1475 EVALUATION OF DENDRITIC CELLS FOR IN VITRO DETECTION OF SENSITIZING CHEMICALS.

S Kerdine, H Lebrec, J Bertoglio and M Pallardy. *Immunotoxicology group, INSERM U461, Faculté de Pharmacie, Chatenay-Malabry, France.*

After application of haptens to the skin, Langerhans cells (LC) that are immature dendritic cells (DC) of the skin, secrete some cytokines and migrate to secondary lymphoid organs leading to activation of naive T cells. To assess which cytokines are produced by LC after an application of haptens, we have isolated $CD34^+$ hematopoietic progenitor cells from human cord blood through positive selection using anti-CD34 mAb and goat anti-mouse IgG coated microbeads. Isolation of $CD34^+$ cells was achieved using Minimacs separation column. After purification, $CD34^+$ cells were cultured with GM-CSF and h-TNF-α for 12 days in order to obtain Langerhans cells /dendritic cells. At day 12, surface marker analysis was performed using cytofluorometry. These LC/DC were positive for $CD1a^+$ (83%), CD83 (23%), B7-1 (40%) and ICAM-1 (80%). These cells showed a good uptake of antigens as measured using FITC-dextran and cytofluorometry. To assess the expression of cytokine mRNA after hapten stimulation, we added to LC/DC dinitrochlorobenzene (DNCB), a chemical agent known to induce cutaneous reactions. Results obtained using quantitative RT-PCR with a standard competitor DNA, showed that DNCB induced a three time fold increase in IL-1β mRNA expression as compared to controls. Maximal expression was obtained 15 min after the application of the hapten. These experiments showed that haptens can induce expression of IL-1β in cultured human dendritic cells. We are currently analyzing the expression of IL-12, MIP-1α and MIP-1β mRNAs in LC/DC after DNCB stimulation.

1476 EXAMINATION OF HUMAN PERIPHERAL BLOOD DERIVED DENDRITIC CELLS FOR USE AS ANTIGEN-PRESENTING CELLS IN AN *IN VITRO* LYMPHOCYTE PROLIFERATION ASSAY.

C A Ryan, B C Hulette and G F Gerberick. *The Procter & Gamble Company, Cincinnati, OH, USA.*

Dendritic cells (DC) are professional antigen presenting cells which are widely distributed in the body and are key for initiating T cell mediated immune responses. Methods for culturing human DCs from peripheral blood precursors have been developed by several laboratories. We were interested in examining DCs produced by these methods and evaluating their ability to present low molecular weight chemical haptens to sensitized T cells in an *in vitro* lymphocyte proliferation assay. Immature DCs (iDCs), were generated by culturing human peripheral blood mononuclear cells for 7 days in the presence of the cytokines Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Interleukin-4 (IL-4). When examined by flow cytometry, these cells were found to be MHC class II $HLA\text{-}DR^+$, $CD80^+$, $CD11b^+$, $CD45RO^+$ and $CD36^-$. iDCs were treated with the test allergen, dinitrobenzenesulfonic acid (DNBS), then cultured with 10^5 autologous T cells at several responder (T-cells) to stimulator (DCs) ratios (R:S). The cultures were incubated for four days, pulsed with ^{3}H-thymidine and harvested 18-20 hours later. T-cells cultured with DNBS haptenized iDCs at a 10:1 R:S demonstrated the greatest proliferation based on ^{3}H-thymidine incorporation compared with control cultures. Mature DCs (mDCs) were generated from iDCs by culturing them in the presence of GM-CSF, IL-4, TNF-α , IL-1β , IL-6 and PGE_2 for 2 additional days. These cells, in addition to being $HLA\text{-}DR^+$, $CD80^+$, were also found to be $CD83^+$. When mDCs haptenized with DNBS were used as antigen presenting cells, sensitized T cells demonstrated the greatest proliferation at an R:S of 50:1. Increased T cell proliferation above controls was also observed at an R:S ratio of 100:1. These initial results suggest that mDCs are better antigen presenting cells for chemical haptens.

1477 mRNA EXPRESSION BY CULTURED HUMAN BLOOD-DERIVED DENDRITIC CELLS: ASSOCIATION WITH SKIN SENSITIZATION POTENTIAL.

J S Pichowski[1], M Cumberbatch[1], R J Dearman[1], D A Basketter[2] and I Kimber[1]. [1]*Zeneca Central Toxicology Laboratory, Macclesfield, Cheshire, UK;* [2]*Unilever SEAC, Bedford, UK.*

