

FOURTH INTERNATIONAL RESEARCH WORKSHOP ON ALOPECIA AREATA

Poster Abstracts: POSSIBLE CAUSES

Poster #1

WNT signals are required for the initiation of hair follicle development and for postnatal hair growth. Thomas Andl¹, Seshamma Reddy¹, Adam Glick² and Sarah E. Millar¹, ¹Departments of Dermatology and Cellular and Developmental Biology, University of Pennsylvania, Philadelphia, PA and ²Laboratory of Cellular Carcinogenesis and Tumor Promotion, N. C. I., Bethesda, MD.

Mutations in downstream effector genes of a conserved WNT signaling pathway cause hair follicle phenotypes, suggesting that WNT proteins control hair follicle development and postnatal hair follicle cycling. To begin to test this hypothesis we surveyed for *Wnt* and WNT receptor (*Frizzled*) gene expression in developing and postnatal mouse skin. We discovered that several *Wnt* and *Frizzled* genes are expressed in developing hair follicles, in specific cell layers of postnatal anagen follicles, and in the dermal papilla and adjacent epithelial cells of hair follicles at anagen onset. To investigate the effects of blocking the actions of WNT proteins in the skin and hair we made use of Dickkopf 1 (DKK1), a potent secreted WNT inhibitor. Transgenic embryos expressing high levels of *Dkk1* in basal epidermal cells under the control of a keratin 14 promoter displayed a complete absence of hair follicle, tooth and mammary gland development. Molecular markers of hair follicle placodes, including the very early markers Ectodysplasin receptor and *Lefty*, failed to show normal patterned expression in transgenic skin, indicating that WNT signals are required for the localized expression of placode markers and for initiation of hair follicle development. To determine whether WNT signals are also necessary for postnatal hair follicle cycling we developed a bi-transgenic system in which expression of *Dkk1* in the epidermis and hair follicle outer root sheath can be induced by oral doxycycline. Doxycycline treatment of embryos throughout gestation caused phenotypes identical to those resulting from constitutive expression of *Dkk1*. In contrast, single transgenic and untreated bi-transgenic pups were normal. Administration of doxycycline to previously untreated bi-transgenic postnatal mice prevented hair growth. WNT signals are therefore necessary for both hair follicle formation and postnatal hair growth.

Poster #2

Decreased serum ferritin is associated with alopecia areata in women. Jonathan Kantor¹, Lisa Jay Kessler², David Brooks², George Cotarelis³. Department of Biostatistics and Epidemiology¹, Division of Medical Genetics² and Department of Dermatology³ University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Our aim was to evaluate whether alopecia areata and alopecia areata totalis/universalis are associated with decreased tissue iron stores (as measured by serum ferritin). Cases were obtained by selecting 18 consecutive patients over the age of 18 treated at the University of Pennsylvania hair and scalp clinic in the years 2000 and 2001. Normal patients were obtained by sampling individuals screened negative in the Hospital of the University of Pennsylvania Division of Medical Genetics for the HFE-1 genotype for hemochromatosis. Using the t-test, we found that the mean ferritin level (stated as ng/ml [95% confidence intervals]) in cases with alopecia areata (25 [17, 33]) was statistically significantly lower than levels in normal controls (61 [38, 84]), with a p-value of 0.0036. The mean ferritin level for patients with alopecia areata totalis/universalis (n = 7) was 54 ([25, 94]) and not significantly different than the normals (p = 0.32). Hemoglobin levels for patients with alopecia areata (13.0 [12.2, 13.7]) were also statistically significantly lower than normals (14 [13.4, 14.6]) with a p-value of 0.012, whereas hemoglobin levels in patients with alopecia areata totalis/universalis were not statistically different than normals (p-value = 0.12). To address the question of whether the alopecia patients were significantly younger than the normals, we performed a one-sided t-test assuming unequal variances which resulted in a p-value of 0.292, suggesting that any differences in ferritin levels between cases and controls were not secondary to a younger patient population. This is the first study to utilize a case-control methodology to address the relationship between iron levels and alopecia areata and alopecia areata totalis/universalis. Our findings suggest that low iron levels may lower the threshold for developing alopecia areata. Future studies to determine whether iron replacement therapy alters the course of alopecia areata will be necessary to evaluate its therapeutic potential.

Poster #3

Interleukin-10 knockout mice are relatively resistant to the experimental induction of alopecia areata. Pia Freyschmidt-Paul¹, Kevin McElwee¹, Margot Zöller¹, John Sundberg², Rudolf Happle¹, Rolf Hoffmann¹, Philipp University Marburg, Germany; DKFZ, Germany¹; The Jackson Laboratory, Bar Harbor, USA.²

Alopecia areata (AA) occurs spontaneously in C3H/HeJ mice, but it can be induced in unaffected C3H/HeJ mice or other histocompatible strains by grafting of AA-affected mouse skin. Because IL-10 expression is increased in scalp from AA-patients, successfully treated with DCP, we wanted to determine whether the absence of IL-10 results in increased susceptibility to the development of AA. For this purpose we tried to induce AA in 20 C3H/HeJ-Bir-IL10^{-/-} mice by skin grafting and as controls we grafted AA-affected skin onto 20 unaffected C3H/HeJ mice. FACS-analysis for a wide panel of cytokines and surface markers was performed on skin-infiltrating leucocytes and cells from skin draining lymph nodes. 13/20 IL-10^{-/-} mice did not develop hair loss, 7/20 IL-10^{-/-} mice developed limited AA, but no mice developed extensive AA. In contrast, 19/20 controls developed AA. FACS-analysis revealed elevated levels of dendritic cells and monocytes in the skin and of dendritic cells in lymph nodes of IL-10^{-/-} mice compared to controls, but a similar level of monocytes in lymph nodes of both groups. IL-10^{-/-} mice had increased levels of IFN- γ and TNF- α in lymph nodes and decreased levels of IL-6 in skin. CD25, CD28, CD40, CD80 and CD86 were increased in skin of IL-10^{-/-} mice and CD44s, CD44v3, CD44v6, CD44v7 and CD44v10 were increased in both, skin and lymph nodes of IL-10^{-/-} mice. In a second part of the study, skin from IL-10^{-/-} mice and from C3H/HeJ mice without AA was grafted onto C3H/HeJ mice with extensive AA. AA developed in skin grafts from normal C3H/HeJ mice, but not in skin grafts from IL-10^{-/-} mice. Contrary to expectations we have found that IL-10 deficient mice are relatively resistant to the induction of AA, despite their elevated levels of proinflammatory cytokines and adhesion molecules. Whether the decreased levels of IL-6 could be responsible for the resistance to AA induction requires further investigation.

