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Characterization of Psoriasiform and Alopecic Skin Lesions in HLA-B27 Transgenic Rats

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We have previously reported a multisystem inflammatory disease in transgenic rat lines with high expression of HLA-B*2705 and human β_2 microglobulin. Skin disease in these rats includes two predominant lesions: 1) marked psoriasiform dermatitis of the tail and digits; and 2) progressive alopecia of face, neck, trunk, and extremities. Here we present the results of a systematic survey of these lesions. Tail and digit skin showed psoriasiform hyperplasia of the epidermis associated with parakeratosis, with marked dermal and epidermal inflammation. The alopecic skin showed perifollicular and follicular mononuclear infiltration and increased numbers of atrophic follicles. Immunohistochemical analysis revealed that B27 expression was prominent on keratinocytes in hyperplastic epidermis where lymphocytic infiltrates were prominent, but was absent in the absence of inflammation. In alopecic lesions, B27 was strongly expressed on follicular epithelium and dermal hair papillae associated with mononuclear infiltrates. T cells, both CD8 and CD4, were most prominent in inflammatory lesions and rat MHC-II expression on keratinocytes, and follicular epithelium was dramatically increased. This study suggests that T cell-mediated immune mechanisms participate in development of cutaneous lesions in HLA-B27 transgenic rats. (Am J Pathol 1995, 147:955–964)

The association between autoimmune/inflammatory disease and certain major histocompatibility complex (MHC) antigens has been extensively investigated. Among these diseases, the chronic inflammatory multisystem diseases collectively termed spondyloarthropathies are known to have strong association with the HLA-B27 allele. These include ankylosing spondylitis, reactive arthritis, juvenile spondyloarthropathy, psoriatic arthropathy, and enteropathic arthropathy.[1] We have previously shown that transgenic rat lines bearing high copy numbers of HLA-B*2705 and human β_2 microglobulin (hβ_2m) develop multisystem inflammatory disease with a strong clinical resemblance to the human spondyloarthropathies.[2,3]

At least two distinct cutaneous manifestations are prominent among the phenotypic traits of the B27 transgenic rats. First, psoriasiform lesions on the tail and digits are characterized by hyperplasia of the epidermis and associated with parakeratosis and exocytosis of lymphocytes into epidermis.[2] These lesions resemble psoriasis vulgaris and keratoderma blennorrhagica (KD), both of which are closely associated with the spondyloarthropathies.[1,4] Second, progressive alopecia is also a prominent cutaneous feature in the transgenic lines that develop disease. In this communication, we present histological and immunohistological studies regarding these two lesions. Previous studies have indicated that the threshold level of B27 expression in lymphoid tissue is critical to the development of inflammatory disease in these rats,[3] as is the presence of T lymphocytes (ref. 5, M Breban, JL Fernandez-Sueiro, JA Richardson, R Hadavard, SD Maika, RE Hammer, JG Taurog, manuscript submitted for publication). The results presented here provide strong evidence for T cell-mediated immune mechanisms as a basis for the two cutaneous lesions seen in the B27 transgenic rats.

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Materials and Methods

Animals

Transgenic rat line 21-4H, bearing 150 copies of HLA-B*2705 and 90 copies of hβ_2m on the inbred LEW background, and line 33-3, bearing 55 copies of HLA-B*2705 and 66 copies of hβ_2m genes on the inbred F344 background, were produced and maintained in our animal colony as previously described.[2] Hemizygous 21-3 line bearing 20 copies of HLA-B*2705 and 15 copies of hβ_2m on the inbred LEW background were mated to produce rats homozygous for the transgene locus. One hundred forty rats between 9 and 70 weeks old were examined for development of skin lesions, and 20 rats from the 21-4H and 33-3 lines were used in immunohistological studies. Nontransgenic LEW and F344 rats purchased from Charles River Inc. (Wilmington, MA) served as negative controls.

Tissue Processing and Staining

Skin was taken from nose, chin, ventral, and dorsal trunk and tail of 14 affected animals. For routine histological examination, skin was fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with Mayer's hematoxylin and eosin solution (Sigma Co., St. Louis, MO). For immunohistochemistry, ~10 mm × 3 mm pieces were embedded in ornithine carbamoyltransferase compound (Miles Scientific, Naperville, IL), snap frozen in liquid nitrogen, and stored at −70°C.

