MARION GIJBELS

CHARACTERISATION OF A MOUSE MODEL WITH CHRONIC PROLIFERATIVE DERMATITIS (*CPDM/CPDM*)

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STELLINGEN

behorende bij het proefschrift

Characterisation of a mouse model with chronic proliferative dermatitis (*cpdm/cpdm*)

1. Onderzoek naar oraal toegediende medicijnen tegen huidziekten geeft sneller resultaat als de slokdarm bestudeerd wordt.
   Dit proefschrift

2. Er is een directe relatie tussen het verschijnen van mitochondriale insluitsels en epidermale hyperplasie.
   Dit proefschrift

3. De flaky skin muis en de cpd muis representeren verschillende aspecten van chronische proliferatieve dermatitis en kunnen daarom beide een bijdrage leveren aan de ontwikkeling van nieuwe geneesmiddelen.
   Dit proefschrift

4. Het kwantificeren van jeuk bij proefdieren is een enorm probleem omdat het niet bekend is of proefdieren krabben wanneer ze jeuk hebben.

5. Bij het testen van geneesmiddelen is het voordeel van een natuurlijk proefdiermodel ten opzichte van een genetisch gemodificeerd model het multifactoriële karakter van het natuurlijke proefdiermodel.

6. Het feit dat de vetophopingen bij hoog vet/cholesterol gevoede apo E-deficiënte muizen abrupt ophouden bij de kliermaag duidt op een verandering van adhesiemolecuulexpressie van monocyten op het lokale endotheel.
   Ree van JH et al. (1995) Atherosclerosis 122:237-243

7. Het lijkt paradoxaal dat na "ontsmetting" van de huid besmet met het blaartrekkend strijdmiddel Lewisiet ernstiger huidschade optreedt dan wanneer geen "ontsmetting" plaatsvindt.
   Hooidonk van C et al. (1995) Proc NATO Research Study Group-3 Prophylaxis and Therapy against Chemical Agents: pp 1-9

8. Het feit dat onderzoeksresultaten tussen de cavia en de rat verschillen komt omdat de cavia geen knaagdier is.
   D'Erchia AM et al. (1996) Nature 381:597-600

9. Het zogenoemde 'Golfoorlogsyndroom' werpt voor de Krijgsmacht nieuw licht op het uitgangspunt 'voorkomen is beter dan genezen'.

10. Bij onderzoek van genetisch gemodificeerde dieren kan belangrijke medische biologische informatie verloren gaan als geen algemeen histopathologisch onderzoek wordt verricht.

11. In personeelsadvertenties zijn arbeidsvoorwaarden altijd uitstekend.

Leiden, 11 september 1996 Marion Gijbels

CHARACTERISATION OF A MOUSE MODEL WITH CHRONIC PROLIFERATIVE DERMATITIS (*CPDM/CPDM*)

PROEFSCHRIFT

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Maria Johanna Josephina Gijbels

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aan mijn ouders,
voor Thijs, Evelien en Thijs

Bij deze wil ik iedereen bedanken, die op welke wijze dan ook, heeft bijgedragen aan het tot stand komen van dit proefschrift.

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Chapter 1

General introduction

GENERAL INTRODUCTION

1 Function and structure of the skin

Famous for its soft, flexible, elastic qualities, the skin is surprisingly resistant to blunt trauma, corrosive liquids, and the effects of sunlight and irradiation. The skin also resists microbial invasion by virtue of its physical properties and by immunologic-inflammatory processes. Located at the interface of self and the external environment, the skin is a major sensory organ receiving such information as touch and temperature; it regulates the heat loss and prevents dehydration. Metabolic functions such as activation of vitamin D precursors, excretion of urea, and storage of carbohydrates and fat occur in the skin. By its appearance, feel and smell, it also plays a major role in socio-sexual communication. These functions in an organ so readily accesible to observation and manipulation make the skin a tempting location to study many basic processes. As a complex organ, the largest of the human body, the skin is composed of two layers: the epidermis and the dermis, resting on the subcutaneous fat.

The epidermis. The epidermis is mainly composed of a single cell type: the keratinocyte. The epidermis is a self-renewing organ and in normal skin most cell divisions occur within the basal cell compartment (stratum basale), where cuboidal, small basophilic cells are tightly attached to the basement membrane by specialized attachment zones, including the hemidesmosomes. As keratinocytes leave the basal layer, they become larger and paler to form the spinous layer (stratum spinosum), where the stability of the skin is maintained by desmosomal interconnections between the cells. Towards the surface of the skin, the spinous cells flatten and acquire keratohyaline granules (stratum granulosum), which contain a histidine rich basic protein, known as filaggrin, involved in protein cross-linking to form an impermeable layer. Activation of proteolytic enzymes with a shift in pH causes the dramatic physical changes in cells moving into the stratum corneum. A rapid cross-linking of envelope and filament proteins takes place and a loss of nuclear structure occurs.

Other cellular constituents of the epidermis include Langerhans cells, melanocytes, Merkel cells and T lymphocytes. The Langerhans cells (2-5% of total epidermal cells) are bone-marrow derived dendritic cells that play a pivotal role in the skin immune system. The major role of Langerhans cells is to process antigens that enter the skin. The antigen-bearing Langerhans cell migrates via the afferent lymphatics, into the paracortex of the draining lymph nodes where antigen presentation to specific T cells may occur. Little is known about the function of the

human epidermal T lymphocytes. Melanocytes are secretory cells mainly involved in melanogenesis. These cells are regularly interspersed among the basal keratinocytes. Merkel cells are innervated by afferent type I mechanoreceptive neurons and are thought to play a role as a transducer element of physical stimuli.
The dermis. The dermis is composed of cellular, fibrous, and ground substance components; the latter two undergo constant synthesis and degradation. The cellular elements include fibroblasts, endothelial cells, mast cells, recirculating T lymphocytes, tissue macrophages and dendritic cells. Also the hair follicles, sebaceous glands, sweat glands, demyelating nerve fibers and their sensory end-organs (e.g. corpuscles of Vater-Pacini) reside in the dermis. Fibers include collagen, reticulin, and elastic fibers. Collagen fibers provide the skin with the bulk of its tensile strength, while elastic fibers contain properties of flexibility of the skin. The dermis has a very rich vasculature which supplies nutrients to the epidermis by diffusion.

Normally the cells in the epidermis and dermis are in a state of equilibrium. In case of an inflammatory reaction this equilibrium is disturbed, leading to an influx of inflammatory cells and stimulation and activation of sessile cells.

Skin inflammation is a transient process as a result of a local damage or pathogen entry. However, skin inflammatory reactions can acquire chronic features. In the next chapters two chronic skin disorders in man will be discussed namely psoriasis and atopic dermatitis. Hereafter, possible experimental animal models that have been or can be used to study skin disorders are presented. One of these animal models, the *cpdm/cpdm* mouse was recently established and the aim of this thesis is to describe this model and to demonstrate its usefulness for the pathophysiological investigation of human skin diseases with a chronic proliferative character and the possibility to test therapies for these human diseases on the *cpdm/cpdm* mouse model.

2 Chronic dermatitis

The two most common skin disorders in man which are characterized by epidermal proliferation and inflammation of the dermis and epidermis are: psoriasis and atopic dermatitis. The etiology of both skin disorders is different, although also similarities are observed (see Table 1).

Table 1. Comparison of human psoriasis[a] and atopic dermatitis[b].

Criteria	*Psoriasis*	*Atopic dermatitis*
General		
Sexual predilection	No	No
Age of onset	0-108 years	0-6 years
Genetic predisposition	Yes	Yes
Pruritus	Occasionally prominent	Prominent
Pathology of the skin		
Epidermal hyperplasia	Yes	Yes
Hyperkeratosis	Yes	Yes
Parakeratosis	Yes (prominent)	Yes (focal)
Spongiosis	Slight	Marked
Apoptosis	No	No
Accumulation of PMN's	Common	Occasionally
Subepidermal fibrin depositions	No	Occasionally
Number of mitotic figures in basal layer	Increased	Normal
Tortuous capillaries	Yes	Yes
Infiltrating cells in dermis (predominant)	T-lymphocytes, macrophages > neutrophils	Eosinophils > T-lymphocytes > neutrophils
Mast-cells	Increased	Increased
IgE (in serum)	Normal	Increased (80% of the cases)
Organs involved		
Liver	In some cases steatosis, periportal inflammation, focal necrosis, fibrosis	No
Lung	No	In some cases associated with asthma
Mouth and upper gastrointestinal tract	Only in some cases with pustular psoriasis	No
Perisynovial connective tissue	In some cases (psoriatic arthritis)	No

Lymph nodes: infiltrated with eosinophils	No	Yes
Biochemical parameters of keratinocytes (in vitro)		
IL-1α	Decreased	Not tested[c]
IL-6	Elevated	Not tested[c]
TNF-α	Elevated	Not tested[c]
PGE_2	Elevated[d]	Not tested[c]
LTB_4	Elevated[d]	Elevated?
12/15-HETE	Elevated[e]	Elevated
9/13-HODE	Elevated[e]	Not tested[c]
Adhesion molecules on endothelial cells		
ICAM-1	Increased	Increased
VCAM-1	Slightly increased	Increased (acute lesions) Normal (chronic lesions)
E-selectin	Increased	Increased
Therapeutic responsiveness		
Cyclosporin A	Yes	Yes
Corticosteroids	Yes	Yes
Calcipotriene	Yes	No
Etretinate	Yes	No
Loratidine (against itching)	Yes	Yes
Capsaicin (against itching)	Yes	Yes
Dapsone	No	?

[a]based on references 1,2,3
[b]based on references 4,5,6,7,8,9,10
[c]not tested for keratinocytes
[d]determined with RIA
[e]determined with HPLC incubated with ^{14}C-Arachidonic-acid

2.1 Psoriasis

Prevalence. Psoriasis is a chronic, genetically predisposed, remitting skin disorder that affects approximately two percent of the population (equally distributed over males and females) in North West Europe and North America. The

disease is less common in the African and Asian races. Although it occurs at all ages, psoriasis has two peaks of onset, one in the adolescents and young adults (at 16 to 22 years of age), the other in older persons (at 57 to 60 years of age) (11).

Macroscopy. The macroscopic manifestations can be described as: circumscribed, erythemato-squamous lesions with hyperkeratosis. The extensor site of the elbows, knees, buttocks, scalp, and sites of local trauma are predilection sites. The severity of involvement is usually estimated by the Psoriasis Area and Severity Index, which takes into account the size of the area involved, redness, thickness, and scaling (12). The lesions can be pruritic.

Microscopy. Histologically, the psoriatic lesion is characterized by epidermal hyperplasia, hyper- and parakeratosis and increased proliferation of the capillary venules in the dermis (13). The proliferation of the keratinocytes can be shown by elongation of the dermal papillae and rete ridges. The infiltrates of the dermis and the deeper layers of the epidermis mostly comprise macrophages and T cells (14,15) although polymorphonuclear leukocytes can be prominent in persistent psoriatic plaques. The proliferating activitity of psoriatic epidermis is much greater than normal; the migration of keratinocytes from the basal layer to the epidermal surface is more rapid. This causes the parakeratosis. The pool of proliferating keratinocytes is expanded, because of the emergence of amplifying suprabasal cells (16,17). Electron microscopic studies indicate that psoriatic keratinocytes show significant abnormalities. The tonophilaments are decreased in number and in diameter and lack their normal aggregation. The size and number of keratohyaline granules is greatly reduced. The intercellular spaces between all epidermal cells are widened because of a deficiency in the glycoprotein-rich cell surface coat, so that intercellular adhesion is limited to the desmosomes. The capillary loops in the dermal pappilae show a hyperplastic endothelium and a multilaminated basement membrane (18).

Pathogenesis. The abnormalities of epidermal cells in human psoriasis are due to the production of several cytokines by the dermal immune reaction (15). It is now widely believed that T cells play an important role in the pathology of the disease (19). This is underscored by the therapeutic effectiveness of cyclosporin and FK506 (20,21), both immunosuppressants that inhibit T-cell function (22) and the predominance of CD4 lymphocytes in the dermis of affected patients. Furthermore, immunotherapy with anti-CD3 (23) and anti-CD4 (24) monoclonal antibodies was effective. Recently, it has been reported that bone marrow transplantation from normal human donors to psoriatic siblings resulted in a cure of the skin disease (25,26). Activated T-cells from lesional skin cause keratinocytes to

proliferate (27). On the other hand, other hypotheses locate the primary defect of psoriasis in fibroblasts or keratinocytes. This is based on the observation that fibroblasts from psoriatic lesions, but not from control skin, induce hyperproliferation of keratinocytes under certain experimental conditions (28,29) and persistent release of autocrine growth factors and insensitivity to growth-inhibitory factors of keratinocytes may cause hyperproliferation as well as infiltration of neutrophils in the epidermis and dermal inflammation (30).

2.2 Atopic dermatitis

Prevalence. Atopic dermatitis is an inflammatory skin disorder that occurs in patients with a personal and/or family history of atopy, although some individuals have typical skin lesions without an atopic history. Although atopic dermatitis is less frequent in adults, it seems to affect 5 per cent of the children in Europe and the USA. Several reports indicate that the prevalence of atopic dermatitis is increasing since 1945 (31,32). Both sexes are equally affected. Although present all over the world, it occurs more frequently in urban areas and hot climates. There is no single diagnostic feature of atopic dermatitis, and the diagnosis is based on a *combination of historical, clinical and histologic features.*

Macroscopy. Clinically, the acute lesions of atopic dermatitis appear as polymorphic lesions i.e. erythemato-squamous lesions with or without papules, vesicles and plaques. Weeping and crusting may be found in the more severe cases. Chronic lesions present as lichenified plaques with increased skin markings. In older children and adults, the lesions are characteristically found on flexural areas of knees and elbows. During infancy, the face is commonly involved. Pruritus is the major symptom in older children and adults, while irritability may predominate during infancy.

Microscopy. The histologic changes of atopic dermatitis are not specific and may be similar to those present in a variety of other skin disorders (33). Acute lesions show slight hyperkeratosis and parakeratosis, marked intercellular edema with spongiotic vesicle formation and a variable epidermal hyperplasia. Chronic lesional sites show severe epidermal hyperplasia with minimal amounts of spongiosis (34). In acute and chronic atopic dermatitis lesions the dermis shows a lymphohistiocytic infiltrate, mainly consisting of T-lymphocytes (35). It has been demonstrated that dermal lymphocytic infiltrates in atopic dermatitis skin lesions are essentially composed of CD4+ helper T-lymphocytes (36). Eosinophils, basophils and neutrophils are occasionally observed. Although the number of eosinophils present in the dermis is limited, the demonstration of relatively large depositions of

eosinophil granule derived major basic protein in the upper part of the dermis suggests active degranulation and therefore involvement of eosinophils in the skin lesions of atopic dermatitis (37). The number of mast cells is normal in acute lesions, but is increased in chronic lesions (34). In acute lesions mast cells appear invariably hypogranulated, whereas most of the mast cells in chronic plaques appear full of granules. The number of capillaries is reported to be increased and the capillary walls are often thickened. Electron miocroscopic examination showed that the keratinocytes are larger than normal, with more irregular nuclei, and they show slight loss of polarity. The mitochondria and Golgi apparatus are increased in both size and number. The tonophilaments are irregularly aggregated and distributed in the cytoplasm. The basement membrane in the acute lesions is noted to be thinner than normal. The lamina lucida is abnormally wide and less electron dense (38).

Pathogenesis. Atopic dermatitis can be transferred by bone marrow transplantation, indicating that hematopoietic cells are the vectors for disease expression in the skin (39). In atopic dermatitis, the majority of patients has increased total IgE levels, whereas a small group (about 20%) has normal total IgE antibody levels (40). Whether this reflects a pathogenetic role for IgE or simply an increased stimulation by inflammatory cells and factors remains uncertain. Patients with atopic dermatitis also have impaired delayed-type hypersensitivity. Allergen presentation is mediated by epidermal Langerhans cells with FcεRI-bound IgE (41). This allergen is presented to Th2 cells which become activated. Interleukin (IL)-4, produced by these Th2, appears to program B-cells for IgE synthesis (reviewed by Hanifin (7)).

3 Experimental models for dermatological research

To study the pathophysiology of dermatological disease and to identify the molecular basis of the disease, animal and experimental *in vitro* models are indispensible. Furthermore, these models can be used to test novel therapies for disease.

3.1 Animal models

Laboratory mice have proven to be extraordinarily useful for biomedical research due to their small size, short breeding time, relatively low husbandry costs, and short life span. The value of animal models depends on how they compare to the human or animal disease of interest. A common approach has

been to use animal models which resemble the human disease to various degrees. But a particular human disease is normally not monomorphic and well-defined. This implies that there are many genetic and epigenetic (environmental-genetic interactions) factors that are responsible for inter-individual variation in the clinical presentation of the disease. Therefore, it is difficult to assemble all those factors of a human disease in one animal model. In the following paragraphs some animal models are described with characteristics of psoriasis (3.1.1.1-3.1.1.3) and atopic dermatitis (3.1.2.1, 3.1.2.2). For research on psoriasis spontaneous mouse mutants and transgenic mouse strains are available. For research on atopic dermatitis a spontaneous model in the dog is available and several transgenic mouse strains with eosinophilia.

3.1.1 Animal models for psoriasis

3.1.1.1 Flaky skin mouse *(fsn)*

History. The skin lesions in the *fsn/fsn* mice resemble the morphologic and biochemical features of some subtypes of psoriasis in humans. This mouse mutation arose spontaneously in the A/J inbred strain at the Jackson Laboratory in 1985 (43). The *fsn/fsn* mouse is also backcrossed onto the BALB/cByJ background.

Macroscopy. After weaning, the mutants develop thick white scales with associated patchy alopecia (44,45,46,47). All *fsn/fsn* mice are mildly anemic at birth, making them visually identifiable. The anemia progresses with age.

Microscopy. Histologically, the epidermis is thicker due to increases in most layers, most notably the stratum corneum. The stratum granulosum is extremely thin in A/J-*fsn/fsn* mice but is very prominent in the BALB/cByJ-*fsn/fsn* mice. In the dermis capillaries are dilated and there is a mixed inflammatory cell infiltrate; in the A/J-*fsn/fsn* mouse mostly neutrophils; in the BALB/cByJ-*fsn/fsn* mouse mostly mononuclear cells. The major ultrastructural alterations include diminished tonofibrils, poor fusion of tonofilaments to keratohyalin granules, intercellular lipid vacuoles in the stratum corneum, and tortuous dermal blood vessels lined by hyperplastic endothelium with a multilaminated basement membrane (48), all features described in human psoriatic lesions (18).

Pathogenesis. Pathologic changes caused by homozygosity for the flaky skin mouse phenotype appear to be determined at the level of bone marrow-derived cells. This hypothesis is based on the observation that bone marrow grafts from *fsn/fsn* mice to *scid/scid* mice induce a skin lesion without other

abnormalities, such as anemia, in recipients (46). This skin lesion does not require the activity of functional lymphoid cells since the skin lesion develops and progressess in double homozygous *(fsn/fsn scid/scid)* mice. Mitotic and DNA synthesis rates are increased in the mutant mouse skin *in situ* as well as when grafted onto nude mouse recipients (49). These findings are consistent with human psoriasis (50,51,52). Cyclosporin A was not effective in treating the epidermal hyperplasia in *fsn/fsn* mice.

3.1.1.2 Asebia mouse *(ab)*

History. Another mouse model with some characteristics of psoriasis is the *ab/ab* mouse. The asebia mutation arose spontaneously in a colony of BALB/c mice (53). The asebia mutation is autosomal recessive and maps to chromosome 19 (54).

Macroscopy. *Ab/ab* mice exhibit epidermal hyperplasia, alopecia and fine epidermal scaling all increasing with age. Mutant mice exhibit pruritus of the eyelids, and develop a sticky exudate that encrusts the eyelids.

Microscopy. Histologically, the sebaceous glands are hypoplastic (55,56,57). The sebaceous glands lack the orderly maturation of the peripheral reserve cells into vacuolated holocrine cells that rupture and secrete their contents into the sebaceous duct. The epidermis becomes moderately hyperkeratotic which increases in severity with age (55,58). The dermis is thicker and shows increased vasculature compared to littermate controls, with various degrees of inflammation (58). The inflammation is due to the presence of numerous macrophages that contain lipid and mast cells (59,60). Defects in the inner and outer follicular root sheaths have been found, suggesting impaired hair development (61).

Pathogenesis. Although some dermatologists have commented that the *ab/ab* mice might be a useful model for human psoriasis (59), the rupture of crystal-laden macrophages, which subsequently induces the release of a variety of inflammatory mediators and chemoattractants, has been proposed to be the cause of epidermal hyperplasia (44,59). Since the general opinion is that this is not the cause of epidermal hyperplasia in human psoriasis, the asebia mouse is not considered a very useful model for hyperproliferative skin disorders (44,57,62).

3.1.1.3 Transgenic mouse models

Various transgenic mice have been described with some characteristics of psoriasis.

a) Transforming growth factor (TGFα) transgenic mice.

History. These transgenic mice overexpress TGFα in basal cells of orthokeratinizing stratified squamous epithelium under the control of the cytokeratin 14 promotor (63) or only in the suprabasal cells of the epidermis, using the HK1 vector (64).

Macroscopy. Both types of transgenic mice have many similarities. The skin is more wrinkled than normal, the outer epidermal layers are flaky, and hair growth of these animals is stunted. Papillomas appear at sites prone to wounding or repeated trauma.

Microscopy. Histopathological examination reveals that the stratum spinosum, stratum granulosum, and stratum corneum are proportionally thickened in comparison to normal epidermis. In addition the keratinocytes are clearly hypertrophic and dermal thickness is reduced.

Pathogenesis. Difference between HK1.TGFα and K14.TGFα mice is the persistence of the hyperplastic/hyperkeratotic phenotypic epidermis in adults with high expression of HK1.TGFα, whereas the epidermis returns to a normal pattern in K14.TGFα adult mice. This is probably due to higher levels of TGFα expression in HK1.TGFα mice. In K14.TGFα mice, EGF receptor levels are unaffected while in HK1.TGFα mice the EGF receptor levels are decreased. This is in contrast to some *in vitro* findings that TGFα upregulates EGF receptor expression (65,66,67). Although there exist some similarities between this mouse model and psoriasis, some aspects are different. Dermal atrophy, for example, is not commonly observed in psoriasis, and overexpression of TGFα alone is not sufficient to evoke psoriatic phenotypes, such as lymphocytic infiltration and EGF-receptor upregulation which is overexpressed in psoriatic epidermis (68).

b) Negative fibroblast growth factor (FGF)-receptor transgenic mice.

History. These transgenic mice overexpress a dominant negative FGF receptor under control of the cytokeratin 10 promotor (69). The receptor is only suprabasally expressed, which leads to interruption of the FGF signalling pathway in the final differentiation process of keratinocytes. In these transgenic mice a mutant receptor for FGF is formed, which is able to dimerize with the wild-type receptor but due to a mutation in the tyrosine kinase domain, forms an inactive hybrid which has lost its kinase activity. This kinase activity mediates the intracellular response of FGF. *In vitro*, FGFs have been found to be powerful stimulators of both mesenchymal or epithelial cell proliferation. Receptors are present both on basal and suprabasal keratinocytes, suggesting an autocrine and paracrine mode of action.

Macroscopy. None of the transgenic mice have obvious macroscopic abnormalities.

Microscopy. The skin of the transgenic mice shows epidermal hyperplasia, various degrees of thickening of the squamous epithelium and several layers of nucleated, undifferentiated cells. In addition, the basal and suprabasal layers show a high degree of disorganization and an irregular border between dermis and epidermis.

Pathogenesis. In these transgenic mice, where the FGF action in suprabasal keratinocytes is blocked, the full differentiation of the keratinocytes is inhibited. Accordingly, FGF appears to act more as differentiation-inducing factor than a mitogenic factor to suprabasal keratinocytes. This is not necessarily in contradiction to the *in vitro* finding that FGF stimulates the proliferation since the keratinocytes which are affected in the transgenic mouse are suprabasal, whereas cultured keratinocytes exhibit a basal-type phenotype. FGF might therefore act as a mitogen on basal keratinocytes and as a differentiation-promoting factor on suprabasal keratinocytes (69).

c) Keratinocyte growth factor (KGF) transgenic mice.

History. These transgenic mice overexpress KGF in basal cells of orthokeratinizing stratified squamous epithelium under the control of the cytokeratin 14 promotor (70).

Macroscopy. The skin is more wrinkled than normal and the animals have age-independent sparsity of hair.

Microscopy. Hyperproliferation and marked epithelial thickening is observed, and the terminal differentiation of keratinocytes is incomplete. The animals have a marked suppression of hair follicle morphogenesis and suppression of adipogenesis.

Pathogenesis. Normally, KGF is only expressed in fibroblasts and its level is increased during skin injury (71). In the skin, KGF seems to perturb mesenchymal-epidermal interactions, leading to remarkable suppression of hair follicle morphogenesis, accompanied by gross epidermal proliferation.

In conclusion, these transgenic mice have only the epidermal hyperproliferation in common with human psoriasis.

3.1.2 Animal models for atopic dermatitis

3.1.2.1 Spontaneous atopic dermatitis in dogs.

History. Except for man, the dog is the only animal in which spontaneously occurring atopic disease has been documented and studied (reviewed by Willemse (72). Of 208 dogs tested by Willemse and Van den Brom (73), boxers, terriers, German Shepherd dogs and poodles were most commonly found with characteristics of atopic dermatitis. Male and female dogs were equally represented and the median age at which the animals were admitted was 4 years (range between 9 months and 12 years).

Macrosopy. The clinical criteria of atopic dermatitis in dogs is pruritus, face rubbing, and paw-and-feet licking. The most frequently observed skin morphology includes erythema, papular reactions, crusts and lichenification. The most commonly affected areas are the muzzle, the periocular and axillary areas, and the feet. Rhinitis, reverse sneezing and conjunctivitis are often observed in conjunction with atopic dermatitis in dogs.

Microscopy. Histopathological examination of lesional skin from atopic dogs reveals varying degrees of chronic non-suppurative dermatitis. Angiocentric accumulations of inflammatory cells are confined to the superficial dermal blood vessels. Plasma cells and neutrophils are the predominant inflammatory cells. Eosinophils are observed in only 15% and then only in very small numbers. Mast cell numbers are increased in 35% of atopic dogs.

Pathogenesis. In dogs with atopic dermatitis, no elevated IgE levels are observed neither in blood nor in skin (74). Of dogs with clinical manifestations of atopic dermatitis together with the presence of immediate skin test reactivity, 89% have allergen specific IgGd antibodies against one or more allergens (75). Till now, no research has been done on IgE on Langerhans cells. The relevance of changes in lymphocyte activity as a fundamental feature of atopy is little understood in the dog. *In vitro*, an altered cell-mediated immune function was found in atopic dogs (76). In comparison with humans, not all aspects of the disease have been investigated in dogs. In its simplest form the sequence of events is as follows (reviewed by Willemse (72): in animals with a hereditary basis for atopic dermatitis, contact with inhalant allergens initiates the formation of mostly IgGd. Subsequently, these antibodies are fixed to the surfaces of basophilic granulocytes and mast cells. Upon re-exposure to the allergen, vasoactive mediators are released from these cells and cause the clinical manifestation of atopic disease. Mast cell release of preformed granule-associated mediators includes histamine, proteolytic enzymes and leukotrienes. Although there exist similarities between this dog model and human atopic dermatitis, no elevated IgE levels, no eosinophils and in most cases no mast cells are found. Beside this, nothing is known about interleukin production in the dog. A disadvantage of a dog model in comparison with a rat or mouse

model is the lack of experimental tools to study the immune system of this species.

3.1.2.2 Transgenic mouse models

There are two transgenic mouse models described with some characteristics of atopic dermatitis.

a) IL-4 transgenic mice.

History. These transgenic mice overexpress IL-4 exclusively in the lymphoid tissues by attenuating the transgene promotor through the insertion of E. coli *lac* operator sequences (77).

Macroscopy. All animals with IL-4 overexpression in the T cell compartment exhibit a striking inflammatory lesion of the external eye characterized grossly by marked swelling and erythema of the eyelid. The thymus shows hypoplasia.

Microscopy. The lines in which the IL-4 transgene was expressed in the T cells shows a striking abnormality in T cell development. Thymus lacks a cortex and shows an increase in medullary thymocytes. The population of immature $CD4^+CD8^+$ thymocytes and peripheral T cells is reduced while the population of $CD8^+$ thymocytes is increased. Histologic analysis of the eyelid reveals a dense inflammatory infiltrate that invades the subepithelial stroma, composed of lymphocytes and a striking number of eosinophils. Furthermore, an excessive number of tissue mast cells is observed in the inflamed eyelid.

Pathogenesis. Overexpression of IL-4 results in a marked increase in serum IgE levels. This transgenic mouse model demonstrates that IL-4 can induce a complex inflammatory reaction resembling that observed in human allergic disease. However, infiltration of eosinophils is not observed in other organs and even not in other parts of the skin.

b) IL-5 transgenic mice.

History. These transgenic mouse overexpress IL-5 exclusively in the thymus, Peyer's Patches and subcutaneous lymph nodes (78). A genomic fragment of the IL-5 gene is coupled to the dominant control region from the gene encoding human CD2.

Macroscopy. The mice appear normal apart from splenomegaly.

Microscopy. Large number of eosinophils are present in spleen, bone marrow and peritoneal exsudate, and are highest in the line with the greatest transgene copy number. Furthermore, eosinophilia is observed in transgenic lungs, Peyer's patches, mesenteric lymph nodes and gut lamina propria.

Pathogenesis. These transgenic mice show that induction of the IL-5 gene

is sufficient for production of eosinophilia, and that IL-5 can induce the full pathway of eosinophil differentiation. Infection of these transgenic mice with *Mesocestoides corti*, which in itself is a potent inducer of eosinophilia, increases serum IL-5 to very high levels. However, the numbers of eosinophils decrease during the first 4 weeks of infection. These results suggest that a control mechanism is operating to limit the numbers of eosinophils produced. This mechanism is not related to the high levels of IL-5 (79). Although these transgenic mice have large numbers of eosinophils in various organs, the animals remain normal (78). Release of toxic mediators by eosinophils during allergic inflammation is considered to be a first pathogenetic step in the pathophysiology of these disorders. Obviously, the eosinophils in these IL-5 transgenic mice are not activated to release these mediators. It is known that IL-5 has a well-characterized activity on B and T cells. However, in these transgenic mice there were only marginal increases in lymphocyte numbers compared with non-transgenic controls.