Poster #4

A polymorphism of the autoimmune regulator (AIRE) gene with potential functional significance is strongly associated with alopecia universalis. Rachid Tazi-Ahmini, Michael J. Cork, David J. Gawkrodger, Dave Wengraf, Andrew G. Messenger, Andrew J.G. McDonagh, Dermatology Immunogenetics Group, Division of Genomic Medicine, University of Sheffield, UK

Alopecia areata (AA) is a chronic inflammatory disease characterised by patchy hair loss with T cell infiltration of hair follicles. The lifetime risk of AA is approximately 1% in the general population, but this is increased to 30% or more in the autoimmune polyendocrinopathy candidiasis ectodermal dysplasia syndrome (APECED), a recessive disorder due to mutation of the autoimmune regulator (AIRE) gene on chromosome 21q22.3. We have therefore studied the possible involvement of AIRE in the pathogenesis of AA. On screening the AIRE coding sequence, we identified 22 variants. Two variants at positions G961C and T1029C gave amino acid changes S278R and V301A located in the DNA-binding segment (SAND) and PHD1 zinc finger motif respectively. We have developed genotyping assays for both G961C and T1029C variants. The AIRE T1029C variant was not informative and the frequency of the rare allele (961C) of the AIRE G961C variant was 0.08 in our control group. We developed differential double enzyme assays to discriminate the AIRE G961C alleles and genotyped 202 AA patients and 175 matched Caucasian controls. There was a significant increase in the frequency of the 961C allele in AA patients overall to 0.13. This increase was higher in severe disease (alopecia universalis) 0.20. We found no association between the AIRE G961C variant and mild (patchy) AA or alopecia totalis. However, the AIRE 961C allele presents a strong risk factor (more than three times) for development of the most severe form of AA [(OR) = 3.27 (1.56,6.88) p = 0.0012]. This allele is also a risk factor for disease of early age at onset (<30 yrs) [OR 2.22 (1.22,4.03) p = 0.0082]. The amino acid change from serine to arginine in the SAND domain of AIRE may have a significant effect on AIRE DNA-binding activity. Moreover, our results could provide a rational explanation of the unusually high frequency of AA in APECED patients which also supports the concept of AA as an autoimmune disease.

Poster #5

Thymosin β 4 promotes wound repair and hair regeneration via an actin binding sequence. D Philp, M Elkin, and HK Kleinman, Craniofacial Developmental Biology and Regeneration Branch, National Institutes of Dental and Craniofacial Research, NIH, Bethesda, MD, USA

Thymosin β 4 (T β 4) is a 43-amino acid polypeptide that is an important mediator of cell migration and differentiation. It promotes *in vitro* and *in vivo* angiogenesis and accelerates dermal wound healing in animal models. Using synthetic peptide fragments we found that the 7-amino acid peptide containing the actin-binding motif (T-3) of T β 4 polypeptide is essential for its angiogenic activity. Migration assays with human umbilical vein endothelial cell (HUVEC) primary cultures and vessel sprouting assays using chick aortic arches conclusively show that T β 4 and the T-3 display near identical activity in the nanogram range. While studying accelerated dermal wound healing by T β 4 or T-3, we found that they also stimulate hair growth in rat and mouse models. Preliminary studies show that HUVECs and human foreskin fibroblasts (HFFs) display increase in MMP-1, -2, and -9 expression when exposed to nanogram quantities of T β 4. Immunohistochemical staining showed increased levels of MMP-9 in T β 4-treated wounds. These results demonstrate that the actin-binding motif of T β 4 is an essential site for angiogenic and hair regeneration activity. It is likely that the wound healing activity of T β 4, in part, resides in its ability to increase protease activity. These studies provide insights into the mechanism of T β 4 activity in wound repair.

Poster #6

The histopathology of alopecia areata: a new look. D. A. Whiting-Baylor Hair Research and Treatment

Purpose: Establish the evolving histopathology of alopecia areata, using follicular counts to relate biopsy findings to the stage at which the patient presents. **Methods:** 4 mm punch biopsies were taken from a scalp area of active, recent or old hair loss, or recent regrowth, in 50 consecutive new patients. In horizontal sections, terminal anagen, catagen and telogen hairs, vellus or miniaturized hairs, follicular units and follicular stela (streamers) were counted; inflammation and fibrosis were rated as nil, mild, moderate or dense. Anagen and telogen percentages and terminal to vellus hair ratios were calculated. The duration of the current episode and total duration of alopecia areata were recorded. The follicular counts and inflammation ratings were correlated with patient sex, age, race, type of alopecia areata, percentage hair loss, current duration, total duration and regression during current episode. Follicular counts and inflammation ratings from normal controls and patients with diffuse alopecia were available for comparison. **Results:** A discernable pattern of the evolving histopathology was found only by relating the biopsy findings to the duration of the current attack of alopecia areata; this provided compelling data regarding the extent, site and timing of peribulbar lymphocytic inflammation and the changing anagen and telogen percentages and terminal to vellus ratios. These findings are summarized according to the stage of the disease: **Acute stage:** Bulbar lymphocytes involve terminal hairs in early episodes and miniaturized hairs in repeated episodes. **Subacute stage:** Decreased anagen and increased catagen and telogen hairs are characteristic. **Chronic stage:** Decreased terminal and increased miniaturized hairs are found with less inflammation. **Recovery:** Increasing terminal anagen hairs from regrowth of miniaturized hairs and a lack of inflammation are noted. **Conclusion:** Alopecia areata should be suspected when high percentages of telogen hairs and/or miniaturized hairs are present, even in the absence of a peribulbar, lymphocytic infiltrate.

