Full-Thickness Skin Grafts from Flaky Skin Mice to Nude Mice: Maintenance of the Psoriasiform Phenotype

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Flaky skin (fsn) is an autosomal recessive mouse mutation with papulosquamous disease features similar to human psoriasis. In fsn/fsn skin, one sees marked acanthosis and hyperkeratosis with focal parakeratosis, subcorneal pustules, dermal capillary dilation, and a marked diffuse dermal infiltration of mixed inflammatory cells, predominantly lymphocytes. To determine if these pathologic features are a characteristic of the skin or a chronic autoimmune attack, we placed full-thickness skin grafts from affected homozygous (fsn/fsn) and normal littermate control (+/?) mice on the dorsal skin of genetically athymic nude (nu/nu) mice. After 10 weeks of observation, the grafts maintained the histologic phenotype of the donor animal. In the fsn/fsn grafts, there was persistence of both epidermal proliferation and dermal inflammation, characteristics of the mutation. The fsn/fsn phenotype was also confirmed by immunohistochemical evaluation for specific mouse keratinocyte marker expression. Based on tritiated thymidine uptake, we found DNA synthesis rates elevated threefold or more in fsn/fsn epidermis compared to littermate control mouse skin. Elevated rates of DNA synthesis remained a feature of the fsn/fsn grafts but not that of littermate control skin grafts. This study demonstrates that the psoriasiform phenotype of this mouse mutation can persist independent of the host thymic-derived immune system.

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Abbreviations: fsn, flaky skin mouse mutation; nu, nude mouse mutation; +/? , flaky skin heterozygote or wild type littermate control.

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Psoriasis is believed to have a genetic basis, although the mode of inheritance and number of genes involved remain to be defined [1-3]. Progress in understanding the mechanisms of psoriasis has long been hampered by the obvious difficulty in obtaining samples from human subjects and the lack of a suitable animal model system [4]. Although sporadic cases of psoriasiform dermatitides have been described in non-human primates [5,6], dogs [7], and pigs [8], the spontaneous nature and infrequent occurrence of these diseases discourage their use for detailed scientific investigation.

Establishment of an animal model system of a psoriasiform dermatitis using an inbred mouse strain would resolve many of the problems associated with other purported animal models of psoriasis, particularly the availability of large numbers of affected animals. Although there are several spontaneous mouse mutations in which hyperkeratosis is part of the phenotype, such as asebia (ab) and ichthyosis (ic) [9], the hyperkeratosis in these animals is not associated with inflammation. Therefore, they are not suitable to investigate the interaction of the epidermis and inflammatory cells, which is central to defining the pathogenesis of psoriasis. Recently, there have been reports of transgenic mice and rats with inflammatory and hyperkeratotic skin disease suggestive of psoriasiform dermatitis [10-12]. The relevance of these lesions to human psoriasis awaits further characterization.

In the fall of 1985, a spontaneous autosomal recessive mouse mutation, designated flaky skin (gene symbol fsn), was found at The Jackson Laboratory [9,13,14]. Extensive longitudinal characterization studies of skin from fsn/fsn mice have demonstrated a high degree of correlation between the mouse skin pathology and human psoriasis [13,14]. The flaky skin mouse has changes in the stratum corneum characteristic of guttate psoriasis and a prominent dermal inflammatory cell infiltrate similar in composition to psoriasis in humans.

Current hypotheses suggest that psoriasis arises as a result of a complex interaction between cytokines and various cells of the immune system [15,16]. Discriminating between these hypotheses requires experimental isolation of the psoriasiform skin and maintaining it in an environment free of an immune system. Although this is precluded by both ethical and practical issues in humans, the role of host inflammatory cells in the maintenance and progression of the disease in flaky skin mice can be analyzed by grafting affected fsn/fsn skin onto genetically athymic nude (nu) mice. Homozygous nu/nu mice lack a thymus and thus do not have T cells necessary to reject cutaneous allografts or xenografts of wide phylogenetic disparity [17,18]. This method has been used to study the biology of permanent skin grafts of normal adult and fetal human skin and adnexa [19-21]. Human allografts onto nu/nu mice have also been used to define the pathogenesis of a number of human skin diseases including psoriasis [22-24]. Transplantation of skin from fsn/fsn to nu/nu mice would test the hypothesis that an abnormal immune system was required for maintenance of the psoriasiform phenotype and would also provide a comparison of the fsn/fsn phenotype with human psoriasis.

