Experimental Induction of Alopecia Areata-Like Hair Loss in C3H/HeJ Mice Using Full-Thickness Skin Grafts

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Alopecia areata (AA) is a common cause of hair loss, affecting almost 2% of the general population (Safavi et al, 1995). Evidence suggests that AA is an autoimmune disease with inflammatory cells, primarily lymphocytes, in and around dystrophic anagen hair follicles (Perret et al, 1984) and the production of autoantibodies specific for hair follicle antigens (Tobin et al, 1994). Although not life threatening, the disease can be so psychologically devastating for those affected that depression or suicide may occur (Colon et al, 1991; King, personal communication).

Progress in understanding the pathogenesis and genetics of AA and developing new therapies has been hampered by the lack of an animal model for this disease. Recently, rat and mouse models, with phenotypic, histologic, and immunologic similarities to human AA, have been identified (Michie et al, 1991; Oliver et al, 1991; Sundberg et al, 1994b, 1995; Zhang and Oliver, 1994; McElwee et al, 1996, 1998b; McElwee and Oliver, 1997; Tobin et al, 1997). Aging C3H/HeJ mice develop a spontaneous, polygenic, semidominant, inflammatory-based, AA-like hair loss. Expression of hair loss rises to 20% in some colonies of mice 18+ mo of age (Sundberg et al, 1994b). Initially, all C3H/HeJ mice produce a normal coat of agouti hair. The onset of disease typically develops as early as 4 mo of age in females with rapid, extensive progression. Males can be affected in equal numbers with similar patterns of balding, but the initial onset occurs at 6–12 mo of age. Typically, alopecia initially develops on the ventral surface, expanding to cover the entire abdomen in a symmetrical pattern. Distinct foci of alopecia may develop elsewhere on the dorsal surface, bilaterally symmetrical or asymmetrical in appearance. Extensive hair loss, which may approach total body or universal alopecia, develops in 17% of AA-affected male and female mice. A diffuse type of AA can occasionally develop. Complete, spontaneous remission is recognized in up to 3% of affected mice (Sundberg et al, 1994b). This low incidence of alopecia with an unpredictable time of onset is typical of polygenic, multifactorial diseases in mice and not unlike that observed in humans. Such variable presentation, however, limits the experimental use of the C3H/HeJ model.

In spontaneous C3H/HeJ mouse AA anagen hair follicles are affected by an intense peri-follicular inflammation of CD4+ and CD8+ lymphocytes in a ratio of 1:2, respectively. Some dendritic cells are also observed in the inflammation along with variable numbers of granulocytes. Peri-follicular inflammation is observed from the bulb to the sebaceous gland with the greatest infiltrate density observed around the infundibulum. Some T cells, primarily CD8+ cells, penetrate to intrafollicular positions in the bulb and infundibulum. Long-term AA-affected mice show the same infiltrate distribution but with a reduced total number of inflammatory cells.

Full-thickness skin grafts from humans to immunodeficient mice, such as those homozygous for nude (Hfh11nu) or severe combined immunodeficiency (Prkdcscid), which accept xenografts, have been used to study a variety of cutaneous diseases including psoriasis (Kreuger et al, 1975, 1981; Hafiek et al, 1981) and AA (Gilhar and Kreuger, 1987; Gilhar et al, 1992, 1998). An allograft approach can also be used between mice either to investigate the pathogenesis of a mutation with a cutaneous phenotype, as with flaky skin (fsn) and chronic proliferative dermatitis (pdmh), or to expand limited numbers of mice for testing.