3.2 Experimental models for psoriasis

In vitro models are not discussed in this thesis, but the experimental transplantation models whereby human psoriatic skin is transplanted onto nude mice (80,81) need to be mentioned. Relative to normal skin, the human epidermis of both involved and uninvolved skin of psoriatic subjects becomes equally hyperproliferative upon transplantation onto nude mice. However, the scaling and erythema that typify a lesion of psoriasis do not persist. A disadvantage of these models is that the systemic character of the disease is difficult to study.

4 Aim of this thesis

Chronic proliferative dermatitis is a spontaneous mutation in C57BL/KaLawRij mice (*cpdm/cpdm*) first observed in 1991 in the specific pathogen-free-breeding facility of TNO in Rijswijk, The Netherlands. Because of the possible contribution of this model to study the pathophysiology of human skin diseases with a chronic proliferative character and the possibility to test therapies for these human diseases on the *cpdm/cpdm* mouse model, we have further characterized the *cpdm/cpdm* mice.

In **chapters 2 and 3**, we describe the morphology of this mouse model in detail. In **chapter 2** the similarities and dissimilarities to other murine models for hyperproliferative skin diseases and to human hyperproliferative skin diseases are discussed. In **chapter 3** the lesions are investigated in detail using electron

microscopy.

Chapter 4 describes the expression of adhesion molecules in *cpdm/cpdm* skin. Using an *in vitro* adhesion assay, the functional significance of this expression is determined. These findings are compared with those in human hyperproliferative skin disorders.

Chapter 5 describes whether the pathologic features observed in the skin of the *cpdm/cpdm* mouse originate from the skin or are the result of a systemic disorder. For this purpose transplantations of full-thickness skin grafts from affected *cpdm/cpdm* mice and control (C57BL/Ka) mice to *cpdm/cpdm*, C57BL/Ka and athymic nude *(nu/nu)* mice were performed.

Chapter 6 describes a study to elucidate the pathogenesis of the chronic skin disease in the *cpdm/cpdm* mouse in more depth. Therefore, the skin and other affected organs of 1, 2, 3, 4, 5 and 6 week old *cpdm/cpdm* mice and appropriate controls were examined. Here, we report which histological changes occur in the various organs and how these changes relate to macroscopic observable skin lesions.

Chapter 7 describes that this mouse model is a suitable *in vivo* model to test drug treatments for epidermal hyperproliferation, eosinophil infiltration and pruritus. To test drug efficacy the following criteria were used: macroscopic appearance of the lesions (cpd score), microscopic evaluation of the inflammatory cells in skin, epithelial thickness and epithelial cell proliferation of skin and esophagus.

In **Chapter 8**, the findings are discussed in light of the usefulness of the *cpdm/cpdm* mutant mouse strain for dermatological research.

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Chapter 2

A sponaneous mutation characterized by chronic proliferative dermatitis in C57BL mice

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Chapter 2

Animal Model

A Spontaneous Mutation Characterized by Chronic Proliferative Dermatitis in C57BL Mice

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Chronic proliferative dermatitis is a new spontaneous mutation in C57BL/Ka mice. Breeding results suggest an autosomal recessive mode of inheritance. Mutant mice develop skin lesions at the age of 5 to 6 weeks. The lesions occur in the ventral and dorsal skin of the body, whereas ears, footpads, and tail are not involved. The lesions are characterized by epidermal hyperplasia, hyper- and parakeratosis, and single cell necrosis of keratinocytes. The dermis and epidermis are infiltrated by granulocytes and macrophages, and occasionally subcorneal and intracorneal microabscesses are formed. The number of mast cells in the dermis progressively increases with age. There is dilatation and proliferation of dermal capillaries. Similar lesions develop in the mouth, esophagus, and forestomach, which, in the mouse, are all lined by orthokeratinizing stratified squamous cell epithelium. Studies with bromodeoxyuridine confirm the increased rate of epithelial cell proliferation. Most inflammatory cells in the affected skin express Mac-1, and few express the T lymphocyte marker CD3. There is increased expression of intercellular adhesion molecule-1 on keratinocytes and endothelial cells. Infiltration of neutrophils and macrophages are also seen in the liver, lung, and several joints. The disease could not be transferred by bone marrow or spleen transplants into irradiated normal syngeneic hosts. Treatment of the mice with triamcinolone, a long-acting corticosteroid, resulted in nearly complete regression of the lesions over a period of 4 weeks, whereas systemic cyclosporin A treatment was ineffective. (Am J Pathol 1993, 143:972–982)

The detection and study of hereditary disorders in animals have greatly contributed to our understanding of the complex mechanisms underlying disease processes in general as well as specific human diseases. Such defects in rodents have been particularly useful because of the relative ease of maintenance and breeding of laboratory rodents and the extensive knowledge of their biology and physiology.[1] Mouse models are especially useful because of rapid advances in gene mapping in this animal species.[1] Although nearly 100 mutations are known that affect the skin of mice, few have been investigated in detail.[2]

Local inflammatory reactions elicited by topical application of chemical compounds to the skin of laboratory animals have been extremely valuable in studies of mechanisms of skin inflammation and inflammation in general.[3] These inflammatory responses are generally acute and may be less relevant as models of chronic inflammation. Here, we describe a new spontaneous mutation, which results in the appearance of chronic inflammatory reactions in the skin and in several internal organs. The skin disease, which will be addressed as chronic proliferative dermatitis (cpd), is characterized by hyperproliferation and neutrophil infiltration of orthokeratinizing stratified squamous cell epithelia, including that of the skin and esophagus, and by a chronic persistent inflammatory reaction in the dermis. In this report, we will present data from our morphological studies of this mouse

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mutant and will discuss its similarities and dissimilarities to other murine models of hyperproliferative skin disease and to human hyperproliferative skin diseases.

Materials and Methods

Animals

The cpd mutation arose spontaneously in the breeding stock of inbred C57BL/KaLawRij mice in the specific pathogen-free-breeding facility of TNO in Rijswijk, the Netherlands. Mice in this breeding colony are housed in Macrolon cages and are provided autoclaved pelleted food and acidified, sterilized bottled drinking water *ad libitum*. The microbiological status is checked regularly by routine serological, bacteriological, and histological procedures. Mice were killed at various ages by ether inhalation. Control mice were C57BL/KaLawRij mice from the same colony.

Treatment with Corticosteroids and Cyclosporin A (CsA)

Two groups (n = 4) of 10-week-old cpd mice were treated with triamcinolone (Lederspan, Lederle, Zoeterwoude, the Netherlands), a long-acting corticosteroid, for 4 weeks. One group received two subcutaneous injections of 10 mg/kg, diluted in saline, per week for 3 weeks and one injection of 10 mg/kg in week 4. The second group was given one injection of 5 mg/kg per week. The animals of both groups were killed at the end of week 4. Four control cpd animals were injected with saline only.

Three groups of 10-week-old mice were treated with cyclosporin A (CsA; Sandimmun, kindly provided by Sandoz, Uden, the Netherlands). One group (n = 3) received intraperitoneal injections of 15 mg/kg CsA, and a second group (n = 2) received intraperitoneal injections of 100 mg/kg CsA, three times a week. The third group (n = 3) was given 100 µl diluent (olive oil) and served as control group.

Hemopoietic Cell Transfer

Bone marrow cells and spleen cells from 5-week-old cpd mice were injected intravenously into lethally irradiated (8.7 Gy at 0.87 Gy/minute from a ^{137}Cs source, type γ cell 20) 8- to 12-week-old syngeneic C57BL/Ka mice. They received 1 × 10^6 bone marrow cells plus 4 × 10^6 spleen cells (n = 6) or 2 × 10^7 spleen cells (n = 10) only. In a second set of experiments, 2 × 10^7 spleen cells from cpd mice were injected intraperitoneally into 12 neonatal nonirradiated syngeneic C57BL/Ka mice. The recipients were monitored daily for clinical signs of cpd lesions and were euthanized after 2 months, after which a complete necropsy was performed.

Histology

Tissues were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin Three-µ sections were routinely stained with hematoxylin-phloxine-saffron (HPS). Additional stains used were periodic acid-Schiff, Gomori's silver stain, and toluidine blue.

Immunohistochemistry

Commercial reagents were biotinylated anti-Ia^b (PharMingen, San Diego, CA), fluorescein isothiocyanate (FITC)-labeled anti-CD3ϵ (clone 145-2C11; Boehringer Mannheim, Almere, the Netherlands), FITC-labeled F(ab')$_2$ rabbit anti-mouse immunoglobulin G (IgG, Serotec, Oxford, England), avidin-peroxidase (Serotec), and mouse anti-rat peroxidase (Jackson Immunoresearch Laboratories, West-Grove, PA). Hybridomas M7/14 (anti-lymphocyte function-associated antigen-1 [LFA-1]; TIB 217) and M1/70 (anti-Mac-1; TIB 128) were obtained from American Type Culture Collection. Hybridoma MEL-14 (anti-L-selectin) was the kind gift of Dr. L. Nagelkerken (TNO, Leiden, the Netherlands). Hybridoma YN1/1.7 (anti-intercellular adhesion molecule-1 [ICAM-1][4]) was generously provided by Dr. F. Takei (Terry Fox Laboratory, Vancouver, BC). Supernatant of the YN1/1.7 cells was purified by affinity chromatography over a protein G-column. Hybridoma NLDC-145, which specifically stains dendritic cells,[5] was kindly made available to us by Dr. G. Kraal (Free University of Amsterdam, the Netherlands).

Skin samples were quick-frozen in liquid nitrogen for immunohistochemistry. Frozen sections were incubated overnight at 4 C with biotinylated anti-Ia^b (1:100), anti-ICAM-1 (1:200), M1/70 (1:5 of supernatant), M7/14 (1:25 of supernatant), MEL-14 (1:40 of supernatant), or NLDC-145 (undiluted supernatant), followed by avidin-peroxidase or mouse anti-rat peroxidase for 60 minutes at room temperature. Negative controls consisted of omission of the primary antibody and replacement of the primary rat monoclonal antibody by an irrelevant rat IgG2a (MECA-

367). Peroxidase activity was visualized with diaminobenzidine (Sigma Chemical Co., St. Louis, MO). Alternatively, sections were incubated overnight at 4 C with FITC anti-CD3 (1:200) or FITC anti-mouse IgG (1:100) and examined using a Leitz Orthoplan epifluorescence microscope.

Bromodeoxyuridine (BrdU) Labeling

To determine the rate of cell proliferation, mice received an intraperitoneal injection of 0.625 mg BrdU (Sigma) 30 minutes before being killed. Tissues were fixed for 18 hours in neutral-buffered formalin and stored in 70% alcohol until further processing. Paraffin-embedded sections were deparaffinized, rehydrated, and treated with 0.2% pepsin in 0.1 N HCl for 10 minutes at room temperature, followed by 2 N HCl for 30 minutes at 37 C, before 60-minute incubation with monoclonal anti-BrdU antibody (1:40; Dakopatts, Copenhagen, Denmark) at 37 C. Labeled nuclei were visualized by peroxidase-labeled rabbit anti-mouse Ig (Dakopatts), followed by diaminobenzidine in combination with 1% cobalt-Cl to enhance staining intensity.

Morphometry and Statistical Analysis

BrdU-labeled nuclei were counted per cm basement membrane. The thickness of the combined nucleated epithelial layers of the interfollicular epidermis, ear, esophagus, and forestomach was measured at 10 sites. From these measurements, the mean thickness was calculated for each epithelium. The density of dermal mast cells was determined in toluidine blue sections as number of cells per mm^2 dermis. The area of the dermis was determined by subtracting the area occupied by pilosebaceous units and blood vessels from the total area of the dermis. The measurements were performed with computer-aided morphometry (Kontron-Videoplan, Zeiss, Germany). All data are expressed as mean ± SEM. The statistical significance of observed differences between control animals and affected mice and between treated and untreated mice was determined by Student's *t*-test and one-way analysis of normal variance.

Results

Clinical Investigations

Mice that are affected by the cpd mutation were first identified at 2 weeks after weaning (5 weeks of age) by the development of hair loss and reddening of the skin of the dorsal neck and ventral chest. The lesions gradually spread to involve most of the skin except for the feet, tail, and ear pinnae, which remained unaffected. This situation had been reached at 12 to 15 weeks of age. The lesions were characterized by erythema, severe hair loss, and mild scaling. The mice showed moderate growth retardation with aging, but otherwise remained vital. However, severe pruritus often caused self-inflicted wounds necessitating euthanasia usually before the age of 30 weeks. Hematological evaluation revealed hematocrits in the normal range and a threefold increase of polymorphonuclear leukocytes with a regenerative left-shift.

Male and female mice were affected in equal numbers. Attempts to breed cpd mice to other cpd mice failed. Through a selective breeding program, we identified seven clinically unaffected male-female combinations that yielded cpd progeny. From these breeding pairs, a total of 760 mice was obtained, of which 123 (16%) developed cpd skin lesions. These results suggest an autosomal mode of inheritance.

Pathology

The following description is based on complete necropsies of 40 mice (23 male and 17 female) of 6 to 20 weeks of age. Gross examination revealed skin changes as described above and a three- to sixfold increase in the size of the spleen. The interfollicular epidermis of affected skin was thickened (from 8.8 ± 0.7 μ to 55.6 ± 17.6 μ; significant at $P < 0.05$) as a result of orthokeratotic hyperkeratosis alternating with parakeratotic mounds, and epidermal hyperplasia (Figure 1). The granular cell layer was slightly thicker or of normal thickness and

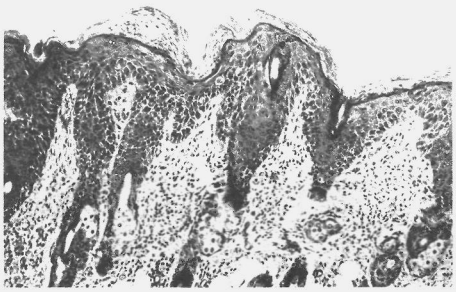

Figure 1. *Hyper- and parakeratosis and acanthosis in the epidermis and inflammatory cell infiltration in the dermis of a cpd mouse (HPS, 104×).*

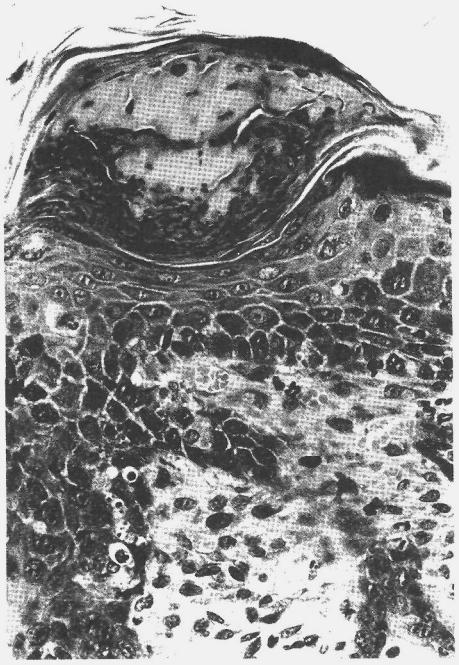

Figure 2. *Intracorneal microabscess and single cell death of keratinocytes in the epidermis and blood vessel proliferation in the dermis of a cpd mouse (HPS, 365×).*

was absent beneath the parakeratotic mounds. Single cell death of a variable number of keratinocytes was present in the stratum basale and spinosum (Figure 2). There was an increased number of mitotic figures in the basal cell layer. Polymorphonuclear granulocytes were present in the epidermis, and they occasionally formed small spongiform pustules just beneath the stratum corneum. Degenerated granulocytes mixed with parakeratotic mounds and formed microabscesses in the keratotic layer (Figure 2). There was occasionally mild spongiosis of the epidermis. The epidermal changes continued along the hair shafts resulting in follicular keratosis and frequent degeneration of hair shafts. The dermis was infiltrated by a mixed population of inflammatory cells, predominantly granulocytes and macrophages. There were tortuous, dilated capillaries in the superficial dermis often associated with mild edema. Special stains for the detection of yeasts and fungi and bacterial cultures of skin samples were consistently negative. The skin of the ears, footpads, tail, and nails were all normal by light microscopy.

The oral cavity, esophagus, and forestomach of the mouse are lined by orthokeratinizing stratified squamous cell epithelium.[6] Similar changes as those in the epidermis were observed to various extents in these epithelia (Table 1). The thickness of the esophageal epithelium was increased from 38.2 ± 4.4 μ in normal C57BL/Ka mice to 75.4 ± 8.3 μ in cpd mice (significant at $P < 0.05$), and the thickness of the forestomach epithelium was increased from 34.1 ± 8.8 μ to 62.9 ± 5.8 μ ($P < 0.05$). An exception was parakeratosis, which was only found in the epidermis. The lesions were particularly prominent in the esophagus, which often contained spongiform pustules (Figure 3). The subepithelial connective tissue was infiltrated by polymorphonuclear granulocytes and macrophages.

Portal and perivenous areas in the liver were infiltrated by neutrophils and macrophages (Figure 4). Similar, but less conspicuous, changes were present in the perivascular and peribronchiolar connective tissues of the lung. In addition, there was an increased number of eosinophilic alveolar macrophages. There was atrophy of the lymphoid tissues, increasing in severity with age, whereas marked extramedullary myelopoiesis accounted for the enlargement of the spleen. The bone marrow was hyperplastic with extensive myelopoiesis. Synovia and periarticular and peritendinous connective tissues of several mice (17 of 40) were infiltrated by a few or moderate number of macrophages and neutrophils (Figure 5). The synovial space was often mildly dilated and contained a few neutrophils and some proteinaceous material. The coxofemoral, tibiofemoral, and intervertebral joints and the knee tendon were most frequently involved. These lesions were more common in the female (12 of 17

Table 1. *Light Microscopic Changes in Orthokeratinizing Stratified Squamous Cell Epithelia of the cpd Mouse*

Changes	Skin	Ear	Tail	Footpads	Tongue	Gingiva	Esophagus	Forestomach
Hyperkeratosis	+++*	–	–	–	+	+	+	+
Parakeratosis	++	–	–	–	–	–	–	–
Hyperplasia	+++	–	–	–	+	+	++	++
Single cell death EC†	++	–	–	–	+	+	++	++
Granulocytes	++	–	–	–	+	+	++	+

* Semiquantitative scoring key: – absent; + mild; ++ moderate; +++ severe.
† EC-epithelial cells.

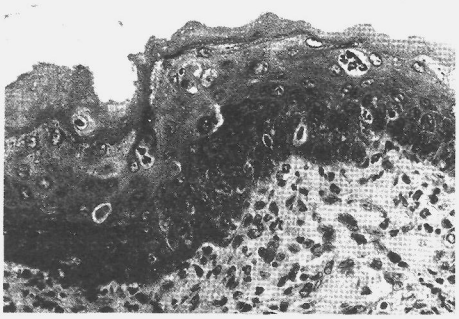

Figure 3. *Hyperkeratosis, acanthosis, intraepithelial spongiform pustules, and mixed inflammatory cell infiltration in the esophagus of a cpd mouse (HPS, 260×).*

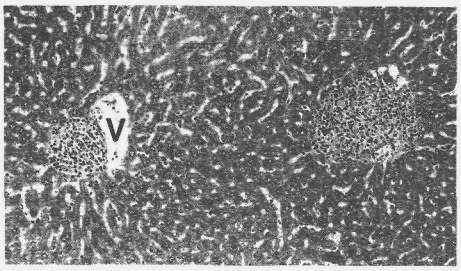

Figure 4. *Liver of a cpd mouse with mixed inflammatory cell infiltration of a portal area and adjacent to a central vein (V) (HPS, 94×).*

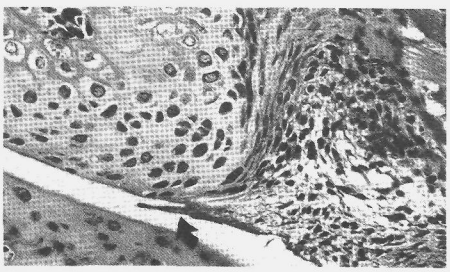

Figure 5. *Intervertebral joint of cpd mouse with mixed inflammatory cells in the synovia and perisynovial connective tissue and proteinaceous material in the synovial space (arrow) (HPS, 250×).*

affected) than in the male mice (five of 23 affected). There was no evidence of cartilage damage in any of the examined joints. Other tissues were normal.

The lesions in five mice (two male and three female) of 28 to 30 weeks of age were essentially the same as in the younger mice. In the skin, fewer neutrophils were present in the epidermis and dermis. Dendritic cells with small melanin granules appeared in the epidermis and dermis, and heavily laden melanophages were present in the dermis and in the draining lymph nodes. The hyperplastic and inflammatory changes in the esophagus and forestomach were less severe than in the younger mice.

BrdU-labeling

In normal skin, BrdU-labeling was limited to a few basal epidermal cells and hair matrix cells. The number of labeled nuclei was higher in the esophagus and forestomach. BrdU-positive nuclei were greatly increased in number in the basal layer of the epidermis (Figure 6) and the hair follicles of cpd mice. Positive nuclei were also found in suprabasal keratinocytes. A few nuclei of dermal cells, including endothelial cells of dermal capillaries and mast cells, were BrdU-positive in cpd mice. There was also a significant increase of BrdU-positive nuclei in the esophagus and forestomach (Figure 6).

Immunohistochemistry

Ia and NLDC-145 expression in normal skin was limited to Langerhans cells in the epidermis and scattered, poorly defined dendritic cells in the dermis. An increased number of Ia-positive cells was present in the epidermis of cpd mice (Figure 7). They were either round and NLDC-145-negative (macrophages) or dendritic and NLDC-145-positive (Langerhans cells). An increased number of Ia-positive cells was also present in the dermis, but there was no evidence of Ia expression on keratinocytes or endothelial cells. Most leukocytes in the dermis and epidermis were Mac-1-positive (Figure 8) and weakly LFA-1-positive. MEL-14 (L-selectin) reactivity was virtually undetectable in skin sections, although T lymphocytes reacted positively in frozen sections of lymph nodes using the same dilution

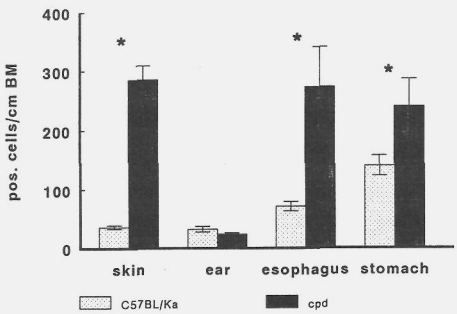

Figure 6. *Number of BrdU-positive cells per cm basement membrane in C57BL/Ka and cpd mice. The bars represent mean ± SEM of five mice. * $P < 0.05$.*

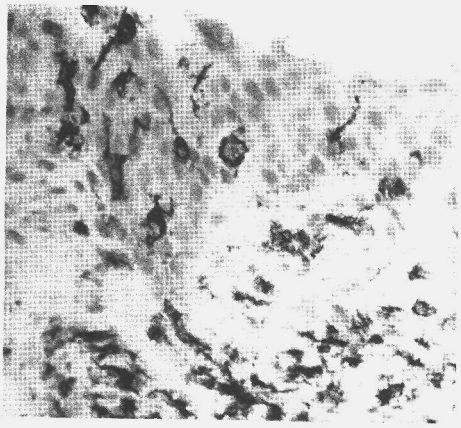

Figure 7. *Ia-positive cells in the epidermis and dermis of a cpd mouse (indirect immunoperoxidase with hematoxylin counterstain, 300×).*

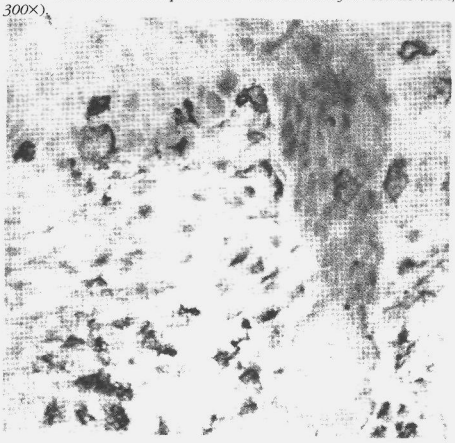

Figure 8. *Mac-1-positive cells in the skin of a cpd mouse (indirect immunoperoxidase with hematoxylin counterstain, 300×).*

(not shown). CD3-positive cells (T lymphocytes) were occasionally observed in the epidermis and were absent from the dermis of normal syngeneic control mice. Few CD3-positive cells were present in the epidermis and dermis of cpd mice.

ICAM-1 was weakly expressed on endothelial cells of blood vessels in the deep dermis of normal skin. In cpd mice, ICAM-1 was strongly expressed on basal keratinocytes and on endothelial cells of dermal blood vessels (Figure 9). The intensity of staining in the epidermis decreased rapidly toward the stratum corneum. Lack of staining with anti-mouse IgG antiserum indicated that there was no

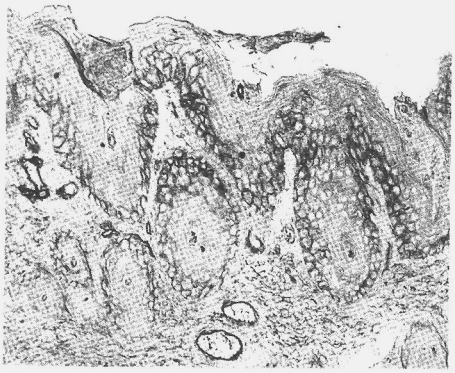

Figure 9. *ICAM-1 expression on keratinocytes and endothelial cells in the skin of a cpd mouse (indirect immunoperoxidase with hematoxylin counterstain, 156×).*

deposition of immunoglobulins or immune complexes in the epidermis and basement membrane.

Mast Cells

In normal skin, a few mast cells (40.6 ± 7.8/mm^2) were present in the dermis as revealed by toluidine blue staining. Their number was significantly increased in the affected skin of 6- to 8-week-old cpd mice (171.3 ± 27.1/mm^2) and even further increased in the 28- to 30-week-old mice (594 ± 110.4/mm^2). Although the mast cells were often in close apposition to the basal membrane, they were never observed in the epidermis.

Corticosteroid and CsA Treatment

Systemic treatment of cpd mice with 5 and 10 mg/kg triamcinolone resulted in a considerable improvement within 4 weeks. The skin became much thinner, there was no scaling, and the mice did not appear pruritic. Light microscopically, hyperkeratosis and parakeratosis were mostly absent. The thickness and BrdU incorporation of the epidermis and esophageal and forestomach epithelium were significantly reduced (Figure 10). Single cell death of keratinocytes was only rarely present. Inflammatory cells had disappeared from the epidermis, were markedly reduced in the dermis of mice treated with 5 mg/kg triamcinolone, and virtually absent from the dermis of mice treated with 10 mg/kg. This was confirmed immunohistochemically by the reduced number of cells staining for Ia-positive and Mac-1-positive cells in the 5-mg/kg group and absence of such cells in the 10-mg/kg group. ICAM-1

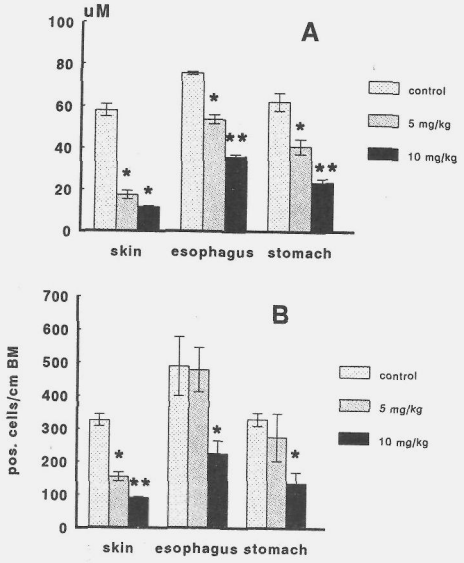

Figure 10. *Effect of corticosteroid (triamcinolone)* treatment on the epidermal and epithelial thickness (A) *and on the number of BrdU-positive cells* (B). *Bars indicate mean ± SEM of four mice. * $P < 0.05$ (treatment vs. control), ** $P < 0.05$ (5 mg/kg vs. 10 mg/kg).*

expression on keratinocytes was greatly reduced, but still high on endothelial cells in the 5-mg/kg triamcinolone group. ICAM-1 was virtually absent from endothelial cells and keratinocytes in the 10-mg/kg group. Inflammatory lesions in the internal organs (esophagus, forestomach, liver, lung, joints) of the 5-mg/kg group were diminished, and they had completely disappeared in the 10-mg/kg group.

Systemic treatment with 15 and 100 mg/kg CsA for 4 weeks did not result in any noticeable improvement of the skin condition either clinically or histologically, except for increase of hair growth over the affected skin. The latter is a well-established effect of CsA treatment[7] and indicates that the concentration of CsA in the skin had reached adequate levels.

Hemopoietic Cell Transfer

Injection of a combination of bone marrow cells and spleen cells or a large number of only spleen cells from cpd mice into lethally irradiated or neonatal syngeneic C57BL/Ka mice did not result in the appearance of cpd lesions for up to 2 months after transfer. This indicates that the disease does not result from abnormal function of mature T cells or cells of the myelomonocytic lineage.

Discussion

The mutation in the C57BL/Ka mouse that is the subject of this report results in a unique combination of proliferative and inflammatory lesions in the skin and proximal digestive tract and inflammatory reactions in a limited number of internal organs. The lesions develop at a young age and persist throughout the allowed lifespan of the animals. The chronic nature of the lesions and their persistence should make this a valuable model to study various aspects of chronic inflammatory disease and to evaluate the efficacy of novel therapeutic strategies.

Both spontaneously occurring and experimentally induced diseases with some resemblance to the cpd lesions have been described in mice. Two mouse mutants with skin lesions characterized by epidermal hyperproliferation and scaling are the asebia (*ab/ab*) and the flaky skin (*fsn/fsn*) mouse. The asebia mouse has hypoplastic sebaceous glands and develops a dermatitis with progressive alopecia and scaling.[8] Rupture of lipid-laden macrophages that have infiltrated the dermis is thought to be the cause of the inflammatory reaction and finally results in epidermal hyperproliferation.[9] The nature of the inflammatory reaction and the time-course of lesion development are clearly different from the cpd mouse. The flaky skin mouse is characterized by progressive hypochromic anemia and hyperproliferative dermatitis.[2] The published information on this mutant is still limited, but it seems to be different from the cpd mouse, because the infiltrating cells are mostly mononuclear and because of the absence of follicular keratosis and single cell death of keratinocytes. Various transgenic mice have been described with proliferative skin lesions.[10–13] The distribution and light microscopy of these lesions do not resemble the skin lesions of the cpd mouse. A diet deficient in essential fatty acids causes dermatitis characterized by hyperproliferation and scaling.[14] However, in contrast to the skin of cpd mice, there is marked hypergranulosis and absence of parakeratosis and single cell death of keratinocytes. Finally, a spontaneous, idiopathic dermatitis occurs in middle-aged and aged mice of the C57BL/6 and related background.[15] Indeed, such dermatitis also occurs in our colony of aged C57BL/Ka mice (HogenEsch and Zurcher, unpublished observations). However, this disease is different from the cpd mutation, as it only emerges in older mice, is more ulcerative in nature, and has a different distribution.