Immunohistochemistry

Ten μm cryosections were placed on poly-L-lysine-coated slides (HCS Inc., Glen Head, NY). Adjacent sections were fixed in 4% paraformaldehyde for 20 minutes, washed twice in 0.5 mol/L Tris-HCl (pH 7.6), and incubated with 20% normal horse serum for 20 minutes. After serum was removed, sections were incubated at 4°C overnight with mouse monoclonal antibody (MAb) listed in Table 1, diluted in 0.5 mol/L Tris/2% horse serum. V65 was purchased from PharMingen (La Jolla, CA), ED1 was purchased from Serotec (Oxford, UK), and OX62 was a gift of Dr. M. Brenan, Oxford, UK.[6–8] The sources of the other MAb were as previously described.[2,3,5] After primary incubation, sections were washed in 2% normal horse serum and incubated 1 hour at room temperature with biotinylated rat adsorbed horse anti-mouse immunoglobulin G (IgG) antibody (Vector Laboratories, Burlingame, CA), 20 μg/ml in 0.5 mol/L Tris/10% normal horse serum. After rinsing and pretreatment with 0.3% H_2O_2, sections were incubated 10 minutes with horseradish peroxidase-conjugated streptavidin (Zymed Laboratories, San Francisco, CA) and the reaction was visualized with amino-ethyl carbazole chromogen kit (Zymed Laboratories). The slides were counterstained with either hematoxylin or methyl green and mounted in Aqua-mount (Lerner Laboratories, Pittsburgh, PA) or 100% glycerol.

Table 1. *Mouse Antibodies Used in Immunohistochemical Analysis of Skin Lesions*

Antibody	Antigen/Cell recognized	Concentration	Reference
B1.23.2	HLA-B,C	1:400*	2
R73	α/β TCR	50 μg/ml	3
V65	γ/δ TCR	20 μg/ml	6
OX33	CD45 on B cell	1:2*	3
ED1	Macrophages and dendritic cells	1 μg/ml	7
OX6	MHC-II, RT1.B	5 μg/ml	3
OX8	CD8	5 μg/ml	5
W3/25	CD4	5 μg/ml	5
OX62	Dendritic cells	1:50 (ascites)	8

*Culture supernatant.
B1.23.2 is IgG2a. All others are IgG1.

Immunofluorescence was used for double labeling of skin sections. Six-μm cryosections were fixed and incubated with 20% normal rabbit serum for 20 minutes, incubated with the first mouse MAb at 4°C overnight, washed, and incubated with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (1:50 dilution) specific for the mouse MAb isotype (Zymed Laboratories) for 3 hours at room temperature. After washing, sections were incubated with 20% normal rabbit serum for 20 minutes and incubated with a biotinylated mouse MAb for 3 hours at room temperature, washed, and incubated with 20 μg/ml of lissamine rhodamine-conjugated streptavidin (Jackson Immuno-Research Laboratories, West Grove, PA) at 37°C for 30 minutes. After washing, slides were mounted in Vectashield (Vector Laboratories) and observed with a Leica DMR fluorescence microscope. Irrelevant IgG1 and IgG2a MAbs served as negative controls.

Results

Macroscopic Observations

Transgenic rats of both 21-4H and 33-3 lines had normal skin and pelage until ~25 weeks of age. The first noticeable change was alopecia of the periorbital skin. Hair loss was bilateral and over time extended toward the ears, snout, ventral neck, and the