Alopecia areata (AA)-like hair loss in C3H/HeJ mice provides an excellent model for human AA disease research. The potential to induce mouse AA in normal haired C3H/HeJ mice at an early age or serially passage the AA phenotype was investigated by exchange of full-thickness skin grafts. Skin grafts from normal male and female C3H/HeJ, or severe combined immunodeficient C3H/SmmC Prkdcscid/J, mice onto AA-affected C3H/HeJ mice became inflamed and lost hair (28 of 28). Successful grafts from AA-affected C3H/HeJ mice induced hair loss in histocompatible C3H/OuJ mice (four of 13) and normal C3H/HeJ mice dependent on age (four of 17 at <31 d and 15 of 15 at >70 d). The AA phenotype was serially transmitted from induced AA mice to normal C3H/HeJ mice (nine of nine). Grafts from AA-affected C3H/HeJ mice onto C3H/SmmC Prkdcscid/J mice resulted in depigmented hair fiber regrowth and perifollicular neutrophil and eosinophil infiltrates but no hair loss (15 of 15). Sham grafting did not induce AA (none of 10). The finding that AA can be serially transferred from AA-affected C3H/HeJ mice to normal littersmates and C3H/OuJ mice, indicates that an immune response against hair follicles can be induced with suitable stimuli. Conversely, skin grafts from normal C3H/HeJ, or C3H/SmmC Prkdcscid/J, mice rapidly lose hair due to lymphocyte, but not neutrophil and eosinophil, mediated inflammation. This AA induction method reproducibly provides large numbers of AA-affected mice to study the pathogenesis and treatment of human AA. Key words: adoptive transfer/autoimmune disease/disease model/inflammation. J Invest Dermatol 111:797–803, 1998

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studies when large production colonies are not available (Sundberg et al, 1994c; Gibson et al, 1995).

AA has successfully been induced by T cell transfer of in vitro stimulated human lymphocytes to human skin grafted onto severe combined immunodeficient Pkld/scid mice (Gilhar et al, 1998). AA-like hair loss has apparently been induced after melanoma immunotherapy in C57BL6/J mice (Becker et al, 1996), with the ability to transfer the phenotype to naïve mice by transfer of T cells. Cutaneous grafting experiments were done between C3H/HeJ mice with and without AA to determine the pathogenic effect on both the graft and the host.

We showed for the first time that full-thickness AA-affected skin grafts onto histocompatible mice, yielded a cutaneous, nonscarring, inflammatory based hair loss (alopecia) with a predictable time course in recipients 70 d of age and older.

MATERIALS AND METHODS

Skin graft technique Donor mice were euthanized by CO2 asphyxiation. Up to six grafts, 1–1.5 cm in diameter, were aseptically removed from each donor by pinch cutting (Billingham and Medawar, 1951), four from the dorsal and two from the ventral surfaces of each mouse. The grafts were placed in sterile phosphate-buffered saline while the recipient mice were prepared.

Recipient mice were anesthetized with tribromoethanol (0.2 ml per 10 g body weight; Aldrich, Milwaukee, WI). A circular piece of skin, 1–1.5 cm in diameter, was aseptically removed from the dorsal anto-posterior midline of the recipient mouse and replaced with a donor skin graft. The graft was oriented 180° from normal in order to have the hair grow in the opposite direction to the recipient’s hair for easy identification. Where possible recipient mice were given a combination of male and female donor-derived skin grafts; however, immunocompetent female recipient mice were only able to accept female donor derived skin grafts due to HY minor histocompatibility antigens.

Four to six simple interrupted sutures (5-O Dexon ''S''; Davis & Geck, Manat, Puerto Rico) were placed at regular intervals around the edges of the graft. Nexaban (Veterinary Products Laboratory, Phoenix, AZ) was used on the opposing edges of the graft site to improve adherence. The grafts were then covered with a sterile Telfa pad (Kendall, Boston, MA), held in place with Micropore surgical tape (3M Medical-Surgical Division, St. Paul, MN), and further bandaged with Coban self-adherent wrap (3M Medical-Surgical Division). Wound clips were placed in the skin over the neck, pelvis, and thorax of each mouse to hold the bandages in place.

