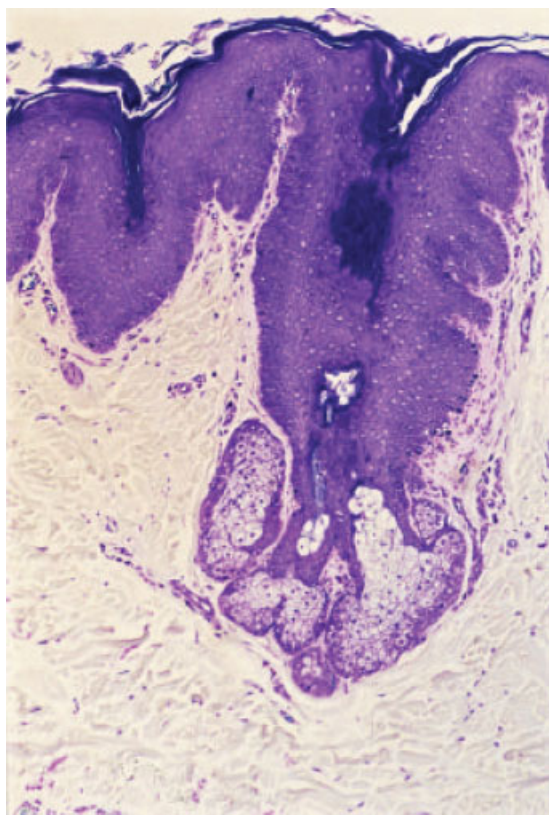


Fig. 7. Microscopic photograph of the skin lesion. Hyperplastic sebaceous glands are seen. $\times 40$, TB stain. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

chromium became a serious sensitizing substance because they were in constant contact with the naked skin of the body. It is suggested that hair coat is a good natural barrier to environmental contactants.

Chromium is one of the most common causes of occupational dermatitis in human males (Keskinen et al., 1980; Hansen, 1983; Bruynzeel et al., 1988; Lee and Goh, 1988; Gad, 1989; Burden and Eedy, 1991; Williams, 1996; Nygren and Wahlberg, 1998; Papa et al., 2000; Kim et al., 2001; Hansen et al., 2002; Bock et al., 2003). In male hairless dogs, we found a similar predisposition to this skin disease.

Although the patch test is not used as frequently in veterinary medicine as in human medicine, definitive diagnosis of contact hypersensitivity should be based on this method. As reported in our previous paper (Kimura and Doi, 1994), patch testing accurately documented positive reactions to dinitrochlorobenzene-induced delayed-type hypersensitivity in the skin of dogs. In the present study, patch testing revealed the sole antigen that caused this dermatosis. It is anticipated that patch tests allow the clinicians to confirm the diagnosis of contact hypersensitivity in dogs.

In experimentally induced contact hypersensitivity in dogs, skin biopsy exhibited varying degree of superficial perivascular dermatitis, with mononuclear cells predominating (Kimura and Doi, 1994, 1999; Kimura et al., 1998). These histopathological findings accorded with those obtained from the sites exposed to chromium in this study. Histopathological examination of patch test reactions may be helpful in verifying the allergic nature of skin lesions in dogs.

Histopathological changes in the lesions of hairless dogs resembled very closely chromium-induced contact dermatitis in human beings (Moller et al., 1986; Fowler et al., 1999). Chromium-sensitized patients exhibited spongiosis and perieccrine, and perivascular inflammation with mononuclear cells and neutrophils (Fowler et al., 1999). Marked thickening, hyperplastic changes, intercellular edema, and acanthosis in the epidermis were common findings. Hyperemia, edema and vascular and perivascular infiltration by inflammatory cells, predominately composed of mononuclear cells intermingled with neutrophils and eosinophils were also observed in the dermis. In chronic lesions, clusters of melanin pigmentation were found in the epidermis. This finding, resulting from severe inflammatory reactions, was noted in experimentally induced contact hypersensitivity in hairless dogs (Kimura and Doi, 1994).