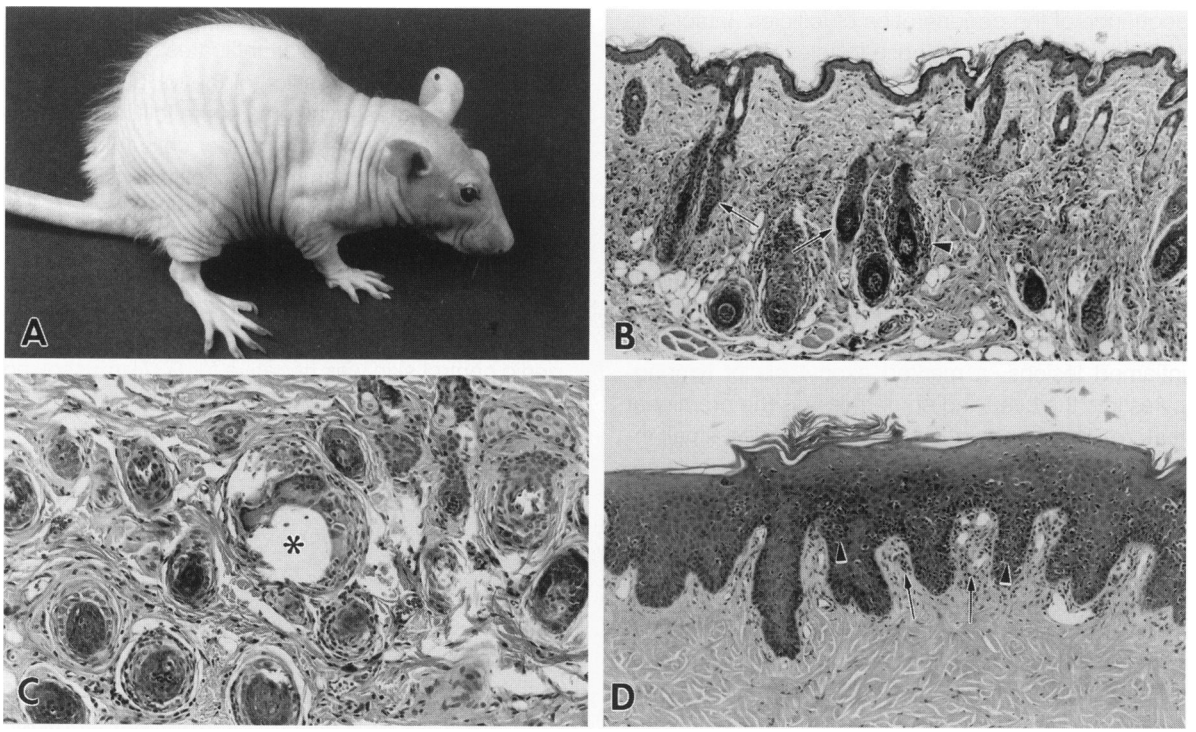


Figure 1. *Gross pathology and histology of the skin of 33-3 and 21-4H rats.* (**A**) *A 60-week-old 33-3 rat showing advanced alopecia of head, trunk and forelimbs.* (**B**) *Alopecic muzzle skin from a 33-3 rat showing multifocal perifolliculitis* (**arrowheads**) *and numerous atrophic follicles devoid of hair shafts* (**arrows**) (88×). (**C**) *Alopecic mandible skin from a 33-3 rat showing a foreign body granuloma* (*) *secondary to follicular rupture* (132×). (**D**) *Psoriasiform tail skin from a 21-4H rat. There is marked epidermal hyperplasia and prominent, elongated rete pegs. There are scattered dermal infiltrates* (**arrow**) *and areas with lymphocytic exocytosis into epidermis* (**arrowheads**) (96×).(**B–D**), *hematoxylin and eosin stained.*

ventromedial surface of the forelegs and dorsally over the carpi. Concurrent with alopecia of the forelegs, hair loss developed along the ventral surface of hind legs and spread over the perineum and craniad along the ventral abdomen. Although the tail is normally relatively hairless, close examination indicated that early in the disease, the sparse hairs of the tail were lost and the skin developed scales and became thickened. Hair loss over the dorsal trunk started ~10 weeks later than that over ventral trunk. By 60 weeks, rats of the 33-3 line developed extensive areas of alopecia over the dorsum, which merged with ventrolateral lesions, resulting in almost total baldness (Figure 1A). In contrast, in rats of the 21-4H line, the alopecic lesions remained as large patchy areas and showed less tendency to coalesce.

The incidence of skin disease between 30–60 weeks of age in the 21-4H line was 94% in males (17/18) and 50% in females (6/12). In the 33-3 line, the corresponding figure was 20% in males (2/10) and 80% in females (28/35). Rats of the 21-3 (LEW) line bear a transgene locus with 20 copies of the B27 gene. Animals homozygous for the 21-3 transgene locus develop multi-systemic inflammation similar to that of the 21-4H line.[3] Hemizygous 21-3 rats remain healthy until ~1 year of age, when they develop alopecia similar to that seen in 21-4H rats. No skin lesions have been seen in the other previously described B27 transgenic lines.[2]

Histology of Alopecic Lesions

Histological examination of alopecic lesions revealed multifocal folliculitis and perifolliculitis (Figure 1B). Inflammatory infiltrates were generally restricted to the deeper portions of the follicle, forming distinct cuffs around the affected follicles. Small numbers of mononuclear cells invaded the follicular epithelium. Occasionally, the follicular integrity was destroyed by the inflammatory process and a focal granulomatous reaction ensued consisting of foreign body giant cells, epithelioid macrophages, and lymphocytes (Figure 1C). In sections from advanced lesions, the number of inflamed follicles was similar but the majority of the follicles were atrophic and contained no hair shaft. Scarring in sections with advanced alopecia was mild and limited to perifollicular areas. In vibrissa, there were large numbers of mononuclear infiltrates surrounding the follicles and occasionally invading through the glassy membrane layer. A local granulomatous folliculitis was also observed in con-

sequence of the degeneration of follicles. Intact hair shafts were not seen in these follicles. The overlying epidermis was mildly hyperkeratotic and focally parakeratotic. The dermal papilla contained small numbers of mononuclear inflammatory cells.

Histology of Tail Skin

In contrast to alopecic skin, the principal change in tail skin was marked epidermal hyperplasia, usually with focal exocytosis of lymphocytes (Figure 1D). In inflamed lesions, a marked exocytosis of lymphocytes into the overlying epidermis was the prominent feature. The invading lymphocytes formed small intraepidermal aggregates and were occasionally associated with necrotic keratinocytes. Whereas the epidermal hyperplasia was diffuse, the intraepidermal inflammatory component was multifocal (Figure 1D). In a minority of lesions, epidermal hyperplasia was found in the absence of lymphocytic infiltration. In these lesions, the epidermis developed prominent diffuse acanthosis, which assumed a multifocal psoriasiform morphology. The epidermis was mildly but diffusely hyperkeratotic and multifocally parakeratotic. There was no hypergranulosis. The dermal papilla and lower dermis contained a mild increase in dendritic-shaped cells but there was no lymphocytic component. Histologically the follicular or epidermal lesions did not differ between the 21-4H and 33-3 lines.