FOURTH INTERNATIONAL RESEARCH WORKSHOP ON ALOPECIA AREATA

Poster Abstracts: GENETICS

Poster #7

The association of major histocompatibility related gene-A (MICA) and familial alopecia areata. Nazila Barahmani, Madeleine Duvic, and Mariza de Andrade. Department of Dermatology, University of Texas M.D. Anderson Cancer Center, Houston, TX and Department of Health Sciences Research, Mayo Clinic, Rochester, MN.

A major histocompatibility locus (MHC) on Chromosome 6p21 encodes human leukocyte antigens (HLA) and is a major genetic susceptibility locus for most of autoimmune diseases. MICA is one of the members of the new MIC (MHC class I chain-related) family of genes located 46.6 kb centromeric to HLA-B. MICA is expressed on epithelial cells in the intestine and skin, monocytes, fibroblasts, and endothelial cells. Case-control studies in different ethnic groups have shown associations between HLA class I and II with Alopecia Areata (AA) patients. Our previous studies also showed a strong association between HLA class II antigens (DQB*302 and DRB4) and AA in multiplex AA families. MICA is known to be associated with other autoimmune diseases such as psoriasis, rheumatoid arthritis and Behcet's syndrome. To further explore the association between HLA class I and AA families, ten large multiplex AA families were studied for associated with MICA. The transmission disequilibrium test (TDT) was applied after using polymerase chain reaction on genomic DNA samples to distinguish between 5 known alleles. MICA was significantly associated with AA ($p=0.012$) and the MICA*3 allele was also associated ($p=0.035$). We are validating these results by genotyping 80 multiplex families and by haplotyping 10 families. **Key words:** MICA (MHC class I chain related gene A)/hair follicles/association tests/Major histocompatibility complex.

Poster #8

Do mutations in lysyl oxidase-like (LOXL) gene cause the rough coat (rc) phenotypes in mice? Tongyu Cao, Claude Jourdan-Le Saux, Howard Passmore, Kimiko Hayashi, Masando Hayashi, Ben Fogelgren, and Katalin Csizsar Pacific Biomedical Research Center, University of Hawaii at Manoa, Honolulu, Hawaii, U.S.A.

The rough coat (π) mutant mice were first described at The Jackson Laboratory in 1966 as a spontaneous mutation. The π mutation was inherited in a recessive mode, and homozygotes of both sexes were fertile. Affected mice were born with no apparent abnormalities, but showed unkempt looking coats by weaning age. The π mutation was later assigned to chromosome 9, yet the gene mutation for the π phenotypes are yet to be identified. One gene that has been assigned to the same region on mouse chromosome 9 is lysyl oxidase-like protein (LOXL). LOXL was first identified through sequence homology to lysyl oxidase, a copper-binding amine oxidase that is required for the extracellular catalysis of lysine-derived cross-links in fibrillar collagens and elastin. The LOXL gene shares extensive homology with lysyl oxidase in the catalytic and copper-binding domains; however, the amino-terminal half of LOXL peptide is quite distinct from lysyl oxidase. LOXL expression was detected at high abundance in the skin, yet the exact functions of LOXL during development and cellular differentiation of the skin are not clear. Based on the proximity of LOXL and π loci on mouse chromosome 9 and high LOXL expression level in the skin, we hypothesized that LOXL and π might be allelic. In this study, we carried out linkage analysis of LOXL and π , and examined LOXL sequence and expression in π mice to determine whether mutations in LOXL caused the π phenotypes.

Poster #9

Characterization of epithelial stem cells isolated from K15/eGFP transgenic mice using cell sorting. Yaping, Liu¹; Rebecca, Morris²; Zaixin, Yang¹; Carol, Trempus³; George, Cotsarelis¹, Department of Dermatology¹ University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; Department of Dermatology, Columbia University, New York²; Environmental Carcinogenesis and Mutagenesis, National Institute of Environmental Health Sciences

Kinetic and lineage studies have shown that epithelial stem cells in the hair follicle bulge differentiate into epidermis, sebaceous gland and hair follicle. Therefore, the isolation and manipulation of bulge cells could lead to a better understanding of cellular differentiation and determination in the cutaneous epithelium, and provide a source of stem cells for gene therapy and reconstitution of skin and its appendages. We previously demonstrated that keratinocytes in the stem cell rich bulge area of the hair follicle preferentially express cytokeratin 15 (K15) in both mouse and human skin. We then isolated the K15 promoter and demonstrated its utility for preferentially targeting putative epithelial stem cells in K15/lacZ transgenic mice. Here we describe the generation of transgenic mice expressing enhanced green fluorescent protein (eGFP) under the control of the K15 promoter. Using FACS, we isolated the brightest eGFP-positive cells from the skin of mice from 3 independent K15/eGFP lines. The isolated cells constituted approximately 7.5% of the total live epithelial cell population. We show that these cells have properties of epithelial stem cells. Firstly, they produce keratinocyte colonies in vitro that are larger and more numerous when compared to non-eGFP expressing basal (α -6 integrin-positive) keratinocytes. Secondly, they are predominantly in the G₀/G₁ stage of the cell cycle with only 0.175% of cells in S-phase, compared to 2.2% of α -6 integrin positive/K15-negative cells ($p < 0.01$). Lastly, K15/eGFP-positive cells also express CD34, a marker of hematopoietic progenitor cells that recently was discovered in hair follicle bulge cells. These data support the concept that cytokeratins are expressed in a differentiation-specific manner, and that K15 is a marker of cutaneous epithelial stem cells. The K15/eGFP transgenic mouse provides a valuable tool for studying epithelial stem cell biology both in vitro and in vivo.