The most common chronic dermatitis in man that is characterized by hyperproliferation of keratinocytes and epidermal infiltration of neutrophils is psoriasis.[16] The pathogenesis of psoriasis has not been resolved and is still the subject of intense research efforts. Virtually every parameter that has been studied was found to be altered, and presumably many of them are epiphenomena.[16,17] Recent studies have focused on the role of T lymphocytes and keratinocytes.[18,19] Autoreactivity of T lymphocytes to some epidermal component may be the initiating event, and these T cells may activate keratinocytes to secrete a variety of cytokines and growth factors.[18] Persistent release of autocrine growth factors and insensitivity to growth-inhibitory factors of keratinocytes may cause hyperproliferation as well as infiltration of neutrophils and dermal inflammation.[19] Still another hypothesis locates the primary defect of psoriasis in fibroblasts.[20] This is based on the observations that fibroblasts from psoriatic lesions, but not from control skin, induce hyperproliferation of keratinocytes under certain experimental conditions [20,21] The lesions of the cpd mouse differ from psoriatic lesions in several respects, such as the paucity of T lymphocytes in the dermal infiltrate and the presence of follicular keratosis and dyskeratosis. Furthermore, the cpd lesions did not regress upon CsA treatment in contrast to the efficacy of CsA in the treatment of psoriasis. Although the pathogenesis of cpd seems to be different from the pathogenesis of psoriasis, further studies of the cpd mouse may provide data relevant to certain aspects of psoriasis, such as the mechanism of neutrophil infiltration into the epidermis and the role of cytokines and their receptors in the inflammatory process.

Keratinocytes can be induced to secrete a variety of pro-inflammatory cytokines. In this manner, they can elicit an inflammatory reaction in the underlying dermis upon injury.[22] Conversely, proliferation and differentiation of epithelia is, at least in part, determined by the subepithelial mesenchymal tissue, and dermatitis is frequently associated with hyperproliferation of keratinocytes.[23] Thus, the primary cellular component responsible for both abnormal epithelial proliferation and subepithelial inflammation may reside either in the epithelium or in the subepithelial connective tissue. Failure to transfer the cpd lesions using hemopoietic cells from spleen or bone marrow indicates that such cells do not play a primary role in the pathogenesis. It is unlikely that the failure to transfer adoptively the disease with spleen and bone marrow cells was caused by rejection of the grafted cells. The syngeneic donor-recipient combination obviously does not allow a verification of the chimeric status, but the dose of total body irradiation and the numbers of cells in the graft exclude the possibility of endogenous hemopoietic reconstitution in these experiments.[24] The absence of immunoglobulin deposition in the epidermis or basement membrane, the paucity of T lymphocytes, and the lack of response to CsA further indicate that (auto)immune phenomena are not primary mechanisms in the development of cpd lesions Skin grafts using cross-recombinations of dermis and epidermis of cpd and syngeneic control mice may determine whether the cell that is primarily responsible for the cpd lesions resides in the dermis or epidermis. Although the skin lesions of the cpd mouse are the most striking and clinically obvious, there were also inflammatory foci in the lungs, liver, and in joint-associated tissues. These lesions could represent secondary phenomena resulting from massive cytokine release from keratinocytes, or they may point toward a generalized systemic condition.

Immunohistochemical studies of cpd mice revealed an increase of Ia-positive cells in the epidermis and lack of Ia expression on keratinocytes. The precise nature of the Ia-positive cells is not known. The round cells in the basal layer lacked the dendritic cell-specific antigen recognized by NLDC-145, suggesting that these cells are not Langerhans cells and are probably macrophages. In psoriasis, an increase of major histocompatibility class II-positive cells has been observed,[25] whereas the number of true Langerhans cells has been reported to be either unaltered[25] or decreased.[26]

Most inflammatory cells in the dermis and epidermis stained strongly for Mac-1 and weakly for LFA-1. This is consistent with these cells being macrophages and neutrophils. Both LFA-1 and Mac-1 are ligands of ICAM-1.[27–29] Increased ICAM-1 expression on keratinocytes and endothelial cells is common in many inflammatory skin diseases[30,31] and was also observed in the cpd lesions. ICAM-1 LFA-1 and ICAM-1 Mac-1 interactions may thus play a role in the infiltration of macrophages and neutrophils in these skin lesions. The expression of ICAM-1 is up-regulated by interferon-γ, interleukin-1, and tumor necrosis factor-α (TNF-α), with combinations of these cytokines often having synergistic effects.[27,32] The absence of Ia expression on keratinocytes and endothelial cells suggests that interferon-γ does not play a role in this inflammatory condition, because

interferon-γ is the major inducer of Ia expression on these cells.[33,34] This is consistent with the small percentage of T lymphocytes in the inflammatory infiltrate. The gp100 antigen recognized by MEL-14 is present on circulating neutrophils and plays a role in the initial adherence to endothelial cells.[35] Upon adhesion, the MEL-14 antigen is shed from the cell surface, thus permitting the penetration of the endothelium, and extravasated neutrophils consequently have a low expression of MEL-14 antigen.[36] This is consistent with our failure to find positive staining with MEL-14 on the surface of neutrophils in the dermis and epidermis. Studies to evaluate the role of these adhesion molecules in this disease by *in vivo* treatment with monoclonal antibodies are under way.

Single cell death of keratinocytes was variably present and often prominent in the lesions of cpd mice. Single cell death of keratinocytes in combination with mild epidermal hyperplasia is a prominent feature of graft-versus-host disease.[37,38] Similar changes can be induced by subcutaneous infusion of TNF-α in susceptible strains of mice,[39] and, indeed, TNF-α has been shown to be an effector mechanism in graft-versus-host disease.[40] Murine keratinocytes can secrete TNF-α under certain conditions,[41] but we have not been able to detect any TNF-α activity in supernatants of cultured keratinocytes from normal or cpd skin *in vitro* (Gijbels and HogenEsch, unpublished observations). Other possible sources of TNF-α in cpd skin include infiltrating macrophages and dermal mast cells.[42]

We observed an increased number of dermal mast cells. Increased numbers of mast cells have also been reported in idiopathic and experimentally induced chronic dermatitis in C57BL/6-related mice,[43] in asebia mice,[9] and in psoriasis in man.[44] Proliferation of mast cells seems to be a common feature of chronic dermatitis, and these cells may play a role in the pathogenesis of the disease when present in such large numbers. The cpd mice may provide a useful model to gain more insight into the mechanisms regulating dermal mast cell proliferation *in vivo*.

The proliferation and inflammation associated with the cpd mutation were responsive to high-dose corticosteroid treatment. Corticosteroids are strong suppressants of the function of various inflammatory cells, including neutrophils and macrophages, and of the production of several cytokines.[45] The decrease of epithelial thickness after corticosteroid treatment could in part be explained by a decrease of proliferation assessed by BrdU incorporation. The reduced rate of proliferation could be the result of the attenuation of dermal inflammation, thereby removing the stimulus for epidermal proliferation. In addition, corticosteroids are known to have a direct antiproliferative effect on keratinocytes.[46] Corticosteroids also cause a shrinking of cellular volume of keratinocytes,[47] thus providing an additional explanation for the reduction of epithelial thickness.

In summary, we have described a new mouse mutant with chronic proliferative inflammation of most orthokeratinizing stratified squamous cell epithelia. The skin lesions undergo nearly complete regression upon treatment with corticosteroids for 4 weeks, but do not respond to CsA. This mouse should be a useful model to study chronic inflammatory reactions in general and chronic dermatitis with hyperproliferation of keratinocytes in particular.

Acknowledgments

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Chapter 3

Ultrastructure of epidermis of mice with chronic proliferative dermatitis (*cpdm/cpdm*)

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Chapter 3

Ultrastructure of Epidermis of Mice with Chronic Proliferative Dermatitis

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C57BL/Ka mice with chronic proliferative dermatitis (*cpdm/cpdm*) develop chronic persistent skin lesions characterized by epidermal hyperplasia, infiltration by granulocytes and macrophages, and vascular dilatation. Similar lesions are present in other orthokeratotic epithelia in affected mice, in particular the esophagus and forestomach. Here, we report on further characterization of epidermal hyperplasia and the granulocytes. Keratinocytes of lesional skin, but not of normal skin, show round and electron-dense mitochondrial inclusions that are present in all layers of the epidermis. Similar inclusions are also present in the esophagus and forestomach of affected mice. There appears to be a direct relation between the presence of intramitochondrial inclusions and epidermal hyperplasia in the mouse. Furthermore, the presence of keratinocyte-derived apoptotic bodies in the epidermis, esophagus, and forestomach was frequently observed in the lesions, which is consistent with previous light microscopic observations of single cell death of keratinocytes. The granulocytes present in the skin, esophagus, and forestomach were mainly eosinophils. There were widespread gaps observed in the lamina densa in the epidermis that were mostly directly associated with dermal or epidermal eosinophils. This type of gap is also observed in psoriasiform diseases in humans. This electron microscopic study demonstrated that this mouse model should be useful to screen potential therapeutic strategies for psoriasiform and other inflammatory skin disorders.

Keywords apoptosis, chronic proliferative dermatitis, epidermis, inclusions, mice, ultrastructure

We have recently described a mouse mutant with chronic proliferative dermatitis (*cpdm/cpdm*) on a C57BL/Ka background [1]. The skin lesions in this *cpdm/cpdm* mouse appeared at 5 to 6 weeks of age, initially involving the skin of the chest and dorsal neck. Macroscopically, these lesions were characterized by erythema, severe hair loss, and mild scaling. The lesions gradually expanded to involve most of the body except for the ears, tail, and footpads. Microscopically, the lesions were characterized by hyper- and parakeratosis, acanthosis, single cell death of keratinocytes, vascular proliferation, and infiltration of dermis and epidermis by granulocytes and macrophages. Similar lesions were also present in other orthokeratotic stratified squamous cell epithelia such as the esophagus and forestomach. In addition, there were mixed cellular infiltrates in the liver, lung, and perisynovial connective tissue. Lymphoid tissues were atrophic, whereas marked extramedullary myelopoiesis accounted for the enlargement of the spleen. The lesions regressed upon systemic treatment with corticosteroids [1].

Two other mouse mutants, the asebia (*ab/ab*) mouse and the flaky skin (*fsn/fsn*) mouse, also have skin lesions characterized by epidermal hyperproliferation and scaling. The inflammatory reaction in the *ab/ab* mouse is of a different nature, with the infiltrating cells being mostly mononuclear. The cause of the inflammatory reaction in the *ab/ab* mouse is thought to be a consequence of the rupture of lipid-laden macrophages that have infiltrated the dermis [2]. It has not yet been proven whether this mouse model is useful as a model for chronic dermatitis in humans [3]. The flaky skin mouse mutation superficially resembles the *cpdm/cpdm* mouse mutation but single cell death and follicular keratosis are absent [4]. Various transgenic mice have been described with proliferative

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skin lesions but the distribution and light microscopy of these lesions do not resemble the skin lesions of the *cpdm/cpdm* mouse [5–9].

The *cpdm/cpdm* mouse may be a useful model to study spontaneous chronic proliferative dermatitis and may thus serve as a useful complement to the more acute models. In order to establish the true value of the *cpdm/cpdm* mouse as a model for chronic proliferative dermatitis, further characterization of its lesions is essential. In this study, we report on electron microscopic studies of *cpdm/cpdm* mouse skin.

MATERIALS AND METHODS

Animals

The cpd mutation (*cpdm/cpdm*) arose spontaneously in the colony of C57BL/KaLwRij mice. *Cpdm/cpdm* mice were bred in the specific-pathogen-free breeding facilities of TNO Rijswijk, The Netherlands. They were housed in Macrolon cages and provided pelleted food and acidified, bottled drinking water ad libitum. The microbiologic status was checked regularly by routine serologic, bacteriologic, and histologic procedures.

Male and female mice aged 6 to 26 weeks were used. They were clipped under ether inhalation when necessary (control and unaffected skin samples) and subsequently killed by cervical dislocation. Control mice were C57BL/KaLwRij mice of the same age derived from the same colony.

Tissue Preparation

Strips of skin samples (5 × 10 mm) were immersed immediately in cold fixative consisting of 2.5% glutaraldehyde, 2% paraformaldehyde in 0.15 M sodium cacodylate buffer (pH 7.4). Pieces of the strips 1 × 2 mm were transferred to fresh fixative for at least 48 hours. After a brief rinse in sodium cacodylate buffer, tissue specimens were postfixed in 1% sodium cacodylate-buffered osmium tetroxide, dehydrated through a graded series of ethanol, and embedded in resin LX-112 (Ladd Research Industries, Burlington, VT). The specimens were positioned with the epidermal plane perpendicular to the cutting surface of the mold. Semithick sections (0.5-μm) were stained with toluidine blue for light microscopy. Properly oriented blocks, with full-thickness epidermis and dermis present, were used for ultrathin sections. Ultrathin sections (100-nm) were stained with uranyl acetate and lead citrate and examined in a Philips 410 electron microscope.

RESULTS

Cellular Ultrastructure of Control Mice

Keratinocytes of the control mice had a normal appearance. They had a modest number of filaments, numerous desmosomes, abundant rough endoplasmic reticulum, normal mitochondria, and an occasional cytoplasmatic lipid droplet. Hemidesmosomes were present along the basal cell membrane of basal keratinocytes. Keratinocytes in the stratum corneum had electron-dense, irregularly shaped keratohyalin granules. Other cells in the epidermis such as Langerhans cells and lymphoid cells could be distinguished easily from the keratinocytes by their less electron-dense cytoplasm and the absence of desmosomes. The occasional presence of Birbeck granules allowed identification as Langerhans cells. However, only one or two granules were observed per Langerhans cell in a particular section, and it is likely that several additional non-keratinocyte cells were actually Langerhans cells. The ultrastructure of keratinocytes in the esophageal and forestomach epithelium was essentially the same as that of the epidermal keratinocytes. Langerhans and lymphoid cells were also present in the esophagus.

Cellular Ultrastructure of *cpdm/cpdm* Mice

The interfollicular epidermis of affected skin was thickened as a result of orthokeratotic hyperkeratosis alternating with parakeratotic mounds, and epidermal hyperplasia. Intramitochondrial inclusions were observed exclusively in the keratinocytes of the affected epidermis, esophagus, and forestomach of *cpdm/cpdm* mice and not in other cell types. The inclusions were round with a slightly fuzzy border, variable in size, and of moderate to high electron density (Figure 1a). Small inclusions were often less electron-dense than the large inclusions (Figure 1b). One to four inclusions were present in a mitochondrium per section. The inclusions were observed in the basal and spinous cell layers, but the number of mitochondria with inclusions and the number of inclusions per mitochondrium appeared to increase toward the stratum corneum. The inclusions occasionally pushed the mitochondrial cristae aside; there was no evidence of cristae fragmentation (Figure 1b). Other organelles and the cytoplasm did not contain similar inclusions. The mitochondrial inclusions were only observed in keratinocytes; they were not present in non-keratinocytic epidermal cells or in any cells in the dermis. Keratinocytes of non-hyperplastic and non-inflamed skin, such as that of the ear and noninvolved skin of the body of young mice, did not have such alterations.

Apoptotic bodies, characterized by nuclear fragments with condensed chromatin surrounded by cytoplasmic remains with intact organelles, were frequently observed in the keratinocytes of the epidermis, esophagus, and forestomach of cpd lesions (Figure 1c). They were remnants of keratinocytes as evidenced by the preservation of at least some desmosomes and filaments. The mitochondria often contained the electron-dense intramitochondrial inclusions described above. Smaller apoptotic bodies were phagocytized by adjacent

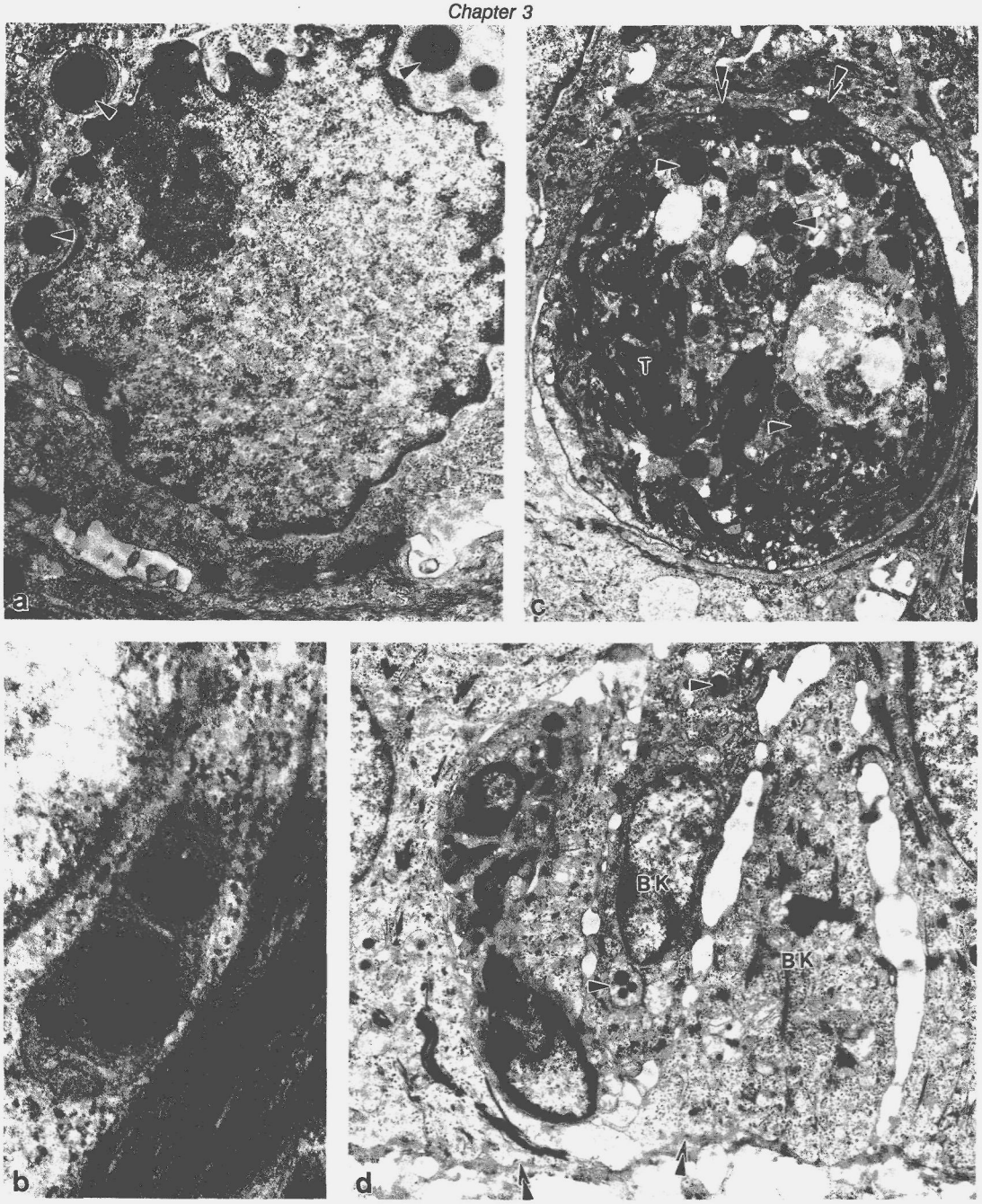

FIG. 1 (a) *Electron micrograph of a keratinocyte of the skin of a* cpdm/cpdm *mouse with intramitochondrial inclusions* (arrowheads), *×22,000.* (b) *Intact cristae within the mitochondria can be seen at higher magnification. Note the variation in size and electron density of these inclusions, ×75,000.* (c) *Electron micrograph of an apoptotic keratinocyte with still-intact intramitochondrial inclusions* (arrowheads). *Remnants of desmosomes* (double arrowheads) *and tonofilaments* (T) *are present, ×10,750.* (d) *Low-power electron micrograph of basal layer of the esophagus. Keratinocytes show intramitochondrial inclusions* (arrowheads). *Between the basal keratinocytes* (BK) *an eosinophil is present. Note that the basal lamina is underbroken* (between double arrowheads). *The presence of the eosinophil is in close association with absence of the basal lamina, ×8,000.*

keratinocytes. There were no apoptotic bodies observed in the keratinocytes of nonaffected skin.

There was a widespread occurrence of gaps observed in the lamina densa of the epidermis, esophagus, and forestomach. These gaps were mostly directly associated with dermal or epidermal eosinophils (Figure 1d). The lamina densa was always closed in the nonaffected skin.

The number of other cells in the epidermis, esophagus, and forestomach, such as Langerhans or lymphoid cells, was not increased and showed a normal appearance when compared with normal control mice.

Granulocytes in Control and *cpdm/cpdm* Mice

The skin and esophagus of the control animals contained almost no granulocytes. Outside the pustules and microabscesses, few granulocytes were found in the epidermis of the *cpdm/cpdm* mouse but the dermis was infiltrated by a mixed population of inflammatory cells, predominantly granulocytes and macrophages. Electron microscopic examination revealed that these granulocytes were mainly eosinophils. The eosinophils in the skin were found most frequently just beneath the basal membrane in the dermis. This is in contrast to the esophagus and forestomach where many eosinophils were observed not only in the lamina propria but also in the epithelium (Figure 1d). In addition, some neutrophils were seen in the lamina propria.

DISCUSSION

The results of this and our previous studies indicate that the keratinocyte alterations in the skin lesions of *cpdm/cpdm* mice consist of hyperplasia, intramitochondrial inclusions, and apoptosis [1]. These alterations are not limited to the keratinocytes of the skin, but are also present in the epithelial cells of the esophagus and forestomach. The epithelium of the esophagus and forestomach of mice is orthokeratotic keratinizing, similar to that of the epidermis. Although there are more known mouse models with intramitochondrial inclusions, this triad of keratinocyte alterations appears to be unique to this inherited mouse model. We have been unable to find any reports on the occurrence of these inclusions in the mitochondria of epithelial cells of the esophagus and forestomach. The flaky skin mouse (*fsn/fsn*), a possible mouse model for psoriasis, has lesions characterized by epidermal hyperplasia and intramitochondrial inclusions [4]. The epidermis of transforming growth factor (TGF)-α transgenic mice resulted in a thicker, hyperproliferative epidermis with cytoplasmatic inclusions in the keratinocytes [8].

The type of intramitochondrial inclusions described here have been reported in murine keratinocytes after topical application of the tumor promotor agents croton oil [10], 12-0-tetradecanoyl-phorbol-13-acetate (TPA) [11, 12], mezerein, and n-dodecane [12]. These compounds also cause hyperplasia of the epidermis. TPA and other phorbolesters interfere with mitochondrial respiration in mouse fibroblasts and it has been suggested that this may be related to the formation of intramitochondrial inclusions [13]. Whether the inclusions interfere with mitochondrial function has not been established.

Treatment of the back of mouse with the carcinogens 7,12-dimethyl-benz[a]anthracene (DMBA) and 3-methylcholanthrene also gave electron-dense inclusion bodies within the mitochondria and epidermal hyperplasia [14]. A single study has reported the presence of intramitochondrial inclusions in the keratinocytes of mice that had been exposed to ultraviolet light over a 6-month period [15]. The ultraviolet radiation caused hyperplasia of the epidermis. Because of the involvement of free radicals in ultraviolet light–induced tissue damage, it was suggested that free radical–induced lipid peroxidation of the inner mitochondrial membrane may result in the formation of the inclusions. There seems to be a direct relationship between the appearance of intramitochondrial inclusions and epidermal hyperplasia in the mouse. Only the *ab/ab* mouse had a hyperplastic epidermis without inclusions [2]. In these mice, the rupture of crystal-laden macrophages, which subsequently induces the release of a variety of inflammatory mediators and chemoattractants, has been proposed as the cause of epidermal hyperplasia [2, 3]. Therefore, the *ab/ab* mouse has not proven to be useful as a model for hyperproliferative diseases [3].

Spherical electron-dense intramitochondrial inclusions have been described in various cell types of several other animal species, including the brown fat cells of rats and some human tumors [16, 17]. Although their chemical composition has not been determined, they are thought to resemble lipid inclusions. In these other species there was no relationship observed between intramitochondrial inclusions and epidermal hyperplasia.

Apoptotic bodies as observed in the *cpdm/cpdm* mouse were not found in other mouse mutants such as the *fsn/fsn* mouse [4], *ab/ab* mouse [2], and TGF-α transgenic mouse [8, 9].

Apoptosis has been recognized in a variety of skin diseases in humans, including lichen planus, fixed drug eruption, graft-versus-host disease, Bowen's disease, after ultraviolet irradiation in so-called sunburn cells [18–21], and it also plays a role in the involution of the hair follicle during the catagen phase [21]. Apoptosis is a controlled event, regulated by the sequential activation and expression of certain genes, that eventually results in cell death. Some of the genes that are activated in the apoptotic process are similar to the genes that play a role in cell proliferation. This has led to the suggestion that the replicative cycle and apoptosis initially share a common pathway, which at some point diverges to result in either cell proliferation or cell death. TGF-β_1 has been identified as a factor that may shift the balance from proliferation to apoptosis in certain epithelial tissues [21, 22].

It seems unlikely that the intramitochondrial inclusions and keratinocyte apoptosis share the same pathogenesis since these changes have not been observed in the same studies.

Gaps in the lamina densa, like those we have observed in the cpd-like lesions, were also seen in psoriasis and in psoriasiform diseases in humans. It is suggested that these basement membrane gaps may be the nonspecific consequence of the inflammatory process [23, 24]. Proteolytic enzymes released from macrophages, neutrophils, and endothelial cells are capable of causing basement membrane dissolutions and may be responsible for gaps in the basal lamina. Focal areas with an inapparent basement membrane were also observed in the *fsn/fsn* mouse [4].

The exact role played by the eosinophils in the cpd lesions is a subject for further investigation. Serum IgE levels of *cpdm/cpdm* mice are not elevated with respect to control C57BL/Ka mice (Gijbels, unpublished data). This suggests that the disease is not caused by an allergic response. Dermatitis herpetiformis in humans is characterized by eosinophil infiltration, but the disease in *cpdm/cpdm* mice differs from the human disease by the absence of immune complex deposition along the basement membrane and a different histologic pattern. Furthermore, the bone marrow transplantation studies disclosed that the lesion was not transferable by hemopoietic cell transfer [1]. Therefore, the lesions are most probably not due to an immunologic dysfunction (e.g., allergic reaction). Full-thickness *cpdm/cpdm* skin transplants to normal C57BL/Ka recipients remained actively inflamed for several months after transplantation (unpublished data). Therefore, it seems likely that locally produced cytokines are responsible for the mobilization of eosinophils. A possibility could be an increased production of interleukin-5. Eosinophils migrate into tissues where interleukin-5 is produced. Therefore, the exact nature and source of chemokines that act as eosinophil attractants remains to be determined.

In conclusion, we described electron microscopy of the *cpdm/cpdm* mouse from which it appears that this mouse should be a useful model to screen potential therapeutic strategies for psoriasiform and other chronic inflammatory skin disorders. Furthermore, detailed studies on the mechanisms underlying this chronic inflammatory skin disorder will allow the development and evaluation of novel therapeutic strategies.

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Chapter 4

Chronic Proliferative Dermatitis in mice. Neutrophil-endothelium interactions and the role of adhesion molecules

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Chronic Proliferative Dermatitis in Mice: Neutrophil-Endothelium Interactions and the Role of Adhesion Molecules

Key Words
Hyperproliferation
Skin
Neutrophil
Adhesion molecules

Abstract
The murine chronic proliferative dermatitis mutation (*cpdm/cpdm*) is characterized by epidermal hyperplasia and hyperproliferation of ventral and dorsal skin sites. The expression of endothelium-associated adhesion molecules was studied in combination with the binding capacity of various cell types on frozen sections of the affected skin. In correlation with the relative absence of lymphocytes in the *cpdm/cpdm* skin no lymphocyte binding could be observed, but avid adhesion of neutrophils was seen. Binding of neutrophils could be blocked with antibodies against L-selectin, LFA-1, CR3 and anti ICAM-1. No expression of vascular addressins or E-selectin on endothelium in the dermis was found. The *cpdm/cpdm* mutation has therefore characteristics of a psoriasis-like as well as a more generalized inflammatory skin condition.

Introduction

Chronic proliferative dermatitis (*cpdm/cpdm*) has recently been discovered as a new mutation in the C57BL/Ka mouse strain [1]. This chronic persistent dermatitis leads to erythema, severe hair loss, and mild scaling on the ventral and dorsal side of the body beginning at the age of 5 weeks, which is microscopically characterized by epidermal hyperplasia caused by hyperproliferation of keratinocytes. Parakeratosis, acanthosis, and apoptosis of keratinocytes are observed [2]. In addition infiltrations in the dermis can be found of mast cells, macrophages and granulocytes, being mainly eosinophils [2]. Only a small percentage of the inflammatory infiltrate consists of T lymphocytes. There is extensive proliferation and dilatation of dermal capillaries. Infiltration of the skin with eosinophils is already present at 1 week of age, at which time macroscopic lesions are not yet present [3]. Six-week-old *cpdm/cpdm* mice had an increased number of mast cells [1, 3]. At the age of 1 week, these mast cells were weakly IgE-positive whereas 3- and 6-week old *cpdm/cpdm* mice showed strongly IgE-positive mast cells [3]. At 8 weeks, serum IgE levels were decreased compared to the control animals [3]. E-selectin was expressed on endothelial cells of blood vessels in the dermis of *cpdm/cpdm* mice on the age of 1, 3 and 6 weeks while no P-selectin was observed in these mice [3]. Based on these observations this model may serve as a relevant alternative to study chronic inflammatory hyperproliferative skin disease and specific aspects of allergic inflammatory skin disease, such as eosinophil tissue infiltration, leukocyte-endothelial cell interactions and mast cell proliferation.

Here we have in more detail studied the expression of adhesion molecules on the skin microvasculature and leu-

kocytes present in the more chronic skin lesions of the *cpdm/cpdm* mice. Using an in vitro adhesion assay, the functional significance of this expression was determined.

