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HB1

EXPRESSION OF CCR-5, BUT NOT CXCR-4, ON FRESHLY ISOLATED LANGERHANS CELLS CORRELATES WITH RESTRICTED TRANSMISSION OF MACROPHAGE-TROPIC HIV. A Blauvelt,[1] M Zaitseva,[2] JP Zoeteweij,[1] C Lapham,[2] J Manischewitz,[2] V Klaus-Kovtun,[1] H Golding.[2] [1]Dermatology Branch, NCI and [2]Division of Viral Products, FDA, Bethesda, MD.

Sexual transmission of HIV is restricted to macrophage (Mø)-tropic strains of virus, although the basis for this phenomenon is unknown. Recently, the chemokine receptors CCR-5 and CXCR-4 have been shown to be the major HIV co-receptors for Mø-tropic and T cell line-tropic variants of HIV, respectively. Since Langerhans cells (LC) have been proposed to be the first cell type infected following mucosal exposure to HIV, the aim of this study was to determine whether restricted transmission of Mø-tropic HIV correlated with the pattern of HIV co-receptor expression on LC. We generated polyclonal rabbit sera containing antibodies directed against the extracellular N-termini of CCR-5 and CXCR-4, and confirmed the specificity of the antisera using a functional fusion assay. We then immunolabeled freshly isolated and cultured human epidermal LC (derived from suction blister roofs) for cell surface expression of CCR-5 and CXCR-4 with these antisera, using a sensitive 3-step staining protocol and flow cytometric analyses. Freshly isolated LC expressed CCR-5 (24-49%), but not CXCR-4. The absence of CXCR-4 on freshly isolated LC was not due to trypsin sensitivity. In contrast, 1 day cultured LC expressed both HIV co-receptors (28-53% for CCR-5 and 16-43% for CXCR-4). On 2 day cultured LC, γ-interferon (20 ng/ml) prevented expression of CXCR-4, whereas TNF-α (20 ng/ml) and GM-CSF (1,000 U/ml) did not significantly affect either CCR-5 or CXCR-4 expression. This is the first study to examine HIV co-receptor expression on freshly isolated LC, and to examine cytokine regulation of co-receptor expression. The fact that freshly isolated epidermal LC (which resemble mucosal LC in situ) express CCR-5, but not CXCR-4, provides a possible explanation for the restricted sexual transmission of Mø-tropic strains of HIV.

HB2

MURINE CUTANEOUS MASTOCYTOSIS AND EPIDERMAL MELANOCYTOSIS INDUCED BY KERATINOCYTE STEM CELL FACTOR EXPRESSION. Takahiro Kunisada[1], Shu-Zhuang Lu[2], Hisahiro Yoshida[3], Satomi Nishikawa[2], Shin-ichi Nishikawa[3], Masako Mizoguchi[4], Shin-ichi Hayashi[1], Lynda Tyrrell[2], B. Jack Longley[2]; [1]Dept. Immunol, Tottori Univ., Yonago, Japan; [2]Dept. of Derm and Skin Disease Research Ctr., Yale Univ., New Haven, CT; [3] Dept. Mol. Gen., Kyoto Univ., Kyoto, Japan; [4] Dept. Dermatol., St. Marianna Univ., Kawasaki, Japan.

The growth and differentiation of mast cells and melanocytes require stem cell factor (SCF), the ligand for the kit receptor tyrosine kinase. SCF is produced by human but not murine epidermal keratinocytes, and may exist as a membrane-bound or soluble molecule. Abnormalities of the SCF-kit signalling pathway have been implicated in the pathogenesis of the human disease mastocytosis, but the cause of mastocytosis has not been demonstrated experimentally. To investigate both the potential of SCF to cause mastocytosis and its role in epidermal melanocyte homeostasis, we targeted the expression of SCF to epidermal keratinocytes in mice with transgenes controlled by the human keratin 14 promoter. The transgenes contained cDNAs which either produced SCF which is both membrane-bound and soluble, or SCF that is predominantly membrane-bound. Epidermal keratinocyte expression of both membrane and soluble SCF reproduced the phenotype of human cutaneous mastocytosis in mice, with dermal mast cell infiltrates and epidermal hyperpigmentation, and caused the maintenance of a population of epidermal melanocytes. Keratinocyte expression of membrane-bound SCF did not by itself cause mastocytosis but resulted in the presence of melanocytes and excess melanin in the interadnexal epidermis, an area where they are found in human skin but not usually in murine skin. We conclude, first, that a phenotype matching that of human mastocytosis can be produced in mice by keratinocyte overproduction of both membrane and soluble SCF, suggesting a mechanism for the cause of this disease. Second, we conclude that murine keratinocyte expression of membrane-bound SCF results in the maintenance of epidermal melanocytes.

HB5

THE MOLECULAR BASIS OF COWDEN'S SYNDROME. H. C. Tsou, X. Ping, X. Xie, Y. Yao, C. Schrager, A. Gruener, A. M. Christiano, D. Liaw, R. Parsons, C. Eng*, and M. Peacocke; Departments of Dermatology, Medicine and Pathology, Columbia University, New York, N.Y., and the Dana-Farber Cancer Institute*, Boston, MA.