Bandages were removed 4–6 d after the surgery. Mice were housed two or three per cage and all received autoclaved food pellets (NIH 31 modified with 6% fat, diet code 5K52, PMI, Richmond, IN) and acidified water ad libitum. Mice were given acidified water containing an antibiotic (Sulfamethoxazole & Trimethoprim Oral Suspension, USP, Schein Pharmaceutical, Florham, NJ) for three in every seven days for 2 wk after surgery to reduce the chance of infection. Immunodeficient C3H/SmnC Pkld/scid mice were also maintained indefinitely on the antibiotic acidified water 3 d per week to minimize complications with Pneumocystis carinii infection (Shulite et al, 1989).

Skin graft studies All mice were supplied from stocks at The Jackson Laboratory (Bar Harbor, ME) specific pathogen-free production facility. In total, 11 skin graft recipient mice were analyzed in this study.

Immunodeficient mice The C3H/SmnC Pkld/scid/J (hereafter referred to as C3H/sid/sid) congenic strain has proved to be consistently immunodeficient and unable to produce functional T and B cells in response to antigenic challenge (Nonoyama et al, 1993). Skin from adult AA-affected male and female C3H/HeJ mice was grafted onto 15 female immunodeficient recipient C3H/sid/sid mice with an age range of 30–81 d. Skin from C3H/sid/sid female mice was also grafted onto 18 female AA-affected C3H/HeJ mice 209–294 d of age.

Immunocompetent mice Skin from young unaffected C3H/HeJ mice was grafted onto 10 female, AA-affected C3H/HeJ mice with an age range of 331–359 days. To reciprocate, skin from AA-affected C3H/HeJ mice was grafted to 18 normal, adult C3H/HeJ mice (eight female, 10 male) with an age range of 70–91 d. Skin from AA-affected male and female mice was also grafted to 17 normal, normal haired C3H/HeJ mice (eight female, nine male) aged 21–31 d. Using these mice with induced AA, a further study was done by grafting of induced AA-affected skin onto 10 more normal haired C3H/HeJ mice (two female, eight male), all 104 d old.

Histocompatible mice The C3H/OuJ (C3H/HeOuJ) mouse strain is immunocompetent, has no prior history of AA-like hair loss in Jackson Laboratory production colonies, and C3H/OuJ mice are histocompatible (H-2K haplotype) with C3H/HeJ mice. Full-thickness, AA-affected C3H/HeJ mouse skin was transferred to 13 C3H/OuJ mice (four female, nine male) with an age range of 63–84 d.

Sham graft control mice Four C3H/OuJ (one female, three male) and six normal haired C3H/HeJ mice (four female, two male) had skin taken from their dorsal surface using the technique described above and then returned to the same mouse.

Histologic analysis At the same time as grafting, skin from graft donors was taken immediately adjacent to excision sites on the dorsal surface on day 0. Skin removed from recipients prior to receiving donor skin was also collected and fixed for histologic analysis. Upon completion of each study, each mouse was euthanized by CO2 asphyxiation and necropsied. Skin was collected across each graft, including adjacent host skin. Grafts were not taken during the study due to the hair wave induction effect of surgery and concern about complications due to scarring from wounding (Sundberg et al, 1997). Biopsies were fixed in Fekete’s acid-alcohol-formalin solution and processed routinely for hematoxylin and eosin staining. Mice were examined weekly to evaluate healing and progression of disease. Dorsal, ventral, and flank macro photographs were taken using ISO400 slide film (Kodak, Rochester, NY) of each donor and recipient mouse under anesthesia on day 0 and of recipients at the completion of each study.

RESULTS

Several observations were common to all grafting studies. Graft contraction was frequently observed, particularly when graft recipients were normal haired mice and ventral skin contracted less than dorsal skin. Initial stimulation of host hair follicles adjacent to the graft site to progress from telogen into an anagen hair growth state was observed as expected (Sundberg et al, 1997). Anagen induction in adjacent recipient skin was an advantage in these studies because inflammatory cells only infiltrate anagen hair follicles in humans. Dundee experimental bald rats, and C3H/HeJ mice with AA (Perrett et al, 1984; Sundberg et al, 1994b; Zhang and Oliver, 1994). The hair wave induction phenomenon also permitted comparison of adjacent donor and recipient hair follicles known to be in the same stage of hair growth.