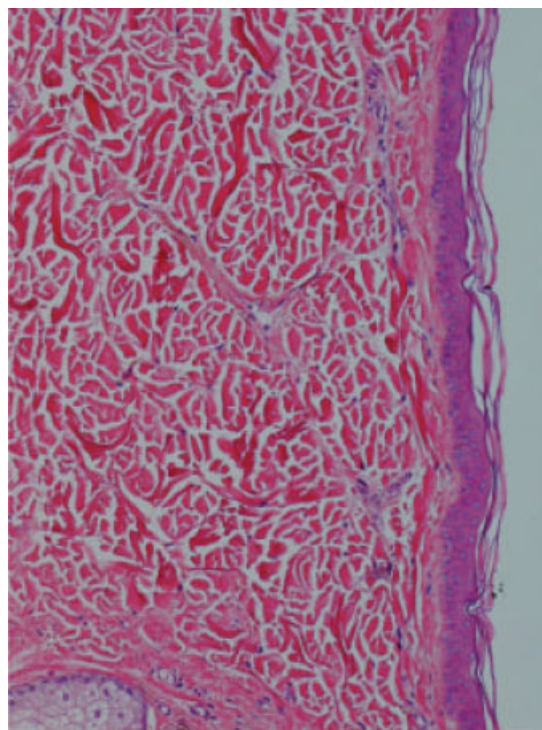


Fig. 8. Microscopic photograph of the nonlesional site. No changes are seen. $\times 100$, H & E. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

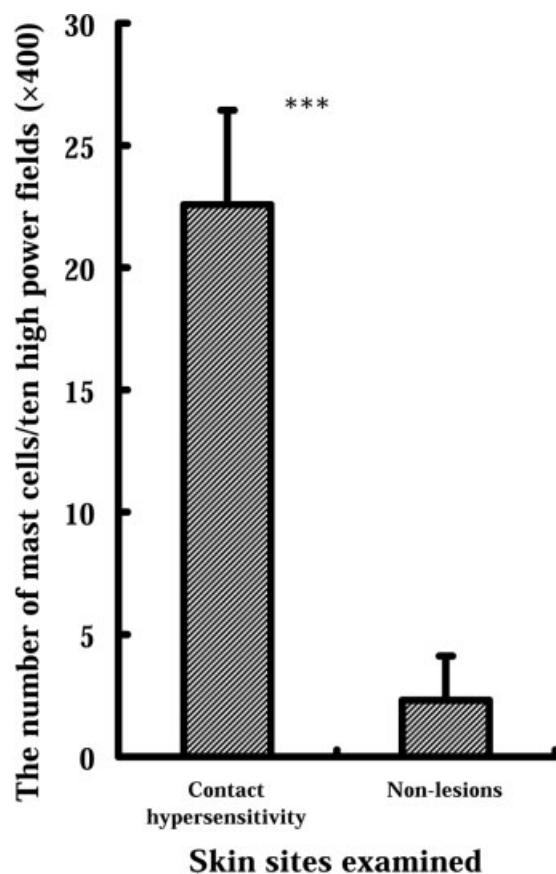


Fig. 9. The number of mast cells. *** $P < 0.001$ (significantly different from nonlesional sites).

The development of chromium contact hypersensitivity has been described as occurring in four phases as follows: phase I, the refractory phase; phase II, the induction phase; phase III, the elicitation phase; and phase IV, the persistence phase (Bagdon and Hazen, 1991). Hexavalent chromium penetrates epidermal cells and intracellular organelles, and it is converted to trivalent chromium intracellularly. The trivalent chromium generated within epidermal cells reacts with antigenic proteins to evoke the release of the cascade of inflammatory mediators, which results in expression of contact hypersensitivity (Bagdon and Hazen, 1991). Histopathological findings appeared to reflect a series of inflammation reactions. Persistent severe dermatitis suggested a manifestation of contact hypersensitivity to chromium ions released during the corrosion of stainless steel.