Poster #10

Human gene expression profiling by microarray delineates disease specific signatures in alopecia areata. K Luettich, H King, J Xiang, J Baumgarten, AA Sinha. Department of Dermatology, Weill Medical College of Cornell University, NY, NY

High density oligonucleotide microarrays, so called "gene chips", allow for simultaneous monitoring of vast numbers of known and/or unknown sequences. We have undertaken genome wide documentation of gene expression by microarray to further study the genetic basis of alopecia areata (AA). There is clear evidence for a genetic predisposition to AA and there is increasing support for autoimmune activation. However, the exact mechanisms of disease initiation and progression are not well understood. Although expression of a number of genes has been reported as altered in affected scalp, a broad perspective on the gene expression abnormalities in AA has thus far been unavailable. Total RNA was prepared from lesional and site-matched non-lesional scalp biopsy samples from 9 patients with patchy, persistent AA. The RNA was then reverse transcribed and labeled using biotinylated nucleotides, followed by hybridization to Affymetrix Human Genome U95A Arrays (>12,500 sequences). We report 173 differentially expressed genes between lesional and non-lesional samples: 84 up- and 89 down-regulated sequences. Hierarchical cluster analysis clearly distinguishes lesional from non-lesional scalp. Non-hierarchical K-means clustering identifies 9 further sub-patterns of expression. We are currently comparing differentially expressed genes in AA with other genetically based autoimmune skin diseases such as psoriasis and vitiligo.

Poster #11

Differential expression of Rho signaling molecules in the stem cell compartment of human hair follicles. Q Tao, G Cotsarelis, and S Lyle. Dept. of Pathology, Beth Israel Deaconess Med. Ctr., Boston, MA and Dept. of Dermatology, Univ. of Pennsylvania, Philadelphia, PA.

We previously showed that human hair follicle stem cells reside in the hair follicle bulge, which is delineated by keratin 15 expression. The purpose of this study was to identify genes which are specifically expressed in the stem cell compartment of the human hair follicle so that we may better understand the molecular requirements for the maintenance of the stem cell phenotype. Hair follicles were plucked from Dispace-treated human scalp skin. Total RNA from telogen hair follicle bulges and from anagen bulbs was labeled and hybridized to cDNA expression arrays (Clontech). Two members of the Rho signaling pathway, RhoA and NET1 (a RhoA-specific guanine exchange factor), were present in the stem cell-enriched telogen follicle and not in the anagen bulb. Immunostaining shows that RhoA is co-expressed in the K15 bulge area of human hair follicles at all phases of the hair cycle, however, NET1 appears to be restricted to the bulge area at the onset of anagen. In addition, RhoA and NET1 co-localized in clonogenic keratinocytes, cultured from telogen follicles. Therefore, RhoA appears to be differentially expressed in the keratinocyte stem cells of the human hair follicle, while NET1 is differentially and temporally expressed in the stem cell region at anagen onset when the stem cells are activated to proliferate. Rho GTPases are small protein switches which mediate a variety of essential cellular responses such as gene transcription, cell-cycle progression, cell adhesion and actin reorganization. Future characterization of RhoA signaling within hair follicle stem cells should lead a better understanding of the signaling pathways involved in stem cell maintenance.

Poster #12

LIG-1 is expressed in keratinocyte stem cells. A. Tanemura, K. Ozawa, and S. Itami Department of Dermatology, Course of Molecular Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan

Despite the central role of keratinocyte stem cells in tissue homeostasis, wound healing, and cancers, the precise in vivo localization of the cells is an unsettled issue because of the lack of appropriate molecular markers. LIG-1 is a transmembrane glycoprotein of which the extracellular region is uniquely organized with leucine-rich repeats and immunoglobulin-like domains. We have reported that targeted disruption of LIG-1 gene resulted in psoriasisiform epidermal hyperplasia. LIG-1 was expressed in basal cells of the epidermis and outer root sheath cells of hair follicles in mice. The LIG-1 expression was apparently decreased in the psoriatic lesion, suggesting that LIG-1 inversely correlates with proliferative ability of epidermal keratinocyte. (FEBS Letters 521: 67-71, 2002). In the present study, we further examined the expression of LIG-1 in mice and human keratinocytes in vitro. Rapidly adherent keratinocytes (RACs) isolated from epidermis and hair follicles strongly expressed LIG-1 mRNA. All of the RACs expressed β 1 integrins, and a subpopulation of the cells showed strong LIG-1 expression on their cell surface. About 50% of the sorted cells with LIG-1 antibodies were label-retaining cells in mice skin. LIG-1 would be a new cell-surface marker for keratinocyte stem cells.

FOURTH INTERNATIONAL RESEARCH WORKSHOP ON ALOPECIA AREATA

Poster Abstracts: GENETICS & TREATMENT

Poster #13

Onset of alopecia areata in C3H/HeJ mice involves transient, high expression of CD44 variant isoforms and low CD4⁺/CD25⁺ suppressor cell activity. K.J. McElwee, M. Zöllner*, P. Engel*, and R. Hoffmann. Dept. of Dermatology, Philipp University, Marburg, Germany. *Dept. of Tumor Progression and Tumor Defense, German Cancer Research Center, Heidelberg.