Materials and Methods

Animals

C57Bl/KaLawRij mice were bred and maintained in our colonies as described previously [1]. BALB/c mice and C57Bl/6 mice were purchased from Harlan-CPB (Zeist, The Netherlands). They were kept under conventional conditions with water and food ad libitum. All animal experiments were performed under authorization of the Ethical Commitee for Animal Experiments of the Vrije Universiteit in accordance with the Dutch law on animal experiments.

Immunohistochemistry

Blocks of skin tissue from affected sites or control animals were frozen in liquid nitrogen and stored at −20 °C. Cryostat sections of 8- to 10-μm thickness were fixed in acetone for 10 min and air-dried for at least 30 min. After washing in 0.01 *M* PBS (pH 7.4) the sections were incubated for 1 h with the various monoclonal antibodies as indicated, followed by a peroxidase-conjugated swine antirat Ig (DAKO, Glostrup, Denmark) for 30 min. After washing again, the peroxidase activity was demonstrated with 3,3′-diaminobenzidine-tetrahydrochloride (Sigma) in 0.5 mg/ml Tris HCl buffer (pH 7.6) containing 0.01% H_2O_2.

Monoclonal Antibodies

The following rat antimouse monoclonal antibodies were used: MECA 79 and MECA 367 (vascular addressins [4, 5]; anti-ICAM-1 (CRL1878, ATCC), anti-VCAM-1 ([6]; kindly provided by Dr P.W. Kincade); anti-E-selectin ([7]; kindly provided by Dr B. Wolitzky); MECA 325 (specific for HEV in lymphoid tissues, but does not recognize active ligands involved in leukocyte adhesion [8]; MECA 32 (pan endothelium marker; a kind gift of Dr E.C. Butcher, Stanford, Calif., USA); BR2 (panendothelium marker, a kind gift of Dr Rupert Hallman, Erlangen, Germany); 5C6 and M1/70 against the murine C3 receptor (CD11b/CD18 [9]); anti-LFA-1 (CD11a/CD18; clone *H154.163*, directed against the a chain [10]); anti-L-selectin (CD62L; MEL-14 [11]); RB6 8C5 (antigranulocyte marker, a kind gift of Dr R. Coffman, DNAX, Calif., USA).

Isolation of Neutrophilic Granulocytes

Mouse neutrophils were isolated from femurs according to Lewinsohn et al. [12]. Bone marrow suspensions were washed and contaminating erythrocytes were lysed with NH_4Cl. Adherent cells were removed by culturing the cells for 1 h at 37 °C and the remaining nonadherent fraction was depleted from lymphocytes using magnetic beads in combination with a cocktail of anti T and B cell antibodies (anti-CD4, anti-CD8 and anti-κ light chain). The suspension which was obtained this way consisted of >95% neutrophilic granulocytes as judged by morphology and staining with the RB6 8C5 antibody. FACS analysis on isolated granulocytes was performed using a FACScan (Becton Dickinson, Mountain View, Calif., USA).

Adherence Assay on Skin Sections

Cell adherence to skin sections in vitro was assayed according to the method of Butcher et al. [13], developed for lymphocyte adherence to high endothelial venules in lymphoid organs. Briefly, mixtures of cells as indicated in Results were incubated at $1 \cdot 10^6$ cells/100 μl at 4 °C on unfixed frozen sections of skin under constant rotation for 30 min. Determination of the cell binding capacity was assessed microscopically either directly by counting the number of bound cells under dark-field illumination, or by comparison with an internal standard population. To this isolated granulocytes were labeled with rhodamine (1–2 g TRITC/10^6 cells/ml) and mixed with equal amounts of cells to be tested. The mixture was then immediately added onto unfixed, frozen tissue sections, incubated for 30 min and fixed in 1% formaldehyde. The number of cells bound was determined under dark-field illumination, and by changing to epi-illumination the ratio of cells to be tested and rhodaminated internal standard cells was assessed. The ratio of labeled over unlabeled cells in the original suspension was then used to calculate the specific adherence ratio (SAR): SAR = ratio (sample cells/internal standard cells) bound to HEV divided by the ratio (sample cells/internal standard cells) of the incubation mixture. This is a direct measure for the capacity of a given population of cells to adhere in comparison with the internal standard cells. For a full description of the calculation method and statistics, see the original paper by Stevens et al. [14].

Results

Expression of Adhesion Molecules in the Skin of the cpdm/cpdm *Mouse*

This study was undertaken to investigate the presence of various adhesion-associated endothelial markers on the microvasculature of the *cpdm/cpdm* skin and to correlate the expression with the inflammatory condition. Table 1 summarizes the markers that were investigated and the expression that was found in the skin of *cpdm/cpdm* and control animals.

Table 1. Expression of endothelial cell markers on small vessels in dermis of *cpdm/cpdm* and control skin

	cpdm/cpdm	Control
E-selectin	–	–
VCAM-1	–	–
ICAM-1	+++[a]	+[a]
BR2	+++[b]	++
MECA 32	+++	++
MECA 325	++[c]	+
MECA 79	–	–
MECA 367	–	–

[a] ICAM-1 was also expressed on epithelial keratinocytes in epidermis.
[b] Prominent networks in papillary dermis.
[c] On some subdermal vessels.

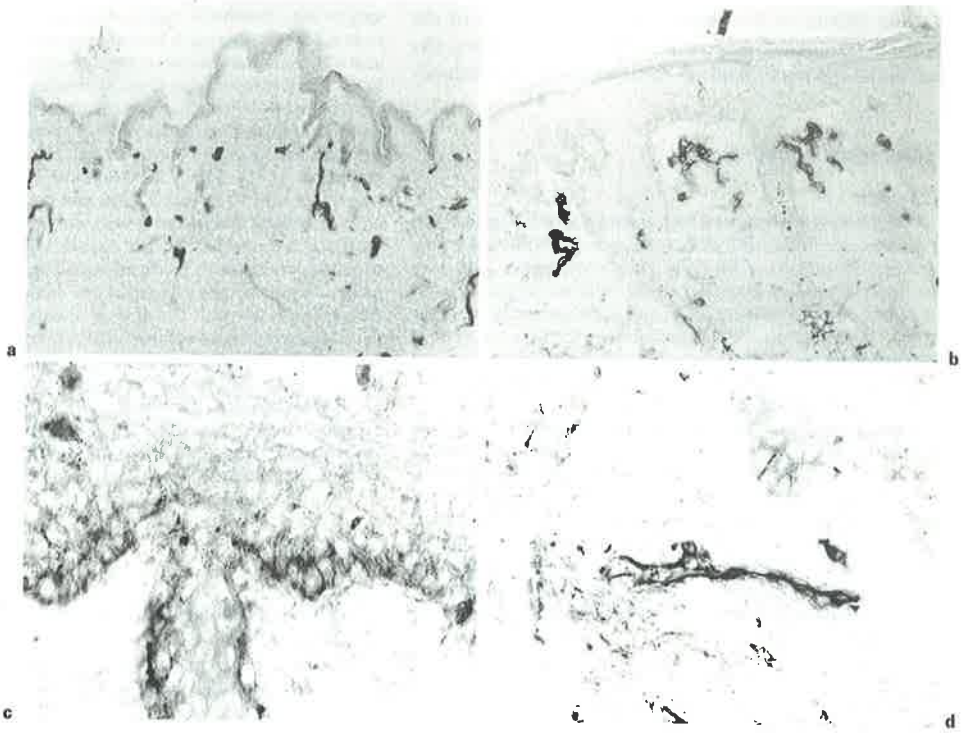

Fig. 1. Staining of skin sections with the panendothelial marker MECA 32 showing the extensive papillary networks in the *cpdm/cpdm* skin (**b**). Note the considerable thickness of the *cpdm/cpdm* epidermis (**b**) compared to the thin layer of epidermis from the same dorsal area of a control animal (**a**). **c** Increased expression of ICAM-1 is seen in the basal layers of the epidermis of a mouse with *cpdm/cpdm*. **d** From the same skin a detail with an ICAM-1-positive small vessel is shown. **a**, **b** × 10. **c**, **d** × 20 objective.

The panendothelial antigens recognized by BR2 and MECA 32 dramatically illustrate the extensive vascularization of the dermal papillae of the affected skin (fig. 1). Of the many capillary and venular loops in the dermis several showed the characteristic elevated morphology of activated endothelium.

The MECA 79 and MECA 367 antibodies recognize GLyCAM and MAdCAM, respectively. These two adhesion molecules are associated with high endothelial venules in lymphoid tissue and play an important role in the extravasation of blood-borne lymphocytes into the parenchyma of lymphoid tissues. However, no expression of both antigens was found in *cpdm/cpdm* or control skin.

The MECA 325 antibody recognizes epitopes expressed on high endothelial venules as well, but its function is less well understood. Interestingly, this antibody recognized some vessels in both *cpdm/cpdm* and control skin. These were not papillary vessels but were located more deeply in the reticular dermis. The expression was always moderate, compared to expression on high endothelial venules in e.g. lymph nodes [data not shown]. In the *cpdm/cpdm* skin these vessels were invariably surrounded by infiltrates of inflammatory cells.

In addition the endothelium activation markers VCAM-1, ICAM-1, and E-selectin were studied for their expression on the *cpdm/cpdm* skin. Of these, only ICAM-1 showed vascular expression on various, but not all, papillary vessels and subdermal vessels of the cpdm/cpdm skin. This expression was quite substantial, in contrast to the weak ICAM-1 expression which was occasionally seen in

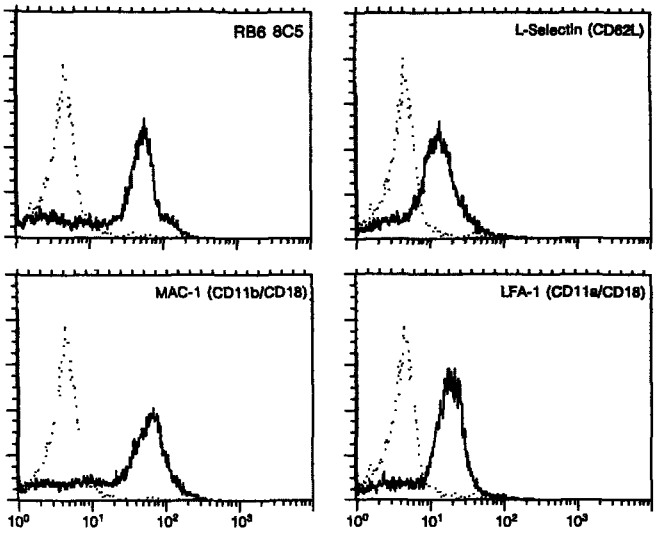

Fig. 2. FACS profiles of the purified bone marrow-derived neutrophilic granulocytes. The majority of the cells are positive for the granulocyte marker RB6 8C5 and express L-selectin, the CR3 receptor (MAC-1) and LFA-1. ····· = Fluorescence intensity of the population after staining with second stage antibodies only.

control skin. In addition the keratinocytes of the basal layers of the epidermis from *cpdm/cpdm* skin showed ICAM-1 expression (fig. 1). No expression at all was found for VCAM-1 and E-selectin in the skin of these mice.

Functional Adherence to Vessels in the Skin

To study whether the vascular changes and expression of adhesion molecules observed in the *cpdm/cpdm* skin were in some way related to functional changes, the ability of the dermal microvasculature to harbor leukocyte binding was studied in a modified in vitro frozen section assay.

When the ability of lymphocytes to bind to dermal structures was tested this way little or no binding was observed either on *cpdm/cpdm* or control skin. We then tested the binding capacity of isolated neutrophilic granulocytes. Using sections from control skin limited binding was observed, but when skin sections from *cpdm/cpdm* skin were assayed clear binding to various parts of the dermal structures was observed. Neutrophils showed a more or less random distribution over the epithelial cells, with a preference for the more basal layers of keratinocytes. Neutrophils also bound to confined areas in the dermal papillary regions. In addition binding was observed in the deeper regions of the dermis.

To demonstrate that the binding observed in the dermal regions resulted from endothelium-neutrophil interactions, the binding was performed on sections that had previously been pretreated with MECA 32. After the cell binding procedure and mild fixation of the sections with 1% formaldehyde this panendothelial marker was then visualized using a fluoresceinated second stage antibody. This way we could demonstrate that the structures to which the neutrophils bound in the dermal layers were indeed small blood vessels.

To study the role of adhesion molecules in the binding process a series of experiments were performed in which the adhesion was tested in the presence of monoclonal antibodies against adhesion molecules. The antibodies used in the assays were selected for their known blocking or nonblocking activity in other assays [data not shown].

First, the expression of various adhesion molecules on the neutrophil preparation was determined by FACS analysis. It was found that, based on the expression of the granulocyte-specific antibody RB6 8C5, the bone marrow-derived neutrophils were >95% pure. The majority of the cells showed prominent expression of CD62L (L-selectin), CD11a (LFA-1), and CD11b (CR3) (fig. 2). Incubation of the cells with antibodies against these adhesion molecules resulted in a decreased binding to endothe-

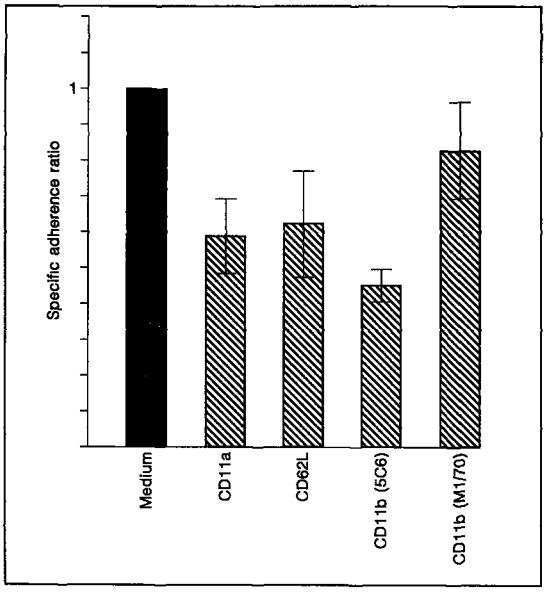

Fig. 3. Binding of neutrophils on *cpdm/cpdm* skin endothelium. Isolated neutrophils were incubated with various antibodies against adhesion molecules as indicated. The cells were washed and mixed with an equal amount of rhodamine-labeled neutrophils. The specific adherence ratio was calculated from the specific binding of incubated cells over fluorescence-labeled cells. Incubation in medium was determined as unity. Per experiment three separate skin sections were analyzed. Per section 50–100 cells were counted. The results are expressed as the mean (SD) of three separate experiments.

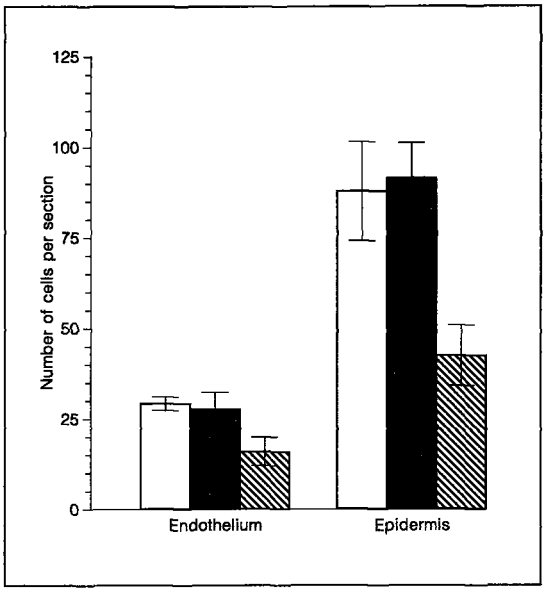

Fig. 4. Skin sections of *cpdm/cpdm* mice were incubated with anti-ICAM-1 antibodies (▨), MECA 325 (nonblocking antiendothelium control antibody; ■), or medium (□). The sections were thereafter incubated with neutrophils and the number of bound cells on either endothelium of small blood vessels in the papillary regions or on the epidermis was determined. Using serial sections which were alternatively incubated with the different antibodies identical stretches of skin were used for the determinations. For each incubation three sections were counted and the results are expressed as the mean number of cells per section.

lial components of the *cpdm/cpdm* skin (fig. 3). In addition, incubation of the skin sections with anti-ICAM antibodies prior to incubation of the sections with neutrophils led to a reduced adhesion (fig. 4).

Discussion

The chronic proliferative dermatitis mutation is characterized by epidermal hyperplasia, hyper- and parakeratosis and extensive infiltration of epidermis and dermis with macrophages, mast cells, neutrophilic, and especially eosinophilic granulocytes. Similar lesions can be seen in the tongue, esophagus and forestomach, which, in the mouse, are all lined by orthokeratinizing epithelium. There is granulocyte infiltration in liver, lung, and in various joints [1]. The absence of T cell infiltration in the affected skin and the inability to transfer the disease by bone marrow or spleen transfer into irradiated hosts do not point to an immunological disorder. Full thickness grafts of affected skin from *cpdm/cpdm* mice and normal skin from C57BL/Ka mice transplanted onto *cpdm/cpdm*, C57BL/Ka mice or athymic nude mice maintain the donor phenotypes [15]. These findings suggest that the pathological features of the *cpdm/cpdm* mice are the result of a disorder in the dermis or epidermis and not caused by a systemic defect. In psoriasis, dermal lymphocytic infiltrates are quite common [16, 17] and binding of T cells, in particular CD4+ T cells, to frozen biopsy sections from psoriatic skin has been demonstrated [18]. This is in contrast with the situation in the *cpdm/cpdm* skin where very few lymphocytes can be found, an interesting correlation with the fact that in our adhesion studies little or no binding of overlaid lymphocytes was found

on affected skin sections, whereas avid binding of neutrophils could be observed. This also corresponds with the absence in the skin of expression of the HEV-related vascular addressins, which are intimately involved in the arresting and transmigration of lymphocytes, but not neutrophils [4, 5]. The absence of E-selectin and VCAM-1 expression in these chronic lesions of the *cpdm/cpdm* mice may fit with the notion that these endothelial adhesion receptors appear after more acute activation [19, 20]. Yet the expression of both VCAM-1 and E-selectin has been described in lesional psoriatic human skin [21, 22]. However, in the chronic lesions of atopic dermatitis, where eosinophils play a more dominant role, an increased expression of E-selectin but no expression of VCAM-1 was observed [23].

Although in the *cpdm/cpdm* skin predominantly eosinophils are present, for practical reasons our experiments were performed with neutrophilic granulocytes. In contrast to eosinophils, mouse neutrophils are relatively easy to obtain in sufficient numbers and we assumed that both cell types would make use of similar adhesion pathways. The adhesion of neutrophils to the inflamed skin of the *cpdm/cpdm* mouse reveals a role for the LFA-1/ICAM-1 (CD11a/CD54) interaction. This pair of molecules is involved in many cellular interactions and is considered to function as a strengthening bond after initial adhesion events that are mediated by members of the selectin family of adhesion molecules and their ligands [24, 25].

From our binding studies a clear role for ICAM-1 could be seen both in binding to endothelium as well as to the keratinocytes, where increased ICAM-1 expression can be found in the *cpdm/cpdm* skin. In line with this is the inhibitory effect of anti-LFA-1 treatment of granulocytes in the binding studies, as well as of anti-CR3 incubation. Although CR3 has been described as a ligand for ICAM-1 it has also been found to associate with other ligands [26–28]. The CR3-ligand interaction has furthermore been implicated to play a major role in monocyte extravasation, and blocking of this receptor has proven an efficient way to stop monocyte influx in inflamed tissues [29–31].

Although the LFA-1 and ICAM-1 interactions are also an important adhesion pathway for lymphocytes it is interesting to see that with the *cpdm/cpdm* skin no in vitro binding of lymphocytes can be seen, in correlation with the absence of lymphocytes in the affected skin in vivo. This suggests that granulocytes use additional adhesion pathways to bind to the *cpdm/cpdm* skin endothelium, or express more activated integrin molecules on their surface compared to lymphocytes. The fact that in our experiments we were never able to completely block granulocyte binding to skin endothelium argues for additional adhesion mechanisms.

In conclusion we have demonstrated that the expression and function of adhesion molecules in *cpdm/cpdm* mice are in agreement with the chronic inflammatory character and the influx of eosinophils found. The *cpdm/cpdm* mouse should therefore be a useful model to screen potential therapy strategies for hyperproliferative and other inflammatory skin disorders.

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Chapter 5

Maintenance of donor phenotype after full-thickness skin transplantation from mice with chronic proliferative dermatitis (*cpdm/cpdm*) to C57BL/Ka and nude mice and vice versa

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Chapter 5

Maintenance of Donor Phenotype After Full-Thickness Skin Transplantation from Mice with Chronic Proliferative Dermatitis (*cpdm/cpdm*) to C57BL/Ka and Nude Mice and Vice Versa

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Chronic proliferative dermatitis is a spontaneous mutation in C57BL/Ka mice (*cpdm/cpdm*) and is characterized by epithelial hyperproliferation, infiltration by eosinophils and macrophages, and vascular dilatation. To elucidate whether these pathologic features are the result of a local (skin) process or a consequence of a systemic disorder, transplantations were performed of full-thickness grafts of affected skin from *cpdm/cpdm* mice and normal skin from control (C57BL/Ka) mice on the back of *cpdm/cpdm*, C57BL/Ka and athymic nude mice. After 3 months, the grafts maintained the histologic phenotype of the donor animal. Intercellular adhesion molecule-1 continued to be expressed by basal keratinocytes of the *cpdm/cpdm* grafts after transplantation. In contrast, the basal keratinocytes of the C57BL/Ka grafts onto *cpdm/cpdm* mice remained negative for intercellular adhesion molecule-1 3 months after transplantation. An increased number of proliferating keratinocytes was present in the *cpdm/cpdm* skin-graft transplanted to nudes or to C57BL/Ka mice based on short-term bromodeoxyuridine labeling. The bromodeoxyuridine incorporation in the keratinocytes of the control C57BL/Ka skin grafts transplanted to *cpdm/cpdm*, nude, or C57BL/Ka mice was the same as in the keratinocytes of normal C57BL/Ka mice. This study demonstrates that the pathologic features found in the *cpdm/cpdm* mice are the result of a disorder in the epidermis or dermis and not due to a systemic defect.

Key words:* *dermatitis/mouse model/transplantation/skin.

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We have recently described a mouse mutant with chronic proliferative dermatitis (gene symbol *cpdm*) on a C57BL/Ka background [1,2]. The skin lesions in this *cpdm/cpdm* mouse are macroscopically characterized by erythema, severe hair loss, and mild scaling. Microscopically, the lesions are characterized by hyper- and parakeratosis, acanthosis, apoptosis of keratinocytes, vascular proliferation, and infiltration of dermis and epidermis by mast cells, macrophages, and granulocytes (mainly eosinophils). Only a small percentage of the inflammatory infiltrate are T cells. Based on these characteristics, this mouse mutant may serve as a useful model to study chronic proliferative dermatitis. Similar lesions as found in the skin were also observed in the esophagus and forestomach, which, in the mouse, are lined by orthokeratinizing epithelium.

Two other mouse mutants, the asebia (*ab/ab*) and the flaky skin (*fsn/fsn*) mouse, also have skin lesions characterized by epidermal hyperproliferation and scaling. The inflammatory reaction in the asebia mouse is different from that of the *cpdm/cpdm* mouse because the infiltrating cells in the dermis and epidermis are mostly mononuclear cells. The cause of the inflammatory reaction in the asebia mouse is thought to be rupture of the lipid-laden macrophages that have infiltrated the dermis [3]. So far, this mouse mutant has not yet been proved to be a representative model for chronic dermatitis in humans [4]. The flaky skin mouse mutation superficially resembles the *cpdm/cpdm* mouse mutation, but apoptosis and follicular keratosis are absent and the mice develop anemia [5]. Certain transgenic mice also develop proliferative skin lesions. However, the distribution and light microscopy of these lesions do not resemble the skin lesions of the *cpdm/cpdm* mouse [6–10]. Another attempt to study chronic proliferative dermatitis in an animal model has been the transplantation of human allografts of diseased skin onto *nu/nu* mice [11–13]. A disadvantage of these models is that the systemic character of the disease is difficult to study.

Although the skin lesions of the *cpdm/cpdm* mice are the most apparent, inflammatory foci were also present in the lungs, liver, and joint-associated tissues. These lesions suggest that the dermatitis is part of a systemic condition, but they could also result from massive cytokine release of keratinocytes. Failure to transfer the *cpdm/cpdm* lesions to syngeneic control animals using hemopoietic cells from spleen or bone marrow from affected mice suggests that such cells do not play a primary role in its pathogenesis [1].

To further elucidate whether the pathologic features observed in the *cpdm/cpdm* mouse originate in the skin or result from a systemic

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Abbreviations: *cpdm*, chronic proliferative dermatitis mouse mutation; *nu*, nude mouse mutation; *fsn*, flaky skin mouse mutation; BrdU, bromodeoxyuridine.

disorder, we performed transplantations of full-thickness skin grafts from affected *cpdm/cpdm* mice and control (C57BL/Ka) mice to *cpdm/cpdm*, C57BL/Ka and athymic nude (*nu/nu*) mice. Here we report that after transplantation the donor phenotype was maintained in the recipients.

MATERIALS AND METHODS

Animals Skin graft donor mice consisted of female *cpdm/cpdm* mice and C57BL/KaLawRij control mice 6–8 weeks old. Recipients consisted of 8–12-week-old C57BL/KaLawRij control mice, *cpdm/cpdm* mice and BALB/c *nu/nu* mice. Mice were individually housed in Macrolon cages and were provided autoclaved pelleted food and acidified, sterilized bottled drinking water *ad libitum*.

Transplantation The grafts were performed according to the methods described earlier [14]. Briefly, donor mice were sacrificed by CO_2 inhalation, dorsal skin (dermis and epidermis) was removed, and excess fat and blood vessels were stripped off. The recipient mice were anesthetized with Hypnorm (1:20, 120 μl/mouse; Janssen Pharmaceutica, Tilburg, The Netherlands). Two circular pieces of dorsal skin (dermis and epidermis), approximately 1 cm in diameter, were aseptically removed down to the pannus carnosus. The donor skin was cut to fit the transplant bed and held in place using six sutures. The transplant was covered with Op-Site (Smith & Nephew, Hull, England) and protected by gauze held in place with 3M surgical foam tape. The tape was kept on for at least 2 weeks. *Nu/nu* recipients (4) were each transplanted with two skin grafts: one from a *cpdm/cpdm* mouse and one from a C57BL/Ka control mouse. C57BL/Ka recipients (3) were similarly transplanted with skin grafts from a *cpdm/cpdm* mouse and a C57BL/Ka control mouse. One *nu/nu* mouse and two C57BL/Ka mice received two skin grafts from a *cpdm/cpdm* mouse. Three *cpdm/cpdm* donor mice received only one graft for transplantation from a C57BL/Ka mouse. The direction of the hairs of the C57BL/Ka graft on the C57BL/Ka recipient was turned 90° to allow the graft to be distinguished from the donor skin by the orientation of the hairs.

Bromodeoxyuridine (BrdU) Labeling and Histology Three months after transplantation, the recipient mice were administered 0.625 mg BrdU (Sigma) intraperitoneally to determine the rate of cell proliferation. Thirty minutes after injection, the mice were sacrificed by ether inhalation. The transplanted and peripheral skin of the donor mice were removed and trimmed into three pieces. One part was immediately frozen and stored in liquid nitrogen. The other parts were fixed for 18 h in neutral-buffered formalin, stored in 70% alcohol, and later embedded in paraffin. Paraffin-embedded sections were deparaffinized, rehydrated, and incubated with monoclonal anti-BrdU antibody (Dakopatts, Copenhagen, Denmark) or stained with hematoxilin-phloxine-saffron or with toluidine blue. The labeled nuclei of the slides incubated with anti-BrdU were visualized by peroxidase-labeled rabbit anti-mouse Ig (Dakopatts), followed by diaminobenzidine in combination with 1% cobalt-chloride to enhance staining intensity.

Immunohistochemistry Cryostat sections of the transplanted skin were stained using the indirect peroxidase method. Sections were fixed in acetone, washed in phosphate-buffered saline, and incubated overnight at 4°C with the monoclonal antibody against anti-intercellular adhesion molecule-1 (ICAM-1) purified as described earlier [1]. Thereafter, sections were incubated with mouse anti-rat peroxidase (Jackson Immunoresearch Laboratories, West-Grove, PA) for 60 min at room temperature followed by staining with diaminobenzidine and hydrogen peroxide. The sections were counterstained with Mayer's hematoxylin (Merck, Darmstadt, FRG)

Morphometry and Statistical Analysis BrdU-labeled nuclei were counted per centimeter of epidermal basement membrane. The density of dermal mast cells was determined in toluidine blue sections as cells/mm^2 dermis. The area of the dermis was determined by subtracting the area occupied by pilosebaceous units and blood vessels from the total area of the dermis. The measurements were performed with computer-aided morphometry (Kontron-Videoplan, Zeiss, Germany). All data are expressed as mean ± SD. Statistical analysis was performed using the Student t test.

RESULTS

Maintenance of Donor Phenotype After Transplantation Transplantation of the 10 C57BL/Ka grafts to the *nu/nu*, *cpdm/cpdm*, and C57BL/Ka mice was macroscopically characterized by a thin graft and normal fur with the exception of the development of long, white hairs in some C57BL/Ka grafts transplanted to the *nu/nu* mouse. At the time of transplantation, the *cpdm/cpdm* fur was

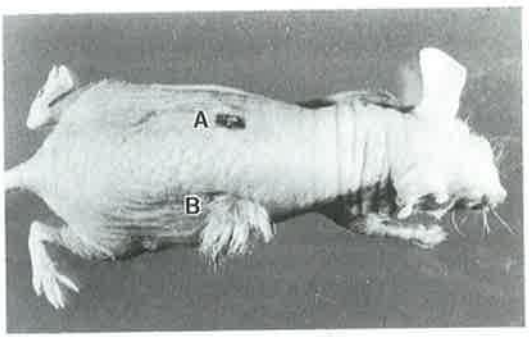

Figure 1. The macroscopic appearance of *cpdm/cpdm* and C57BL/Ka skin is maintained 3 months after transplantation onto *nu/nu* mice. Full-thickness skin from a *cpdm/cpdm* (*A*) and a C57BL/Ka mouse (*B*) was transplanted to a *nu/nu* host mouse. The *cpdm/cpdm* skin graft showed fine scaling.

normal but the skin was slightly thickened. In the 3 months after transplantation, the 11 *cpdm/cpdm* grafts transplanted to the *nu/nu* and C57BL/Ka mice lost their hair, became thicker, and developed fine scale. **Figure 1** gives a representative photograph of a *nu/nu* host mouse with a full-thickness skin graft from a *cpdm/cpdm* and a C57BL/Ka mouse. One *cpdm/cpdm* skin transplantation onto a *nu/nu* and onto a C57BL/Ka mouse was not succesful.