Immunohistochemistry of Folliculitis

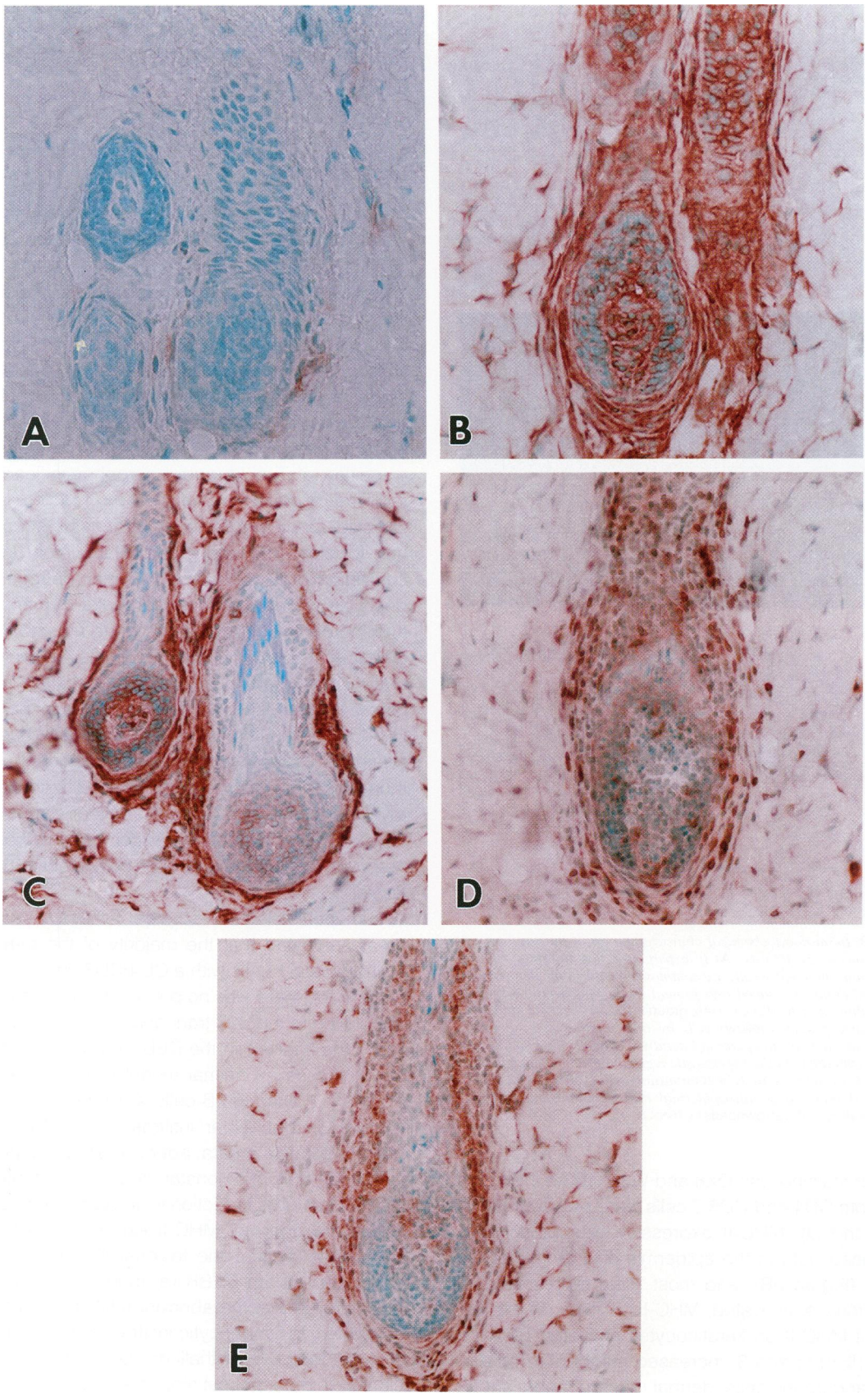
Cryosections of non-alopecic skin from young transgenic rats and of alopecic lesions from older rats were stained with MAb to HLA-B (B1.23.2) to determine expression of the B27 transgene. Non-alopecic skin showed small numbers of B27-positive mononuclear cells scattered throughout the upper dermis and aggregated surrounding intact follicles. There was no staining in the follicular epithelium (Figure 2A). Sections from completely alopecic skin showed marked perifollicular and follicular inflammatory cell infiltrates. Perifollicular infiltrates were strongly positive for B27 and both hair papilla and follicular epithelium, where there were lymphocytic infiltrates, also expressed B27 (Figure 2B). Immunohistochemical staining with a MAb (OX6) to the MHC-II antigen revealed that perifollicular infiltrates were strongly positive, and the expression of the MHC-II molecule RT1.B was increased in some of the follicular epithelium associated with inflammatory cells (Figure 2C). The perifollicular inflammatory cells consisted primarily of T cells (α/β TCR$^+$, Figure 2D; OX33$^-$, data not shown) and macrophages (ED1$^+$, Figure 2E). Where perifolliculitis evolved to frank folliculitis, the inflammatory cells invading into the follicular epithelium were identified as T cells (Figure 2D). Nontransgenic control skin was negative for B27 and showed only scant T cells, macrophages, or MHC-II expression.

