A COMPARATIVE STUDY OF ALLERGIC AND PRIMARY IRRITANT CONTACT DERMATITIS WITH DINITROCHLOROBENZENE (DNCB) IN DOGS

D. R. Krawiec, B.S., M.S., and S. M. Gaafar, D.V.M., Ph.D.

Department of Veterinary Microbiology, Pathology and Public Health, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana

Attempts were made to induce allergic contact dermatitis in dogs, a species generally considered poorly responsive to experimental allergic contact dermatitis. Young Beagles were sensitized to 2,4 dinitrochlorobenzene (DNCB) by multiple intradermal injections. Two weeks after sensitization, these dogs were challenged topically with 0.1% DNCB by a standard closed-patch technique. Sensitization evidenced by various degrees of reaction following challenge was established in all of 14 pups used, while 7 nonsensitized control pups did not react to challenge. Primary irritant contact dermatitis was induced in the skin of nonsensitized Beagle pups by 1%, 5%, and 10% solutions of DNCB.

In allergic contact dermatitis the sites of challenge were grossly indurated, erythematous, and edematous. Histologically at these sites there was an infiltration of mononuclear cells which reached maximum intensity at 3 to 4 days. Accumulations of lymphoid cells were marked around sweat glands and hair follicles. Penetration of leukocytes into these cutaneous adnexa was associated with degenerative processes in their cellular structures. Mononuclear cell infiltration into the epidermis was mild. Spongiosis was observed in the epidermis, but vesicle formation was rare.

In primary irritant contact dermatitis gross lesions were characterized by severe erythema, edema, and gangrene of the skin. Microscopically, the main lesions were necrosis of the epidermal cells, separation of the epidermis from the dermis, dermal edema, and massive infiltration of the dermis with polymorphonuclear cells.

The extensive use of dogs in experimental transplantation and in other investigations on cell-mediated immunity suggests the importance of understanding delayed hypersensitivity in these animals. Allergic contact dermatitis (ACD) as a clinical syndrome in dogs is referred to in the literature [1-6]. These reports, however, are based mostly on clinical observations alone and it is often difficult to differentiate between ACD and primary irritant dermatitis. The most dependable method for differentiation is by the use of the patch test, a technique unfortunately seldom performed in veterinary clinics [3,5].

Two experimental studies were reported where investigators attempted to induce ACD in the dog. Using dinitrochlorobenzene (DNCB) as the contact sensitizer, Rostenberg and Haeberlin [7] concluded that dogs had questionable evidence of allergic contact sensitization. Likewise, Hoey [8], following extensive investigations with DNCB and poison ivy, indicated that dogs have a lower degree of sensitivity to these compounds than do guinea pigs or man. It is currently believed, therefore, that the dog is a poor and inconsistent responder to contact allergens [9].

The purposes of the present investigations were: (1) to determine whether dogs can be consistently sensitized with contact allergens, (2) to characterize the gross and histologic appearance of lesions of ACD in this animal, and (3) to compare the gross and histologic appearance of the lesions of ACD with those of primary irritant dermatitis.

MATERIALS AND METHODS

Purebred Beagle pups used were 8 to 12 weeks of age when they were included in the present investigations. A total of 14 sensitized and 7 nonsensitized control pups were used for the experiments on ACD. Six pups were used to produce lesions of primary irritant dermatitis.

The chemical used was 2,4 dinitrochlorobenzene (DNCB). When used for intradermal inoculation, DNCB obtained in crystalline form (Matheson Coleman and Bell Company, Norwood, Ohio) was dissolved in propylene glycol, and when used for patch testing it was dissolved in 95% ethanol.

Two methods were used to sensitize the pups. Twelve pups were sensitized by intradermal inoculation of 0.1 ml of 0.1% DNCB in the skin of the scapular area every other day for a total of 10 injections. The remaining 2 pups were sensitized by a regimen developed by Magnusson and
Kligman [10] which they termed the "guinea pig maximization test."

The method used for challenge was the closed-patch technique [10]. All pups were challenged on the skin of the ventral area 2 weeks after the last sensitizing application of DNCB. Six to eight patches were placed on each pup and left for 24 hr. In addition, a patch containing only the vehicle was applied on all pups. Similar patches were secured on 7 control nonsensitized animals to observe the toxic effect of the chemical and vehicle as well as the irritating effect of the patch. Skin biopsy specimens from the sites where the patches were applied were obtained one location at a time each day at 1 to 7 days post application of the patch. The skin biopsy specimens were processed using conventional embedding and sectioning techniques, and the histologic sections were stained with hematoxylin and eosin, periodic acid–Schiff, and Giemsa stains.

To study the primary irritant toxic effects of DNCB, solutions of 1%, 5%, and 10% in ethanol were applied by the closed-patch technique on the ventral skin of nonsensitized dogs. Two animals were used for each concentration. The lesions were evaluated grossly, and skin biopsy specimens obtained at 1 to 5 days post application were processed and histologic sections prepared as described above.

RESULTS

Gross Observations

Of the 14 pups sensitized with DNCB, 4 developed lesions consisting of slight erythema and edema, 8 developed lesions of moderate erythema and edema, and 2 developed severe erythema and edema at the site of application of challenge patches. There was a sharp line of demarcation between the bright red, reactive, round area where the patch was applied and the adjacent normal skin. The most severe lesions were raised 2 to 3 mm above the level of the adjoining skin and were indurated. The intensity of erythema and edema was maximal within 2 days following application of the patches and the lesions were dissipated by 7 days post challenge. None of these lesions appeared to be pruritic. No lesions were observed upon examination of the control nonsensitized animals.