Immunodeficient mice Results of the various studies are summarized in Table I. Alopecic skin from AA-affected C3H/HeJ mice was grafted onto 15 recipient C3H/sid/sid mice (Fig 1). All recipients readily accepted the grafts without complications and all mice had normal hair regrowth in their AA-affected skin grafts (Fig 1C). First hair growth was noted in one mouse by day 28 and hair growth was apparent in all grafts by 42 d post-surgery. Hair typically first regrew from the periphery of the skin graft, with follicles at the center of the graft producing visible hair a few days later. Recipient C3H/sid/sid hair follicles were not macroscopically affected for the duration of the study and no hair dystrophy was observed.

Histology of donor skin at day 0 typically revealed a dense mononuclear cell infiltrate in and around dystrophic anagen hair follicles in all AA-affected C3H/HeJ donor mice. Catagen and telogen hair follicles had no associated inflammatory infiltrate. C3H/sid/sid recipients typically showed no infiltrated telogen follicles and normal waves of anagen hair growth. On completion of the study, the histology of biopsy site confirmed that hair regrew around the periphery with some regrowth of isolated hair follicles toward the center of the grafts (Fig 2). Some isolated mononuclear cells were detected within the graft and infiltrating around adjacent anagen hair follicles of the recipient; however, the inflammatory cell infiltrate around graft hair follicles, and recipient C3H/sid/sid anagen hair follicles, also included large numbers of neutrophils and eosinophils (Fig 2B). Occasional multinucleated giant cells were observed in peripheral sid/sid anagen hair follicles.

Skin from normal haired C3H/sid/sid mice was also grafted onto 18 adult AA-affected C3H/HeJ mice (Fig 3). All grafts were readily accepted by C3H/HeJ recipients. Hair loss within grafted skin was rapid, beginning 14 d post-surgery in one mouse. In other mice, hair was first lost from the periphery of the graft with gradual encroachment of hair follicle dystrophy toward the graft center between 14 and 21 d. All mice exhibited hair follicle dystrophy over the entire graft 35 d post-surgery (Fig 3C). There was no apparent hair regrowth or other macroscopic change in status of the grafts up to 70 d post-surgery. Histology of these sites revealed scarring associated with surgery and
Table I. Summary of results from skin graft protocols

<table>
<thead>
<tr>
<th>Skin graft donors</th>
<th>Skin graft recipients</th>
<th>Successful /total transplants</th>
<th>Hair loss in graft</th>
<th>Hair loss in recipient</th>
<th>Permanent graft hair regrowth</th>
<th>Mean days between surgery and hair loss</th>
<th>Mean days between surgery and hair growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-affected C3H/HeJ</td>
<td>C3H/SmnC Prkdc&lt;sup&gt;scid&lt;/sup&gt;/J</td>
<td>15/15</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>–</td>
<td>36.8 ± 2.8 SD</td>
</tr>
<tr>
<td>C3H/SmnC Prkdc&lt;sup&gt;scid&lt;/sup&gt;/J</td>
<td>AA-affected C3H/HeJ</td>
<td>18/18</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>21.5 ± 9.7 SD</td>
<td>–</td>
</tr>
<tr>
<td>Normal C3H/HeJ</td>
<td>AA-affected C3H/HeJ/Prkdc&lt;sup&gt;scid&lt;/sup&gt;/J</td>
<td>10/10</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>21.5 ± 4.7 SD</td>
<td>–</td>
</tr>
<tr>
<td>AA-affected C3H/HeJ/Prkdc&lt;sup&gt;scid&lt;/sup&gt;/J</td>
<td>Normal C3H/HeJ</td>
<td>15/18</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>63.3 ± 5.6 SD</td>
<td>–</td>
</tr>
<tr>
<td>AA-affected C3H/HeJ</td>
<td>Normal C3H/HeJ</td>
<td>17/17</td>
<td>4</td>
<td>4</td>
<td>13</td>
<td>68.8 ± 9.4 SD</td>
<td>42.2 ± 7.6 SD</td>
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<tr>
<td>Induced AA-affected C3H/HeJ</td>
<td>Normal C3H/HeJ</td>
<td>9/10</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>60.0 ± 9.3 SD</td>
<td>–</td>
</tr>
<tr>
<td>AA-affected C3H/HeJ</td>
<td>Normal C3H/OuJ</td>
<td>10/13</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>56.5 ± 10.5 SD</td>
<td>39.8 ± 4.4 SD</td>
</tr>
<tr>
<td>Normal C3H/HeJ</td>
<td>Self, sham graft</td>
<td>10/10</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>–</td>
<td>20.8 ± 3.4 SD</td>
</tr>
</tbody>
</table>