Immunohistochemistry of Psoriasiform Lesion

Tail skin specimens were stained for HLA-B27. Skin from transgenic animals that had not yet developed cutaneous lesions had only rare B27-positive cells in the epidermis. In sections where epidermal hyperplasia had developed but was not associated with epidermal inflammatory infiltrates, B27-positive cells were found primarily within the dermis. The staining pattern (ED1$^+$, OX6$^+$, V65$^-$) indicated that these were macrophages or dermal dendritic cells. There was also a distinct set of B27-positive cells observed in hyperplastic epidermis. These cells were most likely Langerhans cells, as judged by their dendritic morphology, suprabasal location (Figure 3A), and surface phenotype of OX6$^+$ (Figure 3B), OX62$^-$ (data not shown). In contrast, where marked lymphocytic infiltration accompanied the epidermal hyperplasia, keratinocytes also expressed a large amount of the B27 molecule (Figure 3C). Both the number of B27 positive keratinocytes and the intensity of the B27 expression per cell were most predominant within the foci of inflammation.

The inflammatory cell population was characterized as follows. Macrophages (ED1$^+$ cells) were observed primarily in the superficial dermal papillae and infiltrated in small numbers into the epidermis (Figure 4A). Intraepidermal lymphocytes were strongly R73$^+$ (Figure 4B) and V65$^-$, OX33$^-$ (data not shown), indicating that they were α/β T cells.

Figure 2. *Immunocytochemical characterization of alopecic skin lesions in 33-3 rats. Immunoreactive cells are red and sections were counterstained with methyl green.* (A) *Non-alopecic trunk skin of a 16-week-old rat and* (B–E) *alopecic trunk skin of a 60-week-old rat.* (A) *Some perifollicular cells are HLA-B immunoreactive (B1.23.2 MAb), whereas the follicular epithelium is negative (240×).* (B) *In alopecic skin, perifollicular inflammatory cells, follicular epithelium, and dermal cells are HLA-B immunoreactive (204×).* (C) *The follicular epithelium has prominent MHC-II immunoreactivity (OX6 MAb) (156×).* (D) *α/β T cells (R73 MAb) are present around the hair follicle and invade the follicular epithelium (168×).* (E) *Macrophages and/or monocytes (ED-1 MAb) cluster around the hair follicle and are evident within the dermal papilla, but do not invade the follicular epithelium (144×).*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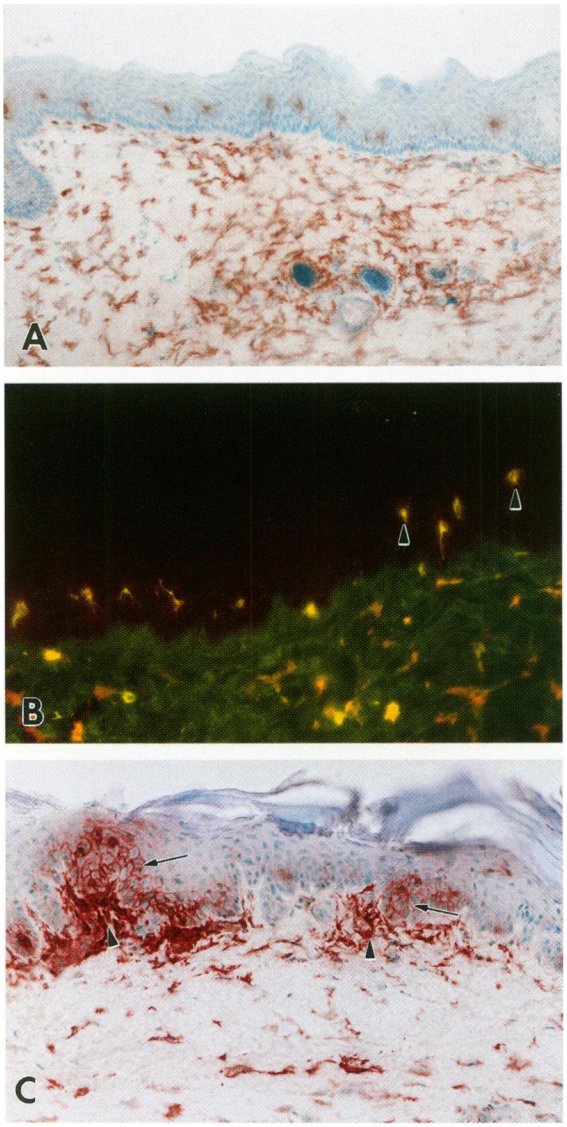

Figure 3. *Immunocytochemical characterization of psoriasiform tail skin lesions in 21-4H rats.* (**A**) *In hyperplastic non-inflamed regions, HLA-B immunoreactive cells are commonly found in the dermis, with increased dendritic-shaped cells present in the epidermis* (*96×*). (**B**) *Localization of MHC-II* (*Ox6 MAb,* green), *HLA-B* (*B1.23.2 MAb,* red) *and double positive* (yellow) *cells by immunofluorescent analysis. There is an increase in epidermal dendritic-shaped cells* (*double positive,* arrowheads) *in the hyperplastic region* (*120×*). (**C**) *In inflamed regions, keratinocytes are HLA-B immunoreactive* (arrow) *and there is a marked increase of intraepidermal and dermal HLA-B staining inflammatory cells* (arrowheads) (*168×*).