Microscopically, the C57BL/Ka grafts could be distinguished from the *nu/nu* recipient mouse by differences in hair follicles and epithelial thickness. The hair shafts of the *nu/nu* mice were distorted and malacic [15], and the epidermis was about five cell layers thick. The epidermis of the C57BL/Ka grafts was three cell layers thick. The C57BL/Ka grafts could be distinguished from the C57BL/Ka recipient mice by the different orientation of the hair shafts **(Fig 2)**.

All 10 normal C57BL/Ka grafts onto *cpdm/cpdm*, C57BL/Ka, and *nu/nu* mice showed no differences compared with C57BL/Ka control skin except for variable loss of melanin pigment from hair bulbs and occasionally scattered dermal macrophages and granulocytes **(Fig 2)**, probably a result of the surgical procedure. In contrast, all 11 *cpdm/cpdm* skin grafted on C57BL/Ka and *nu/nu* mice showed marked acanthosis **(Fig 3)**. Ten of 11 *cpdm/cpdm* grafts showed spongiosis **(Fig 4)**, and in five of 11 *cpdm/cpdm* grafts eosinophils were observed in the epidermis. Only two *cpdm/cpdm*

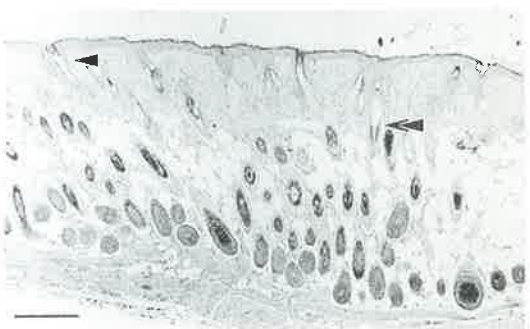

Figure 2. The phenotype of C57BL/Ka skin grafted onto a C57BL/Ka recipient is maintained 3 months after transplantation. Sections were stained with hematoxylin-phloxine-saffron. Note the different orientation of the hair shafts between the graft (*arrowhead*) and the recipient (*double arrowhead*). No lesions were observed in the graft, except for the occasional scattered dermal infiltrate (*bar*, 0.3 mm).

Figure 3. The phenotype of *cpdm/cpdm* skin grafted onto a C57BL/Ka recipient is maintained 3 months after transplantation. Full-thickness skin of a *cpdm/cpdm* mouse (*right*) was transplanted to a C57BL/Ka mouse (*left*). Sections were stained with hematoxylin-phloxine-saffron. The *cpdm/cpdm* skin graft showed marked acanthosis (*bar*, 0.3 mm).

grafts showed hyperkeratosis, eight showed parakeratosis, and in four *cpdm/cpdm* grafts apoptosis of keratinocytes was observed **(Fig 4)**. The dermis of all 11 grafts was infiltrated by a mixed population of inflammatory cells, predominantly eosinophils, mast cells, and macrophages. There were significantly more mast cells present in the dermis of the *cpdm/cpdm* grafts (532 ± 108 and 527 ± 31, respectively) than in the surrounding dermis of the *nu/nu* (170 ± 64) recipients ($p < 0.025$) and C57BL/Ka (115 ± 44) recipients ($p < 0.001$). Ten *cpdm/cpdm* grafts showed tortuous, dilated capillaries in the superficial dermis. **Table I** summarizes the results of the *cpdm/cpdm* transplantation to *nu/nu* and C57BL/Ka mice. Only a few lymphocytes were observed in the epidermis and dermis of the *cpdm/cpdm* grafts. These characteristics of the *cpdm/cpdm* grafts were similar to the *cpdm/cpdm* donor skin **(Table I)**. Neutrophils were occasionally present in the subepidermal adventitial dermis as a consequence of the grafting procedure. There were only minor differences between the *cpdm/cpdm* grafts transplanted to the C57BL/Ka and to the *nu/nu* mice **(Table I)**.

No gross lesions were observed in the other organs of the recipient mice.

BrdU Labeling Remains High After Grafting **Table II** summarizes the results of the BrdU-labeling studies. The grafts from the *cpdm/cpdm* to the C57BL/Ka and *nu/nu* mice had signif-

Table I. Microscopical Lesions of *cpdm/cpdm* Skin Remain After Grafting to *nu/nu* and C57BL/Ka Mice

	Grafts (Skin) with Lesion/Total Examined Grafts (Skin)			
	Graft			Donor
Lesion	*cpdm/cpdm* on *nu/nu*	*cpdm/cpdm* on C57BL/Ka	Total	*cpdm/cpdm*
Acanthosis	5/5	6/6	11/11	6/6
Inflammatory cells in epidermis	4/5	1/6	5/11	1/6
Apoptosis	2/5	2/6	4/11	6/6
Spongiosis	5/5	5/6	10/11	5/6
Parakeratosis	3/5	5/6	8/11	3/6
Hyperkeratosis	3/5	0/6	3/11	6/6
Dilated capillaries	4/5	6/6	10/11	6/6
Inflammatory cells in dermis	5/5	6/6	11/11	6/6

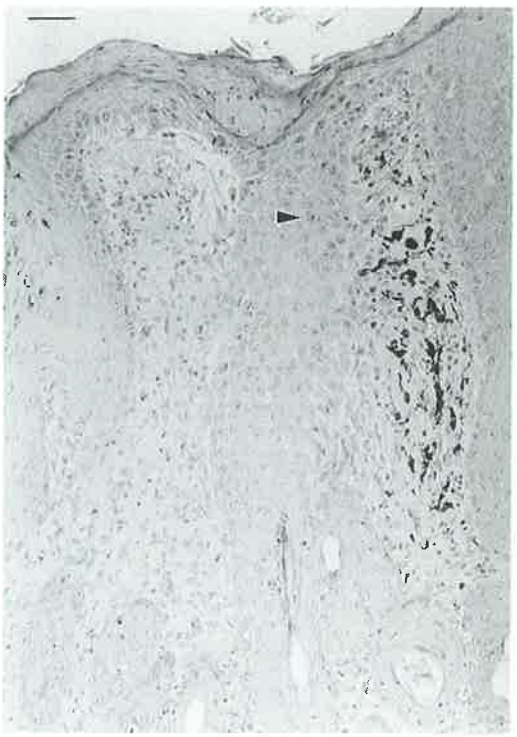

Figure 4. The characteristics of *cpdm/cpdm* grafts are similar to those of untransplanted *cpdm/cpdm* donor skin. Full-thickness skin of a *cpdm/cpdm* mouse was transplanted to a *nu/nu* mouse. Sections were stained with hematoxylin-phloxine-saffron. Note spongiosis, parakeratosis, and apoptosis (*arrowhead*) of keratinocytes, and blood vessel dilatation and inflammatory infiltration in the dermis of a *cpdm/cpdm* skin graft (*bar*, 50 μm).

icantly more BrdU-positive nuclei than C57BL/Ka control grafts ($p < 0.025$) **(Fig 5*a,b*)** and the number of BrdU-positive cells was comparable with the number of BrdU-positive cells in the epidermis of *cpdm/cpdm*. The grafts from C57BL/Ka to *cpdm/cpdm* did not show an increase of the BrdU-positive nuclei compared with the number of BrdU-positive nuclei of the normal C57BL/Ka epidermis. There was no difference in BrdU incorporation in the nuclei of the C57BL/Ka grafts transplanted to the *nu/nu* or to the C57BL/Ka mice. The number of positive nuclei in the epidermis of the

Table II. BrdU Incorporation in Nuclei of Grafts and Recipients Remains Comparable to Donor Skin

	BrdU Incorporation in Nuclei[a]	
	Graft	Recipient
cpdm/cpdm graft on *nu/nu* recipient	286 ± 62[b,c]	89 ± 36
C57BL graft on *nu/nu* recipient	47 ± 21	165 ± 45
cpdm/cpdm graft on C57BL recipient	365 ± 118[d]	40 ± 18
C57BL graft on C57BL recipient	36 ± 4	34 ± 15
C57BL graft on *cpdm/cpdm* recipient	56 ± 9	338 ± 84

[a] BrdU-labeled cells per cm basement membrane length.
[b] Standard deviation.
[c] $p < 0.025$ *cpdm/cpdm* graft versus C57BL/Ka graft on *nu/nu* recipient.
[d] $p < 0.001$ *cpdm/cpdm* graft versus C57BL/Ka graft on C57BL/Ka recipient.

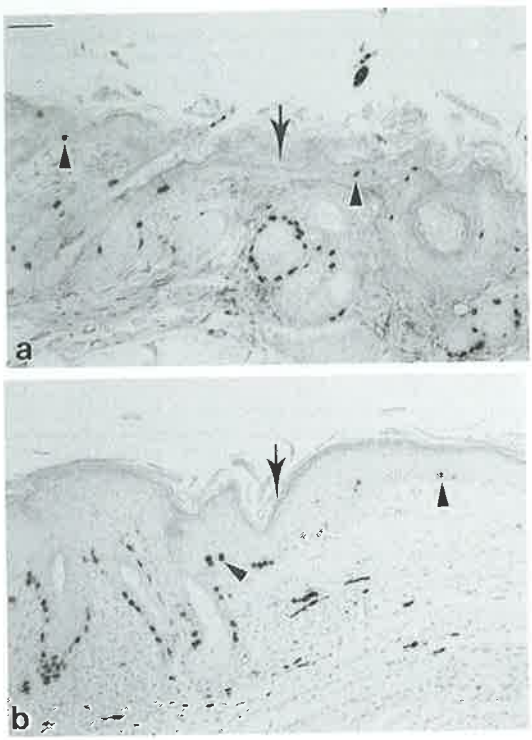

Figure 5. BrdU labeling of *cpdm/cpdm* and C57BL/Ka skin grafted onto *nu/nu* recipients is unaltered 3 months after transplantation. The recipient mice were injected intraperitoneally with 0.625 mg BrdU 30 min before sacrificing. Paraffin-embedded sections were stained immunohistochemically using an anti-BrdU antibody. BrdU-positive nuclei (*arrowheads*) in a C57BL/Ka (*a*) and a *cpdm/cpdm* (*b*) skin graft on a *nu/nu* recipient. Skin grafts are left of the *arrow* (*bar*, 0.1 mm).

recipient BALB/c *nu/nu* was relatively high when compared with that of recipient normal skin.

ICAM-1 Remains Present in *cpdm/cpdm* Skin Grafts The ICAM-1–staining pattern of the keratinocytes of the grafts was similar to the staining-pattern observed in the keratinocytes of the normal C57BL/Ka and *cpdm/cpdm* skin. ICAM-1 was weakly expressed on endothelial cells of blood vessels in the deep dermis of skin of C57BL/Ka mice. In *cpdm/cpdm* mice, ICAM-1 was expressed on basal keratinocytes and on endothelial cells of dermal blood vessels. The basal keratinocytes of the *cpdm/cpdm* grafts also stained for ICAM-1 whereas the basal keratinocytes of the C57BL/Ka recipient did not. The keratinocytes of the C57BL/Ka grafts did not stain, whereas the *cpdm/cpdm* recipient keratinocytes remained reactive. The endothelial cells in *cpdm/cpdm* and C57BL/Ka grafts also stained. As expected, the endothelial cells in *cpdm/cpdm* recipient skin surrounding the graft stained for ICAM-1, but the endothelial cells in the skin of the C57BL/Ka and *nu/nu* recipient surrounding the transplant also stained.

DISCUSSION

Transplanted *cpdm/cpdm* full-thickness skin grafts onto *nu/nu* or C57BL/Ka control mice maintained the *cpdm/cpdm* phenotype. Only apoptosis and hyperkeratosis were observed in a limited number of *cpdm/cpdm* grafts, probably due to the restricted size of the graft. Based on BrdU incorporation, we found a comparable proliferation rate of the *cpdm/cpdm* keratinocytes in the grafts and the keratinocytes of *cpdm/cpdm* mice [1]. These observations indicate that the pathologic features found in the *cpdm/cpdm* mice are the result of a disorder within the epidermis or dermis and not due to a systemic defect.

Keratinocytes can be triggered to secrete a variety of pro-inflammatory cytokines and thus elicit an inflammatory reaction in the underlying dermis [16]. Persistent release of cytokines could result in a chronic dermatitis. Alternatively, subepithelial mesenchymal components, such as fibroblasts or endothelial cells, can release factors that induce proliferation and differentiation of epithelia [17]. We have transplanted full-thickness grafts to *nu/nu* and C57BL/Ka mice, and it is not clear whether the vascularization of the graft is achieved by penetration of new vessels from the host into the transplant or by anastomosis of host blood vessels with pre-existing graft vessels. Data from the literature are conflicting in this respect [18–20]. Endothelial cells may cause or contribute to chronic inflammation by persistent expression of adhesion molecules.

The endothelial cells in the *cpdm/cpdm* grafts and *cpdm/cpdm* recipients stained for ICAM-1, consistent with previous observations on the *cpdm/cpdm* mice [1]. However, the endothelial cells in the C57BL/Ka grafts and in the skin of C57BL/Ka and *nu/nu* recipients were also ICAM-1 positive in contrast to earlier studies in which we could not detect ICAM-1 staining on dermal endothelium of C57BL/Ka mice [1]. The expression of ICAM-1 is rapidly increased upon endothelial activation [21]. Thus, the endothelial cells of the C57BL/Ka and *nu/nu* recipients and grafts in this transplantation study are activated, presumably as a result of the injury inflicted by the transplantation.

This study shows that the inflammatory cells found in the dermis and epidermis of the *cpdm/cpdm* mouse do not play a primary role in its pathogenesis. The eosinophils infiltrated in the *cpdm/cpdm* graft should originate from the host because of the estimated short survival time of these cells (approximately 14 d) in the skin [22,23]. Transplantation of human psoriatic skin onto nude mice showed that psoriatic epidermis did not contain polymorphonuclear cells [13]. This could indicate that the *cpdm/cpdm* skin, in contrast to the psoriatic skin, produces cytokines that are responsible for the mobilization or attraction of eosinophils. A role for T cells in the development and maintenance of the *cpdm/cpdm* lesion seems improbable. Transplantation of skin from human proliferative diseases such as psoriasis to *nu/nu* mice has suggested that natural killer cells or immature T lymphocytes of nude mice might infiltrate the human skin grafts and release mediators that stimulate proliferation of potentially hyperreactive epidermal cells from psoriatic patients [24]. However, C57BL/Ka skin grafted onto *nu/nu* mice did not show any increase in BrdU labeling indicating that, if there was a low-level graft rejection reaction in these animals, it was too limited to affect epidermal proliferation and unlikely to play a role in the *cpdm/cpdm* lesion. Similarly, human breast skin transplanted onto *nu/nu* mice shows no increase in proliferative index up to 3 months after transplantation (G. Elliott, personal communication). Moreover, only a few T lymphocytes are present in the dermis and epidermis of the *cpdm/cpdm* mouse and transplantation of the *cpdm/cpdm* skin to the athymic *nu/nu* mouse implies that these T lymphocytes are not required for maintenance of the lesion. In addition, transfer of hemopoietic cells from *cpdm/cpdm* mice failed to cause *cpdm/cpdm* lesions in the recipient, indicating that hemopoietic cells are unlikely to play a primary role in the pathogenesis [1].

Full-thickness skin transplantation from *fsn/fsn* and littermate control mice to *nu/nu* mice resulted in maintenance of the mutant phenotype [25]. These authors previously found that bone marrow grafted from *fsn/fsn* mice to severe combined immunodeficiency (*scid/scid*) mice resulted in the development of a proliferative skin disease in the recipients [26]. Double mutants (*fsn/fsn, scid/scid*) were created specifically to remove parts of the immune system, and these mice still developed a psoriasiform dermatitis. Apparently, the difference between the *cpdm/cpdm* and *fsn/fsn* mice is situated in the hemopoietic progenitor cells. In the *fsn/fsn* mouse,

these cells are necessary for production of the *fsn* phenotype, whereas the functional lymphoid cells are not required for development of the skin lesions. However, the bone-marrow–derived cells residing in the dermis or epidermis in the full-thickness grafts are sufficient to maintain the *fsn* phenotype.

We have demonstrated that *cpdm/cpdm* skin grafted onto *nu/nu* and C57BL/Ka mice maintain the *cpdm/cpdm* phenotype in the recipient as measured by histopathology, proliferation rate, and ICAM-1 expression. The *cpdm/cpdm* mouse should therefore be a useful model for screening potential therapy strategies for psoriasiform and other chronic inflammatory skin disorders.

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Chapter 6

Pathogenesis of skin lesions in mice with chronic proliferative dermatitis (*cpdm/cpdm*).

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Chapter 6

Pathogenesis of Skin Lesions in Mice with Chronic Proliferative Dermatitis (*cpdm/cpdm*)

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Chronic proliferative dermatitis is a spontaneous mutation in C57BL/Ka mice (cpdm/cpdm), showing alopecia, epithelial hyperproliferation, infiltration by eosinophils and macrophages, and vascular dilatation. To further elucidate its pathogenesis, organs of 1-, 2-, 3-, 4-, 5-, and 6-week-old cpdm/cpdm mice were examined. At 4 weeks, the epidermal thickness was increased, whereas already at 3 weeks, the bromodeoxyuridine incorporation was increased in the basal keratinocytes. However, already at the age of 1 week, skin, lungs, and lymph nodes were infiltrated by eosinophils although no macroscopic lesions were present. Compared with control animals, 6-week-old cpdm/cpdm mice had decreased serum IgE levels and increased numbers of mast cells. From the age of 1 week these mast cells became increasingly IgE positive. In contrast, the mast cells of the control animals remained IgE negative. Mast cells of control and cpdm/cpdm mice were interleukin-4 and tumor necrosis factor-α positive. A likely explanation for the tissue infiltration of eosinophils could be the release of interleukin-4 and tumor necrosis factor-α from activated mast cells. Tumor necrosis factor-α may lead to the expression of E-selectin on endothelial cells, facilitating interleukin-4-mediated eosinophil transendothelial migration. Although various pathogenetic aspects of the cpdm/cpdm mouse need further elucidation, this model can be a tool to study eosinophil infiltration, leukocyte-endothelial cell interactions, and mast cell proliferation. Furthermore, the cpdm/cpdm mouse can be used to study chronic inflammatory skin disease because of the severe epidermal proliferation.

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The detection and study of hereditary disorders in animals have greatly contributed to our understanding of the complex mechanisms underlying disease processes. We recently described a mouse mutant (*cpdm/cpdm*) with chronic proliferative dermatitis on a C57BL/Ka background.[1–4] The skin lesions are characterized by erythema, severe nonscarring hair loss, and scaling at the age of 5 weeks. Light microscopically, the lesions are hyper- and parakeratosis, hyperplasia and apoptosis of keratinocytes, vascular proliferation, and infiltration of mast cells, macrophages, and granulocytes, mainly eosinophils. Only few T cells are present in the skin. Similar lesions as found in the skin are observed in the esophagus and forestomach. In addition, there are mixed cellular infiltrates in the liver, lungs, and perisynovial connective tissue. Both the draining lymph nodes of the skin and the spleen are enlarged due to heavy infiltration of eosinophils, whereas marked extramedullary myelopoiesis accounts for the enlargement of the spleen. The lesions regressed upon systemic treatment with corticosteroids.[1]

Transfer of hemopoietic cells from *cpdm/cpdm* mice to lethally irradiated syngeneic mice failed to induce *cpdm/cpdm* lesions in the recipients, suggesting that hemopoietic cells do not play a primary role in the pathogenesis.[1] Full-thickness grafts of affected skin from *cpdm/cpdm* mice and normal skin from C57BL/Ka mice transplanted onto *cpdm/cpdm*, C57BL/Ka mice or athymic nude mice maintain the donor phenotypes.[3] These findings suggest that the

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pathological features of the *cpdm/cpdm* mice are the result of a disorder in the dermis or epidermis and not caused by a systemic defect.

To elucidate the pathogenesis of the chronic skin disease in the *cpdm/cpdm* mouse, additional insight in the development of the lesions is necessary. Therefore, the skin and other affected organs of 1-, 2-, 3-, 4-, 5-, and 6-week-old *cpdm/cpdm* mice and appropriate controls were examined. Here, we report which histological changes occur in the various organs and how these changes relate to macroscopic observable skin lesions.

Materials and Methods

Animals

The cpd mutation (*cpdm/cpdm*) arose spontaneously in the colony of the inbred C57BL/KaLawRij mice in the specific-pathogen-free breeding facility of TNO in Rijswijk, The Netherlands. The microbiological status is checked regularly by routine serological, bacteriological, and histological procedures. Two breeding models were used. 1) Females, heterozygous for the cpd mutation (+/*cpdm*) were bred with homozygous males (*cpdm/cpdm*). These males cannot be older than 11 weeks because the fertilizing capacity decreases. This was not due to atrophy of the reproductive organs but to an overall weakened systemic condition resulting from severe pruritus. 2) Heterozygous females (+/*cpdm*) were bred with heterozygous males (+/*cpdm*). The current investigation was carried out with the offspring of both breeding models. Eight mice (four *cpdm/cpdm* and four littermate controls) per week were macroscopically examined at the age of 1, 2, 3, 4, 5, and 6 weeks, weighed, killed, necropsied, and microscopically examined. Mice used for serum IgE determination were sacrificed at 8 weeks.

Histology

All tissues were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin. Three-micron sections were routinely stained with hematoxylin and eosin (H&E). The selected tissues with *cpdm/cpdm* lesions, ie, skin, tongue, esophagus, forestomach, lung, liver, spleen, and lymph nodes, were given a score corresponding to the degree of cellular (granulocytic and monocytic) infiltration as follows: no infiltration, 0; minimal infiltration, 1; mild, 2; moderate, 3; severe, 4; and marked, 5.

Immunohistochemistry

Skin samples were quick-frozen in liquid nitrogen for immunohistochemistry. Cryostat sections of the skin of animals at the age of 1 and 6 weeks were stained using an indirect peroxidase method. Sections were fixed in acetone, washed in phosphate-buffered saline, and incubated with anti E-selectin (10E6[5]; kindly provided by Dr. B Wolitzky), anti P-selectin (RW40 34/4[6]), anti-IgE (EM-95[7]; kindly provided by Dr. M. Baniyash), biotinylated anti-tumor-necrosis-factor (TNF)-α (61E71[8]) anti-interleukin (IL)-4 (11B11[9]), or biotinylated anti-IL-5 (TRFK4[10]) for 60 minutes at room temperature. Thereafter, sections were washed and incubated with avidin-peroxidase (Dakopatts, Copenhagen, Denmark) or rabbit anti-rat peroxidase (Dakopatts) for 60 minutes at room temperature. Peroxidase activity was visualized with diaminobenzidine (Sigma Chemical Co., St. Louis, MO). Some sections incubated with anti-IgE were counterstained with alcian blue

Bromodeoxyuridine (BrdU) Labeling

Thirty minutes before being killed, mice were administered 0.625 mg of BrdU (Sigma) intraperitoneally to determine the rate of cell proliferation in the epidermis. Skin was fixed for 18 hours in neutral-buffered formalin, stored in 70% alcohol, and later embedded in paraffin. Paraffin-embedded sections were deparaffinized, rehydrated, and incubated with monoclonal anti-BrdU antibody (Dakopatts). The labeled nuclei of the slides incubated with anti-BrdU were visualized by peroxidase-labeled rabbit anti-mouse Ig (Dakopatts), followed by diaminobenzidine in combination with 1% cobalt chloride to enhance the staining intensity.

Total Serum IgE Determination

Total serum IgE levels were measured in nine control C57BL/Ka and three *cpdm/cpdm* mice of 8 weeks old by isotype-specific enzyme-linked immunosorbent assay as described previously.[11] Plates were coated with monoclonal RaAM/IgE (EM95; 2μg/ml) and incubated overnight at 4°C with diluted serum samples. Detection was based upon addition of biotinylated RaAM/IgE (2 μg/ml; clone R35–118, Pharmagen, San Diego, CA) and, after washing, the conjugate streptavidin-peroxidase (Jackson Immunoresearch Laboratories, West Grove, PA). The ELISA was developed by using 2,2′-amino-bis(3-ethylbenz-thioazoline-6-sulfonic acid (Sigma). The detection limit of this enzyme-linked immunosorbent assay was 0.5 ng/ml.

Morphometry and Statistical Analysis

BrdU-labeled nuclei were counted per centimeter of basement membrane. The thickness of the combined nucleated epithelial layers of the esophagus and interfollicular epidermis was measured at 10 sites From these measurements, the mean thickness was calculated for each epithelium. The density of dermal mast cells was determined for the 1-, 3-, and 6-week-old animals in toluidine blue sections as number of cells per square millimeter of dermis. The area of the dermis was determined by subtracting the area occupied by pilosebaceous units and blood vessels from the total area of the dermis. The measurements were performed with computer-aided morphometry (Kontron-Videoplan, Zeiss, Germany). Data are presented as mean ± SEM. Statistical analysis was performed by Student's *t*-test.

Results

Breeding Efficacy

Two breeding models were used in this study. Breeding model 1 (+/*cpdm* female × +/*cpdm* male) resulted in 26% *cpdm/cpdm* descendants, with equal numbers of males and females. Breeding model 2 (+/*cpdm* female × *cpdm/cpdm* male) resulted in 45% *cpdm/cpdm* descendants, 58% males and 42% females. These results are consistent with an autosomal mode of inheritance and indicate that breeding method 2 results in the highest breeding efficiency (during the relatively short period of male mating activity).

Clinical Symptoms

The first *cpdm/cpdm* symptom consisted of thickening of the eyelids. This was observed not earlier than week 2. Three out of four animals demonstrated this symptom and could also microscopically be identified as *cpdm/cpdm* mice. At the age of 3 weeks the *cpdm/cpdm* mice had a thinner fur than control mice, and reddening of the axilla was observed in such a way that all *cpdm/cpdm* mice could be recognized At the age of 4 weeks the dorsal neck and ventral chest developed hair loss and mild scaling and this subsequently became more severe. After 5 weeks, the animals suffered from severe pruritus demonstrated by severe scratching.

From the age of 5 weeks, the *cpdm/cpdm* animals showed 7% weight reduction compared with the control animals At the age of 6 weeks this increased to 12% weight reduction.

Pathology

No lesions were observed in the control animals. Gross examination revealed skin changes in the *cpdm/cpdm* mice as described above. Furthermore, at the age of 3 weeks the deep and superficial cervical, thoracic, axillary, and brachial lymph nodes and the spleen were mildly enlarged. Detailed examination of the skin, lymph nodes, lung, tongue, esophagus, forestomach, liver, and spleen led to the observations described below. In other organs, no macroscopic or microscopic abnormalities were observed. Special stains for the detection of yeasts, fungi, and parasites were consistently negative. Also, serological, bacteriological, and histological measurements for the microbiological status were consistently negative. The results of semiquantitative measurements of cellular infiltration at different locations are represented in Figure 1, a–h.

Skin

At the age of 1 week, the dermis of the *cpdm/cpdm* mice was already mildly infiltrated with inflammatory cells, predominantly eosinophils and some monocytes (Figure 2a). This picture was similar at the age of 2 weeks. At 3 weeks, a folliculitis with some degenerated hairshafts, some apoptosis of the keratinocytes, dilatation of the capillaries, and a moderate infiltration of inflammatory cells in the dermis were present. This became more severe at 4 weeks, at which age also acanthosis (Figure 2b), multifocal parakeratosis, and hyperkeratosis had developed. The lesions were more advanced in 5-week-old mice with intracorneal microabscesses present in two animals. At 6 weeks, the lesions were quite extensive and severe.

Lymph Nodes

At the age of 1 week, the deep and superficial cervical, thoracic, axillary, and brachial lymph nodes showed acute lymphadenitis and perilymphadenitis mainly characterized by eosinophil infiltration in the cortex and medulla sometimes obscuring the normal architecture (Figure 3). The lymphadenitis varied from mild to severe, but the severity did not change with age.