We describe here the successful transplantation of full-thickness skin grafts from fsn/fsn and +/? littermate control mice to nu/nu mice. The mutant phenotype was maintained, as determined by histopathology, immunohistochemistry, and epidermal kinetic studies. No influence on the normal littermate control grafts was identified.
MATERIALS AND METHODS

**Animals** Skin graft donor mice consisted of six female CBy.A N1F2-fsn/fsn and six female +/+ (may be either +/fsn or +/+ ) littermate control mice that were 7–9 weeks old. In addition, unmanipulated female fn/fsn and +/+ mice at 6 weeks of age (three each) and 16 weeks of age (two each) were necropsied for comparison to the donor and 10-week-old grafted mice. Mice were obtained from the small research colony developed and maintained at The Jackson Laboratory, Bar Harbor, ME. Recipients consisted of twelve 7–8 week old, male CByB6F1/-nu/nu mice obtained from production colonies of The Jackson Laboratory. Mice were fed Wayne sterilizable Rodent Blox (TecLab, Premier Laboratory Diets, Bartonville, IL), provided chlorinated water (10–20 ppm residual chlorine) ad libitum, and maintained on a 12:12 light:dark cycle.

**Skin Grafts** The grafts were performed according to the methods described by Briggaman [25]. Briefly, recipient and donor mice were each anesthetized with pentobarbital (80 mg/kg body weight; Nembutal, Abbott Laboratories, North Chicago, IL) diluted in phosphate-buffered saline (pH 7.2) administered intraperitoneally (IP). A circular piece of skin (epidermis and dermis), approximately 1 cm in diameter, was aseptically removed from the dorsal cervical and lumbar regions of the donor mice. Grafts were held in place with tape cut to form a vest around the mouse. Each recipient received one skin graft from a fn/fsn and one from a +/+ littermate control mouse. Each donor provided grafts for transplantation to two nu/nu recipients. At the same time, additional samples of skin were fixed from the donor mice for histopathologic comparison of the donor skin with transplanted skin.

**Epidermal Kinetics** Seventy days after transplantation, the recipient mice were administered 1 μCi/gm body weight of 3H-thymidine (specific activity 20 μCi/mM; New England Nuclear, Boston, MA) IP, to label cells entering the “S” phase of the cell cycle. Forty minutes after injection, the mice were euthanized by CO2 asphyxiation. The transplantation sites and peripheral skin from the nu/nu mouse host were removed, attached by gentle pressure to heavy paper, and fixed by immersion in Fekete’s acid alcohol formalin for 12 h. The tissues were subsequently transferred to 70% ethanol, trimmed for histology, embedded in paraffin, and sectioned at 5 μm. Serial sections were either stained with hematoxylin and eosin, prepared for immunohistochemistry, or processed for autoradiography.

Autoradiographs were prepared by immersing the slides in a liquid photographic emulsion (Kodak NTB-2; Eastman Kodak Co., Rochester, NY) under darkroom conditions, exposed at 4°C for 39 days, developed (Kodak D19 developer; Eastman Kodak Co.), and stained with Mayer’s hematoxylin. On microscopic examination, an epidermal cell was defined as labeled (synthesizing DNA) if it contained five or more silver grains over the nucleus. Two labeling indices were defined for each transplantation site: 1) the epidermal basal cell labeling index, which represents the labeled epidermal basal cells/total epidermal basal cells X 100 (estimated by counting 2000 labeled and unlabeled basal cells); and 2) the epidermal nucleated cell-labeling index, which represents the total labeled epidermal nucleated cells/epidermal nucleated cells X 100 (established by counting 5000 labeled and unlabeled nucleated cells [26]). The cell-renewal time was estimated from these data using the method of Leblond et al [27]. Data were analyzed by the t test; differences were considered significant at p < 0.05.

**Immunohistochemistry** Serial sections, obtained as described above, were stained with a panel of rabbit affinity-purified polyclonal antibodies directed against mouse specific keratinocytic markers (K1, K5, K6, K10, K13, K14, filaggrin, and loricrin) using the avidin-biotin method. Diaminobenzidine was used as the chromagen and Mayer’s hematoxylin as the counterstain as previously described [28]. Dark brown cytoplasmatic staining was scored as positive with blue as a negative result. Results were compared to staining of host skin taken adjacent to the excision site.

**RESULTS**

**Skin Grafts** Representative photographs of host mice and photomicrographs of full-thickness skin graft from fn/fsn and +/+ littermate controls are presented in Figs 1 and 2. The histologic and immunohistochemical results, as well as labeling index data, were essentially identical for the duplicate grafts even though they were on separate recipients. The data are summarized in Tables 1 and II.