Further staining with OX8 and W3/25 MAb indicated that both CD4 and CD8 T cells were present (Figure 4, C and D). MHC-II expression was prominently increased both in the epidermis and dermis of the lesion (Figure 5B), and most likely represented 1) exocytosis of activated, MHC-II$^+$ T cells; 2) expression of MHC-II on keratinocytes activated by interferon (IFN)-γ; and 3) increased numbers of epidermal Langerhans cells, dermal macrophages, and/or dendritic cells. In young transgenic rats that had not yet developed lesions, MHC-II-positive cells were sparse and the keratinocytes were MHC-II-negative (Figure 5A).

Discussion

As part of a spontaneous systemic inflammatory disease, transgenic rats with high expression of HLA-B*2705 and hβ_2m developed two characteristic skin changes after 25 weeks of age, namely, extensive alopecia and psoriasiform dermatitis. In the alopecic lesion, the principal histological alterations were folliculitis and perifolliculitis. Despite the generalized alopecia seen grossly, there was little dermal scarring, necrosis, or loss of follicles. Most likely, the arrest of hair production is related to peribulbar aggregation of T cells and macrophages leading to cytokine-mediated involution of the follicles, and alopecia supervenes without significant destruction of hair follicles. The peribulbar inflammatory infiltrate consisting of lymphocytes and macrophages suggests an underlying immune-mediated mechanism and morphologically bears a strong resemblance to alopecia areata in humans. Alopecia areata is generally considered to be a tissue-restricted autoimmune disease and an association with particular HLA-DR alleles, including DR4 and DR5, has been reported.[9,10] It may coexist with additional autoimmune diseases such as autoimmune thyroiditis and myasthenia gravis.[11] However, neither a specific antibody to any part of follicular components nor autoreactive T cells have been consistently demonstrated.

Immunohistological studies in active alopecia areata revealed that the majority of the peribulbar infiltrates are T cells with a CD4/CD8 ratio of ~4:1.[12] In contrast, there was no predominance in the T cell population in the B27 transgenic rats. This was similar to the findings in the DEBR rat, which has been described as an animal model of human alopecia areata,[13,14] although B cells were also shown to be involved in peribulbar infiltration in DEBR rats. In human alopecia areata, aberrant MHC-II expression in the hair bulb is a constant feature.[15] Although the mechanism of its induction is unclear, one possibility is that the aberrant MHC-II expression in follicular epithelium triggers the lesional insults,[16] which is supported by the DEBR rat model. The alternative possibility is that the aberrant MHC-II expression is secondary to lymphocytic infiltration. MHC-II expression on follicular epithelium was found only occasionally in recent-onset alopecia areata, whereas it is