Lungs

Already at the age of 1 week lesions in the lungs were observed. Predominantly perivascularly, but in two animals also interstitially, mild cellular infiltration

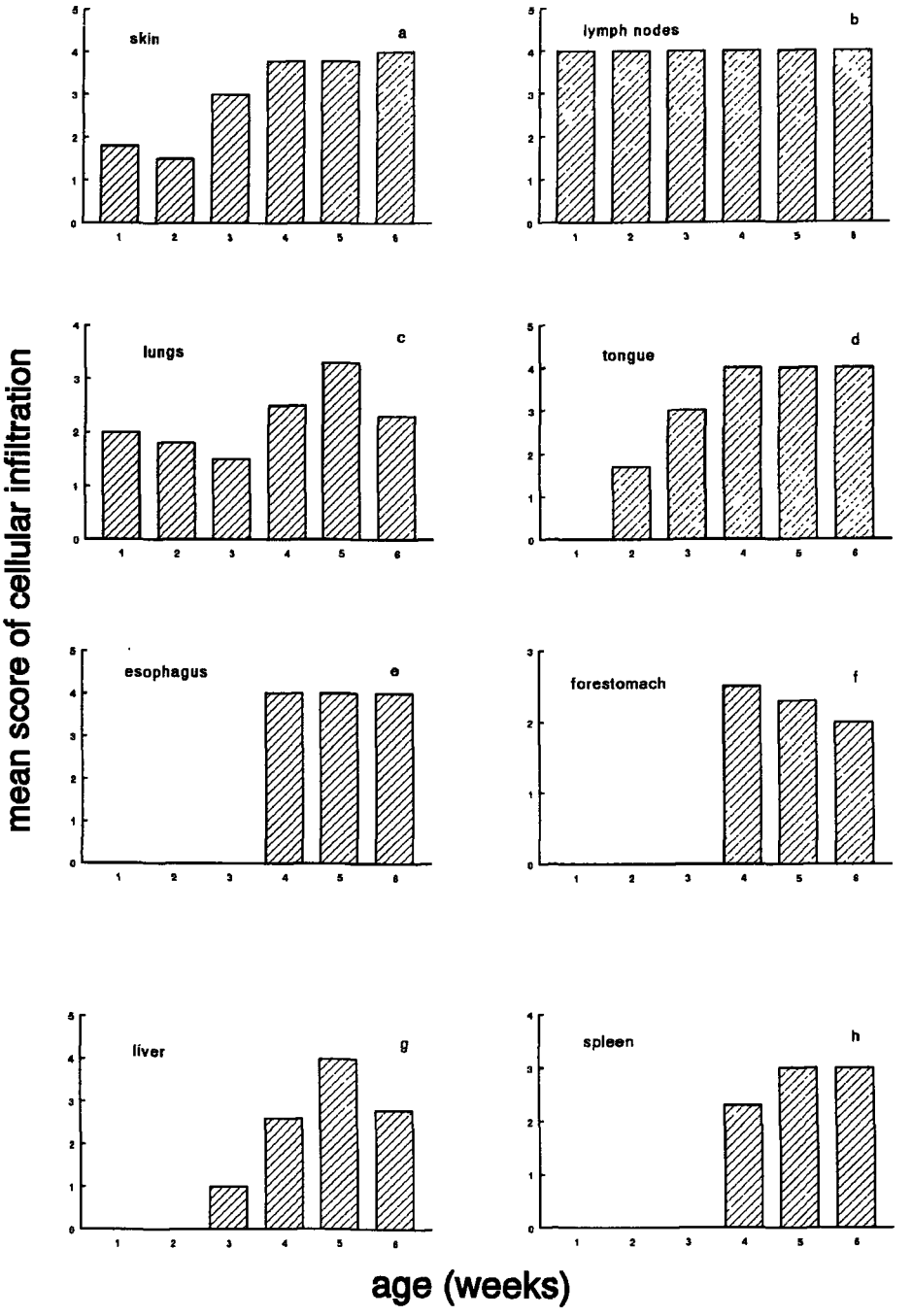

Figure 1. *Mean scores (n = 4) of cellular infiltration (monocytes and granulocytes), in excess of (negligible) values obtained in controls, in the skin* (a), *deep and superficial cervical, thoracic, axillary, and brachial lymph nodes* (b) *lungs* (c) *tongue* (d) *esophagus* (e) *forestomach* (f) *liver* (g) *and spleen* (h) *of* cpdm/cpdm *mice at the age of 1 to 6 weeks*

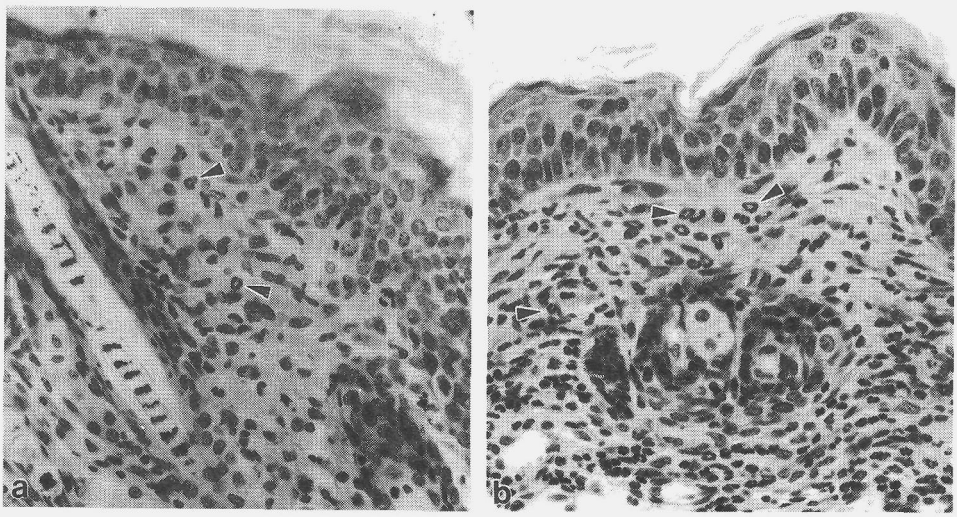

Figure 2. *Skin of a 1-week-old* (a) *and a 4-week-old* (b) cpdm/cpdm *mouse. At the age of 1 week, the dermis of the* cpdm/cpdm *mouse is mildly infiltrated with predominantly eosinophils* (a, arrowheads), *whereas at 4 weeks a severe cellular infiltration is observed* (arrowhead) *and acanthosis has developed* (b). *H-E, magnification, ×380.*

was present. These inflammatory cells were predominantly eosinophils with some monocytes. At the age of 2 and 3 weeks the lesions had not changed appreciably. However, at the age of 4 weeks, the four *cpdm/cpdm* mice had developed a mild to moderate diffuse granulomatous pneumonia. This became worse thereafter.

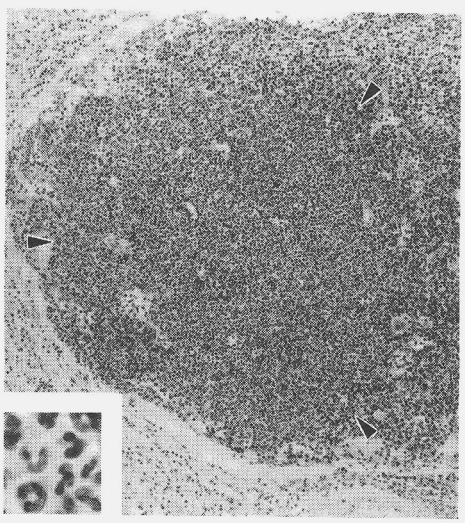

Figure 3. *Lymph node of a 1-week-old* cpdm/cpdm *mouse with acute lymphadenitis* (arrowheads) *mainly characterized by eosinophil infiltration* (inset). *H-E, magnification, ×90.* Inset: *H-E, magnification, ×900.*

Proximal Intestinal Tract

The tongue, esophagus, and forestomach of the mouse are lined by orthokeratinizing squamous epithelium. Although similar changes were observed as those in the skin, the time of occurrence was different. The first lesions were observed at the age of 2 weeks in the tongue, whereas lesions in the esophagus and forestomach were absent at week 3 (Figure 4a) and appeared at the age of 4 weeks (Figure 4b). The lesions were comparable to the lesions observed in the skin. There were two exceptions: infiltrating cells in the epithelium of the proximal digestive tract forming intraepithelial and intracorneal spongiform pustules, and no parakeratosis was observed. These infiltrating cells were mainly eosinophils. Once the lesions had developed in the tongue and the esophagus, they were immediately moderate to severe. The lesions in the forestomach were not as pronounced as in the esophagus, and they were most severe at the junction of the forestomach and glandular stomach.

Liver

Normal extramedullary myelopoiesis was observed in the liver of 1-week-old control and *cpdm/*

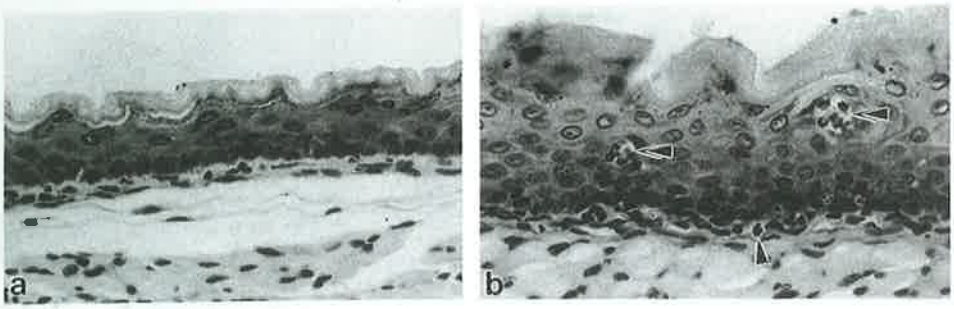

Figure 4. *Esophagus of a 3-week-old* (a) *and a 4-week-old* (b) cpdm/cpdm *mouse. No lesions are observed at 3 weeks, whereas at 4 weeks hyperkeratosis, acanthosis, intraepithelial spongiform pustules* (double arrowheads), *and subepithelial cellular infiltration* (arrowheads) *are present. H-E, magnification, ×380.*

cpdm animals. However, at the age of 2 weeks, only mild myelopoiesis was observed in the control mice, whereas the *cpdm/cpdm* mice had developed moderate myelopoiesis. At 3 weeks of age, the *cpdm/cpdm* mice showed small perivascular infiltrates consisting of eosinophils and macrophages. These lesions subsequently became more severe.

Spleen

At the age of 1 and 2 weeks, control and *cpdm/cpdm* mice both had extramedullary hematopoiesis in the spleen, but at 3 weeks of age only the *cpdm/cpdm* mice showed moderate myelopoiesis. This became more severe with age, and severe eosinophil infiltration was observed in the red and white pulp starting at the age of 4 weeks. Together with this infiltration, atrophy of the white pulp occurred.

Epithelial Thickness and Proliferation

The epidermal thickness of the skin of the *cpdm/cpdm* mice was significantly increased ($P < 0.025$) compared with the control mice at 4 weeks of age (Figure 5a) while the BrdU incorporation was significantly increased ($P < 0.025$) in the basal keratinocytes of the *cpdm/cpdm* mice already at 3 weeks (Figure 5c). The epithelial thickness (Figure 5b) and BrdU incorporation (Figure 5d) in the basal keratinocytes of the esophagus were significantly increased ($P < 0.025$ and $P < 0.05$, respectively) at 4 weeks of age.

Mast Cell Count, Serum IgE Level, and Immunohistochemistry

At the age of 1 and 3 weeks the normal (68 ± 7 and 123 ± 23 cells/mm^2, respectively) and *cpdm/cpdm* skin (75 ± 10 and 85 ± 9 cells/mm^2, respectively) had the same number of mast cells, whereas 6-week-old *cpdm/cpdm* mice had a significantly increased ($P < 0.025$) number of mast cells compared with 6-week-old control mice (213 ± 22 and 82 ± 7 cells/mm^2, respectively).

Total serum IgE levels of the *cpdm/cpdm* mice (0.05 ± 0.03 ng/mg) were decreased approximately 10-fold compared with total IgE levels of control animals (0.66 ± 0.15 ng/ml; $P < 0.025$).

No E-selectin, P-selectin, IgE, or IL-5 expression was observed in skin of control animals (Table 1). E-selectin was expressed on endothelial cells of blood vessels in the dermis of *cpdm/cpdm* mice at the age of 1, 3, and 6 weeks, whereas no P-selectin was observed in these mice (Table 1). IgE-positive mast cells were observed in the dermis at the age of 1, 3, and 6 (Figure 6) weeks of *cpdm/cpdm* mice. The mast cells of the 1-week-old *cpdm/cpdm* mice were weakly IgE positive, whereas 3- and 6- (Figure 6) week-old animals showed strongly IgE-positive mast cells (Table 1). In mast cells of 6-week-old *cpdm/cpdm* mice, there seemed to be a negative correlation between alcian blue positivity and IgE positivity; strongly alcian-blue-positive mast cells showed less dense or even negative IgE surface staining and vice versa. The mast cells of the control and *cpdm/cpdm* mice were IL-4 and TNF-α positive (Table 1). No tissue IL-5 expression was observed (Table 1), although T lymphocytes reacted positively in frozen sections of spleen using the same dilution.

Discussion

In this paper, we describe the development of the *cpdm/cpdm* phenotype in the mutant mouse on a C57BL/Ka background. The most striking observa-

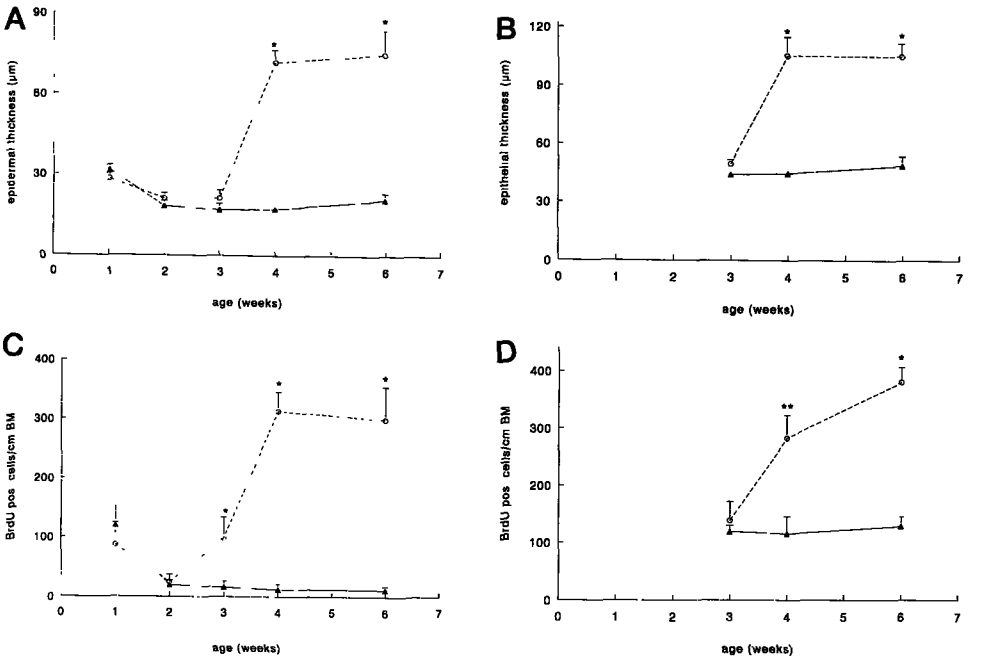

Figure 5. *Epidermal thickness* (a) *and number of BrdU-positive cells* (c) *in the skin and epithelial thickness* (b) *and number of BrdU-positive cells* (d) *in the esophagus* ▲, *control mice*. O, cpdm/cpdm *mice BM, basement membrane* Bars *indicate mean ± SEM of four mice* $^{*}P < 0.025$, $^{**}P < 0.05$ (cpdm/cpdm *versus control*)

tion of this study was that, already at the age of 1 week, skin, lungs, and lymph nodes were infiltrated by eosinophils, whereas at that time point no macroscopic lesions were present. At 1 week later, similar observations were made in the tongue, and 2 weeks later, cellular infiltrations were also observed in the esophagus and forestomach This coincided with the moment that the animals received pelleted food. At 3 weeks, the *cpdm/cpdm* lesions became macroscopically visible

These observations leave us with the intriguing question of what mechanism leads to this selective accumulation of eosinophils Our results seem to indicate that mast cell activation is involved in this process. Mast cell number alters and high levels of bound IgE are observed, which are not observed in control mice. An explanation for the low serum IgE levels observed in the *cpdm/cpdm* mouse could be that serum IgE is maintained at a low level due to its rapid adsorption to tissue mast cells and circulating basophils [12,13] With the alcian blue staining, it seems that most mast cells in the *cpdm/cpdm* mice that demonstrate detectable IgE have lost their granules, especially prominent in the animals of 3 and 6 weeks old Taken together, these observations indicate mast cell activation through IgE resulting in the loss of granule-containing mediators like histamine, proteases, and cytokines and even secretion of membrane-derived mediators like leukotrienes and prostaglandins.[14–16] However, so far no mast cell

Table 1. *Immunohistochemistry of Skin of 1- 3-, and 6-Week-Old Control and* cpdm/cpdm *Mice*

	Control			*cpdm/cpdm*		
Age in weeks	1	3	6	1	3	6
IgE on mast cells	–	–	–	+	+++	+++
TNF-α	ND	ND	++	ND	ND	++
IL-4	+	+	+	+		+
IL-5	–	–	–	–	–	–
E-selectin on endothelial cells	–	–	–	++	+ r	++
P-selectin	–	–	–	–	–	–

ND, not done

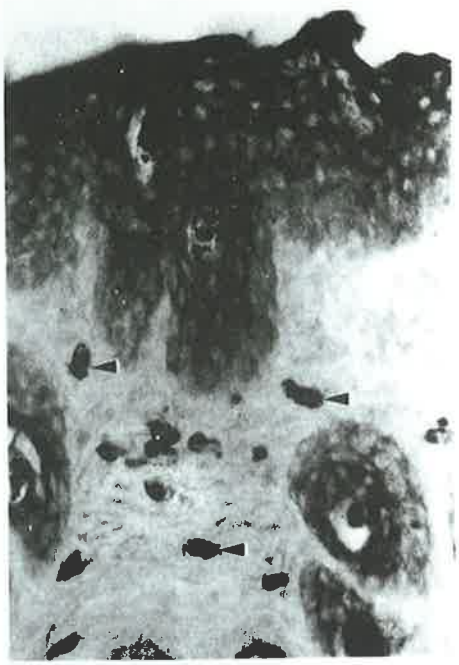

Figure 6. *IgE-positive mast cells in the dermis of a 6-week-old* cpdm/cpdm *mouse* (arrowheads). *Indirect immunoperoxidase with hematoxylin counterstain; magnification,* ×380.

products have been measured. It has been reported that IL-1, TNF-α, IL-3, IL-4, IL-5, granulocyte/macrophage colony-stimulating factor, IL-8, and RANTES are of importance with respect to eosinophil tissue mobilization.[17] Although we could not demonstrate IL-5 on the protein level, IL-4 and TNF-α were present in mast cells of control and *cpdm/cpdm* mice. Thus, upon IgE-mediated activation the mast cells of *cpdm/cpdm* mice may be triggered to release IL-4 or TNF-α. The release of those cytokines, but in particular that of IL-4, may provide the explanation for the selective tissue mobilization of eosinophils. Injection of IL-4 in mice recruits eosinophils selectively.[18] Furthermore, IL-4 is only chemotactic for eosinophils from the peripheral blood of patients with atopic dermatitis and not for eosinophils from normal individuals.[19,20] These arguments support the view that IL-4 could play a role in the observed eosinophil recruitment. However, as it is known that IL-4 stimulates human B cells to produce IgE[15,21] and elevated IgE levels are not observed in the *cpdm/cpdm* mouse, the possibility of another factor cannot be excluded at this moment. The involvement of cytokines that can be produced by monocytes and keratinocytes like RANTES and IL-8 have to be investigated.[22,23] TNF-α released from the mast cells can play a role in the transendothelial migration of eosinophils into the dermis. Transendothelial migration of eosinophils into the tissue involves a cumulative series of interactions in which adhesion molecules on endothelial cells play a crucial role. E-selectin expression is observed already at the age of 1 week and is still seen at the age of 6 weeks in *cpdm/cpdm* mice. On the other hand, P-selectin was not observed. E-selectin is transcriptionally induced by cytokines such as TNF-α or IL-1β.[24] Therefore, our findings concerning the observed E-selectin expression in the developing *cpdm/cpdm* mice indicate the release of TNF-α, probably from mast cells. It was hypothesized by Bosse[25] that P-selectin mediates very early adhesion events, whereas E-selectin would act later and maybe even replace P-selectin at later steps in the inflammatory process. Therefore, the age of 1 week of the *cpdm/cpdm* mice may even be too late for the detection of P-selectin, as this adhesion molecule on the endothelial cells may have been replaced by E-selectin. In a more chronic stage of the inflammatory process of the *cpdm/cpdm* mice, E-selectin could not be observed (H. I. Gallardo Torres, personal communication). It has been reported that in patients with atopic dermatitis E-selectin is a critical adhesion molecule, not only in the acute stage but also in the chronic skin lesions. In this paper, E-selectin is considered responsible for the tissue infiltration of memory T cells,[26] whereas in the *cpdm/cpdm* mouse it should be involved in eosinophil tissue infiltration. Taken together, in the early development of the *cpdm/cpdm* lesion, IgE-mediated mast cell activation leading to the release of the cytokines IL-4 and TNF-α could be responsible for the observed eosinophil tissue infiltration. In this concept IL-4 should lead to the endothelial expression of an as yet unidentified adhesion structure, allowing selective eosinophil tissue infiltration.

The earlier finding that no lesions could be observed after transfer of hemopoietic cells from spleen or bone marrow from *cpdm/cpdm* mice to syngeneic control animals suggested that cells such as mast cells do not play a primary role in its pathogenesis.[1] The maturation of mast cells is regulated by many factors including certain cytokines.[27,28] What cell type and which factors are most important for the development of the *cpdm/cpdm* mouse therefore remains a subject for additional investigation.

Microscopically as well as macroscopically, no epidermal thickening can be observed in the *cpdm/cpdm* mice at the age of 3 weeks, whereas the BrdU incorporation is already increased. At the age of 4 weeks the increased epidermal thickness becomes

obvious microscopically as well as macroscopically A possible explanation for the proliferation of the keratinocytes could be that accumulation of recruited eosinophils leads to enhanced TGF-α concentrations and thus to epidermal proliferation and thickening [29]

In conclusion, the *cpdm/cpdm* mouse can be a valuable tool to study eosinophil tissue infiltration, leukocyte-endothelial cell interactions, and mast cell proliferation Furthermore, the *cpdm/cpdm* mouse can be used to study chronic inflammatory skin disease because of the severe epidermal proliferation

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Chapter 7

Therapeutic interventions in mice with chronic proliferative dermatitis (*cpdm/cpdm*)

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ABSTRACT

Chronic proliferative dermatitis (*cpdm/cpdm*) is a spontaneous mutation in C57BL/Ka mice. The dermatitis is characterized by redness, hairloss, scaling, pruritus and histologically by epithelial hyperproliferation, infiltration of eosinophils, macrophages and mast cells. Lesions similar to those in the skin occur in the esophagus and forestomach. In this paper, we describe the effect of drug treatments directed against epidermal hyperproliferation (calcipotriene and etretinate), against inflammation (corticosteroids and dapsone) and against pruritus (loratidine and capsaicin). The criteria used to objectively estimate the effect of the treatment were 1) macroscopic evaluation of the lesions (cpd score), 2) degree of epithelial hyperproliferation assessed by BrdU incorporation and epithelial thickness, and 3) microscopic evaluation of the inflammatory cells in the skin samples.

Topical calcipotriene treatment (5 μg/day for 3 weeks) inhibited epidermal proliferation and the number of eosinophils. Systemic etretinate treatment (30 μg/g/day for 3 weeks) was not very effective. Topical corticosteroids (0.05 μg/day, for 3 weeks) exerted a therapeutic effect on the cpd score, hyperproliferation and the number of eosinophils. Oral dapsone treatment (34 μg/g/day, for 5 weeks) reduced the BrdU incorporation in the skin and the epithelial thickness in the esophagus. The anti-histamine loratidine (orally, 1.71 μg/g/day, for 4 weeks) reduced the severity of the lesions macroscopically, probably by suppressing the pruritus. Capsaicin (topically, 30 mM, for 5 weeks) also reduced the severity of the macroscopic observable lesions. Moreover, capsaicin reduced the dorsal and ventral epidermal thickness.

The results from this and previous studies indicate that steroids (topically and systemically) and less strongly calcipotriene are the most effective treatments for the lesions observed in the *cpdm/cpdm* mice, since both hyperproliferation and the influx of eosinophils are reduced. Although the pathogenesis of the cpd lesions remains to be determined, our results indicate that the *cpdm/cpdm* mouse can be used to investigate new drugs for their possible application in chronic dermatitis.

INTRODUCTION

We recently described a mouse mutant (*cpdm/cpdm*) with chronic proliferative dermatitis on a C57BL/Ka background [1,2,3,4]. The skin lesions are characterized by erythema, severe hair loss, and scaling beginning at the age of 5 weeks. The

animals are severely pruritic based on intense scratching of the animals. This often results in ulceration of the skin. Light microscopically, there is hyper- and parakeratosis, hyperplasia and apoptosis of keratinocytes, vascular proliferation, and infiltration of mast cells, macrophages and granulocytes, mainly eosinophils. Only a few T-cells are present. Similar lesions as found in the skin are observed in the esophagus and forestomach. The lesions regressed upon systemic treatment with corticosteroids but not with cyclosporin A (CsA) [1]. Infiltration of the skin with eosinophils is already present at 1 week of age, at which time macroscopic lesions are not yet present [5]. One week later increased number of eosinophils are present in the tongue and this is followed by cellular infiltration of the esophagus and forestomach. Six week old *cpdm/cpdm* mice had an increased number of mast cells [1,5]. At the age of one week, these mast cells were weakly IgE-positive whereas 3 and 6 week old *cpdm/cpdm* mice showed strongly IgE-positive mast cells [5]. At 8 weeks, serum IgE levels were decreased compared to the control animals [5]. Based on these characteristics the *cpdm/cpdm* mouse can be used to study chronic inflammatory hyperproliferative skin disease and specific aspects of allergic inflammatory skin disease, such as eosinophil tissue infiltration, leukocyte-endothelial cell interactions and mast cell proliferation.

The most common chronic dermatitis in man characterized by epidermal proliferation and inflammation of the dermis and epidermis, is psoriasis [6]. The abnormalities of epidermal cells in human psoriasis may be caused by cytokines secreted in dermal immune reaction involving T-cells [7,8]. Autoreactivity of T lymphocytes to some epidermal component may be the initiating event, and these T cells may activate keratinocytes to secrete a variety of cytokines and growth factors [9]. However, the exact mechanism has not yet been resolved. Although the pathogenesis of the *cpdm/cpdm* mouse and human psoriasis are most likely different, the final result characterized by hyperproliferation of keratinocytes is similar. For this reason, treatments used in psoriasis such as *calcipotriene*, *etretinate* and *triamcinolon*, were tested for their effect in the *cpdm/cpdm* mouse.

Calcipotriene is a vitamin D analogue that inhibits epidermal cell proliferation and enhances cell differentiation [10]. Furthermore, calcipotriene may interact with and regulate a cytokine pathway responsible for the accumulation of leukocytes during skin inflammation [11]. Topical (twice-daily) treatment with a calcipotriene ointment (50 μg of calcipotriene/g) over an eight-week period caused substantial improvement in 60 percent of patients with chronic plaque psoriasis. *Etretinate*, a retinoic acid derivate, stimulates epithelial cell differentiation and inhibits malignant transformation in the skin [12]. It restores the depleted population of Langerhans' cells in psoriatic lesional skin. Only 50% of the patients with extensive psoriasis

show moderate improvement upon oral etretinate treatment with 0.3 to 0.4 mg/kg/day [13]. *Corticosteroids* are very potent immunosuppressive agents and are widely used to treat psoriasis. The more potent topical corticosteroids are consistently effective with a very fast mode of action [13]. In 60% of the psoriasis patients the skin condition improves after treatment with 0.025 - 0.1 % triamcinolone (once daily for three weeks) .

Another reason to treat the mice with topical steroids is that some characteristics of the lesions of the *cpdm/cpdm* mouse are also present in atopic dermatitis. Atopic dermatitis is considered an inflammatory disorder involving T-lymphocytes and eosinophils, and therefore anti-inflammatory treatment with topical steroids is successful [14,15]. Itching is severe in *cpdm/cpdm* mice and is a symptom of psoriasis and atopic dermatitis in human patients. Itching may be suppressed by anti-histamines and therefore treatment with *loratidine* was carried out. Loratidine, a H_1-antagonist, inhibits the histamine induced effects i.e. vasodilatation, increased permeability of small blood vessels, smooth-muscle contraction and itching. The dose used in man is 10 mg daily, p.o.. Another anti-pruritic agent is *capsaicin*. Capsaicin depletes neuropeptides such as substance P from local sensory terminals. The release of substance P in the dermis causes vasodilatation and plasma extravasation in rats and humans [16,17]. In addition, substance P release has been associated with activation of cytokines, mast cell degranulation resulting in release of histamine, and macrophage chemotaxis [18,19]. Although data from human studies are limited, it appears that capsaicin cream (0.025%, 6 weeks treatment) may have anti-pruritic effects in psoriasis [20,21].

Dapsone (4,4'-diaminodiphenyl sulfone) an anti-inflammatory agent used for treatment of dermatitis herpetiformis and a number of other non-infectious diseases that have in common the presence of neutrophils or eosinophils as the preponderant infiltrating cell [22]. Dapsone has an effect on various granulocyte functions such as myeloperoxidase-mediated iodination, neutrophil-lysosomal activity, and the generation of active oxygen metabolites [23,24,25]. Dapsone suppresses the leukocyte chemotactic and cytotoxic functions by suppression of leukocyte integrin function [22]. Since eosinophils are abundantly present in various tissues of the *cpdm/cpdm* mouse, the effects of this treatment were evaluated. The dose used in man is 50 mg (3-4 x daily, p.o).

The above mentioned treatments were tested in the *cpdm/cpdm* mice to find out whether this mouse model is a suitable *in vivo* model to test drugs for epidermal hyperproliferation, eosinophil infiltration and pruritis. Besides, some of these treatments were chosen to study whether interference with the disease

process was possible and so unravel the pathogenesis of the *cpdm/cpdm* mouse. Based on earlier investigations [1] the following criteria were used to test the therapeutic effectiveness: macroscopic appearance of the lesions (cpd score), microscopic evaluation of the inflammatory cells in skin, epithelial thickness and epithelial cell proliferation of skin and esophagus.

MATERIAL AND METHODS

Animals The cpd mutation (*cpdm/cpdm*) arose spontaneously in the colony of the inbred C57BL/KaLawRij mice in the specific pathogen-free-breeding facility of TNO in Rijswijk, The Netherlands. The colony of *cpdm/cpdm* mice is being maintained by heterozygous x heterozygous breeding. Mice were housed in Macrolon cages and were provided autoclaved pelleted food and acidified, sterilized bottled drinking water *ad libitum*. The animals were 8-12 weeks of age at the initiation of treatment unless otherwise specified.

The oral dosages (etretinate, loratidine and dapsone) were based on human concentrations extrapolated to the mouse. The dose equivalent from man to mouse is 12.5 (kg^{-1}) [26]. This is based on the surface area. Oral dosages for loratidine and dapsone used in the *cpdm/cpdm* mice were human concentration x 4 (lowest dose) and x 12.5 (highest dose). For etretinate was a concentration used of human concentration x 12.5 (lowest dose) and x 37.5 (highest dose). The topical calcipotriene cream and the corticosteroid cream were both used at concentrations used on human patients. The protocol for skin desensitisation by capsaicin was based on pilot studies performed by W. van den Hoven (Yamanouchi Europe B.V., Leiderdorp, The Netherlands).

Experimental design Calcipotriene. Mice (n=3) were treated on the dorsal skin with 100 μl of cream with calcipotriene (50 μg of calcipotriene per gram). The control animals (n=3) received 100 μl placebo cream. The skin of the back was shaved when necessary for topical treatment. The duration of the treatment was 7 days a week, one treatment a day for 3 weeks.

Etretinate. Mice received orally by gavage 10 μg/g/day (n=3) or 30 μg/g/day (n=3) etretinate. The etretinate was dissolved in olive oil. The control animals (n=3) received olive oil (2 μl/g/day). The duration of the treatment was 7 days a week for 3 weeks.

Corticosteroids. Mice (n=5) received topical 50 μl/mouse of a cream with

triamcinolonacetonide (1 mg/g) (Bufa BV, Uitgeest, The Netherlands) on the dorsal skin. The control animals (n=5) received 50 μl/mouse of a placebo cream (cetomacrogol). The age of the animals in each group was 11 weeks (n=3) and 24 weeks (n=2). Mice were treated daily from Monday to Friday for 3 weeks. The skin of the back was shaved when necessary for topical treatment.