Alopecia areata (AA), a suspected autoimmune disease of anagen stage hair follicles, AA can be induced in normal haired C3H/HeJ mice by grafting AA-affected skin from littermates. Standard CD44s and CD44 isoforms are adhesion molecules required for leukocyte extravasation during inflammatory processes. CD4⁺/CD25⁺ cells are known to be important for T cell homeostasis. By flow cytometry, we examined skin and lymph node expression levels of cytokines, CD44 variant isoforms, CD4, CD25, and other cell surface molecules, at 2, 6, and 12 weeks post grafting as compared to sham grafted mice, normal haired mice and chronic AA affected mice. Leukocytes in the skin of mice 2 weeks after transplanting AA affected skin highly expressed CD44v3, CD44v6, CD44v7 and CD44v10 as compared to sham grafted control mice. By 12 weeks, expression of CD44 isoforms had returned to normal. With chronic AA expression, CD44s and CD44v6 were slightly elevated, but expression of CD44v3 was reduced. CD4⁺/CD25⁺ cells were present in significantly reduced numbers in mice grafted with AA-affected skin both at 2 weeks after grafting and in chronic AA-affected mice as compared to controls. In association, CD40 and CTLA-4 expression was reduced. Expression of cytokines IL-2, IL-4, IL-6, IL-10 and IL-12 was elevated in mice receiving AA affected skin at all times whereas sham grafted mice exhibited only transient increases post surgery. Taken together, expression of CD44 variant isoforms appears most important for the migration of leukocytes during the initial onset of AA, but is less significant in maintenance of the disease state that is characterized by high cytokine production levels and an increased number of CD4⁺ and CD8⁺ cells. The low level of CD4⁺/CD25⁺ cells, CD40 and CTLA-4 in the skin of AA-affected mice are in support of AA as an autoimmune disease on the basis of an altered regulation of immune cell homeostasis.

FOURTH INTERNATIONAL RESEARCH WORKSHOP ON ALOPECIA AREATA

Poster Abstracts: TREATMENT

Poster #15

Stimulation of CD8 cell apoptosis through p75 kD neurotrophin receptor as a new approach for alopecia areata treatment. A.A. Sharov¹, T.N. Palkina^{1,2}, M. Yaar¹, P. Freyschmidt-Paul¹, W. Syska¹, A.Y. Baryshnikov³, R. Hoffmann¹, M. Maurer¹, J.P. Sundberg², B.A. Gilchrist¹, and V.A. Botchkarev¹. Depts. of Dermatology, ¹Boston University School of Medicine, Boston, MA, ²University of Marburg, Germany, ³University of Mainz, Germany, ²Cancer Research Center, Moscow, Russia, ³Jackson Laboratory, Bar-Harbor, ME.

Neurotrophins fulfill a variety of non-neurotrophic functions in postnatal skin including stimulation of murine hair follicle regression (catagen) and keratinocyte apoptosis via p75 kD neurotrophin receptor (p75NTR). Here, we investigated a possible role of neurotrophins in autoimmune hair loss (alopecia areata, AA). In the C3H/HeJ mouse model for AA, we showed by ELISA that brain-derived neurotrophic factor (BDNF) and neurotrophin-4 are upregulated ($p < 0.05$) in affected skin, compared to unaffected control. BDNF and NT-4 immunoreactivity was found in MOMA-2 positive macrophages of perifollicular inflammatory infiltrates, while p75NTR expression was seen on CD8 lymphocytes. By FACS analysis, 45-75% of CD8 cells isolated from the skin and peripheral blood of mice affected by AA expressed p75NTR, while no p75NTR was seen on CD4 cells and macrophages. p75NTR message was detected in isolated CD8 cells using semi-quantitative RT-PCR. BDNF induced apoptosis in CD8 cells isolated from the skin and peripheral blood of AA-affected mice and cultured *in vitro*. BDNF administration into AA-affected skin was associated with hair regrowth accompanied by the reduction ($p < 0.01$) in number of CD8 cells, which co-expressed p75NTR and TUNEL. Conversely, treatment of AA-affected mice with cyclic peptide that blocks signaling through p75NTR markedly increased a number of CD8 cells in skin. These data suggest a previously unrecognized role for neurotrophins in eliminating auto-reactive CD8 cells from skin affected by AA via stimulating CD8 cell apoptosis. Stimulation of neurotrophin signaling through p75NTR may prove a new approach for treatment of autoimmune hair loss.

Poster #16

Effect of Aldara 5% cream on anagen:telogen and terminal: vellus ratios in extensive alopecia areata. LP Steiner, CM Boeck, ME Ericson, MJ Deeths*, DA Whiting**, MK Hordinsky. Departments of Dermatology, University of Minnesota, *University of Colorado and **University of Texas Southwestern and Baylor Hair Research and Treatment Center.

Eleven Caucasian patients with extensive long-standing alopecia areata (>95% scalp hair loss, 5M, 7F, ages 30 to 68, average disease duration 14 years) applied one packet of Aldara 5% cream to the right half of the scalp for six months. Two 4-mm punch biopsy specimens were taken from the right parietal scalp before and after treatment. Scalp biopsy specimens were vertically and horizontally sectioned and stained using standard techniques. Biopsy sections were analyzed by a dermatopathologist (DW) who was blinded to any patient information. Observations were made for the upper follicle (infundibulum and isthmus above arrector pili muscle insertion) and lower follicle (below arrector pili muscle insertion and including the hair bulb) on each patient's pre- and post-treatment biopsy specimens. After six months of Aldara 5% cream treatment, we found: anagen:telogen ratios increased in 7/11 patients. terminal:vellus ratios increased in 9/11 patients. follicular structures per mm² increased in 5/11, remained unchanged in 5/11 and decreased in one patient. Aldara 5% cream is known to indirectly augment interleukin-2 production and enhance Th1 responses. This drug effect appears to influence the hair cycle. Although no patient in this study had significant clinical improvement, Aldara 5% cream treatment did impact the hair cycle, as 5/11 patients demonstrated increased numbers of anagen follicles. Our results demonstrate a probable correlation between histologic improvement and cytokine production with the use of 5% Aldara cream in patients with extensive long-standing alopecia areata.