Figure 1. AA-affected C3H/HeJ skin when transplanted to a C3H/SmnC Prkdc<sup>scid</sup>/J mouse. (A) Day 0 immediately before surgery; (B) day 0 immediately after surgery; (C) 80 d post-surgery with white hair growth from the graft.

Figure 2. Histology of AA-affected C3H/HeJ skin grafts and C3H/SmnC Prkdc<sup>scid</sup>/J graft recipients 80 d post-surgery. Transverse section with induced anagen host hair follicles (left) and graft with isolated follicles and scarring (right, A, scale bar: 100 µm). Infiltrate of neutrophils and eosinophils plus isolated mononuclear cells evident in boxed area (B, scale bar: 10 µm).

Immunocompetent mice  Skin from young unaffected C3H/HeJ mice was grafted onto 10 AA-affected C3H/HeJ mice and all allografts survived the duration of the study. Hair loss was apparent within 21 d in all skin grafts and eventually all hair follicles within grafts were dystrophic after 35 d. As with C3H scid/scid skin grafted to AA-affected C3H/HeJ mice, hair at the periphery of grafts was lost first, hair in the center survived longer. Microscopically, a mononuclear cell infiltrate in and around anagen hair follicles within the graft was evident upon completion of the study, 70 d post-surgery.

Skin from AA-affected C3H/HeJ mice grafted to normal adult C3H/HeJ mice consistently induced AA in all transplant recipients both at the graft site and also distal from the graft on the ventral and/or dorsal surface (Fig 5). No difference in ability to induce AA was observed using dorsal or ventral, male or female, derived AA-affected skin grafts. Of 10 female and eight male normal grafted C3H/HeJ...
adults, 15 developed patchy AA. The two female and one male mice that did not lose hair failed to accept their grafts. Graft rejection was clearly apparent within 7 d from transplantation surgery. Typical presentation of successful grafts involved some initial white hair regrowth from the AA-affected graft skin first seen 21 d after grafting. This hair growth was first observed around the periphery of the graft with the center becoming rehaired a few days later. Regrowth was short lived as hair was lost from all grafts by 49 d post-surgery. Hair loss developed in recipient mice by 77 d with the first observation of hair loss made in one mouse on day 53. The first evidence of hair loss was frequently noted as a narrow ring around the graft site, swiftly followed by ventral hair loss (Fig 5C, D). Dorsal hair loss distal to the graft site typically took longest to develop.

Microscopically, grafted donor skin contained dystrophic anagen hair follicles with a peri- and intrafollicular mononuclear cell infiltrate (Fig 6). Recipients had typical anagen waves of hair growth and telogen hair follicles with no mononuclear cell involvement. Histologic tissue sections showed scarring associated with graft healing, isolated dystrophic hair follicles within the graft, and a mononuclear cell infiltrate. Host hair follicles immediately adjacent to the graft, and on the ventral surface, were dystrophic, and infiltrated with mononuclear cells when in anagen (Fig 6C), but when in catagen or telogen there was no inflammation. The distribution of inflammation was the same as observed for spontaneous AA. Intense peri-follicular lymphocyte inflammation was observed from the bulbs to sebaceous glands with greatest intensity of cells focusing on the infundibulum. As with some stages of spontaneous mouse AA, some individual anagen hair follicles of graft recipients adjacent to the graft site had peri-follicular inflammation that contained a number of granulocytes in addition to lymphocytes. Individual lymphocytes had penetrated to intrafollicular positions.