Loratidine. Mice received 0.43 μg/g/day (n=5) or 1.71 μg/g/day (n=5) loratidine (Schering/Plough, Kenilworth, USA) in water. The control animals (n=5) received non-acidified drinking water. The duration of the treatment was 4 weeks.

Capsaicin. Mice (n=5) received 100 μl on the dorsal and 100 μl on the ventral skin of 30 mM capsaicin (Sigma) dissolved in ethanol. The schedule of the treatment was: the first week twice a day during 2 days, the next 5 weeks one treatment a week. The control animals (n=3) received dorsal and ventral ethanol. The age of the animals at the beginning of the experiment was 5 weeks. The skin of the back was shaved when necessary for topical treatment.

Dapsone. Mice received 8.5 μg/g/day (n=5) or 34 μg/g/day (n=4) dapsone (Bufa BV, Uitgeest, The Netherlands) in water. The control animals (n=5) received non-acidified drinking water. The age of the mice was 14 weeks at the beginning of the experiment. The duration of the treatment was 5 weeks.

Male and females were randomized in the groups. The mice of all groups were weighed each week. The amount of drinking water was established per cage of the animals which received the treatment in the drinking water.

Cpd-score The cpd-score is a quantification of the severity of the gross lesions. Therefore, the affected percentage body surface is calculated (with computer-aided morphometry) multiplied by the grade of severity of the individual lesions. 0: no lesions, 1: erythema, 2: mild scaling/hairloss, 3: moderate/severe scaling/hairloss, 4: ulceration. The cpd-score of the animals treated with topical corticosteroids, loratidine, capsaicin and dapsone was established each week.

Bromodeoxyuridine (BrdU) labeling and histology Thirty minutes before being killed, mice were administered 0.625 mg BrdU (Sigma) intraperitoneally to determine the rate of cell proliferation in the epidermis and esophageal epithelium. The dorsal and ventral skin and the esophagus were fixed for 18 hours in neutral-buffered formalin, stored in 70% alcohol and later embedded in paraffin. Paraffin-embedded sections were deparaffinized, rehydrated and incubated with monoclonal anti-BrdU antibody (Dakopatts, Copenhagen, Denmark) or stained with hematoxilin-phloxine-saffron (HPS). The labeled nuclei of the slides incubated with

anti-BrdU were visualized by peroxidase-labeled rabbit anti-mouse Ig (Dakopatts), followed by diaminobenzidine in combination with 1% Cobalt-Cl to enhance the staining intensity. Routine HPS stained sections of skin and esophagus were examined for cellular infiltration, hyperkeratosis, acanthosis and parakeratosis.

Morphometry and statistical analysis BrdU-labeled nuclei were counted per cm of epidermal basement membrane. The thickness of the combined nucleated epithelial layers of the esopagus and interfollicular epidermis were measured at 10 sites. From these measurements, the mean thickness was calculated for each epithelium. The cpd-score was calculated based on morphometric analysis of skin surface multiplied by severity grade of the gross cpd-lesions. The measurements were performed with computer-aided morphometry (Kontron-Videoplan, Zeiss, Germany). All data are expressed as mean ± SD. Statistical analysis was performed using the Student's *t*-test.

RESULTS

None of the treatments modified the body weight of the mice (data not shown).

The results of the various treatments including those of systemic cyclosporin A and corticosteroids [1] are presented in Table 1.

Calcipotriene. There was no difference in cpd-score between the control (n=3) and calcipotriene treated animals (n=3). Light microscopy of the dorsal skin revealed reduced hyperkeratosis and cellular infiltration in 2 of the calcipotriene treated animals. No difference was observed in the epidermal thickness of the skin and esophagus of the control and calcipotriene treated animals. The BrdU-incorporation was decreased in the dorsal skin ($p<0.05$) of the calcipotriene treated animals but remarkably also in the (non-treated) ventral skin ($p<0.025$). Cell proliferation in the esophagus was equal in the control and calcipotriene treated animals (Figure 1a).

Etretinate. There was no difference in cpd-score between the control (n=3) and etretinate treated animals (n=3). Light microscopically a decreased cellular infiltration in the upper dermis and more eosinophils in the subcutaneous fat was present in the etretinate treated animals. The dorsal epidermal thickness of the animals treated with 30 μg etretinate was diminished compared with that of the control animals whereas the ventral skin ($p<0.05$) and esophagus were equal (Figure 2a). No difference in BrdU-incorporation was observed between the treated and untreated animals (data not shown).

Table 1. Summary of the effects of various drugs on cpd-index, epidermal thickness and cell proliferation. Treatment of calcipotriene, etretinate, corticosteroids (topical and systemic), capsaicin and dapsone have beneficial effects on the lesions in the *cpdm/cpdm* mouse.

Test compounds	cpd-index	epidermal thickness	cell-proliferation	cellular infiltration
calcipotriene (topical dorsal 5 μg/day)	-	-	↓ dorsal ↓ ventral	↓ dorsal
etretinate (oral 30 μg/g/day)	-	↓ dorsal	-	↓?
triamcinolone (topical dorsal 0.05 μg/day)	↓	↓ dorsal	↓ dorsal	↓ dorsal
loratidine (oral 1.7 μg/g/day)	?	-	-	-
capsaicin (topical dorsal + ventral, 30 mM)	?	↓ dorsal ↓ ventral	-	-
dapsone (oral 34 μg/g/day)	-	↓ esophagus	↓ dorsal ↓ ventral ↓ esophagus	-
triamcinolon (systemic, 10 μg/g twice a week) [1]	↓	↓	↓	↓
cyclosporin A (systemic, 100 μg/g three times a week) [1]	-	-	-	-

?: questionable effect
-: no effect compared with control animals
↓: decreased effect compared with control animals

Corticosteroids. The dorsal skin of the control group (n=5) was thickened, contained fine white scales and showed partial alopecia. The dorsal skin of the triamcinolone treated animals (n=5) became much thinner and the scaling disappeared resulting in a diminished cpd-score (Figure 3). In two animals (11 weeks old) the dorsal skin lesions had completely resolved after 10 days of treatment. Light microscopy of the dorsal skin of the 11 week old animals (n=3) treated with triamcinolone showed no or only mild hyperkeratosis and mild cellular infiltration whereas 24 weeks old triamcinolone treated animals (n=2) showed moderate hyperkeratosis and mild cellular infiltration. There was no difference in the other tissues between the control group and the triamcinolone treated group. The epidermal thickness of the dorsal skin was decreased in the triamcinolone treated animals mainly in the 11 weeks old and less in the 24 weeks old animals (Figure 2b). One animal of 11 weeks was omitted because the sample taken for histology was from a severe lesion on the back. This animal was also excluded for the BrdU-

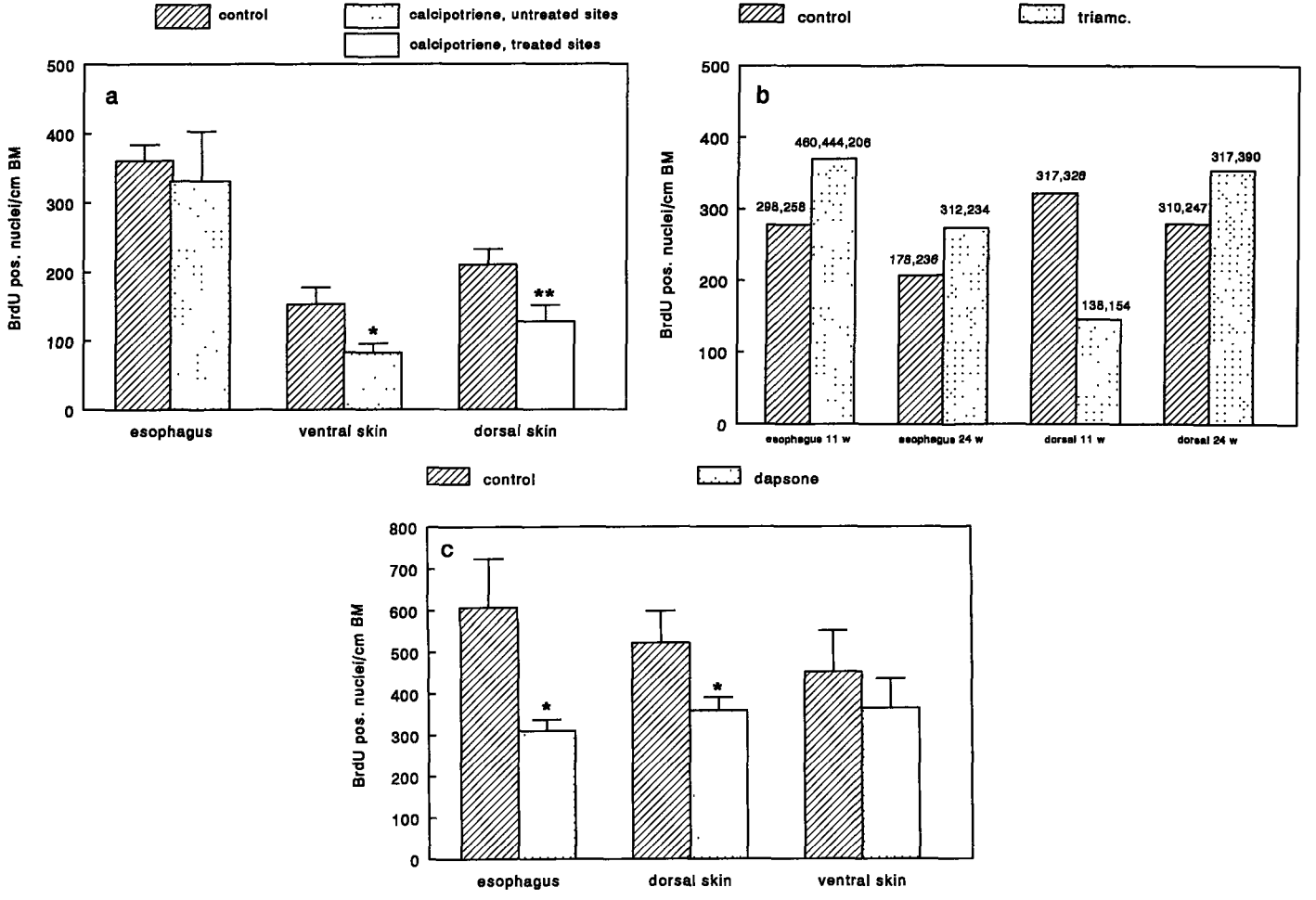

Figure 1 BrdU labeling of esophagus and/or skin of *cpdm/cpdm* mice is decreased after treatment with calcipotriene (topical, dorsal) (a), triamcinolone (11 weeks old, topical, dorsal) (b) and dapsone (oral) (c). Bars indicate mean ± SD. *: $p<0.025$, **: $p<0.05$ (*cpdm/cpdm* vs. control). Individual data are given for the triamcinolone treatment because there were only 2 animals per group.

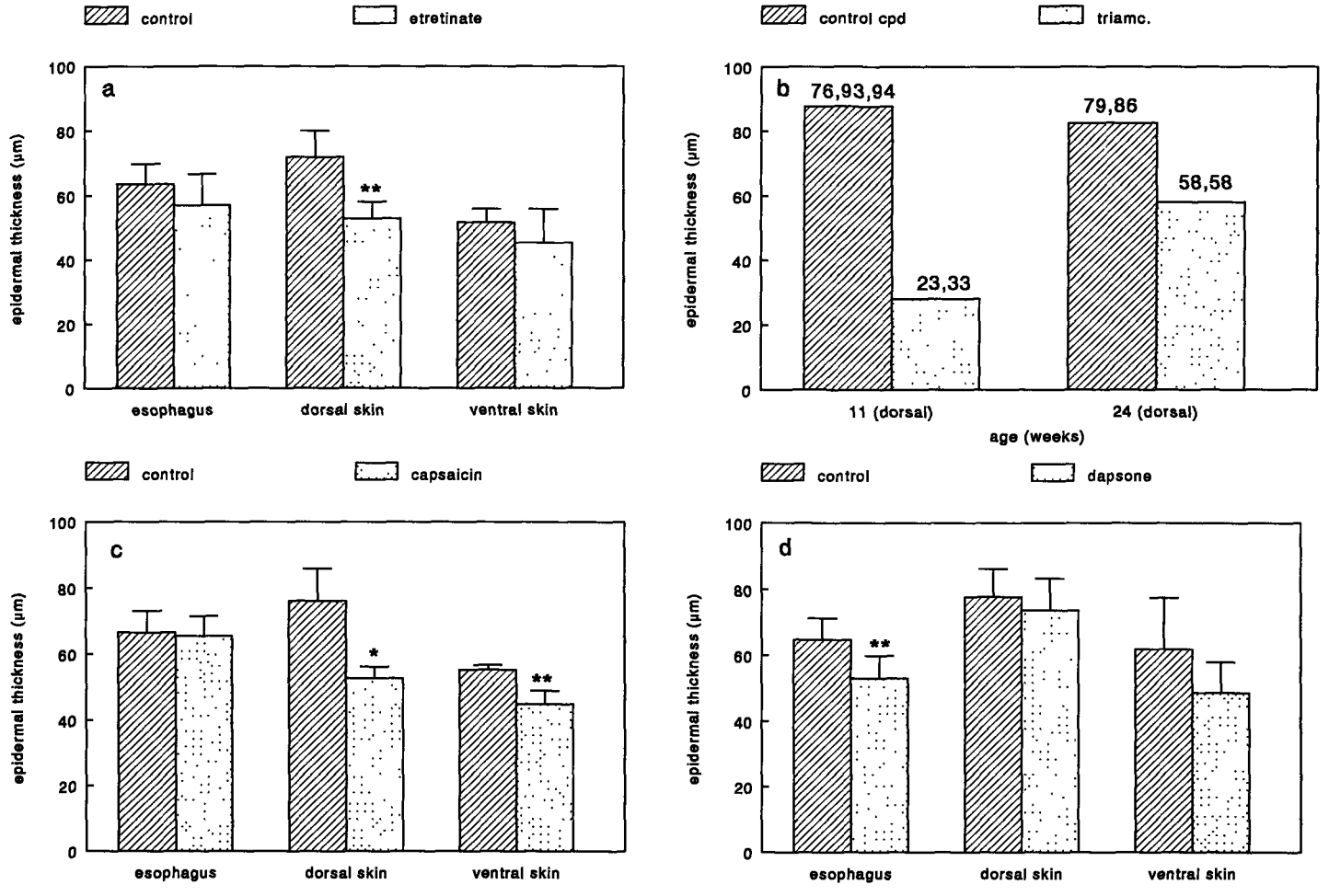

Figure 2 Epithelial thickness of esophagus and/or skin of *cpdm/cpdm* mice is decreased after treatment with etretinate (oral) (a), triamcinolone (topical, dorsal) (b), capsaicin (topical, ventral and dorsal) (c) and dapsone (oral) (d). Bars indicate mean ± SD. *: $p<0.025$, **: $p<0.05$ (*cpdm/cpdm* vs. control). Individual data are given for the triamcinolone treatment because there were only 2 animals per group.

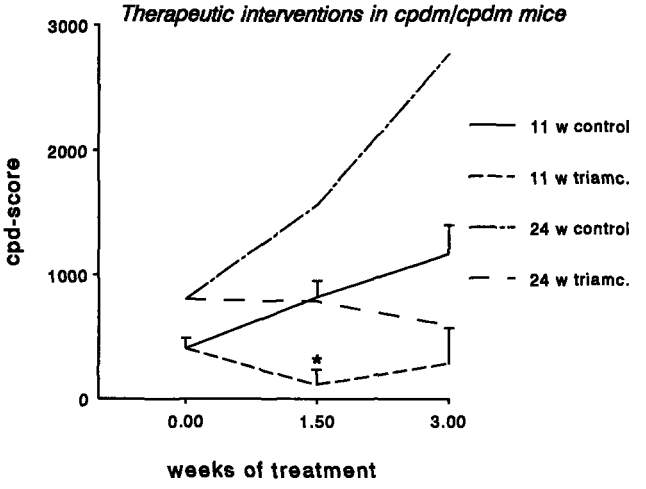

Figure 3 Cpd-score is decreased in 11 and 24 weeks old triamcinolone (topical, dorsal 0.05 μg/day) treated animals. Bars indicate mean ± SEM. *: $p<0.025$ (*cpdm/cpdm* vs. control).

incorporation. The BrdU data are presented in Figure 1b with the omission of the triamcinolone treated animal mentioned above and one control animal (11 weeks old) which did not have BrdU-positive nuclei, most likely due to inappropriate injection of BrdU. A decreased proliferative effect could only be demonstrated in the dorsal skin of triamcinolone treated animals of 11 weeks old.

Loratidine. Macroscopically less severe lesions were observed (almost no ulceration and only mild scaling) in the loratidine treated animals but this did not result in a significant lower cpd-score. There were no differences in epidermal thickness, BrdU-incorporation and infiltrating cells between the control animals and the loratidine treated animals (data not shown).

Capsaicin. Macroscopically less severe lesions were observed (almost no ulceration and only mild scaling) in the capsaicin treated animals but this did not result in a significant lower cpd-score. The cellular infiltrate was not reduced compared to control animals. The dorsal and ventral ep idermal thickness of the animals treated with capsaicin was diminished ($p<0.025$ respectively $p<0.05$) compared with the control animals, whereas the epithelial thickness of the esophagus was unaltered (Figure 2c). No difference in BrdU-incorporation was observed between the treated and untreated animals (data not shown).

Dapsone. There was no difference in cpd-score between the control (n=5) and dapsone treated animals (n=5). The cellular infiltration was not diminished in the skin of dapsone treated animals. No difference was observed in epidermal thickness of the skin between the control and dapsone treated animals. The epidermal thickness of the esophagus was decreased in the mice treated with 34

μg/g/day dapsone ($p<0.05$) (Figure 2d) but not in the mice treated with 8.5 μg/g/day dapsone. The cell proliferation was decreased in the dorsal skin ($p<0.025$) and esophagus ($p<0.025$) of the with 34 μg/g/day dapsone treated animals (Figure 1c) but not in the mice treated with 8.5 μg/g/day dapsone.

DISCUSSION

The skin lesions of the *cpdm/cpdm* mice are characterized by hyperproliferation of the keratinocytes resulting in increased thickness of the epidermis and influx of eosinophils in the dermis. The animals are severe pruritic based on intense scratching of the animals. In this paper, we describe the effect of treatments that putatively inhibit hyperproliferation and inflammation and reduce pruritus. Criteria used for testing the effect of treatment were 1) macroscopic evaluation of the lesions (cpd score), 2) degree of epithelial hyperproliferation assessed by BrdU incorporation and epithelial thickness, and 3) microscopic evaluation of the inflammatory cells in the skin samples.

Topical *corticosteroids* had a therapeutic effect on both hyperproliferation and infux of eosinophils. In an earlier study [1], we already described the improvement of the skin after treatment with the systemic corticosteroid triamcinolone. The thickness and BrdU incorporation were reduced and inflammatory cells were absent after systemic treatment with 10 mg/kg triamcinolone (200 μg/mouse). Topical treatment with corticosteroids (1 mg/g; 50 μl/mouse) reduced the epidermal thickness, the BrdU incorporation and the presence of inflammatory cells (eosinophils) of the treated skin when the animals were young (11 weeks), whereas in older animals (24 weeks) inflammation was reduced without an effect on BrdU incorporation. The tissue infiltration of eosinophils appears to be more sensitive to the action of corticosteroids than epidermal hyperproliferation. Furthermore, young animals were more sensitive to treatment with steroids on hyperproliferation than older animals. Young *cpdm/cpdm* animals (age range 7-18 weeks) should preferably be used when the effects of drugs on epidermal thickness is investigated.

After a 5 week oral treatment with *dapsone*, the BrdU incorporation was significantly reduced in the highest dose-group. Also the epithelial thickness of the esophagus, where the dapsone concentration was probably highest (because of oral application), was decreased. A possible explanation for the fact that dapsone has only a small effect on epidermal thickness could be the relatively short period of treatment. In the skin of dapsone treated animals infiltration of eosinophils was not reduced. In human dermatitis herpetiformis, treatment with comparable oral

dapsone concentrations leads to complete inhibition of influx of neutrophils [27]. It has been suggested that dapsone acts by suppressing neutrophil recruitment to lesional sites through interaction with the CD11/CD18 family of integrins [22] which includes MAC-1, LFA-1 and p150,95 receptors, adhesion molecules which also contribute in recruitment of eosinophils. Our observations do not support this suggestion. However, Anderson [28] demonstrated eosinophils in tissues of a CD18 deficient patient. Therefore, eosinophils were likely to adhere by a CD18-independent mechanism as well.

Topical *calcipotriene* treatment inhibited epidermal proliferation in the *cpdm/cpdm* mice not only at the (treated) dorsal skin but remarkably also at the ventral skin which received no topical treatment. An explanation may be that calcipotriene cream through scratching has been replaced from the dorsal to the ventral side. In the esophagus, where no cream was applied, no difference was observed between controls and treated animals. The fact that this treatment did not decrease the epithelial thickness suggests that the duration of the treatment period (3 weeks) may have been too short. In human psoriasis, the improvement is maximal after a treatment period of 6-8 weeks [11].

Etretinate was not very effective in the treatment of the cpd lesions. No effect was observed on the BrdU incorporation. The epidermal thickness of the dorsal skin was slightly diminished although the route of application (orally) of the drug should lead to such an effect in the esophagus, where it did not. However, also in the treatment of psoriasis the efficacy of etretinate alone is limited [29]. Among patients with extensive plaque psoriasis, approximately half of the patients show moderate improvement [30]. Treatment of other mouse mutants with retinoic acids did not result in any decrease of epidermal thickness. Topical treatment of the rhino mouse ($hr^{h}hr^{h}$) with retinoic acid can even led to an increase of the epidermal thickness [31,32]. A similar effect was observed in hairless albino mice fed with aromatic retinoids [33,34].

The only effect of the anti-histamine *loratidine* was reduction of macroscopic lesions. This is most likely due to the itching depressing effect of the drug. Although no good reliable objective parameter is yet available to quantify this, our observations of the treated animals strongly suggested this. Microscopically no improvement was observed. In humans, anti-histamine medication in atopic dermatitis prevents further aggravation of the dermatitis by depressing the pruritus [35]. The other anti-pruritic treatment *capsaicin* reduced also the macroscopic lesions. Moreover, after treatment with capsaicin, the dorsal and ventral epidermal thickness in these animals was reduced. Topical capsaicin, which can deplete neuropeptides such as substance P from sensory neurons in the skin, has been

succesfully used to treat pain and/or itch, associated with human diseases such as diabetic neuropathy, postherpetic neuralgia, hemodialysis-related pruritus [reviewed by 36] and psoriasis [20,21]. These pruritic disorders are believed to be mediated by neuropeptide transmitters, in particular substance P. Pruritic stimuli are conveyed to central processing centers via small, substance P-containing, unmyelinated, type C fibers in the skin for which capsaicin has selective activity. Taken together, the relief of severe macroscopic lesions and epidermal thickness in the *cpdm/cpdm* mouse suggest that neuropeptides are involved.

Taken together, our studies indicate that corticosteroids and less strongly calcipotriene exert an effect on both epidermal hyperproliferation and the influx of eosinophils. By other drug treatments (etretinate, dapsone and capsaicin) only epidermal hyperproliferation is influenced without influencing the degree of influx of eosinophils. In case of anti-pruritus (loratidine) treatments only the severity of the macroscopic lesions in influenced, wheras both epidermal hyperproliferation and eosinophil influx are not influenced. With our choice of drugs, it is difficult to conclude whether epidermal hyperproliferation and eosinophil influx are separated entities or whether they are interrelated. In some instances only one of those two entities was influenced by drug therapy which suggests that no interrelationship exists. However, longer treatment periods could change this view. Further investigations should provide more insight in the relation between epidermal hyperproliferation and eosinphil infiltration.

Taken together, the presented results indicate that the *cpdm/cpdm* mouse is a suitable *in vivo* model to test treatments against epidermal hyperproliferation, eosinophil infiltration and possibly against pruritus. The model allows to study the effect of drug treatment on the separate entities although a method to quantitate the pruritus still needs to be developed.

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Chapter 8

General discussion

GENERAL DISCUSSION

Characteristics of the cpdm/cpdm mouse

Chronic proliferative dermatitis (gene symbol: *cpdm*) was identified in 1991 as a spontaneously occurring disease in the C57BL/KaLawRij strain at the TNO-breeding facility in Rijswijk, the Netherlands. It is an autosomal recessive mutation, as suggested by the incidence of the disease in the offspring of various combinations of breeding pairs. The aim of this thesis was to investigate whether this mouse model has similarities with human skin diseases so that the model can be used to study the pathophysiology of human skin disorders with a chronic proliferative character. Furthermore, the *cpdm/cpdm* mouse model was used to test therapies developed for skin disorders with a chronic proliferative character.

The skin lesions in *cpdm/cpdm* mice become apparent at 5 weeks of age and they are characterized by erythema, partial alopecia, and fine scaling starting in the dorsal neck and ventral chest area. The animals are severely pruritic and intense scratching often results in ulceration of the skin.

Light microscopically, there is epidermal hyperplasia with hyper- and parakeratosis. There is dilatation and proliferation of dermal capillaries. Besides some small pustules and microabscesses in the stratum corneum, only a few inflammatory cells are observed in the epidermis. In contrast, the dermis is infiltrated by a mixed population of inflammatory cells, predominantly granulocytes and macrophages. Electron microscopic examination revealed that these granulocytes are mainly eosinophils. Only a few T-lymphocytes are present. Also the mucosa of the oral cavity, esophagus, and forestomach which are lined by orthokeratinizing squamous epithelium in the mouse, are afflicted by similar lesions in *cpdm/cpdm* mice.

Although the skin lesions of the *cpdm/cpdm* mice are most obvious, inflammatory foci are also present in the lungs, liver and peri-synovial connective tissue. Both the draining lymph nodes of the skin and the spleen are enlarged due to heavy infiltration of eosinophils, whereas marked extramedullary myelopoiesis also accounts for the enlargement of the spleen.

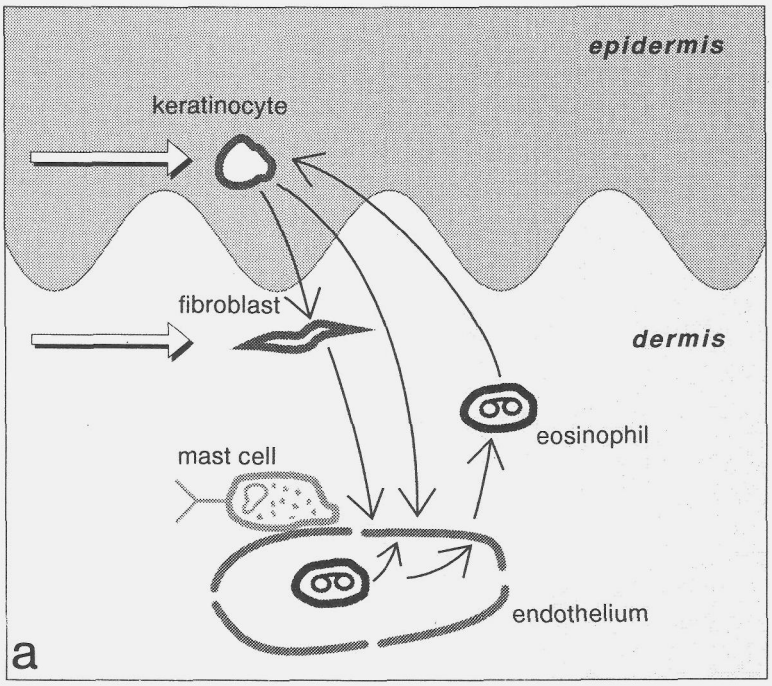
Transfer of hemopoietic cells from *cpdm/cpdm* mice to lethally irradiated syngeneic mice failed to induce *cpdm/cpdm* lesions in the recipients, suggesting that hemopoietic cells do not play a primary role in the pathogenesis (chapter 2). Full thickness grafts of affected skin from *cpdm/cpdm* mice and normal skin from C57BL/Ka mice transplanted onto *cpdm/cpdm*, C57BL/Ka mice or athymic nude mice, maintain the donor phenotype (chapter 5). These findings suggest that the pathological features of the *cpdm/cpdm* mice are the result of a disorder in the

dermis or epidermis and not caused by a systemic defect. It has become clear that eosinophils play an important role. Already at the age of 1 week skin, lungs, and lymph nodes are infiltrated by eosinophils, whereas at that time point no macroscopic lesions are present. One week later similar observations are made in the tongue and two weeks later cellular infiltrations are also observed in the esophagus and forestomach. The hyperproliferation of the basal keratinocytes of the epidermis and the basal cells of the esophagus does not start before the age of 3 and 4 weeks respectively. The number of mast cells starts to increase in the *cpdm/cpdm* mice from the age of 6 weeks while IgE positive mast cells are already observed at 1 week of age. It seems that most mast cells in the *cpdm/cpdm* mice which demonstrate detectable IgE have lost their granules, which is especially prominent in animals of 3 and 6 weeks old. E-selectin expression is observed already at the age of 1 week and can still be seen at the age of 6 weeks (chapter 6).

Pathogenesis

From the skin transplantation studies, it can be concluded that the primary defect of the chronic proliferative dermatitis in the *cpdm/cpdm* mouse is localized in the epidermis or dermis. The first abnormality observed in the *cpdm/cpdm* mouse is the increasing number of eosinophils in the skin. Yet, from the hemopoietic cell transplantation study it can be concluded that the primary defect is not localized in these cells. Another early observation is the IgE positive mast cells while these mast cells have lost their granules at 3 weeks. Endothelial cells can also play an important role in the pathogenesis because these cells are activated in a very early stage of the disease. They express E-selectin, an adhesion molecule for eosinophils. Only later, at the age of 3 weeks, the hyperproliferation of the keratinocytes is observed in the disease process. However, this does not necessarily rule out the possibility that the primary defect could not be localized in the keratinocytes.