Poster #14

Children with alopecia areata – analysis of clinical experience. Berna K. Remzi MD, Wilma F. Bergfeld MD. Department of Dermatology, The Cleveland Clinic Foundation

Alopecia Areata is characterized with a non-scarring hair loss which affects children and adults of both gender. We aimed to analyze and assess available treatment options for children with alopecia areata while the search of etiology and pathophysiology is continuing. We reviewed 41 patients who had an onset of alopecia areata before 16 years of age. Patients were divided into two groups: Group I (20 patients) had alopecia areata with no known associated autoimmune diseases. Group II (21 patients) had alopecia areata with at least one associated disease such as atopy, Hashimoto's thyroiditis, etc. Demographic, disease and treatment related data were collected and severity of alopecia areata (with photographs) was classified according to the National Alopecia Areata Foundation's investigational assessment guidelines. Of the 41 patients, 24 (59%) were female and 17 (41%) were male with mean age of onset 8 (± 4.2) years. 31 (76%) of 41 patients had a positive family history of alopecia areata. 19 (46%) patients had baseline scalp hair loss severity of ≥ 3 , 16 (39%) of 41 patients experienced cosmetically acceptable response to various treatment combinations. 8 (50%) of 16 patients, who had cosmetically acceptable response to treatments, had baseline scalp alopecia severity of ≥ 3 , 9 (56%) of 16 patients had at least one associated disease such as atopy and/or thyroid dysfunction. Atopy as an associated disease was found not significant for severity of scalp alopecia areata.

Poster #17

Cytokine profiles in animal models of alopecia areata. L. Tang, L. Cao,* J. P. Sundberg, H. Lui and J. Shapiro Division of Dermatology, The University of British Columbia and Vancouver General Hospital, *The Jackson Laboratory, ME

An imbalance between pro-inflammatory cytokines and cytokine antagonists or inhibitors has been one of the factors predisposing to initiation or perpetuation of various inflammatory and autoimmune disorders. Alopecia areata (AA) is a common inflammatory disease targeting anagen hair follicles. There are two well-known AA animal models: C3H/HeJ mice and Dundee experimental bald rat (DEBR). The cytokine profile of AA has not been extensively investigated in these AA models. Using real time kinetic reverse transcription polymerase chain reaction and RNase protection assay, we compared the cytokine expression profile between normal mice and AA-affected C3H/HeJ mice, between normal Wistar rats and AA-affected DEBR rats. Proinflammatory cytokines like interferon-gamma (IFN- γ), tumor necrosis factor- α/β (TNF- α/β), and interleukin-12 (IL-12) are dramatically increased in the AA mice. No significant changes were observed in the expression of IL-1 α/β and IL-10. Interestingly, the expression of TNF- α/β , IL-12 and IFN- γ were consistently and significantly decreased upon successful treatment with topical mechlorethamine in AA-affected C3H/HeJ mice. In AA-affected DEBR rats, increased expression of IL-1 α/β , TNF- α , IFN- γ was observed as compared to normal Wistar rats. The expression of TNF- α and IFN- γ was down regulated on the treated sides in DEBR rats after therapeutic hair restoration with topical anthralin treatment. In contrast, IL-10 and IL-1 receptor antagonist (IL-1RA) were consistently up regulated upon successful treatment in DEBR rats. Our data illustrate that various cytokines are involved in the inflammatory and destructive changes during the development of AA as well as in reversing hair follicle activity during therapy. It is likely that targeted cytokine intervention may be beneficial for AA.

Poster #18

Mechlorethamine-treated alopecia areata mice as a model system to study therapeutic mechanisms for restoring autoimmune-arrested hair follicle activity. L. Tang, L. Cao, O. Bernado, J. P. Sundberg*, H. Lui, and J. Shapiro

(AA) is an autoimmune disease targeted at hair follicles with infiltrative T lymphocytes likely playing an important role in its pathogenesis. It was reported in 1985 that mechlorethamine was effective on AA patients. This has never been confirmed. The aims of the study were to investigate the effects of mechlorethamine on C3H/HeJ mice affected by AA and to explore the underlying mechanisms. Mice were treated on half of the dorsal skin with mechlorethamine and the contralateral side with the vehicle ointment. A full pelage of hair covered the treated side in all the mice and was maintained during the experiment, while the vehicle-treated sides showed either no change or continued hair loss. Immunohistochemistry revealed that infiltrative CD4⁺ and CD8⁺ lymphocytes were eliminated from the treated side. Furthermore, *in vitro* cell viability assay showed that lymphocytes were much more sensitive to the cytotoxic effects of mechlorethamine than other native skin and hair follicular cells. Our findings support that mechlorethamine restores follicular activity by selectively targeting infiltrated lymphocytes *in vivo* in AA-affected mice. To further explore the possible molecular mechanisms, RNA and proteins were extracted from both treated and control skins. RNase protection assay showed that interleukin-12, tumor necrosis factor- α/β , and interferon- γ were consistently and significantly inhibited by mechlorethamine upon successful treatment. Protein kinase screening revealed that growth factor mediated signaling pathway involving Erk1/2, Raf, Mek 1/2, p70^{S6K} and p90^{S6K}, as well as cell cycle kinases (CDK4/5/6/7) all showed increased expression of the treated side. Another pathway mediated by PKB and GSK-3 kinases was also up regulated. In contrast, the cell adhesion mediated FAK kinase was dramatically inhibited by the treatment. We conclude that the molecular mechanism underlying the efficacy of mechlorethamine on hair regrowth in AA mice might be mediated by the interplay of cytokines produced locally in the skin. The signal transduction pathways involving various protein kinases and their regulators may represent the initial molecular events in mouse skin upon successful mechlorethamine treatment.