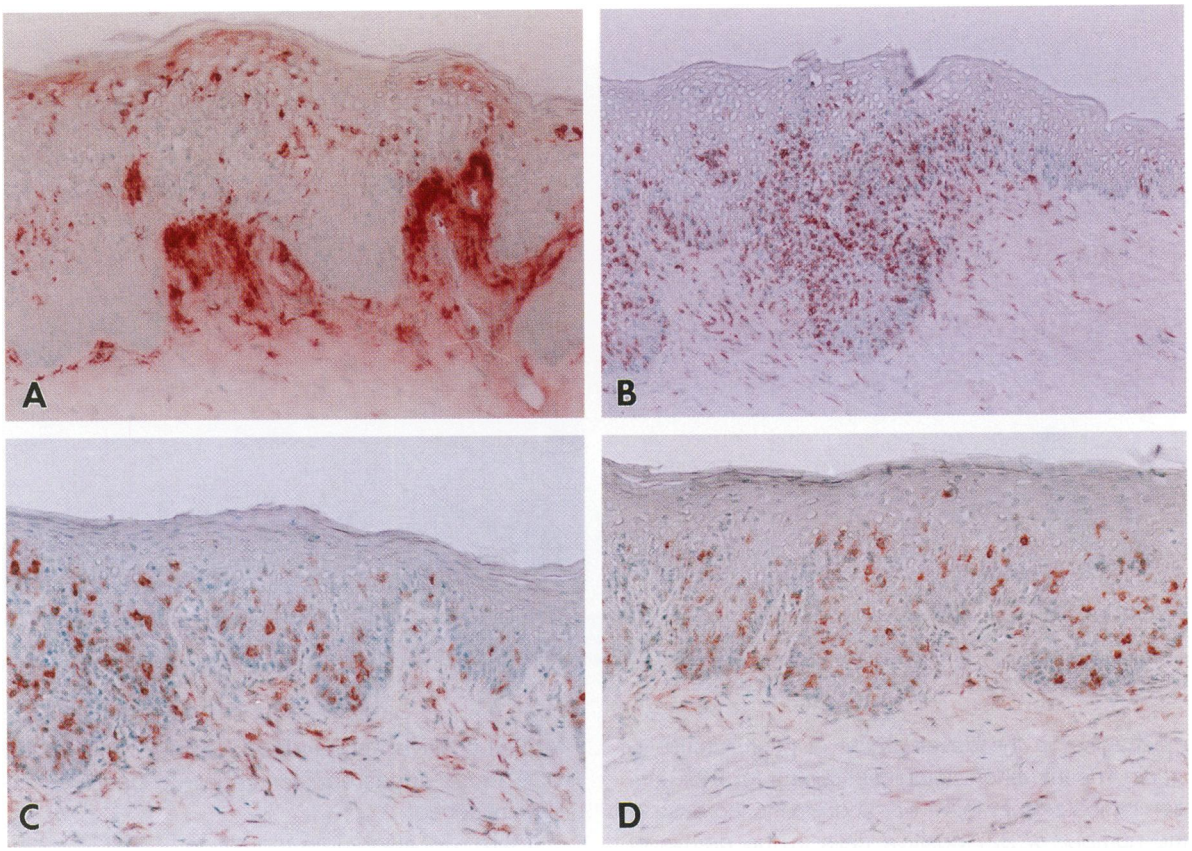

Figure 4. *Characterization of inflammatory infiltrates in psoriasiform tail skin of 21-4H rats.* (A) *Macrophages and/or monocytes (ED1 MAb) are abundant in the dermis and less frequent and scattered within the epidermis (168×).* (B) *α/β T lymphocytes (R73 MAb) are abundant, both in the dermis and epidermis (120×).* (C) *CD4 cells (W3/25 MAb) and* (D) *CD8 cells (OX8 MAb) are scattered throughout the epidermis (168×).*

more frequently seen in the lesions with perifollicular T cell infiltrates and in longstanding cases.[17] The B27 rats seem to show the latter temporal pattern of MHC-II expression in alopecic lesions.

The other type of skin change in B27 rats, psoriasiform dermatitis, was evident in the tail and digits. It is instructive to compare these lesions with human disease. In humans, epidermal hyperplasia is a characteristic feature of psoriasis vulgaris. Its histological manifestations include typical regular hyperproliferation of the epidermis, thinning of the suprapapillary plates, and overlying hyperkeratosis with focal parakeratosis. Neutrophils are present, often scattered throughout the epidermis, and a superficial perivascular infiltrate of lymphocytes is seen in the dermis. KD, which most often occurs in the setting of B27-associated reactive arthritis, is a macropustular skin lesion closely related to pustular psoriasis.[18] Its histological features include hyperkeratotic, scaling papules, and plaques, often with a vesicular or pustular component. There is significant clinical overlap between psoriasis vulgaris and KD, both of which may be exacerbated by HIV infection.[19] Psoriasis and psoriatic arthritis show MHC class I associations most prominently with Cw6, B13, and B57 but also in a subgroup of patients with B27.[20,21] Although there are strong similarities, some features of the skin lesions in the B27 transgenic rats distinguish them from psoriasis and KD. Regular elongation of rete ridge is less prominent and suprapapillary plates are much thicker in B27 rats. Spongiform pustules are not as massive as those seen in KD. There is no significant atrophy of sebaceous glands, as is frequently observed in psoriasis.[22] In addition, the Koebner phenomenon was not seen in B27 rats, which was indicated by the absence of psoriatic changes after wound healing from repetitive tape-stripping or needle scratching on non-lesional skins (data not shown).

In psoriasis vulgaris, activated T cells, monocytes, and neutrophils are demonstrated in psoriatic lesions, and increased expression of local cytokines suggests that immunological mechanisms are involved in the pathogenesis of psoriasis.[23,24] In B27 rats, there are several lines of evidence that indicate a potentially similar underlying immune-mediated

