ABSTRACT

Erythema is a hallmark skin response to excessive ultraviolet radiation (UVR) and is associated with cutaneous inflammation. Both are mediated by inflammatory mediators including nitric oxide (NO), prostaglandin E_2 (PGE_2) and chemoattractants such as 12-hydroxyeicosatetraenoic acid (12-HETE) leading to vasodilation and increased leukocyte infiltration. The erythematous response is more pronounced in individuals with low basal melanin levels or who fail to respond to UVR with a robust up-regulation of melanogenesis. While melanin production is a key function of melanocytes, these cells can also produce NO and PGE_2, and are located in close proximity to the dermal vasculature. It has been hypothesized that melanocytes with poor melanogenic capacity may participate in the inflammatory response to UVR.

The aim of this project was to investigate the inflammatory response in the skin of individuals with either skin phototype (SPT) 1 or 4 to UVR. Sixteen normal healthy individuals were selected for study (8 SPT-1 & 8 SPT-4). Buttock skin was investigated by immunohistochemistry for leukocyte subtypes, eicosanoid producing enzymes and NO synthases under basal and UVR-stimulated conditions. In addition primary cultures of epidermal melanocytes (EM) were established from 16 individuals (8 SPT-1 & 8 SPT-4) and assessed for the presence of eicosanoid-producing enzymes, melanogenic enzymes and NO synthases, by immunocytochemistry, Polymerase Chain Reaction and Western Blotting and for the production of the main pro-inflammatory eicosanoid PGE_2 by ELISA and Mass Spectrometry. Moreover, the fatty acid composition of cultured melanocytes was assessed by Gas Chromatography.

Results showed that individuals with SPT-1 had significantly greater neutrophil infiltration into the epidermis than those with SPT-4 at 24 hrs post-UVR. Moreover,

$CD3^+$ lymphocyte infiltration into the dermis was significantly greater in individuals with SPT-4 than those with SPT-1 at 24 and 72 hrs post-UVR. NOS-1, NOS-3, 12-LOX and COX-2 expression were significantly increased in SPT-1 skin, while NOS-2 and 15-LOX were significantly increased in SPT-4 skin. As 12-LOX and COX-2 products are chemoattractive (for neutrophils) and pro-inflammatory respectively these data could explain the greater observed neutrophil infiltration in SPT-1. The 15-LOX product (15-HETE) is anti-inflammatory and may suggest that 15-LOX up-regulation in SPT-4 skin may aid resolution of the sunburn response, which in part may be mediated by $CD3^+$ lymphocytes and a class-switch in eicosanoid production from COX to LOX products.

Melanocyte primary cultures surprisingly showed that SPT was not correlated with melanin content or melanogenic enzyme expression/activity suggesting that all melanocytes *in vitro* contained the necessary cellular machinery to produce melanin. This finding may reflect also their equal treatment under these enriched culture conditions, which may or may not be available to these cells *in situ*. Moreover, all melanocytes expressed the necessary machinery (PLA_2, COX-1, cPGES) to produce PGE_2. However, only some cultures did so at baseline and in response to UVR, and this was not correlated with SPT. A positive correlation was found however between expression level of dopachrome tautomerase (DCT) and protection against PGE_2 production in response to UVR, which may suggest a novel role for DCT unrelated to melanogenesis.

In summary this research project has generated data that highlights differences between the skin of individuals with SPT-1 and those with SPT-4, and may provide evidence that the keratinocyte partner contributes significantly to the SPT-associated response. This research may also suggest DCT as a novel therapeutic target to protect EM from participation in the UVR-associated inflammatory response in skin.

KEYWORDS

Erythema, ultraviolet radiation, skin, inflammation, prostaglandin E_2, leukocyte, melanocyte, melanin, phototype, dopachrome tautomerase.

SUPERVISOR NAMES

Prof. D. J. Tobin

Prof. A. Nicolaou