The fn/fsn and +/+ grafts could be distinguished from adjacent nu/nu mouse skin by microscopic differences in the hair follicles (Fig 2). The donor grafts were from an albino background and accordingly had hair follicles with normal hair shafts devoid of melanin pigment. The nu/nu mouse background is pigmented and the hair shafts in the follicular infundibulum were characteristically distorted and malacic [29].

Normal +/+ control skin and +/+ grafted onto nu/nu mice had no substantive differences other than mild dermal and panniculitis fibrosis and rare, scattered lymphocytes and macrophages. These were judged to be a consequence of the surgical procedures.

In contrast, fn/fsn donor skin and grafts had marked laminated orthokeratosis alternating with mounds of parakeratosis and occasionally contained neutrophils (Fig 2). The nucleated epidermal layers had moderate to marked epidermal hyperplasia with prominence of the granular cell zone. The pattern of hyperplasia in the grafts was often more papillated than donor skin and there was prominent keratin plugging of the follicular infundibulum. In both donor and graft biopsies, there was an interstitial and perivascular inflammatory response involving the entire dermis. The reaction was somewhat less severe in the grafts (but much more intense than the scattered inflammatory cells noted in the littermate control grafts). The inflammatory reaction was composed primarily of small lymphocytes and macrophages. Neutrophils were occasionally present in the subepidermal adventitial dermis. There was dermal and panniculitis fibrosis as a consequence of the grafting procedure.

**Immunohistochemistry** Immunohistochemical evaluation of keratinocyte markers of the transplanted and non-transplanted controls (+/+, +/+, and K14 stained the lower half of the epidermis (primarily basal cells) and the keratinocytes of the outer root sheath of the hair follicle (Fig 3a–d). Anti-K6 stained the cells comprising the inner layer of the outer root sheath of the follicular isthmus (Fig 3ef). Staining for K1 and K10 was specific for the upper half of the non-cornified epidermis (Fig 4a–d). Anti-K13, which normally stains only the mucous membranes of the oral cavity, did not stain skin cells. Anti-loricrin and filaggrin staining was limited to the cytoplasm of the epidermal and infundibular granular cell layer, the expression of filaggrin being specific for keratohyalin granules (Fig 4ef).

The immunohistochemical staining patterns of the fn/fsn mice...
Figure 2. Skin grafts from littermate control (+/?) and affected fsnl/fsnl mice. Abrupt change in orientation of follicles identified location of normal grafts (a, bar, 200 μm). Normal unpigmented hair follicles and shafts (arrowheads) of the +/? mice could be differentiated from the pigmented, dystrophic hair shafts (arrows) (b, bar, 100 μm). Heavily pigmented nu/nu bulbs (c, bar, 50 μm) could be easily differentiated from +/? bulbs (b). In the fsnl/fsnl graft there was more intense inflammation, marked epidermal hyperplasia, laminated orthokeratosis, and keratin plugging of the follicular infundibula (d, bar, 100 μm; e, bar, 50 μm).
were consistent between the donor and the grafts, as summarized in Table I. Keratinocyte marker proteins K5, K13, and K14 stained similarly to that of the +/+ controls. Filaggrin and loricrin staining was more prominent than in controls, a change consistent with the hyperplastic epidermis and in the entire outer root sheath.

**Epidermal Kinetics** The results of the ³H-thymidine labeling studies are presented in Table II and illustrated in Fig 5. The grafts from the fn/fn mice had significantly higher cell labeling indices than +/+ control grafts ($p < 0.005$), with calculated cell renewal times of 1.9 d and 6.9 d, respectively. As a consequence of factors associated with surgical grafting, the grafts from both fn/fn and +/+ control mice had significantly more proliferative activity when compared to biopsies from their respective genotype controls ($p < 0.05$).

**DISCUSSION**

Grafting psoriatic human skin onto nu/nu mice has been developed as a system that can be manipulated to study the biology and potential treatment of the disease [22-24]. With the discovery of a new mouse mutation that resembles psoriasis, it was possible to use skin grafting to ask fundamental questions about the pathogenesis of the disease. It was essential to use nu/nu mice as recipients because the fn gene was not on an inbred background, thus precluding syngeneic grafting. This study demonstrated that grafting of fn/fn skin to nu/nu mice would maintain the clinical phenotype for periods of at least 10 weeks. This has advantages as a test system, but also implies that the thymic-derived T lymphocyte part of the immune system is not required for maintenance of the lesion.

