MEETING REPORT

Third International Research Workshop on Alopecia Areata

Kevin J. McElwee
The Jackson Laboratory, Bar Harbor, Maine, U.S.A.

The Third International Research Workshop on Alopecia Areata was held in Washington DC on November 5, 1998. The conference was organized jointly by the National Alopecia Areata Foundation and the National Institute of Arthritis and Musculoskeletal and Skin Diseases with organizers Vera H. Price, Alan Moshell, Kurt S. Stenn, Madeleine Duvic, Lowell A. Goldsmith, Michael J. Cork, and Rudolf Happle. The Workshop brought together dermatologists, immunologists, geneticists, molecular biologists, biochemists, and pathologists for an open exchange of recent advances in alopecia areata, which is an autoimmune disease that causes hair loss. Over 250 individuals from 21 countries in Europe, North and South America, Asia, and the Middle East attended the 1 day meeting. This was a significant increase in participation over the Second International Research Workshop on Alopecia Areata held in 1994. Fourteen speakers gave seminars, and 33 posters were available for review on wide-ranging aspects of hair biology and alopecia areata. The format of the Workshop stimulated enthusiastic interaction and discussion among all the participants who represented academia, government, and the pharmaceutical industry.

ANIMAL MODELS FOR ALOPECIA AREATA

The Smyth chicken model, derived from the SL strain, was identified as a new animal model for alopecia areata (AA). Normally pigmented feathers gradually lose color with age in association with non-scarring inflammation similar to vitiligo. It was noted that some chickens with vitiligo also develop feather loss. The loss is patchy and may expand over time and become universal in some chickens. This new model may help in identifying AA susceptibility genes and may suggest a link between vitiligo, melanogenesis, and development of AA.

The C3H/HeJ mouse model for AA was discovered shortly after the First International Research Workshop on AA. Up to 20% of C3H/HeJ mice spontaneously develop patchy non-scarring inflammatory alopecia (Sundberg et al., 1994). AA-like hair loss has now been discovered in other mouse strains including A/J, CBA/CaHN-B6½/J, BALB/2R-H2h2/Lil, and HRS/J heterozygotes. Different animal models may represent different forms of human AA and analysis of these mouse strains may identify AA susceptibility genes common or unique to each. This information could illuminate the themes common to all forms of AA and help delineate subcategories.

Whereas significant advances were made in defining the nature of animal models with spontaneous AA, induced AA mouse models were also demonstrated. Skin grafts from affected C3H/HeJ mice could be given to normal haired recipients who then developed AA 8–10 weeks after grafting. Investigators also noted that AA could be induced by taking skin-draining lymph node cells from AA affected mice and transferring them to normal haired recipients. Again, hair loss developed 8–10 weeks after the procedure indicating inflammatory cells are capable of inducing hair loss even though hair follicles are potentially immune-privileged sites. This consistent model will be useful to examine what happens in the system before overt hair loss occurs. The ability to induce AA in a model also suggests that whereas individuals may be genetically predisposed towards AA, just having susceptibility genes does not guarantee that the individual will develop the disease. This AA induction technique can also be used to produce large numbers of mice for AA treatment evaluation.

The importance of inflammatory cells in AA has previously been demonstrated using a nude mouse model (Gilhar and Kreuger, 1987, 1992, 1998). Human AA-affected skin grafted to nude mice regrew hair indicating a systemic factor blocks hair growth rather than localized dysfunction of hair follicles. Inflammatory cells taken from AA-affected patients, isolated into different cell types and injected into the grafts of now regrown hair were shown to promote renewed hair loss. CD8+ cytotoxic T cells were particularly potent in their direct action on the hair follicles. These animal model studies provide the first indirect evidence of inflammatory cell mediation of AA and suggest that hair follicle specific auto-antibodies (McElwee et al., 1996; Tobin et al., 1997a) may not be fundamentally required for AA development.

In organ-specific autoimmune diseases the inflammatory response is directed to just a few antigenic epitopes and in turn only those T lymphocytes specific for these antigens are directly involved in disease pathogenesis. Epitope spreading may lead to a broader spectrum of target antigens involved in chronic disease perpetuation (Chan et al., 1998). Lymphocyte cells from C3H/HeJ mice with AA were screened and T cell clones expressing a Vß8.2/Jß2.5 T cell receptor arrangement predominated. The receptor arrangement of these cell clones may be characterized further and this may help to identify the nature of targeted antigen(s) during initial onset and later in chronic lesions.

The collected work of several research groups on cell-mediated activity in AA may eventually lead to new treatments directed against specific pathogenic inflammatory cell subsets. Clearly, animal models will provide an important tool in the investigation of genetic and environmental activation factors, disease pathogenesis, and development of new and improved treatment protocols.