In addition to grafting adult mice greater than 70 d of age, 17 juvenile C3H/HeJ mice up to 31 d of age were also grafted with AA-affected C3H/HeJ skin. Of eight female and nine male mice, four female mice developed extensive AA but no males had any signs of hair loss. Histologic features of skin from recipient males and grafts consisted primarily of telogen or anagen hair follicles with no inflammation or other abnormalities. All dorsal hair follicles were in telogen. Histologic features of AA-affected females included the classic type of mononuclear cell infiltrate seen in AA, similar to the presentation of spontaneous adult AA-affected mouse skin described above.

The four juvenile female mice with induced AA were then used as skin graft donors for additional normal haired, adult C3H/HeJ mice. These skin grafts were equally capable of inducing AA in naïve hosts. Of two female and eight male normal haired, grafted mice, hair loss was first observed in one mouse 49 d after grafting, and nine of 10 total mice were affected with patchy hair loss by 70 d. One male mouse failed to develop hair loss as the graft was rejected by 7 d post-surgery. Some mice first exhibited hair loss within the graft and in a ring of host skin adjacent to the graft. Hair loss was first observed distal to the graft site on the ventral surface, presenting as one or more symmetrically aligned patches of dystrophic hair follicles. Dorsal skin
distant or far removed from the graft site gradually became affected with patchy hair loss, first observed at 84 d post-surgery.

**Histocompatible mice** AA-affected C3H/HeJ mouse skin was transferred to four female and nine male C3H/OuJ mice. Three of 13 mice rejected their grafts by 7 d post-surgery but hair loss was successfully induced in four mice. Hair loss was first observed in two female mice 49 and 63 d after surgery. Patchy hair loss first presented on the ventral surface, later progressing to the dorsum similar to progression of spontaneous AA observed in C3H/HeJ mice. It took 70 d before any host hair loss was apparent from male C3H/OuJ mice. Two males developed patchy hair loss by 84 d. Male C3H/OuJ mice that remained unaffected had white hair regeneration within their skin grafts. Histology of C3H/OuJ mice with graft hair regrowth and no hair loss showed no signs of follicular inflammation. Mice with hair loss had a peri- and intra-follicular mononuclear cell infiltrate in both graft and host skin typical of mouse AA.

**Sham graft control mice** Sham grafting in three male and one female immunocompetent C3H/OuJ mice, or four female and two male normal haired adult C3H/HeJ mice, did not induce AA. No sham grafted mice revealed any untoward phenomena. Hair fibers growing from sham autografts were normal, pigmented agouti hair. In contrast, all hair growth in allograft studies was always depigmented. Scarring from surgery was observed with no inflammatory cell infiltrate in the skin. The seven mice that rejected their grafts in the other studies (three C3H/OuJ and four C3H/HeJ mice) might also be interpreted as a form of negative control. No hair loss was observed in any mouse that had previously rejected its skin graft.