The number of dermal mast cells apparently increased in the lesional sites as compared with nonlesional sites. These results were consistent with those reported in experimentally induced contact hypersensitivity in mice (Cho and Hough, 1986; Kerdell et al., 1987; Ikeda et al., 2000; Yokozeki et al., 2002; Jung et al., 2003, 2004). The onset of dermal edema may be associated with mast cell activation.

Mast cells seemed to be a disease-intensifying element in contact dermatitis.

There are numerous studies on the number and turnover of LCs in contact sensitivity reactions. In this study, an increased number of activated LCs was found in the chronic lesions of hairless dogs. Metal modification by the antigen-presenting LCs showed an important step in contact hypersensitivity. These findings agreed with those reported in previous studies (Sonoda et al., 1985a,b; Watanabe et al., 2002; Cumberbatch et al., 2003). It is known that a low-molecular-weight hapten such as a chromium ion is conjugated with epidermal carrier proteins. The hapten-protein complex is then pinocytosed by epidermal LCs and antigen-presenting LCs stimulate helper T cells (Bagdon and Hazen, 1991). It is likely that this cutaneous procedure (induction and elicitation phase) becomes repetitious following constant contact with chromium. Although contact hypersensitivity proceeds in two phases (induction and elicitation), the line of demarcation between both phases seemed to be clearly unrecognizable.

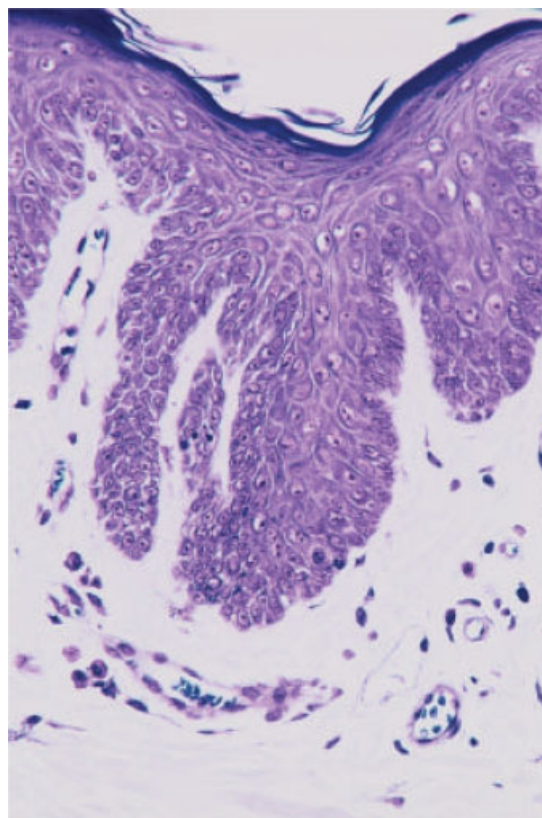


Fig. 10. Microscopic photograph of the skin lesion. A number of mast cells throughout the upper dermis and around the pilosebaceous glands are seen. ×200, TB stain. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Contact hypersensitivity in dogs has a different pathogenesis from that in other species, and clinical signs vary depending on the allergens involved. It has been noted that atopy is present in about 20% of dogs with contact hypersensitivity. The predominance of skin lesions in canine atopic dermatitis occurring in contact areas supports the importance of percutaneous penetration of allergens (Scotto et al., 1995). Additionally, Shanon (1965) reported human pseudoatopic dermatitis resulting from sensitivity to chromium. Matsumoto et al. (2004) demonstrated that repeated elicitation of contact hypersensitivity brought about atopic dermatitis-like lesions. In basic immunologic pathogenesis, there may be some similarities between chromium-induced contact hypersensitivity and atopic dermatitis.