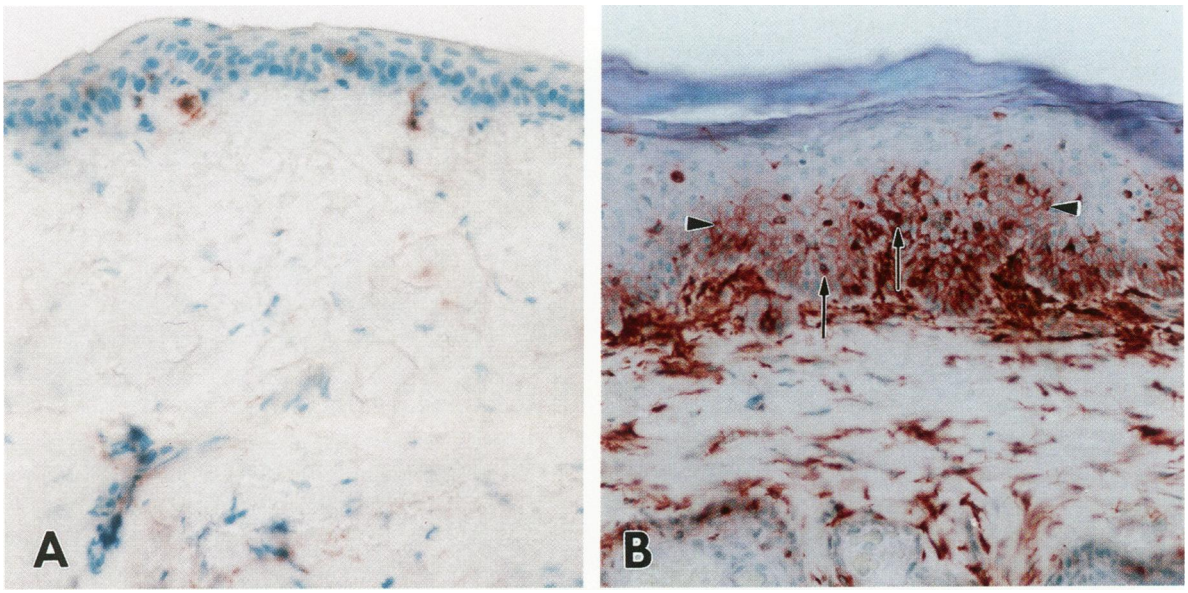

Figure 5. *Characterization of epidermal MHC-II expression in psoriasiform tail skin of 21-4H rats.* (**A**) *In subclinical tail skin, MHC-II (OX6 MAb) cells are sparse and are most frequently located perivascularly. Keratinocytes are not immunoreactive (150×).* (**B**) *In inflamed hyperplastic epidermis, keratinocytes are strongly MHC-II immunoreactive* (**arrowheads**) *and there is an abundance of MHC-II positive intraepidermal inflammatory cells* (**arrows**) *(168×).*

mechanism in the development of psoriasiform lesions. The immunohistological staining for B27 antigen indicated that there was a dramatic increase in the number of B27-positive cells in the dermis of hyperplastic lesions compared with unaffected skin of transgenic animals. These B27-positive cells were largely ED1$^+$, OX6$^+$, a phenotype consistent with MHC-II positive antigen-presenting cells. In associated epidermis, there was also an increase of B27-positive dendritic-shaped cells that were OX62$^-$, OX6$^+$, a pattern consistent with Langerhans cells. The importance of antigen-presenting cells in the development of psoriasis has been shown in human specimens,[25–27] and the numbers of dermal dendritic cells, macrophages, and epidermal Langerhans cells are increased in the psoriatic lesion.[28] The importance of antigen-presenting cells in development of the skin lesions in the B27 rats is underscored by our previous finding that engraftment of transgenic bone marrow from disease-prone lines is necessary and sufficient to reproduce the disease phenotype in syngeneic nontransgenic recipients, whereas transfer of lymphocytes alone is insufficient.[5]

The other significant change in B27 rats was the marked epidermal infiltration. In human psoriasis, there are reports indicating that CD4 T cells[29] or CD4 and CD8 T cells, Langerhans cells, and macrophages[30] are the principal cell types in the epidermal infiltrate. In our animals, these cells were strongly positive for B27 and were identified as MHC-II-positive, activated α/β T cells. Both CD4 and CD8 T cells appeared to contribute to the inflammatory response.

There was also an up-regulation of MHC-II molecules and B27 expression on the keratinocytes in proximity to the epidermal infiltration. An immunoregulatory role for keratinocytes in inflammatory skin disease has been suggested by several lines of evidence (reviewed in refs. 31 and 32). In addition to its primary function in providing structural integrity and barrier formation, the keratinocyte can interact with T cells by presenting antigen and serving as an antigen-specific target cell. The expression of MHC-II on human keratinocytes is known to be induced in psoriasis and other inflammatory skin disorders, including allergic contact dermatitis and systemic lupus erythematosus.[33] It is known that the secretion of IFN-γ from inflammatory T cells up-regulates MHC-II expression on keratinocytes[34] and induces the production of other cytokines, including interleukin (IL)-1α, IL-6, and IL-8.[31,35–37] It also induces the expression of CD54 (intercellular adhesion molecule-1) on keratinocytes.[38,39] Therefore, we speculate that the keratinocytes, when activated by IFN-γ secreted from inflammatory T cells, act to amplify the immune response in cutaneous lesions in B27 rats. In preliminary experiments, we have detected mRNA for several proinflammatory cytokines in inflamed B27 rat tail skin (IL MacLean, RE Hammer, JD Taurog, unpublished data). This supports the concept that cytokine production by T cells

and/or keratinocytes is a significant feature in the pathogenesis of these lesions, and that B27 rats can serve as a useful model to investigate the interaction between antigen-presenting cells, T cells, and keratinocytes.

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