Maintenance and progression of the fn/fn phenotype in grafts may be caused by host factors produced by the nu/nu mouse that support already inherently abnormal fn/fn keratinocytes. Grafting of human psoriatic skin to nu/nu mice has yielded conflicting results and interpretations. Thus, Briggaman et al. [1] were successful in their studies and concluded that host factors were important for supporting the skin disease. Others have achieved intermediate results [22-24] and concluded that only some factors are present in nu/nu mice. Finally, Baker et al. [30] reported loss of the psoriatic phenotype, and by immunohistochemical markers identified putative mouse T cells in the human grafts, raising the specter of graft-versus-host response confounding the interpretation of skin graft proliferation as evidence for abnormal keratinocytes [23]. This latter conclusion must be tempered by the fact that several cell types including NK cells, all T cells, and Thy-1² dendritic epidermal cells also express Thy 1.2 antigen [13,31–33]. The confusion may stem from several sources, such as variability of the human grafts, unidentified species differences in host factors, and substantial differences in genetic backgrounds of the nu/nu mice used for grafting studies [22-24,30]. It is well known that a mutant phenotype can be subtly or dramatically altered by background modifying genes [34].

Variation in endogenous factors may play a role in changes in phenotypes between allograft versus xenografts in this type of iatrogenic model system. Dermal-epidermal recombination grafts of human psoriasis skin onto nu/nu mice duplicated psoriasis only in recombinants composed of psoriatic epidermis and dermis. The human recombination study indicated that generalized (exfoliative) psoriasis resides within the skin and not just the epidermis.† Neontal or fetal fn/fn skin grafts combined with dermal-epidermal recombination grafts are in progress to determine if both mutant dermis and epidermis are required for phenotypic expression.

Patients with severe, generalized psoriasis, who have complete and persistent regression of skin lesions following bone-marrow transplants from unaffected siblings for diseases unrelated to the psoriasis [35,36]. Therefore, it appears that bone marrow-derived cells and growth factors are involved in the pathogenesis of at least some forms of psoriasis. We have found that bone marrow grafted from fn/fn mice to severe combined immunodeficiency (scid/scid) mice resulted in the development of a proliferative skin disease in the recipients [13]. This experiment suggests that the papulosquamous disease in fn/fn mice has a pathogenesis similar to that of some forms of human psoriasis. Alternatively, cells or factors residing in the dermis or epidermis in the full-thickness grafts might be adequate to maintain the phenotype during the observation period. The fn/fn grafts to nu/nu hosts in this study did have lymphocytic infiltrates (probably B cells of nu/nu origin) in the dermis 10 weeks after the surgery. The source of these cells was not identified. Some studies suggest that T lymphocytes mediate keratinocyte proliferation in psoriasis [5]. The scid/scid mutation lacks functional T cells, B cells, and Thy-1² dendritic epidermal cells [37]. The double mutation (scid/scid, fn/fn) maintains the papulosquamous phenotype in spite of the lack of a functional immune system [13]. Therefore, functional lymphocytes are not required for the pathogenesis of the fn papulosquamous disease. These observations initially indicate that the mechanisms underlying the fn phenotype are different from those of human psoriasis. This may be the case with some forms of psoriasis; however, because patients with acquired immunodeficiency syndrome (lacking a functional immune system) have been reported with severe cases of psoriasis [38], this hypothesis should be re-examined.

Immunohistochemical evaluation of keratinocyte markers has proved to be useful for evaluating naturally occurring mouse skin tumors [39] and carcinogen-induced mouse skin tumors [40–42], as well as xenografts to nude mice [43]. Some of the markers, such as K13, are detected in squamous cell carcinomas of the skin and may prove to be valuable in the mouse for differentiating hyperplastic versus neoplastic changes in naturally occurring or induced skin tumors [40,44–46] or various biochemically related processes in mutant mice. K13 was not expressed in either the donor ungrafted fn/fn skin, suggested that there is no inherent transformational defect in this mutant mouse. Another keratinocyte marker, K6, has been associated with nonspecific hyperplasia of the epidermis and papilloma formation [42,47,48], whereas expression of others such as K1 and K10 are decreased or absent [42,49,50]. Expression in the fn/fn epidermis was consistent with these types of observations. K6 in mice without epidermal hyperplasia is only expressed in the inner cell layer of the outer root sheath of the follicular isthmus whereas K1 and K10 are present in normal suprabasal cells. These

