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EXPRESSION AND CYTOKINE MODULATION OF VASCULAR CELL ADHESION MOLECULE-1 IN NORMAL AND DISEASED HUMAN SKIN. RW Groves, E Ross, JNWN Barker, DM MacDonald. Laboratory of Applied Dermatopathology, UMDS, Guy's Hospital, London, UK.

Expression of adhesion molecules by vascular endothelium and other cutaneous cells is likely to be of great importance in the genesis of inflammatory skin disease. Vascular cell adhesion molecule-1 (VCAM-1) is a novel endothelial molecule with adhesive properties in vitro for lymphocytes and eosinophils. Using anti-VCAM-1 monoclonal antibodies, we have performed an immunohistochemical study of its expression in normal and inflamed skin, and have examined ways of modulating its expression in vivo.

In normal skin (n=8) low levels of VCAM-1 were present on perivascular dendritic cells and occasional endothelial cells. In inflamed skin (allergic contact dermatitis [n=6, t=24-96 hrs], chronic atopic dermatitis [n=6], psoriasis [n=8], and lichen planus [n=6]) VCAM-1 was upregulated on dermal endothelium and was also present on interstitial dermal dendritic cells. Three normal volunteers underwent intradermal injection of 100U rHuTNFα and five received 30μg rHuIFNγ. Following TNFα and IFNγ there was marked upregulation of VCAM-1 on dermal endothelium and dendritic cells.

Widespread expression of VCAM-1 in inflamed skin suggests that this molecule may be of importance in the initiation and maintenance of a variety of skin diseases. Both keratinocyte derived (TNFα) and lymphocyte derived (IFNγ) cytokines may be of importance in its control; interference with these pathways may be of future therapeutic benefit.

INCREASED ADHERENCE OF PERIPHERAL BLOOD MONONUCLEAR CELLS AND ENHANCED ICAM-1 EXPRESSION ON FIBROBLASTS FROM PATIENTS WITH SCLERODERMA. S. Majewski, B. Makiela, L. Rudnicka, M. Skopinska, A. Skiendzielewska, N. Hunzelmann, J.P. Johnson, Th. Krieg, S. Jablonska. Department of Dermatology, Warsaw School of Medicine, Warsaw, Poland; Department of Dermatology, University of Cologne, Cologne, Germany; Institute of Immunology, LMU Munich, Germany.

The adherence of peripheral blood mononuclear cells (MNC) from patients with systemic scleroderma (SSc) to fibroblast monolayers was studied by means of ^{51}Cr izotope assay. The attachment was compared to the expression of ICAM-1, LFA-3 and HLA-DR molecules on fibroblasts derived from patients with SSc and from healthy individuals. The adherence of MNC from SSc patients was significanlty increased as compared to that of control MNC. MNC from both SSc patients and healthy individuals showed a higher adherence to fibroblasts from SSc patients than to control cells. This was correlated with an increased spontaneous expression of ICAM-1 on SSc fibroblasts, as detected by ELISA and Northern blot analysis of cellular RNA. The expression of LFA-3 did not differ on both types of fibroblasts. Non-activated fibroblasts did not express detectable amounts of HLA-DR. The expression of ICAM-1 and HLA-DR (but not LFA-3) could be stimulated by IFN-g, TNFα and IL-1. The results confirm our previous findings on an in vivo increased expression of ICAM-1 on SSc fibroblasts, and suggest a role of cell-cell interactions in the fibrotic processes in this disease.

EXPRESSION OF BETA-2 INTEGRIN MOLECULES ON HUMAN KERATINOCYTES IN CYTOKINE-MEDIATED SKIN DISEASES. M. Simon jr., J. Hunyadi; Dept. of Dermatology, University of Erlangen-Nürnberg, Erlangen, FR Germany

Integrins are cell surface molecules of importance in a wide variety of cellular functions, including morphogenesis, cell migration and cell matrix interactions. The beta-2 (B2) integrin subfamily consists of three members, each composed of a shared beta subunit (CD18) noncovalently associated with unique alpha subunits (CD11a, CD11b, CD11c). In the present study, we have analysed the expression pattern of B2 integrins on the surface of human keratinocytes (HKs) in biopsies obtained from healthy volunteers, from positive tuberculin skin tests and from patients with acute urticaria (AU), lichen planus (LP), psoriasis vulgaris (PV), mycosis fungoides (MF) or purpura pigmentosa chronica (PPC). In biopsies obtained from positive tuberculin tests and from the clinically involved skin of patients with LP, PV, MF or PPC, a multifocally occurring, suprabasal peroxidase-positive reaction was observed on the membranes of the HKs when the monoclonal antibodies (MABs) Dako CD11a, Dako-p150,95 or CD18 were used. In contrast, no specific staining of the HKs was observed with the same MABs in biopsies from healthy volunteers, from patients with AU and in the uninvolved skin specimens obtained from the other patients. The HKs from PV, LP, MF, PPC and AU patients and those from the healthy subjects failed to give a positive reaction when the MAB against CD11b (OKM1) was used. Our present findings provide further evidence that HKs may be actively involved in cell adhesion processes.

TIME-COURSE, DOSE-DEPENDENCE AND DISTRIBUTION OF INTERFERON GAMMA-INDUCED ICAM-1 AND HLA-DR EXPRESSION IN A HUMAN RECONSTRUCTED SKIN MODEL IN VITRO : COMPARISON OF NORMAL HUMAN SKIN IN SHORT-TERM ORGAN CULTURE.