Poster #19

Clobetasol propionate 0.025% under occlusion in the treatment of alopecia totalis and universalis. Antonella Tosti, Bianca Maria Piraccini, Massimiliano Pazzaglia, Colombina Vincenzi Department of Dermatology – University of Bologna

We report here the results of a controlled study using clobetasol propionate 0.025% ointment under occlusion in 28 adult patients with AT or AT/AU. All patients, after giving their informed consent to participate in the study, were prescribed clobetasol propionate 0.025% ointment and instructed to apply 2.5 gr of the ointment to the right side of the scalp every night under occlusion with a plastic film. The contralateral side of the scalp was left untreated as control. Patients were instructed to wash the scalp in the morning with a mild shampoo. Treatment was performed daily for 6 days a week for 6 months. When regrowth of terminal hair occurred on the treated side, treatment was extended over the entire scalp maintaining the same total dose (2.5 gr/day). All patients were followed up for a further 6 months, but clobetasol propionate treatment was prolonged with the same regimen only in responders.

Of the 28 patients included in the study, 8 were treated successfully (28.5%). Six of these were females and 2 males. Regrowth of terminal hair started on the treated side 6 to 14 weeks after start of treatment. Extension of treatment to the contralateral scalp produced complete or almost complete (95%) hair regrowth in all of them. Three of these 8 patients however had a relapse and were not able to maintain hair regrowth despite treatment. Our study shows that clobetasol propionate under occlusion is effective in inducing hair regrowth in patients with AT/AU. Although spontaneous regrowth should always be considered when evaluating treatments of alopecia areata, the occurrence of hair regrowth only on the treated side of the scalp confirms the effect of the treatment. Occurrence of hair regrowth only on the treated half of the scalp clearly shows that efficacy of treatment is due to a local and not systemic effect of the drug.

Poster #20

Treatment of alopecia areata in the DEBR model using Cyclosporin A lipid vesicles. D.D. Verma*, S. Verma*, K.J. McElwee[§], P. Freyschmidt-Paul[§], R. Hoffmann[§], and A. Fahr*. *Inst. of Pharm. Tech. & Biopharm, [§]Dept. of Dermatol., Philipp University, Marburg, Germany.

Alopecia areata (AA) is a suspected autoimmune cutaneous disease. Cyclosporin A (CyA), a T cell-specific immunosuppressant, has proven successful for the treatment of AA orally, but the beneficial effect of topical application is limited. However, a topical formulation is desirable given the potential toxicity of systemically applied CyA. Topical liposome formulations have attracted considerable interest concerning their potential utility both as a drug carrier and reservoir for the controlled release of drugs within the skin and especially to the hair follicle. We evaluated the efficacy of CyA in a topically applied liposomal formulation (75% > phosphatidylcholine, 5% lysophosphatidylcholine, 5% > sterol, natural oils) of 10% wt. in ethanol with and without 2% wt. terpenes (d-limonene: citral: cineole: > 10:45:45) as a penetration enhancer using the Dundee Experimental Bald Rat (DEBR) model. Fifteen DEBR were allocated to 3 groups of 5. Groups I, II and III received CyA vesicles with PE, CyA vesicles without penetration enhancer, and CyA in ethanol respectively. All rats were treated twice a day for 6 weeks within a 2 x 2 cm area on one bald flank with 20 µl/cm² on days 0-6, 40 µl/cm² on days 8-14, 50 µl/cm² on days 15-20 and 80 µl/cm² on days 21-42 of 5 mg/ml CyA. The contralateral flank was treated with the equivalent control formulation. All 5 rats in group I showed visible hair regrowth on the drug treated site by day 7 of drug application while in group II all rats had visible hair regrowth by day 14. The hair growth was progressive and reached a maximum density at the site of application by day 42 in both groups. Histological examination revealed a reduced number of inflammatory infiltrate cells within the drug treated area as compared to the contralateral drug vehicle treated flank skin. Group III rats showed no visible signs of hair growth nor diminution of hair follicle inflammation. Topical CyA in a liposomal formulation had a localized hair growth promoting effect in DEBR suggesting that liposomes have significantly greater drug delivery capability and increase CyA potency as compared to CyA in ethanol. The accelerated hair growth response in group I rats suggest that penetration enhancer used in this formulation may also play a role in CyA delivery. CyA in lipid vesicles may have significant potential for the treatment of human AA.

Poster #21

Inducible nitric oxide synthase inhibitors have no significant effect on alopecia areata in C3H/HeJ mice. K.J. McElwee, P. Freyschmidt-Paul, E. Wenzel, and R. Hoffmann. Dept. of Dermatology, Philipp University, Marburg, Germany.