Cowden's Syndrome (CS) or multiple hamartoma syndrome, is an autosomal dominant disorder characterized by a variety of benign skin lesions, including trichilemmomas, benign breast lesions and an increased risk of breast cancer. Tissue specific cancer susceptibility is also increased for the thyroid gland, although this cancer risk is far less than for the breast. A subpopulation of CS patients are also at risk for the development of an unusual form of brain hamartoma, a dysplastic gangliocytoma. Linkage analysis previously identified a locus for CS at chromosome 10q23. A gene encoding a novel protein tyrosine phosphatase has recently been identified at this same locus. As this family of genes has been implicated in tumor suppression, we devised a mutation screening strategy for the different exons of this phosphatase. We have now identified 2 nonsense mutations and 2 missense mutations in certain of the CS families that are linked to this region. These sequence differences are not evident in normal individuals. However, we have a series of CS families with classic skin findings and early onset breast cancer which do appear not appear to be linked to 10q23, and do not have mutations in the coding sequence of this phosphatase. Screening a series of other, isolated CS patients with breast cancer also failed to identify mutations in this gene. These data demonstrate that mutations in this phosphatase appear to associated with certain individuals with CS. There are other families that are not linked to 10q23 and do not have mutations in this phosphatase. The mutations in this phosphatase are the first mutations associated with CS, however, it is likely that CS is genetically heterogenous and other genes are involved in the development of breast cancer in CS.

HB9

GENE INTRODUCTION OF ANGIOSTATIN INHIBITS TUMOR GROWTH IN A MOUSE MODEL OF LIFE-THREATENING HEMANGIOMA. Brian Lannutti and Amy S. Paller, Departments of Pediatrics and Dermatology, Northwestern University Medical School, Chicago, IL.

Angiostatin, a cleavage product of plasminogen, is a newly described potent antiangiogenic agent. Systemic administration of angiostatin protein to mice with malignancies inhibits tumor metastases. Hemangiomas are common endothelial cell tumors of infancy that may be life-threatening, especially if Kasabach-Merritt syndrome develops. Interferon-α, a weak anti-angiogenic agent, is considered the drug of choice, but has recently been shown to cause neurotoxicity. We have used a mouse model of the Kasabach-Merritt syndrome to test our hypothesis that local production of angiostatin inhibits hemangioma growth, decreases the associated thrombocytopenia, and increases survival. Mouse hemangioendothelioma (EOMA) cells were stably transfected by lipofection with mouse angiostatin cDNA, tagged with hemagglutinin, and selected by geneticin followed by clonal selection. Transcription of angiostatin mRNA was demonstrated by RT-PCR and expression of the hemagglutinin tag by Western analysis. EOMA cells transfected with the angiostatin cDNA, vector alone, or parental EOMA cells were injected subcutaneously into athymic nude mice to establish the model. By 5 days after injection, hemangiomas were visible. However, by approximately 3 weeks after injection, control mice died from hemorrhage while mice with tumors that produced angiostatin thrived without any signs of toxicity and with limited thrombocytopenia. At 21 days after injection, control tumor volumes averaged 1700 mm^3 vs. tumor volumes of 110 mm^3 in angiostatin-producing tumors. These studies show that local production of angiostatin dramatically inhibits the growth of life-threatening hemangiomas without toxicity. The antitumor activity in this immunodeficient mouse provides further evidence that the action of angiostatin does not involve T-cell immune activation. Tumor delivery of angiostatin, either through protein introduction or gene therapy, may revolutionize therapy of life-threatening and even uncomplicated hemangiomas.

HB21

IDENTIFICATION OF A NOVEL HUMAN SKIN-DERIVED ANTIMICROBIAL PEPTIDE. J. Harder, J. Bartels, E. Christophers, J.-M. Schröder, Department of Dermatology, University of Kiel, D-24105 Kiel, FRG

Epithelial cells of vertebrates and invertebrates as well as plants are known to prevent bacterial, fungal as well as viral infection by the release of antimicrobial peptides which kill infectious organisms by forming pores. Since little is known about this innate immunity system in man, we addressed the question, whether also human skin is capable of producing similar antimicrobial peptides.

Because psoriatic patients have an unexpected low skin infection rate, we speculated whether active psoriatic skin and scales might be a potential source of antimicrobial peptides. Therefore we separated psoriatic scale extracts by reversed phase HPLC techniques and tested antimicrobial (E.coli) activity in fractions by the use of a plate assay. As a result we obtained several peaks of antimicrobial activity. A major one termed "skin derived antimicrobial peptide (SAP-1)" was purified to homogeneity and further analyzed biochemically. As a result we found SAP-1 to be a novel 4 kD peptide having a sequence homology to mammal antimicrobial ß-defensins. SAP-1 was found to be highly effective in killing gram negative bacteria and Candida albicans ($LD_{90} \approx 10\mu g/ml$). Using degenerated primers we were able to isolate the complete SAP-1 cDNA from the human keratinocyte cell line HaCat proving keratinocytes as a potential cellular source of SAP-1. SAP-1 mRNA is present in human skin, trachea and lung and is upregulated in cultured normal keratinocytes by TNF-α and strongly increased in the presence of heat killed gram positive, gram negative bacteria as well as Candida albicans.

Our observation indicates active participation of human keratinocytes in innate immunity by producing antimicrobial peptides thus allowing homeostasis of bacterial colonization on skin.

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