Microscopic Observations

Pathologic changes due to ACD in the skin of sensitized dogs were observed in both the dermis and epidermis. At the site of reaction there was an intense infiltration of mononuclear cells and dermal edema. The infiltrative cells formed a dense band in the upper dermis (Fig. 1). The vessels were dilated and filled with blood. They also contained a number of polymorphonuclear and mononuclear cells. There was an intense infiltration of mononuclear cells around the base of the hair follicles and the sebaceous glands. Penetration of the infiltrate into these adnexa was associated with their degeneration (Fig. 2). The surface epidermis was acanthotic, parakeratotic, hyperkeratotic, and some rete peg formation was observed. Mononuclear cell infiltration of the surface epidermis was not a

Fig. 1. Skin of a sensitized dog 4 days after challenge with dinitrochlorobenzene (DNCB). Dense infiltration of mononuclear cells in the upper dermis (H & E, × 73).

Fig. 2. Hair follicles from a dog sensitized with dinitrochlorobenzene (DNCB) 48 hr post challenge. Perifollicular infiltration of mononuclear cells, infiltration of monocytes into the base of the follicle (H & E × 94).

Gross lesions of primary irritant dermatitis were obvious upon removal of the patches 24 hr after their application. The lesions developing as a result of application of 1% and 5% DNCB were intense hyperemia and edema associated with numerous papules at the site of the patch. In lesions produced by 10% DNCB, the skin appeared necrotic, dark, and gangrenous. These lesions were raised 3 to 5 mm above the adjacent surface of the skin.
distinctive feature of ACD, and the dermoepidermal junction was generally not disrupted. Mild spongiosis was observed, but vesicle formation was rare. The reaction peaked in intensity 3 to 4 days post challenge and by 7 days the inflammatory response had dissipated.

Histologic examination of skin biopsy specimens from control animals indicated that they were essentially normal except for some thickening of the epidermis.

In primary irritant dermatitis, the epidermis was observed in varying stages of degeneration. The most severe lesions occurred after application of 10% DNCB. In mild lesions there was loss of differential staining ability of the epidermal cells. In the severe lesions there was complete necrosis and loss of structure of the epidermis. In most lesions the epidermis was separated from the dermis and the resulting space was filled with fluid and inflammatory cells. In some instances this space was packed with polymorphonuclear cells (Fig. 3). The dermis was edematous and the collagen fibers were disrupted and fragmented. In this layer of the skin there was intense inflammatory infiltrate and the predominant infiltrating cells at all levels consisted of the polymorphonuclear neutrophil. The vessels were extremely dilated and filled with blood and polymorphonuclear cells. In the hair follicles, intracellular edema of epithelial cells was the primary feature of the mild lesion. In severe lesions there was complete loss of the epidermis surrounding the follicles. Where this occurred, the hair shaft was ensheathed by a thin homogenous necrotic layer containing remains of nuclear material. In other instances polymorphonuclear cells penetrated into the follicle and could be seen surrounding the hair shaft. Necrosis of sebaceous glands and loss of most cellular elements were consistent changes. The remaining cells were unusually foamy and appeared to be in the process of degeneration. By 72 hr post challenge the epidermis seemed to be regenerating.

**Fig. 3.** Skin from a dog 24 hr after application of 5% dinitrochlorobenzene (DNCB) solution. Separation of dermis and accumulation of polymorphonuclear cells (H & E, × 73).

**DISCUSSION**

The results of the present experiments demonstrated that the dog can indeed become sensitized and react to contact allergens. The problems previous investigators encountered in eliciting allergic contact dermatitis (ACD) in the dog probably relate to the fact that they did not challenge their animals using the closed-patch technique. This procedure tends to keep the allergen in solution and in close contact with the skin, enabling better penetration. It is also possible that the consistency of development of sensitivity in the dogs in the present experiments may be related to their young age. Dogs were very readily sensitized to DNCB by using multiple intradermal inoculations to this chemical and by the "guinea pig maximization test" [10] technique. All dogs sensitized to DNCB did not react with the same intensity. This variation in reaction may relate to the individual susceptibility of the animal.

The gross and microscopic lesions of primary irritant dermatitis were different from the lesions of ACD. Grossly, the erythema and edema associated with primary irritant dermatitis were more severe than lesions of ACD. In the dog, papules and necrosis, which commonly occur in primary irritant dermatitis, were not observed in ACD. Microscopically the lesions of primary irritant dermatitis in the dog resembled the reaction seen in both the guinea pig and man [11,12]. The main lesions were necrosis of the epidermal cells, separation of the epidermis from the dermis, dermal edema, and massive infiltration with polymorphonuclear cells. In ACD the main infiltrating inflammatory cell was the mononuclear cell, and the dermal edema was much milder than in primary irritant dermatitis. The other cellular changes seen in primary irritant dermatitis were not observed in ACD.

**REFERENCES**

1. Frederick LD: Non parasitic skin diseases of the dog. JAVMA 95:490-493, 1939
10. Magnusson B, Kligman AM: Allergic Contact Der-
matitis in the Guinea Pig. Springfield, Ill, Thomas, 1970