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**Table I. Keratinocyte Marker Expression in the Epidermis of Grafted and Host Skin**

<table>
<thead>
<tr>
<th>Keratinocyte Marker</th>
<th>fn/fn</th>
<th>+/+</th>
<th>nu/nu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin K1</td>
<td>Patchy to negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Keratin K5</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Keratin K6</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Keratin K10</td>
<td>Patchy to negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Keratin K13</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Keratin K14</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Filaggrin</td>
<td>Increased</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Loricrin</td>
<td>Increased</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* Immunohistochemistry was done on skin from fn/fn and +/+ mice as well as the grafted skin from both mutant and control mice and the nu/nu host skin to determine if phenotypic expression of these keratinocyte markers remained the same in the grafts as donor skin.

* Staining limited to outer root sheath of the hair follicle at the level of the follicular isthmus.
Figure 3. Antibodies directed at mouse keratins K5 (a, b, bar, 100 µm) and K14 (c, d, bar, 100 µm) stained basal and suprabasal epidermal cells and the outer root sheath of the hair follicle. Patterns between controls (a, c) and fsn/fsn (b, d) grafts were similar. Staining for K6 was localized to the follicular isthmus in control grafts (e, bar, 100 µm; inset, bar, 20 µm) but extended to the proliferative suprabasal epidermis in the fsn/fsn grafts (f, bar, 100 µm).
Figure 4. K1 (a, b, bar, 100 μm) and K10 (c, d, bar, 100 μm) were detected in suprabasilar epidermis of control grafts (a, insert, bar, 20 μm, e) but not in the proliferating epidermis of the fsn/fsn skin grafts (b, d). Filaggrin staining was limited to the keratohyalin granules within the cytoplasm of cells in the stratum granulosum of both control (e, bar, 20 μm) and fsn/fsn skin grafts (f, bar, 20 μm). Loricrin staining was limited to the cytoplasm of cells in the stratum granulosum of both controls (g, bar, 20 μm) and fsn/fsn (h, bar, 20 μm) grafts.
observations in both \( fn/fm \) in situ skin and grafted skin parallel similar observations of human psoriatic skin [51,52].‡

The \( ^{3}H \)-thymidine labeling indices of the \( fn/fm \) and \(+/\) litter- mate controls were significantly different (\( p < 0.005 \)); duplicate biopsies grafted onto each of two \( nu/nu \) mice yielded essentially identical results. The labeling indices of the grafted sites were higher than the donor skin of the affected and control mice. This observation can most reasonably be explained by the dermal fibrosis and other processes of wound healing, which can stimulate increased epidermal turnover. There is an inherent variability in the labeling index of humans with and without psoriasis. † This could reflect the background genetic variation between humans (an outbred species) and the variation of the purported human genetic mutation(s) as well [34]. However, by grafting of human psoriatic skin from one individual onto several \( nu/nu \) mice, similar results were obtained within that group of nude mice [22], as was the case with \( fn/fm \) grafts to \( nu/nu \) mice in this study.

There is increasing evidence that supports the flaky skin mouse mutation as a potentially useful animal model that will serve as a useful tool to unravel the mechanisms involved in papulosquamous diseases, such as psoriasis. In this study we have demonstrated that skin grafts onto \( nu/nu \) mice will maintain the mutant phenotype as measured by histopathology, keratinocyte markers, and epidermal kinetics. All of these parameters parallel similar studies utilizing

**Table II.** Cell Kinetic Data Based on Tritiated Thymidine Uptake for 42-Day—Old Flaky Skin Mice or Littermate Controls or Comparable Grafted Skin to Nude Mice

<table>
<thead>
<tr>
<th>Criteria</th>
<th>( fn/fm )</th>
<th>(+/)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cell labeling index</td>
<td>Skin graft</td>
<td>31.7 ± 4.6 †</td>
</tr>
<tr>
<td></td>
<td>Unmanipulated skin</td>
<td>18.6 ± 7.7</td>
</tr>
<tr>
<td>Nucleated cell labeling index</td>
<td>Skin graft</td>
<td>13.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Unmanipulated skin</td>
<td>7.8 ± 2.4</td>
</tr>
<tr>
<td>Calculated cell renewal time in days</td>
<td>Skin graft</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Unmanipulated skin</td>
<td>3.6 ± 0.9</td>
</tr>
</tbody>
</table>

‡ Mice were injected with tritiated thymidine to determine if epidermal kinetics of both the \( fn/fm \) donor and grafted skin remained elevated compared to that of the control skin.

† Standard deviation.

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