GENETICS OF AA

A preliminary study using C3H/HeJ mice examined potential chromosome locations that may contain genes involved in AA and/or in high immunoglobulin production. Three gene loci were identified one of which was common to both high immunoglobulin concentration and AA susceptibility. A region of mouse chromo-
some 6 may contain one or more genes involved in inflammatory events associated with AA. Intriguingly, a separate investigation looking at human AA independently identified the equivalent chromosome region (2p12–13) as being a location for AA susceptibility gene(s). Investigators indicated IL-1 receptor gene polymorphism was involved (Cork et al., 1996). Candidate genes in this chromosome region are being reviewed for their potential involvement in both human and murine AA.

The association of specific human leukocyte antigens (HLA) with autoimmune disease may involve molecular and structural features of the HLA peptide-binding site and/or a general predisposition to overexpression of HLA antigens on target tissue and antigen-presenting cells (Hang and Nakamura, 1997). Two separate investigations independently confirmed the presence of specific HLA antigens in association with AA. The broad HLA antigen DQ3 was identified as a general susceptibility HLA antigen for AA. HLA alleles DQB1*0301, DRB1*0401, and DRB1*1104 were found in highly significantly increased frequency in people with alopecia totalis/aloppecia universalis. DRB1*1104 was also found with significantly increased frequency in those with patchy AA but the other two alleles were not. The investigators suggested that amino acid sequencing of the antigen binding grooves of these HLA antigens may indicate the structure and identity of the elusive AA target antigens. Investigators also suggested DRB3*52a may confer resistance to AA.

Clearly, AA is a polygenic susceptibility based disease involving complex gene interaction and environmental modification. Understanding the genetics behind AA will help explain the disease activation process, may identify susceptible subgroups in the general population, and in the long term may eventually lead to a treatment involving gene therapy.

HAIR FOLLICLE FUNCTION

Immune privilege may play an important part in AA (Paus et al., 1993; Paus, 1997). Some research groups are trying to understand transient immune privilege of the hair follicle and how this protection might break down in AA. Researchers have noted that major histocompatibility complex (MHC) class I and class II antigens are not expressed in normal hair follicles (Paus et al., 1993; Paus, 1997). They have also shown that immunosuppressants such as α-melanocyte-stimulating hormone, adrenocorticotropic hormone, and transforming growth factor β are produced locally in hair follicles and this may further bolster hair follicle immune privilege. The investigators hypothesize there might be upregulation of MHC antigens and/or downregulation of locally produced immunosuppressants and this may allow the immune system to recognize hair follicle antigens leading to onset of AA. Further work on natural immunosuppressants is in progress.

Key genes involved in hair growth and cycling are of increasing interest and will help us understand how hair follicles function and why they may be disrupted in various hair loss conditions. Investigators have identified several families from Pakistan and Ireland with the hairless gene trait equivalent of the hairless IRS/3 mouse (Ahmad et al., 1998). Investigators confirmed that the human hairless gene is associated with a disease now called generalized atrichia and/or atrichia with papules, and erroneously described in previous reports as alopecia universalis congenita. People with the hairless gene are born with hair which is then permanently lost during the first 3 mo of life. It was shown that people with a hairless gene had massive and premature apoptosis in the hair matrix cells suggesting that hair cycling into catagen is totally and irreversibly disrupted. Most intriguingly, the investigators have begun to screen people previously diagnosed as having inflammatory alopecia universalis, who permanently lost all scalp and body hair within the first 3 mo of life, and have found a very small number who apparently have a variation of the hairless gene defect. This is a reminder of the potential for misdiagnosis of AA and emphasizes the need for a simple diagnostic test.

We know that Hox genes are important in defining the position, density, and development of hair follicles in an embryo as well as being involved in growth of the limbs, eyes, and nails. Hoxc13 seems to be involved in controlling several gene products most notably hair keratins. Transgenic mice targeted for Hoxc13 deficiency were found unable to synthesize correctly hair keratins. Clinically, the mice had sparse, brittle hair, brittle tongue papillae, and misshaped, overgrown nails. Identifying genes involved in normal hair follicle function and those important in AA susceptibility will provide an understanding of the potential mechanisms involved in hair disease.

TREATMENTS

Clinical dermatologists at the Second International Research Workshop on AA in 1994 were asked to complete a questionnaire about how they treat AA. In total 38 different treatments including combination treatments were in use 4 years ago. The most common treatment was use of intralesional corticosteroids for adults and topical corticosteroids for children. Extensive AA was most frequently treated with a contact sensitizing agent such as: diphenyl-cyclopentenone, squaric acid dibutyl ester, and dinitrochlorobenzene. The therapy protocols varied markedly in composition, concentration, and delivery, indicating personal preference of the dermatologist plays a significant part in how AA is treated. Studies to identify superior, new treatments are urgently required.