For the pathogenesis of the *cpdm/cpdm* lesions the following hypotheses can be put forward. 1. The lesions originate from abnormal functioning keratinocytes and fibroblasts (Figure 1a). The keratinocytes and fibroblasts can secrete a variety of pro-inflammatory cytokines when activated. These can cause mobilization of eosinophils in the dermis. It has been reported that IL-1, TNF-α, IL-3, GM-CSF (granulocyte-macrophage colony stimulating factor), eotaxin (1,2) and possibly RANTES (3) can be produced by keratinocytes (reviewed by Schröder (4)). Dermal fibroblasts express and secrete RANTES when they are stimulated with TNF-α and IL-1 (5,6) and eotaxin (2). These cytokines may be directly or indirectly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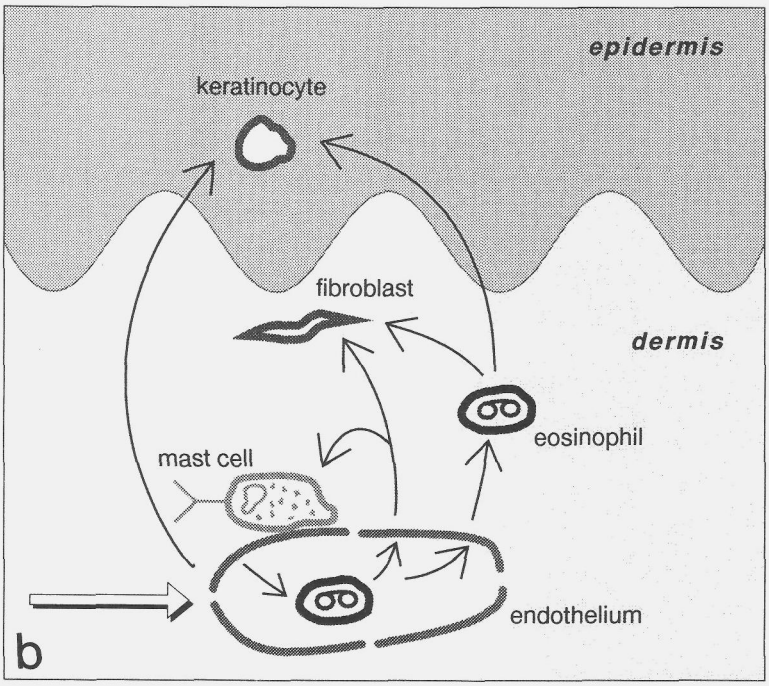
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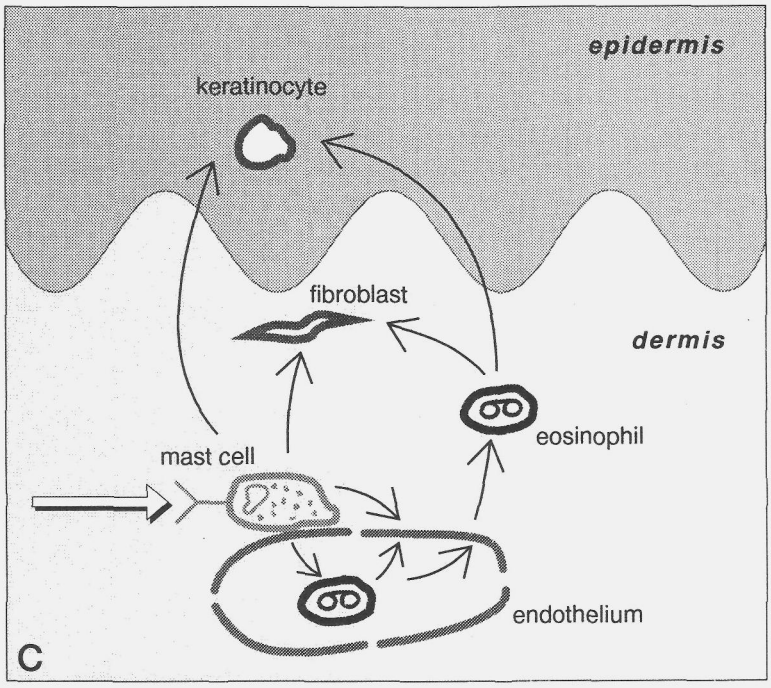

Figure 1
Schematic illustrations of the possible explanations for the pathogenesis in the skin of the *cpdm/cpdm* mouse.
a) A primary disturbance originates from the keratinocytes or fibroblasts. The keratinocytes and fibroblasts may secrete a variety of pro-inflammatory cytokines which can cause mobilization of eosinophils in the dermis. It has been reported that IL-1, TNF-α, IL-3, GM-CSF, eotaxin, and possibly RANTES can be produced by keratinocytes. Dermal fibroblasts secrete RANTES when they are stimulated with TNF-α and IL-1. These cytokines may be directly or indirectly involved in eosinophil tissue mobilization. The accumulation of recruited eosinophils may lead to enhanced TGF-α production which in itself may cause epidermal proliferation and thickening.
b) A primary disturbance originates from the endothelial cells. A primary disturbance, by unknown cause, in endothelial cells may contribute to increased eosinophil tissue infiltration by persistent expression of adhesion molecules. Transendothelial migration of eosinophils into the tissue involves a cumulative series of events in which adhesion molecules on endothelial cells may play a crucial role. Under steady state, non-activating conditions, eosinophils hardly bind to endothelial cells. Cytokines such as TNF-α, IL-1 and IL-4 have been shown to stimulate the transcription and the expression of the endothelial adhesion molecules E-selectin, ICAM-1 and VCAM-1. These adhesion molecules are involved in the interaction between eosinophils and endothelial cells. The accumulation of recruited eosinophils may lead to enhanced TGF-α production which in itself may cause epidermal proliferation and thickening. In turn, the activated keratinocytes can produce IL-1, TNF-α, IL-3, GM-CSF and eotaxin which can stimulate eosinophil tissue mobilization.
c) A primary disturbance originates from mast cells. Mast cell activation through IgE may result in the loss of granules containing mediators like histamine, proteases, and cytokines and even secretion of membrane-derived mediators like leukotrienes and prostaglandins. Cytokines which can be produced by mast cells and may act as chemoattractants for eosinophils are TNF-α, IL-3, IL-4 and IL-5. The release of those cytokines, but in particular that of IL-4, may provide the explanation for the selective tissue mobilization of eosinophils.

involved in eosinophil tissue mobilization (1,7,8,9 and reviewed by Silberstein (10)). The accumulation of recruited eosinophils may lead to enhanced TGF-α production which in itself may cause epidermal proliferation and thickening (11).

2. The *cpdm/cpdm* lesions originate from abnormal functioning endothelial cells (Figure 1b). Endothelial cells may cause or contribute to the increased eosinophil infiltrate by persistent expression of adhesion molecules. Transendothelial migration of eosinophils into the tissue involves a cumulative series of events in which adhesion molecules on endothelial cells may play a crucial role. Under steady state, non-activating conditions, eosinophils hardly bind to endothelial cells. Cytokines such as TNF-α, IL-1 and IL-4 have been shown to stimulate the transcription and the expression of the endothelial adhesion molecules E-selectin, ICAM-1 and VCAM-1 (reviewed by Silberstein (10)). These adhesion molecules are involved in the interaction between eosinophils and the endothelial cells. E-selectin expression on endothelial cells is observed in the *cpdm/cpdm* mice already at the age of 1 week and is still observed at the age of 6 weeks in *cpdm/cpdm* mice. ICAM-1 is strongly expressed on basal keratinocytes and on endothelial cells of dermal blood vessels in the *cpdm/cpdm* mouse. VCAM-1, P-selectin and L-selectin are not observed in the *cpdm/cpdm* mouse. It has been hypothesized by Bosse (12) that P-selectin mediates very early adhesion events while E-selectin would act later and even may replace originally present P-selectin at later stages of the inflammatory process. In *cpdm/cpdm* mice at the age of one week, P-selectin on the endothelial cells may have already been replaced by E-selectin. In a more chronic stage of the disease also E-selectin expression disappears. However, expression of adhesion molecules alone is not sufficient to obtain recruitment of eosinophils. Also endothelial cells can produce cytokines which are chemotactic for eosinophils e.g. IL-1α, GM-CSF (reviewed by Mantovani (13)) and eotaxin (1,2). Activation of accumulated eosinophils may lead to enhanced TGF-α production which in itself may cause epidermal proliferation and thickening. In turn, the activated keratinocytes can produce IL-1, TNF-α, IL-3, GM-CSF and eotaxin which can again stimulate eosinophil tissue mobilization.

3. The *cpdm/cpdm* lesions originate from abnormal functioning mast cells (Figure 1c). Mast cell activation through IgE may result in the loss of granules containing mediators like histamine, proteases, and cytokines and secretion of membrane-derived mediators like leukotrienes and prostaglandins (14,15,16). Cytokines which can be produced by mast cells and may act as chemoattractants for eosinophils are TNF-α, IL-3, IL-4 and IL-5 (17,18,19). Although we could not demonstrate IL-5 on the protein level, IL-4 and TNF-α were present in mast cells of control and *cpdm/cpdm* mice. Thus, through continuous IgE-mediated activation of

mast cells of the *cpdm/cpdm* mice release of IL-4 or TNF-α may take place. The release of those cytokines, but in particular that of IL-4, may provide the explanation for the selective tissue mobilization of eosinophils, since it has been reported that injection of IL-4 in mice selectively recruits eosinophils (20). The bone marrow and spleen cell transplantation from *cpdm/cpdm* to syngeneic control mice suggests that cells such as mast cells do not play a primary role in its pathogenesis. Nevertheless, it cannot be excluded that the maturation of mast cells in the skin, which is regulated by many factors including certain cytokines produced by fibroblasts (reviewed by 21,22,23) could be disturbed in these mice.

The cpdm/cpdm mouse as model for human diseases

The study of the skin disorder in the *cpdm/cpdm* mouse may contribute to our understanding of the complex mechanisms underlying chronic proliferative disease processes in the human skin. The *cpdm/cpdm* mouse has similarities with human skin diseases such as psoriasiform dermatitis and atopic dermatitis (Table 1).

Table 1. Comparison of the *cpdm/cpdm* mouse with human psoriasis and atopic dermatitis.

	cpdm/cpdm mouse	psoriasis	atopic dermatitis
Inflammatory cells			
T cells	-	+++	++
Eosinophils	+++	-	++
IgE positive mast cells	++	-	++
Keratinocytes			
Hyper-proliferation	++	++	++
Endothelium			
ICAM	++	++	++
VCAM	- (chronic lesions)	+	++ (acute lesions) - (chronic lesions)
E-selectin	++ (acute lesions) - (chronic lesions)	++	++

The most common chronic dermatitis in man characterized by epidermal proliferation and inflammation of the dermis and epidermis, is psoriasis. The abnormalities of epidermal cells in human psoriasis may be caused by cytokines secreted in dermal immune reactions involving T-cells (24,25). Autoreactivity of T lymphocytes to some epidermal component may be the initiating event, and these T cells may activate keratinocytes to secrete a variety of cytokines and growth factors (26). However, the exact mechanism has not yet been resolved. Although T-cells do not play a role in the *cpdm/cpdm* mice, and therefore the pathogenesis of the *cpdm/cpdm* mouse and human psoriasis are likely to be different, the manifestation of the disease characterized by hyperproliferation of keratinocytes is similar and the *cpdm/cpdm* mice can be used to study chronic dermatitis with hyperproliferation of keratinocytes.

Since the skin lesions also showed macroscopic and microscopic resemblance with atopic dermatitis, a similar pathogenetic mechanism could be involved in the observed development of the pathological signs. Pathological features like acanthosis, hyperkeratosis, focal parakeratosis, slight spongiosis and subepidermal fibrin depositions underline this similarity. Also the influx of eosinophils and the involvement of IgE, present on mast cells, support this view. The only strongly deviating finding in the *cpdm/cpdm* mouse is the small number of T-lymphocytes in the skin. In atopic dermatitis Th2 cells which produce IL-4, IL-5 and IL-10 play an important role. IL-4 is a potent inducer of B-cell IgE production and may, together with IL-5, induce tissue eosinophilia. Although the pathogenesis of atopic dermatitis and the lesions in the *cpdm/cpdm* mice are different, it is clear that the *cpdm/cpdm* mouse can be a valuable tool to study specific aspects of allergic inflammatory skin disease, such as the above mentioned aspects of the skin lesions, eosinophil infiltration, leukocyte-endothelial cell interactions and mast cell proliferation.

Therapeutic interventions in the cpdm/cpdm mouse

In our opinion, the *cpdm/cpdm* mouse is a suitable *in vivo* model to test novel treatments against epidermal hyperproliferation, eosinophil infiltration and pruritus. Therefore, the *cpdm/cpdm* mouse can be used to test drugs developed for skin disorders with a chronic proliferative character such as psoriasis and atopic dermatitis (Table 2). The data obtained so far suggest that the *cpdm/cpdm* mouse can indeed be used to study therapeutic effects (chapter 7).

Table 2. Effect of various drugs on human psoriasis, human atopic dermatitis and the lesions in the *cpdm/cpdm* mouse.

	psoriasis	atopic dermatitis	*cpdm/cpdm* mouse
calcipotriene (topical)	+++	-	++
etretinate (oral)	+++	-	+/-
corticosteroids (topical)	+++	+++	+++
corticosteroids (systemic)	+++	+++	+++
cyclosporin A (systemic)	+++	++	-
dapsone (oral)	-	?	++
loratidine (oral) (against itching)	++	++	+
capsaicin (topical) (against itching)	++	++	++

Conclusion

In this thesis we have presented data which indicate that the *cpdm/cpdm* mouse can be used to study certain aspects of the pathophysiology of human skin disorders with a chronic proliferative character. Since these human skin disorders encompass a very broad spectrum of clinical manifestations, it would be unrealistic to expect that any single animal model would contain all features of these human diseases. Nevertheless, the *cpdm/cpdm* mouse mutation may prove to be useful as an animal model for exploring the mechanisms of hyperproliferation and inflammation that are present in human chronic proliferative skin disorders. Furthermore, the *cpdm/cpdm* mouse is a suitable *in vivo* model to test novel treatments against epidermal hyperproliferation, eosinophil infiltration and pruritus.

Future perspectives

As has become clear from the previous discussion we have attempted to solve some intriguing questions concerning the pathogenesis of the *cpdm/cpdm* mouse. Unfortunately, at this moment more questions remain than have been answered. The most important question, the cause of the *cpdm/cpdm* lesion, is still unresolved. The question: is the lesion located in the dermis or epidermis can be resolved by dividing the epidermis from the dermis and transplant these separately to a syngeneic control animal. Another question is: what cell type is responsible for

the accumulation of eosinophils; the keratinocyte, the fibroblast, the mast cell or the endothelial cell? To solve this question keratinocytes, fibroblast or mast cells should be isolated, cultured and the supernatant collected and injected in control C57BL/Ka mouse. When it is known what cell type is responsible for the attraction of the eosinophils the next question is: what attractant is released by those cells? With adequate primers, *in situ* RT-PCR could help to solve this question. On the other hand, the proteins of the dermis and epidermis of the *cpdm/cpdm* mice can be compared with the control mice with gel electrophoresis. Through sequencing the changed proteins of the *cpdm/cpdm* mouse, the structure can be elucidated. Possible lesions in the endothelial cell can be studied by blocking the adhesion molecules with antibodies. Because of the very early influx of eosinophils, it is important to know when the first eosinophils infiltrate the dermis, and for this younger animals than 1 week have to be studied. Another important question is: when keratinocytes are triggered by an external factor to become hyperproliferative, what factor is responsible? And by what cell type is it secreted? For this purpose fibroblasts, eosinophils, and mast cells should be isolated and after *in vitro* culture (and stimulation) their supernatant should be injected in control C57BL/Ka mouse.

It could be possible to change the characteristics of the mouse through backcrossing the *cpdm/cpdm* mouse onto a variety of genetic backgrounds such as BALB/c or A/J mice. One point of interest is to alter the infiltrate in the skin from eosinophilic to lymphocytic to make the *cpdm/cpdm* mouse more representative for dermal and epidermal inflammatory processes as observed in chronic skin disorders such as psoriasis and atopic dermatitis.

Taken together, this mouse model in itself offers many intriguing questions for future research. In solving these questions they may contribute to an increased insight in certain pathogenetic aspects of psoriasis and atopic dermatitis.

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SUMMARY

Chronic proliferative dermatitis (gene symbol: *cpdm*) was identified in 1991 as a spontaneously occurring disease in the C57BL/KaLwRij strain at the TNO-breeding facility in Rijswijk, the Netherlands. The aim of this thesis was to investigate whether this mouse model has similarities with human skin diseases so that the model can be used to study the pathophysiology of human skin disorders with a chronic proliferative character. Furthermore, the possibility to use the *cpdm/cpdm* mouse model to test therapies for these human diseases was investigated.

The skin lesions in *cpdm/cpdm* mice become apparent at the age of 5 weeks and are characterized by erythema, partial alopecia, and fine scaling starting in the dorsal neck and ventral chest area. The lesions expand to involve the entire body and head, but ears footpads, and tail remain unaffected. The animals are severely pruritic and show intense scratching. Light microscopically, there is epidermal hyperplasia with hyper- and parakeratosis. Individual cell death (apoptosis) is present to various degrees in the stratum spinosum. There is dilatation and proliferation of dermal capillaries. Beside some small pustules and microabscesses in the stratum corneum, few inflammatory cells are observed in the epidermis but the dermis is infiltrated by a mixed population of inflammatory cells, predominantly granulocytes and macrophages (**chapter 2**). Electron microscopic examination have revealed that these granulocytes are mainly eosinophils (**chapter 3**). Only a few T-lymphocytes are present. The number of mast cells in the dermis progressively increases with age. The mucosa of the oral cavity, esophagus, and forestomach have similar lesions as the skin. The hyperproliferative nature of the skin lesions was confirmed by bromodeoxyuridine (BrdU) labeling. An eightfold increase of BrdU-positve keratinocytes in the interfollicular epidermis and a two- to threefold increase of BrdU-positive epithelial cells in the esophageal and forestomach epithelium are observed. Also, there is an increased expression of intercellular adhesion molecule-1 (ICAM-1) on keratinocytes and endothelial cells. Although the skin lesions of the *cpdm/cpdm* mice are most obvious, inflammatory foci are also present in the lungs, liver and peri-synovial connective tissue. Failure to transfer the *cpdm/cpdm* lesions to syngeneic control animals using hemopoietic cells from spleen or bone marrow from affected mice suggest that such cells do not play a primary role in its pathogenesis. Treatment of the mice with triamcinolone (systemic) resulted in nearly complete regression of the lesions, whereas systemic cyclosporin A is ineffective (**chapter 2**).

Ultrastructurally, electron-dense spherical inclusions were observed in the mitochondria of keratinocytes in skin lesions. The mitochondria otherwise appeared

intact. Although the significance and pathogenesis of these inclusions are unknown, it is of interest that they are observed in a variety of proliferative skin diseases in mice (**chapter 3**).

In **chapter 4**, the expression of endothelium associated adhesion molecules was described in combination with the binding capacity of various cell types on frozen sections of the affected *cpdm/cpdm* skin. In agreement with the relative absence of lymphocytes in the *cpdm/cpdm* skin no lymphocyte binding could be observed; in contrast avid adhesion of neutrophils was seen. Binding of neutrophils could be blocked with antibodies against L-selectin, LFA-1, CR3 and anti ICAM-1. No expression of vascular addressins or E-selectin on endothelium in the dermis was found.

To elucidate whether these *cpdm/cpdm* lesions are the result of a local (skin) process or a consequence of a systemic disorder, transplantations were performed of full-thickness grafts of affected skin from *cpdm/cpdm* mice and normal skin from control (C57BL/Ka) mice on the back of *cpdm/cpdm*, C57BL/Ka and athymic nude mice. After 3 months, the grafts maintained the phenotype of the donor animal. This demonstrates that the pathologic features found in the *cpdm/cpdm* mice are the result of a disorder in the epidermis or dermis and not due to a systemic defect (**chapter 5**).

To further study the pathogenesis of the *cpdm/cpdm* mouse, organs of 1, 2, 3, 4, 5, and 6 weeks old *cpdm/cpdm* mice were examined (**chapter 6**). At 4 weeks, the epidermal thickness was increased while already at 3 weeks, the BrdU incorporation was increased in the basal keratinocytes. However, already at the age of 1 week skin, lungs and lymph nodes were infiltrated by eosinophils although no macroscopic lesions were present. Compared to control animals, 6 weeks old *cpdm/cpdm* mice had decreased serum IgE levels and increased numbers of mast cells. From the age of 1 week these mast cells became increasingly IgE positive. In contrast, the mast cells of the control animals remained IgE negative. Mast cells of control and *cpdm/cpdm* mice were IL-4 and TNF-α positive.

In **chapter 7** the effect of various drug treatments against epidermal hyperproliferation (calcipotriene and etretinate), against inflammation (corticosteroids and dapsone) and against pruritus (loratidine and capsaicin) are described in the *cpdm/cpdm* mice. We noticed that steroids and less strongly calcipotriene were the most effective treatments for the lesions observed in the *cpdm/cpdm* mice. By these treatments both hyperproliferation and the influx of eosinophils were reduced. This indicates that the *cpdm/cpdm* mouse can be used to screen new drugs for their possible application in chronic dermatitis.

The data presented in this thesis are discussed in **chapter 8**. The

cpdm/cpdm mouse mutation may prove to be useful as an animal model for exploring the mechanisms of hyperproliferation and inflammation that are present in human chronic proliferative skin disorders.

SAMENVATTING

Chronische proliferatieve dermatitis (genetische afkorting: *cpdm*) is een spontane huidafwijking in de muizenstam C57BL/KaLwRij. Deze afwijking werd voor het eerst gezien in 1991 in de fokfaciliteiten van TNO te Rijswijk. Het doel van dit proefschrift was om te bestuderen of dit muizemodel overeenkomsten vertoonde met humane huidafwijkingen opdat de *cpdm/cpdm* muis gebruikt zou kunnen worden om de pathofysiologie van humane huidziekten met een chronisch proliferatief karakter te bestuderen. Ook de mogelijkheid om de *cpdm/cpdm* muis te gebruiken bij het testen van geneesmiddelen tegen deze humane huidziekten werd bestudeerd.

Bij een leeftijd van 5 weken zijn bij deze muis de huidlaesies macroscopisch zichtbaar als roodheid, haarverlies en lichte schilfering beginnend in de nek en op de borst. De laesies breiden zich uit over het hele lichaam maar oren, staart en voetzolen blijven onaangedaan. De dieren krabben enorm veel waaruit geconcludeerd werd dat ze veel jeuk hebben. Lichtmicroscopisch werd epidermale hyperplasie met hyper- en parakeratose geconstateerd. Individuele celdood (apoptose) kon worden aangetoond in het stratum spinosum en er is dilatatie en proliferatie van de dermale bloedvaatjes aanwezig. Weinig ontstekingscellen zijn gevonden in de epidermis op enkele kleine pustulae en microabcesjes in het stratum corneum na. Daarentegen is de dermis geïnfiltreerd met een gemengd ontstekingsinfiltraat dat voornamelijk uit granulocyten en macrofagen bestaat (**hoofdstuk 2**). Electronmicroscopisch onderzoek heeft aangetoond dat deze granulocyten hoofdzakelijk eosinofielen zijn (**hoofdstuk 3**). Er zijn maar enkele T-cellen gevonden. Het aantal mestcellen neemt toe met de leeftijd. De mucosa van de mondholte, de slokdarm en de voormaag heeft dezelfde afwijkingen als de huid. De hyperproliferatie van de huid werd vastgesteld met bromodeoxyuridine (BrdU) incorporatie, waarbij een achtvoudige toename van BrdU-positieve cellen in de keratinocyten van de interfolliculaire epidermis en een twee- tot drievoudige toename van BrdU-positieve epitheliale cellen in het epitheel van de slokdarm en voormaag werd gevonden. Ook is er een toename van expressie van intercellulair adhesie molecuul-1 (ICAM-1). Hoewel de huidafwijkingen van de *cpdm/cpdm* muizen het meest in het oog springen, is er ook ontstekingsinfiltraat in longen, lever en perisynoviaal bindweefsel aanwezig. De *cpdm/cpdm* laesies konden niet overgebracht worden door transplantatie van haematopoietische cellen van beenmerg en milt van *cpdm/cpdm* muizen naar genetisch identieke controle dieren. Dit suggereert dat deze cellen geen primaire rol spelen in de pathogenese. Behandeling van muizen met triamcinolon (systemisch) resulteerde in een bijna complete regressie van de laesies maar systemische toediening van cyclosporine A

had geen effect (**hoofdstuk 2**).

Ultrastructureel werden in de huidafwijkingen vaak electronen-dichte bolvormige insluitsels gezien in de mitochondriën van de keratinocyten. De mitochondriën in de niet afwijkende huid waren intact. Hoewel de betekenis voor de pathogenese niet bekend is, is het interessant dat deze insluitsels ook gevonden zijn in een aantal andere proliferatieve huidziekten in muizen (**hoofdstuk 3**).

In **hoofdstuk 4** wordt de expressie van adhesiemoleculen op endotheel in combinatie met de bindingscapaciteit van verschillende celtypen op vriescoupes van de aangetaste *cpdm/cpdm* huid beschreven. Er werd geen lymfocytenbinding aangetoond, dit in overeenstemming met de afwezigheid van lymfocyten in de *cpdm/cpdm* huid. Wel werd adhesie van neutrofielen gezien. De binding van de neutrofielen kon worden geblokkeerd met antilichamen tegen L-selectine, LFA-1, CR3 en anti-ICAM-1. Er werd geen expressie van vasculaire adressines of E-selectine op endotheel in de dermis gevonden.

Om te onderzoeken of de *cpdm/cpdm* laesies het gevolg zijn van een locale afwijking in de huid of een systemische afwijking werden huidtransplantaties verricht van de aangetaste huid van de *cpdm/cpdm* muizen en normale huid van controle (C57BL/Ka) muizen op de rug van *cpdm/cpdm*, C57BL/Ka en naakte muizen. Na 3 maanden hadden de transplantaten nog steeds alle karakteristieken van de donor. Dit betekent dat de afwijkingen gevonden in de *cpdm/cpdm* muizen het gevolg zijn van een afwijking in de dermis of epidermis en niet het gevolg van een systemisch defect (**hoofdstuk 5**).

Om de pathogenese van de *cpdm/cpdm* muis te bestuderen werden organen van 1, 2, 3, 4, 5 en 6 weken oude *cpdm/cpdm* muizen onderzocht (**hoofdstuk 6**). Bij 4 weken was de epidermale dikte toegenomen, terwijl bij 3 weken er al een toename werd gevonden van BrdU incorporatie in de basale keratinocyten. Het meest opvallende was dat al op de leeftijd van 1 week eosinofielen infiltratie gevonden werd in de huid, longen en lymfeklieren terwijl macroscopisch geen afwijkingen zichtbaar waren. Vergeleken met controle dieren hadden 6 weken oude *cpdm/cpdm* muizen verlaagd serum IgE en een toegenomen aantal mestcellen. Vanaf de leeftijd van 1 week werden deze mestcellen in toenemende mate IgE-positief in tegenstelling tot de mestcellen van controle dieren die IgE-negatief waren. Mestcellen van *cpdm/cpdm* en controle muizen waren IL-4- en TNF-α-positief.

In **hoofdstuk 7** wordt de behandeling van verschillende geneesmiddelen tegen epidermale proliferatie (calcipotriëen en etretinaat), tegen ontsteking (corticosteroiden en dapson) en tegen jeuk (loratidine en capsaicine) in de

cpdm/cpdm muizen beschreven. Corticosteroiden en in mindere mate ook calcipotriëen vormden de meest effectieve behandeling voor de laesies in de *cpdm/cpdm* muizen. Met deze behandeling waren de hyperproliferatie en het aantal eosinofielen afgenomen. Dit betekent dat de *cpdm/cpdm* muis gebruikt kan worden om nieuwe geneesmiddelen te testen tegen chronische proliferatieve dermatitis.

De experimenten beschreven in dit proefschrift worden geëvalueerd in **hoofdstuk 8**. De *cpdm/cpdm* is een bruikbaar model om het mechanisme van hyperproliferatie en ontsteking zoals aanwezig in chronische proliferatieve huidziekten bij de mens te bestuderen.

ABBREVIATIONS

ab	asebia mouse mutation
BrdU	Bromodeoxyuridine
CD	cluster of differentiation
cpdm	chronic proliferative dermatitis mouse mutation
CsA	cyclosporin A
CR3	complement receptor type 3
EGF	epidermal growth factor
FITC	fluorescein isothiocyanate
FGF	fibroblast growth factor
fsn	flaky skin mouse mutation
GM-CSF	granulocyte-macrophage colony stimulating factor
HETE	*hydroxyeicosatetraen*-1-*oic acid*
HODE	hydroxyoctadecadien-1-oic acid
ICAM	inter-cellular adhesion molecule
Ig	immunoglobulin
IL	interleukin
KGF	keratinocyte growth factor
LFA	lymphocyte-function-associated antigen
LTB_4	leukotriene B_4
nu	nude mouse mutation
PGE_2	prostaglandin E_2
PBS	phosphate buffered saline
PMN	polymorphonuclear
RT-PCR	reverse transcription polymerase chain reaction
SEM	standard error of the mean
TNF	tumor necrosis factor
TGF	transforming growth factor
Th	T helper
VCAM	vascular cell adhesion molecule

CURRICULUM VITAE

Maria (Marion) Johanna Josephina Gijbels werd op 6 mei 1962 geboren te Veldhoven. In Eindhoven behaalde zij in 1980 het VWO-B diploma aan het Eckart College, waarna zij in datzelfde jaar begon met haar studie aan de toenmalige Landbouwhogeschool (nu Landbouwuniversiteit) te Wageningen. Het doctoraal examen Voeding, met hoofdvakken Experimentele Pathologie en Toxicologie en bijvak Humane Voeding, werd afgelegd in juni 1987.

Van juli 1987 tot januari 1995 was zij werkzaam als proefdierpatholoog bij de afdeling Pathologie van het Instituut voor Experimentele Gerontologie-TNO in Rijswijk (hoofd: Dr. C. Zurcher) dat later fuseerde met een tweetal andere TNO-instituten tot het Instituut voor Verouderings- en Vaatziekten Onderzoek, gevestigd te Leiden (algemeen directeur: Prof. Dr. D. Knook). Onder begeleiding van Dr. C. Zurcher werd in augustus 1991 de post-doctorale opleiding Proefdierpathologie afgerond en in februari 1993 begon zij aan het onderzoek beschreven in dit proefschrift.

Vanaf 1 januari 1995 is zij als proefdierpatholoog betrokken bij een project van de Nederlandse Hartstichting, uitgevoerd aan de Medische Faculteit van de Vrije Universiteit te Amsterdam, afdeling Celbiologie en Immunologie (hoofd: Prof. Dr. Sminia).

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