Langerhans cells (LC) are a specialized form of dendritic cell found within the epidermis which are important for the development of contact sensitization and other cutaneous immune responses. The study of LC has been constrained by the fact that they represent only a minority population. However, methods have been developed recently that allow the expansion in culture of LC-like cells from precursors within a number of human tissues. Human peripheral blood mononuclear cells depleted of T and B lymphocytes by positive selection were cultured in the presence of interleukin-4 and granulocyte/macrophage-colony stimulating factor. After five days of culture, approximately 60% of cells displayed a LC-like phenotype, with characteristic dendritic morphology and cell surface expression of CD83, MHC class II and CD1a (determined by flow cytometry). The majority of the remaining cells was CD14+ monocytes/macrophages, while CD3+ T cells and CD19+ B cells represented minor populations. Cytokine expression was analyzed by semi-quantitative RT-PCR. In common with skin-derived LC, blood-derived DC expressed constitutively mRNA for interleukins (IL) -1β , IL-6 and IL-18. Following exposure of blood-derived DC to the chemical sensitizer 2,4-dinitrofluorobenzene (DNFB; 10.7pM) in 0.01% dimethyl sulfoxide (DMSO), IL-1β mRNA expression was up-regulated in cells derived from 2 out of 5 donors. Under the same conditions, 0.01% DMSO alone was without effect. The variation in IL-1β up-regulation may reflect subtle differences in culture conditions, particularly minor variations in cytokine concentration. These data suggest that with further optimisation, up-regulation of IL-1β mRNA expression by blood-derived DC might prove a useful marker of skin sensitizing activity.

1478 EVALUATION OF HUMAN IRRITANTS AND WEAK TO MODERATE SENSITIZERS USING A MODIFIED LLNA AND AN IRRITANCY/PHENOTYPING ASSAY.

J M Valosen[1], B B Hayes[1,2], M D Howell[1,3], T S Manetz[1,2], M R Woolhiser[1,2], and B J Meade[1]. [1]*National Institute for Occupational Safety and Health (NIOSH), Health Effects Laboratory Division, Morgantown, WV;* [2]*Virginia Commonwealth University, Richmond, VA;* [3]*West Virginia University, Morgantown, WV.*

One reported weakness of the murine Local Lymph Node Assay (LLNA) is its inability to differentiate weak sensitizers and strong irritants. In these studies, we evaluated a panel of irritants and weak to moderate sensitizers in a modified LLNA and an irritancy/phenotypic analysis assay. Compounds initially tested included p-xylenol, thiomerosal, formaldehyde, benzocaine, menthol, and phenol. The LLNA protocol was modified to include pre and post ear measurements as an indication of irritation. The irritancy/phenotypic analysis method also used ear swelling as an indication of irritancy but evaluated sensitization by an elevation of the %B220+ and/or %IgE+B220+ (IgE-mediated) draining lymph node cell populations. In both assays, female BALB/c mice were topically exposed on the ear pinnae for 4 consecutive days. Ear measurements were taken prior to exposure on day 1 and 24 hr after final exposure on day 5. For the LLNA, [^{3}H]-thymidine incorporation was measured on day 5 and for phenotypic analysis cell populations were examined on day 10. Consistent with the human literature, thiomerosal and formaldehyde were identified as irritants and sensitizers in both the modified LLNA and the irritancy/phenotypic assay. Additionally, the IgE+B220+ lymph node cell population was elevated in animals exposed to formaldehyde in agreement with reports of human contact urticaria following exposure to this compound. Phenol, a strong human irritant with a low sensitization index, was positive for irritation and sensitization in these assays. Benzocaine gave a false negative response for sensitization in both assays when tested at a 50% concentration in acetone. Although reported to be human irritants, menthol (50%), and p-xylenol (10%) were negative for both irritation and sensitization at the highest concentrations tested. (These studies were supported in part by the NIOSH/NIEHS interagency agreement # Y02ES10189.)

1479 USE OF THE MURINE LOCAL LYMPH NODE ASSAY TO ASSESS THE RELATIVE SKIN SENSITIZATION POTENCY OF TWO PROTEIN COUPLING REAGENTS.

J T Kozak[1], M Inlow[1], B D Naumann[2], T T Kawabata[2], and R V House[1]. [1]*IIT Research Institute, Chicago, IL and* [2]*Merck & Co., Inc., Whitehouse Station, NJ.*

The murine local lymph node assay (LLNA) was used to evaluate the contact allergenic potency of two protein coupling reagents, N,N-dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide (EDC). Using a standard LLNA protocol, groups of five female CBA/J mice each were treated with concentrations ranging from 0.01, 0.03, 0.1, 0.3 and 1.0% DCC in isopropyl acetate and 0.1, 0.3, 1.0, 3.0 and 10% EDC in acetone/distilled water (1:1). Range-finding irritation studies employing clinical observations and ear thickness measurements were used to guide dose selection. Irritation was also monitored in the main studies. Draining auricular lymph nodes were evaluated in individual animals and a concurrent positive control (DNCB, 0.1%) was included. Separate groups of five animals each

An Official Journal of the
Society of Toxicology
Supplement

38th ANNUAL MEETING

TOXICOLOGICAL SCIENCES

Formerly Fundamental and Applied Toxicology

The Toxicologist

Oxford University Press

Volume 48, Number 1-S, March 1999

The Toxicologist

An Official Publication of the Society of Toxicology

and

Abstract Issues of

TOXICOLOGICAL SCIENCES

An Official Journal of the Society of Toxicology

Published by Oxford University Press, Inc.

Abstracts of the 38th Annual Meeting

Volume 48, Number 1-S

March 1999