Nitric oxide (NO) production can be an important part of inflammation driven diseases. NO can enhance inflammation through vasodilation and is a cytotoxic molecule potentially involved in target cell disruption. It is a membrane permeable gas derived from L-arginine conversion to citrulline by a family of isoenzymes, the nitric oxide synthases (NOS). Inducible NOS (iNOS) production from macrophages, Langerhan's cells, granulocytes, and Th1 type CD4⁺ lymphocytes can be promoted by proinflammatory cytokines such as IL-1 β , TNF α and IFN γ . By immunohistology, iNOS is highly expressed in association with inflammatory alopecia areata (AA) affected hair follicles of C3H/HeJ mice. Pentoxifylline (PTX) has been used in autoimmune disease models to inhibit iNOS as well as modulate proinflammatory cytokines TNF α and IFN γ . Similarly, S-methyl-isothiourea (SMT) is a selective iNOS inhibitor. PTX and SMT were injected intraperitoneally at 50mg/kg/day/mouse and 20mg/kg/day/mouse respectively, three times per week for ten weeks into 2 age matched groups of 7 AA affected mice. A third group of 7 matched mice received the 0.1 ml/day/mouse PBS vehicle alone. The high doses used failed to produce any notable hair growth effect by comparison of photographic images taken at initiation and completion of the study. Histology of treated mice also indicated no apparent changes in hair follicle inflammation as compared to the control group. In conclusion, iNOS inhibitors PTX and SMT had no significant effect on AA. However, given high iNOS expression in AA foci, iNOS modulators may yet have a role as part of a multi drug therapy for AA.

Poster #22

Successful treatment of alopecia areata (AA) with diphencyprone (DPCP): analysis of inflammatory cells in the scalp and peripheral blood, dendritic cell (DC) function and DPCP-specific proliferation of peripheral blood leukocytes (PBL) Rudolf E. Schopf, Andrea Tüntenberg, Vanessa Löttsch, Ester von Stebut Dept. Dermatology, Johannes Gutenberg Univ., Mainz, Germany

Diphencyprone treatment has the highest success rate in AA. The mechanism of action, however, is not clear. In order to better understand the beneficial effects of DPCP we examined the cellular infiltrate (CD3, CD4, CD25, CD45RO, CD1a, HLA-DR, ICAM-1) in the scalp in 8 patients before and after treatment. Moreover, we also phenotyped PBL (CD2, CD14, CD19, CD3, CD4, CD8, CD56, HLA-DR) in 3 patients successfully treated compared to healthy controls; in these individuals we also tested the proliferation of PBL to DPCP and the capacity of mature DCs generated from PBL to stimulate allogeneic CD4⁺ and CD8⁺ cells. In the dermis CD1a⁺ and CD25⁺ cells significantly decreased while CD8⁺ cells increased after successful treatment (p < 0.025, Wilcoxon test). Compared to healthy controls AA patients after treatment exhibited 2-3 times more CD14⁺, CD19⁺, CD56⁺ and HLA-DR⁺ cells among PBLs. PBLs also showed increased proliferation to DPCP compared to untreated donors; the response to the recall antigens tetanus toxoid and PPD was unchanged. Mature DCs generated from PBLs of AA patients showed normal allostimulatory capacity. We conclude that successful treatment of AA with DPCP is associated with a down regulation of the number of activated T cells and antigen-presenting CD1a⁺ cells as well as an increase in CD8⁺ cells in the scalp. In the blood there is an increased percentage of monocytes, B cells, NK cells and HLA-DR⁺ cells as well as a capacity of PBLs to proliferate to stimulation with DPCP that is not due to altered function of antigen-presenting dendritic cells.

FOURTH INTERNATIONAL RESEARCH WORKSHOP ON ALOPECIA AREATA**Poster Abstracts: PSYCHOLOGY****Poster #23**

Attitudes and perceptions of school-aged children toward alopecia areata. Heidi McMillan,¹ Marshall Jones,² Jeffrey Miller,³ ¹Department of Pediatrics, Hershey Medical Center, DA, Hershey, PA, U.S.A. ²Department of Behavioral Science, Hershey Medical Center, Hershey, PA, U.S.A. ³Department of Dermatology, Hershey Medical Center, Hershey, PA, USA

Alopecia areata (AA) is a psychologically devastating medical condition. This study examined the attitudes and perceptions of school-aged children (kindergarten [K] – 8th grade) to AA. Subjects were shown a photo of a child with AA and asked a series of questions relating to hair loss. Younger children (K-3rd grade) were more likely to display visible discomfort when viewing a photo of a child with AA compared to older children (5th – 8th grade). Younger children (K-2nd grade) more often believe a child with AA is very sick and contagious compared to older children (4th – 8th grade). Younger children (K-3rd grade) were more likely to be afraid to get close to a child with AA compared to older children (5th – 8th grade). The concept of cancer as a cause of hair loss emerges in 3rd – 8th grade children. Children across all grade levels demonstrate feelings of sadness and want to know "what happened." Children's perception of hair loss representative of AA changes markedly across grade levels. Knowledge of the differing perceptions is helpful in counseling and educating a child with new onset AA.

*Oral Presentation on Saturday morning

Poster #24

Psychological distress: A profile of alopecia areata patients. Sandy Venneman^{1,2*}, Elaine Siegfried³, John Cross¹ ¹Department of Psychology, St. Louis University; ²Departments of Psychology and Biology, University of Houston-Victoria; ³School of Medicine, St. Louis University, St. Louis, Missouri, USA

Stress has been popularized as a common cause of hair loss. While physical and psychological stressors are accepted as precursors to telogen effluvium, the association has not been clarified for AA. Genetic factors, rather than stress, are assumed to be the primary cause of AGA and linked to AA. We examined indicators of stress in AA and AGA subjects seeking medical evaluation or treatment for hair loss. Patients (N = 46) completed questionnaires that included measures of stress, stress correlates and personality factors. Data from these factors were statistically analyzed in a discriminate function analysis with the following results; males with AA and AGA had comparable scores for all variables, including significant depression; females with AA exhibited anxiety, while those with AGA exhibited depression and low Positive Affect. The results do not support a causal role of stress in AA, but do emphasize that anxiety and/or depression are a common co-morbidity for patients with hair loss. This suggests that medical personnel recommend the possibility of psychological assistance for hair loss patients through psychological/psychiatric avenues and/or support groups.