In conclusion, the present results showed that chromium included in stainless steels caused naturally occurring contact hypersensitivity in hairless dogs. Histopathological examinations revealed superficial perivascular dermatitis wherein inflammatory mononuclear cells predominated. In addition, it is probable that increased numbers of mast cells and LCs are associated with the development of the skin lesions.

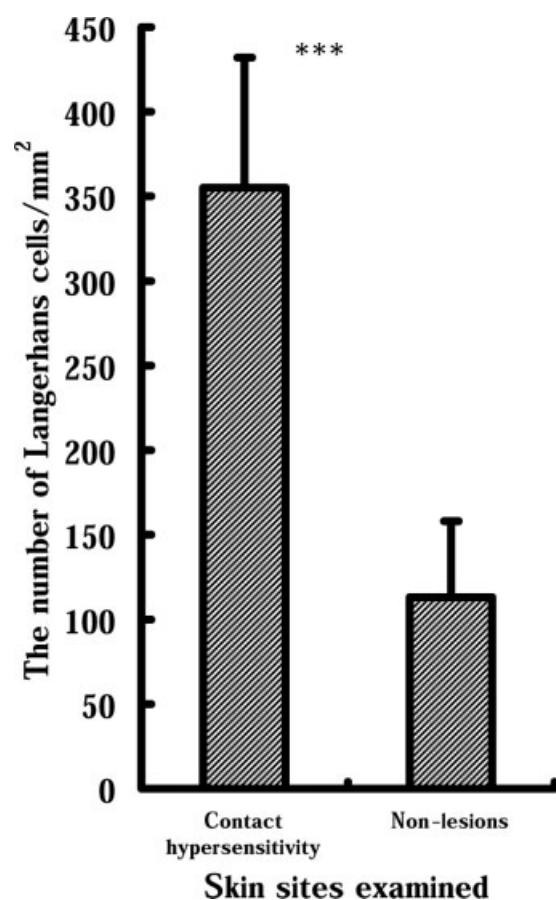


Fig. 11. The number of LCs. *** $P < 0.001$ (significantly different from nonlesional sites).

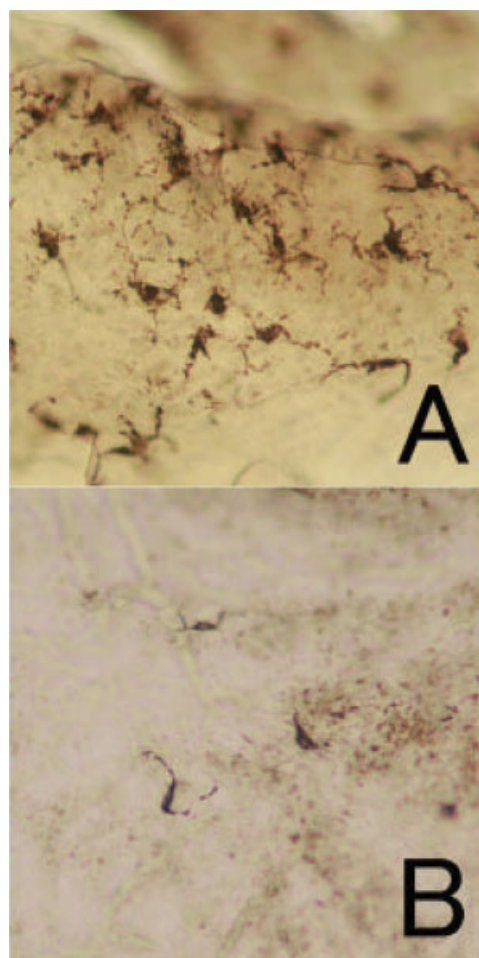


Fig. 12. LCs in the epidermal split sheet. A: Lesional sites. Dense LCs showed dendritic processes stretching from the cell bodies. B: Nonlesional sites. $\times 200$, ATP-MgSO₄-Pb(N₂O₃) stain. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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