**DISCUSSION**

**Immunodeficient mice** Examination of the immunodeficient mouse response to AA-affected skin grafts is important in understanding the roles of lymphocyte activation in AA pathogenesis. Lesional skin biopsies from humans or rats are capable of regrowing hair when transplanted onto immunodeficient animals (Gilhar and Kreuger, 1987; Gilhar et al., 1992, 1998; Oliver, University of Dundee, Dundee, Scotland, personal communication). The grafting of AA-affected C3H/HeJ mouse skin to immunocompromised C3H sid/sid mice resulted in white hair regrowth in the grafted skin. This hair regrowth suggests AA-affected hair follicles (i) are in a dystrophic anagen or telogen state, (ii) are not permanently destroyed, and (iii) may regrow hair. From the results it seems AA is a reversible non-scarring disease in C3H/HeJ mice, although discrete limited scarring can be associated with follicular inflammation in C57BL/6 mice (Eichmüller et al., 1998).
Microscopically, the C3H scid/scid recipient mice response to grafting consisted of a dense infiltration of neutrophils and eosinophils around anagen hair follicles in the C3H scid/scid skin adjacent to the AA-affected graft. There was no overt hair loss but a C3H scid/scid compromised immune system will attempt to respond to the graft activation factor(s) in AA-affected C3H/HeJ mouse skin. Failure to induce actual hair loss in C3H scid/scid mice suggests that an immune system, activated by histocompatible grafts, is incapable of inducing hair loss without the presence of T and/or B lymphocytes and their products. Conversely, hair regrowth in AA-affected grafts onto C3H scid/scid recipients shows normal function in the absence of B and/or T inflammatory cell activity. Lack of hair loss in C3H scid/scid mice suggests an induced viral form of AA is very unlikely in mice. The skin graft technique effectively inoculates recipient mice with any infectious agents that may reside in AA-affected C3H/HeJ mouse skin; however, the studies do not rule out the possibility of an abnormal hair follicle function or local pathogen infection inducing secondary inflammation that leads to overt hair loss. Cytomegalovirus has been suggested in association with AA in humans (Skinner et al., 1995a, b), but C3H/HeJ mice consistently tested negative for murine cytomegalovirus DNA or antibodies against this virus (Sundberg et al., 1994b; McElwee et al., 1998a). More than 30 different mouse strains are housed in close proximity within the same breeding room under the same conditions. Of these, only C3H/HeJ mice spontaneously develop AA. Normal haired C3H/HeJ scid/scid or C3H/HeJ skin when grafted to AA-affected C3H/HeJ mice became infiltrated with mixed, primarily mononuclear inflammatory cells and hair loss developed. Hair loss from normal haired grafts suggests an immune system, exposed to dystrophic hair follicles, is fully capable of responding to anagen hair follicles not previously affected by AA. That the skin grafts rapidly conform to the systemic state of the recipients also infers that the regulation of AA hair loss lies with the immune system as opposed to development of hair follicle abnormalities.

Hair follicles are regarded as an immunologically privileged site (Barker andBillingham, 1977; Westgate et al., 1991). Several hypotheses for alopecia areata onset are based on the loss of hair follicle immune privilege through upregulation of MHC class I and class II expression in normally negative hair bulbs with exposure of previously sequestered antigens to reactive T cells (Paus et al., 1994). In C3H/HeJ mice the purported immune privilege of hair follicles breaks down quickly with little resistance to the pathogenic mechanisms of AA. Overt hair loss developed in normal haired grafts on alopecia recipients within 28 d of surgery.

Immunocompetent mice The spontaneous expression rate of AA is 0.25% for C3H/HeJ mice aged 5 mo, and rises to 20% for mice aged 18 mo (Sundberg et al., 1994b); however, induction of AA by skin grafting suggests 100% of C3H/HeJ mice greater than 70 d old are susceptible to hair loss. It is possible that some humans have similar genetic susceptibility to AA but require an event to trigger hair loss. The requirement for several different factors to collectively contribute to AA has previously been suggested (Paus et al., 1994).

In support of an autoimmune cell-mediated disease, AA-like hair loss has been induced using other mouse models. AA-affected human skin grafted to a Prkdcscid mouse model regrows hair. In vivo stimulated T cells derived from AA-affected skin injected into such grafts on Prkdcscid mutant mice induces hair loss (Gilhar et al., 1998). Lymphocyte-mediated hair loss has also apparently been induced in C57BL6/J mice after immunotherapy for melanoma (Becker et al., 1996). Passive transfer of T cells, specifically CD8+ cells, to naïve mice transferred the alopecia phenotype; however, any suggestion of inflammatory alopaece induction in C57BL6/J mice must be tempered by the knowledge that this strain is predisposed to barbering and alopecia through ulcerative, inflammatory dermatitis (Mizurte and Wecker, 1986; Sundberg et al., 1994a) that is believed to be based on an autoimmune mechanism (Andrews et al., 1994).