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Many inflammatory skin diseases, including allergic contact dermatitis, are associated with leukocyte infiltration in the epidermis and expression of class II histocompatibility antigens by keratinocytes. A crucial step in these reactions may be leukocyte retention in the epidermis by keratinocyte-expressed Inter-Cellular Adhesion Molecule 1 (ICAM-1), a ligand for Leukocyte Functional Antigen 1 (LFA-1) : this attachment may initiate numerous contact-dependent processes such as sensitization or cytotoxicity.

In order to assess the suitability of in vitro models for the study of ICAM-1 and HLA-DR expression by keratinocytes and its modulation by various agents, we compared the time-course and dose-dependence of IFN Gamma-induced expression of these markers using an immuno-fluorescence method in two systems ; a simplified reconstructed skin model (outer root sheath cells on a fibroblast-collagen lattice) and normal human skin in short term organ culture. In both systems, IFN-Gamma induced a strong dose-dependent ICAM-1 staining in basal, non differentiated keratinocytes, and a lower signal in suprabasal layers. A similar pattern was observed with HLA-DR expression by IFN Gamma pretreated keratinocytes. Time-course experiments performed in the two models (incubation periods from 4h to 168h with IFN Gamma) indicated a maximal marker expression after 24h contact for ICAM-1, and 72h for HLA-DR. The minimal contact time necessary for marker induction (IFN Gamma withdrawal after selected time periods of contact and examination at 48h) was found to be 4h for ICAM-1 and 16h for HLA-DR. Marker disappearance after stimulus suppression was also studied : ICAM-1 staining decreased 24h after IFN Gamma removal, while HLA-DR signal was left unchanged up to 48h. Both markers were still visible at 120h.

In conclusion, similar distribution, time-course and dose-dependence of IFN Gamma-induced ICAM-1 and HLA-DR expression were observed in the simplified reconstructed skin and in normal human skin in short-term organ culture. Thus, our culture system would appear to be a potential in vitro tool for the investigation of the role of keratinocytes in immunological processes in the skin and its modulation by cytokines or pharmacological agents.

THE SEQUENCE OF ADHESION MOLECULE EXPRESSION AND THE COMPOSITION OF THE CELLULAR INFILTRATE IN TIMED, INDUCED LESIONS OF DELAYED PRESSURE URTICARIA. R.J.Barlow, E.Ross, A.Kobza Black, D.M.MacDonald*, M.W.Greaves, St.John's Institute of Dermatology, St. Thomas' Hospital and *Department of Dermatology, Guy's Hospital, London, U.K.

In delayed pressure urticaria (DPU) persistent, painful, erythematous swellings develop 30 minutes to 9 hours after applying pressure to the skin. Lesional histopathology may vary from dermal oedema with a mild mononuclear cell infiltrate to an infiltrate containing neutrophils, eosinophils and lymphocytes.The pathogenesis is unknown and we aimed to study the extent of expression of cytokine inducible adhesion molecules in association with the cellular infiltrate in timed skin biopsies.Lesions were induced on the thighs in 13 DPU patients using weighted steel rods. Three punch biopsies were taken from each patient from uninvolved skin or at 0, 2, 6, 24, 48 or 120 hours after pressure application. Immunohistochemical analysis was made with monoclonal antibodies detecting Endothelial Leucocyte Adhesion Molecule 1 (ELAM 1), Intercellular Adhesion Molecule 1 (ICAM 1) and different leucocyte and mast cell antigens.Using a visual grading score, upregulation of ELAM 1 was greatest at 6 hours and of ICAM 1 at 48 hours. Results of cell counts per high power field are tabulated below.

	Neutrophils	Eosinophils	T Lymphocytes	Mast cells
Uninvolved skin	0.4 ± 0.8	0.0 ± 0.0	4.9 ± 2.4	0.8 ± 0.9
6 hours	23.0 ± 23.3 *	1.9 ± 4.3	4.0 ± 4.3	0.8 ± 0.9
24 hours	28.3 ± 12.1 *	4.7 ± 4.4 *	8.6 ± 3.4 *	1.7 ± 1.0 *

(mean ± S.D.) * = significant, $p < 0.05$

These results suggest that expression of leucocyte adhesion molecules is an early response to pressure challenge in DPU although the effect of pressure challenge in normal skin of healthy subjects needs to be examined.

BETA2-INTEGRIN ANTIGEN EXPRESSION IN DIFFERENT TYPES OF URTICARIA. N.Haas, W.Iwen, C.Wesendahl, J.Grabbe, +C.Figdor, B.M.Czarnetzki, Dept. of Dermatology, UKRV, Free University, Berlin and +Dept. of Immunology, The Netherland Cancer Hospital, Amsterdam, The Netherlands

In order to explore the mechanisms involved in inflammatory cell recruitment in certain types of wealing reactions, we studied biopsies from lesional (ls) and normal (ns) skin of patients with acute (AU,5), chronic recurrent (CRU,1), cold (CU,2) and delayed pressure urticaria (DPU,3) for the expression of different subsets of $\beta_2$integrins, using the APAAP technique. Prick test reactions to common inhalant allergens and ns of 4 patients with nonurticarial type I allergy served as control. There were no significant findings in prick test weals and ns of non-urticaria patients, in ls of 2 patients with AU and in ns of all AU patients. There were intense reactions of CR3 and LFA β_2 in 3 ls of AU, and in addition of LFA 1α, and anti-LFA 1α in ls of CRU and DPU, with increased LFA 1α and LFA β_2 in ns of CRU. In CU, no reactivity was seen in ns and ls 5' after provocation. After 20', LFA β_2 marked the middermal vessels. After 30', strong staining of adnexae and the deep horizontal vessels with CR3, LFA β_2 and anti-LFA 1α was observed. The data demonstrate thus a correlation between inflammatory infiltration and intensity of tissue reactions to $\beta_2$integrins in lesional and often also in normal appearing skin of patients with diverse types of urticaria.