Animal models are now used in research for new and improved treatments (McElwee et al., 1997). The drug Leflunomide is an immunosuppressant used to treat rheumatoid arthritis that acts to block IL-2 cytokine activity. The drug was given orally to Dundee Experimental Bald Rats and was found to promote limited hair growth. Some rats responded much better than others and whereas some had good hair regrowth others responded with little or no regrowth. Leflunomide may not be a superior therapy for AA but the project demonstrates the potential for animal models in rapid treatment evaluation. Rodent models were also subjected to treatment with the topical contact sensitizers diphenylcyclopentenone and squaric acid dibutyl ester yielding a variable hair regrowth response similar to that observed in humans. CD44v10 is involved in activating CD4+ and CD8+ cells to migrate into tissue and attack antigenic targets. Normal C3H/HeJ mice grafted with AA affected skin to induce AA in the recipient were given an anti-CD44v10 specific monoclonal antibody shortly after surgery. It was shown that induction of AA could be blocked using this monoclonal antibody. Use of CD44v10 in humans may not be practical but the experiment points towards CD4+ and/or CD8+ cells being the key pathogenic cell subsets in AA.

THE FUTURE OF AA

Research has progressed considerably in the 4 years since the Second International Research Workshop on AA. We have now drawn the conclusion that while auto-antibodies may play an important part in disease, they are probably not the fundamental cause of AA. Investigations are much more focused and directed towards the understanding of AA pathogenesis and demonstration of AA as an autoimmune, cell-mediated disease. Using animal models, investigators are honing in on inflammatory cells as promoters of hair loss. The CD8+ T cell type in particular is receiving attention as the candidate key pathogenic cells with CD4+ cells in their classic helper/supporter role. Genetics research has now begun in earnest. New chromosome locations beyond the HLA region have been suggested as potential areas harboring AA susceptibility genes. Genetic research may ultimately explain why, how, and who develops AA.

Hair follicle function, immune system activity, and genetic susceptibility in AA are all receiving interest from laboratory investigators. One clearly neglected area of research, however, is defining the disease induction process and activation factors involved, particularly contributions from the environment. The
confounding variables that may influence onset, severity, and duration of AA must be systematically examined. Most investigators now realize that AA is not a homogeneous disease. Different forms of AA may indicate different genetic and environmental factor contributions. Different presentations may also indicate future prognoses and response to treatment.

The need for universally accepted criteria to select subjects for clinical and basic laboratory studies of AA was recognized after the 1994 Workshop. As a result the National Alopecia Areata Foundation sponsored an AA consensus meeting in Brussels in 1995 to establish investigational guidelines to facilitate collaborations, comparison of data, and sharing of patient-derived tissue. A central repository for clinical data and a DNA/tissue bank for patients with AA accessible to all investigators will be established. The AA investigation guidelines were presented at the Third International Research Workshop and are being published in the *Journal of the American Academy of Dermatology*.

To help guide future AA research the Workshop included keynote speakers Kai Wucherpfennig and Edward Wakeland reviewing the latest developments in their respective fields of molecular mimicry in T cell mediated autoimmunity, and genetic dissection of autoimmune pathogenesis in murine lupus nephritis. Investigators involved in AA research will need to monitor such relevant and well-funded fields, learn from their mistakes, and adapt research protocols to accelerate our understanding of AA.

In 4 years time, the Fourth International Research Workshop on AA may reveal a hypothetical framework for the induction and progression of AA with essential supporting evidence in place including: identification of the antigenic epitope(s) targeted by inflammatory cells in AA, identification of pathogenic T cell subsets, and the ability to induce AA in models by transfer of lymphocyte subsets, antibodies, and/or antigen epitopes. Pathogenic cell groups may be characterized for their receptors and process of activation. Multiple susceptibility and severity modifying gene loci may be identified if not the actual gene functions involved in AA. Although few new treatments were reported at this Workshop, the development of animal models with the potential for screening new therapies has gained the attention of several academic centers and pharmaceutical companies. Future Workshops will reveal new treatments with improved success rates and fewer side-effects. Understanding disease pathogenesis will reveal key links in the process that can be targeted with new, specific interventions.

The author wishes to thank all the Workshop delegates whose research contributed to this Conference Report. The author is supported by a Glaxo Dermatology/Dermatology Foundation research fellowship. The Third International Research Workshop on Alopecia Areata was sponsored by the National Alopecia Areata Foundation and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR45704-01). Generous support for the Workshop was gratefully received from Sandy Fruym, Debbie and Rich Harris, Lawrence and Sandra Small, Malbert and Alisa Smith, Merck U.S. Human Health, Pharmacia and Upjohn, Pocter & Gamble Europe, Pocter & Gamble USA, Califderma Laboratories, Novartis Pharmaceuticals, Ortho Pharmaceuticals, and Westwood Squibb.

REFERENCES