Grafts of skin from AA-affected C3H/HeJ mice placed on unaffected C3H/HeJ hosts induced hair loss. The association of hair loss with predominantly lymphocytic inflammation would circumstantially sup-port immune mediation of AA. AA-affected mouse skin may contain factors capable of activating hair loss in immunocompetent hosts by promoting host-derived mononuclear cells and triggering an immune response against hair follicles in the grafted skin and host hair follicles. Most likely activated lymphocytes and/or antigen-presenting cells are transferred with the skin graft and prime naïve host lymphocytes. Failure of AA-affected C3H/HeJ skin grafts to induce AA in C3H scid/scid mice suggests that hair loss in immunocompetent C3H/HeJ, and C3H/OuJ, mice is not due to passive transfer of inflammatory cells and/or autoantibodies within AA-affected skin grafts. If AA was transferred, as opposed to actively induced by full-thickness skin grafts, hair loss would be expected to occur in C3H scid/scid recipients of AA-affected C3H/HeJ skin.

It was also possible to induce AA by grafting AA-affected skin to C3H/HeJ female mice as young as 21 d of age; however, male, and to a lesser degree female, juvenile C3H/HeJ mice were more resistant to development of AA by graft induction. Only 30% of the grafted juvenile mice developed AA. Apparently an immature mouse is less susceptible to activation and mediation of an attack on hair follicles leading to AA. Maturation of the skin, hair cycles, hair structure, and/or immune system may be important in determining susceptibility to AA.

Histocompatible mice It is particularly interesting that skin grafts from AA-affected C3H/HeJ mice had the ability to induce nonscarring, inflammatory hair loss in C3H/OuJ mice. C3H/OuJ mice were derived from C3H/HeJ mice in advance of the apparent onset of the AA phenotype in the C3H/HeJ mouse production colony at The Jackson Laboratory (Sundberg et al., 1994b). C3H/OuJ mice differ in their genetic composition, notably C3H/OuJ mice are lipopolysacchiride susceptible whereas C3H/HeJ mice are lipopolysaccharide resistant (Doolittle et al., 1996) The ability to induce AA in the histocompatible C3H/OuJ strain that is not prone to spontaneous AA-like hair loss suggests that an individual with no apparent inherited history of hair loss can develop AA if given a strong trigger factor. These results suggest that the immune system is the regulatory factor in AA rather than hair follicle defects, although we cannot rule out the possibility that the hair follicles from C3H mice are different from hair follicles in other entirely unrelated strains.

C3H/HeJ mice will continue to be invaluable in the investigation of AA as skin graft induction of AA can be employed in the analysis of disease pathogenesis. Taken together, our studies support the notion that AA hair loss is mediated by the immune system and that AA can be actively induced in an immunocompetent mouse using appropriate stimuli. Affected AA skin from donor females successfully grafted onto normal, haired C3H/HeJ females at 70 d of age yields onset of AA in 100% of recipients. The data also document the age and sex dependence of susceptibility towards AA, where induction of AA in mice less than 31 d old was more variable. The skin graft technique will allow studies on the influence of gender and the nature of the maturation required before an individual is predisposed to development of AA. The consistent induction of AA in adult C3H/HeJ mice provides a rapid, reliable evaluation model to examine the systemic and local pathogenic events taking place from trigger factor to onset of AA hair loss. The technique makes prospective studies possible with even greater consistency and precision than previously possible. The ability to transfer previously induced AA from one C3H/HeJ mouse to another and perpetuate the hair loss will be of considerable practical use in producing large numbers of AA-affected mice for use in treatment development.
REFERENCES
Andrews AG, Dyska RC, Spilman SC, Kunkel RG, Brammer DW, Johnson KJ: Immune complex vasculitis with secondary ulcerative dermatitis in aged C57BL/NSNu Mice. Vitr Pathol 31:293–300, 1994