Skin Immune Systems and Inflammation: Protector of the Skin or Promoter of Aging?

Mary F. Bennett[1], Michael K. Robinson[2], Elma D. Baron[1] and Kevin D. Cooper[1]

The immune system may either have a protective role against sunburn and skin cancer or, conversely, promote solar damage. The skin is poised to react to infections and injury, such as sunburn, with rapidly acting mechanisms (innate immunity) that precede the development of acquired immunity and serve as an immediate defense system. Some of these mechanisms, including activation of defensins and complement, modify subsequent acquired immunity. An array of induced immune-regulatory and pro-inflammatory mediators is evident, at the gene expression level, from the microarray analysis of both intrinsically aged and photoaged skin. Thus, inflammatory mechanisms may accentuate the effect of UV radiation to amplify direct damaging effects on molecules and cells, including DNA, proteins, and lipids, which cause immunosuppression, cancer, and photoaging. A greater understanding of the cutaneous immune system's response to photo–skin interactions is essential to comprehensively protect the skin from adverse solar effects. Sunscreen product protection measured only as reduction in redness (current "sun" protection factor) may no longer be sufficient, as it is becoming clear that protection against UV-induced immune changes is of equal if not of greater importance. Greater knowledge of these processes will also enable the development of improved strategies to repair photodamaged skin.

Journal of Investigative Dermatology Symposium Proceedings (2008) **13,** 15–19; doi:10.1038/jidsymp.2008.3

INTRODUCTION

Aging is a multistep process resulting in a decline in biological functions and the ability to adapt to metabolic stress. The skin and the immune system are both subject to chronological aging. In addition, in sun-exposed sites, the skin is also subject to photoaging. Clinically, these changes in the skin manifest as mottled dyspigmentation, sagging of the skin, wrinkling, fragility, loss of elasticity, easy bruisability, accumulation of precancerous lesions, and epithelial and melanocytic neoplasms. The normal skin immune system protects the skin from infection, provides surveillance for emerging cancers, removes damaged cells, and prevents undesirable autoimmune reactions against self proteins. However, upon sun exposure of the skin, the immune system may also promote the aging process. UV-induced inflammatory reactions may participate in the promotion of skin aging through reactive oxygen species (ROS) generation, proteolytic enzyme production, suppressed immune surveillance, and production of growth factors for incipient cancer cells.

HISTOLOGY OF AGING AND PHOTOAGING SKIN

Epidermal changes of chronological aging include thinning of the stratum spinosum and flattening of the dermo-epidermal junction (Wulf *et al.*, 2004). In contrast to sun-protected epidermis, epidermal thickness is increased in sun-exposed skin (El Domyati *et al.*, 2002). Although the mechanism of thickness is unknown, senescent keratinocytes and melanocytes may become resistant to apoptosis and survive for a long time giving time for DNA damage to accumulate, with UV inflammation-associated growth factors contributing to tumor promotion (Bode and Dong, 2003). Melanocyte growth (development of nevi, solar lentigo, and lentigo maligna) or decrease (idiopathic guttate and hypomelanosis) in photo-exposed areas may be due to direct apoptosis or immunological activity. The number of dendritic Langerhans cells (LCs) also decreases with age, and the cells have fewer dendrites and reduced antigen-trapping capacity (Grewe, 2001; Wulf *et al.*, 2004). Both acute and chronic UV exposures further deplete this population (Delo *et al.*, 1981; Gilchrest *et al.*, 1983; Thiers *et al.*, 1984; Toyoda and Bhawan, 1997).

The dermis of aged skin contains decreased numbers of fibroblasts, which have a reduced capacity to produce type I procollagen when compared with the cells in young skin (Varani *et al.*, 2000). With chronological aging, cross-linking of collagen is increased as a result of an enzyme-controlled process and a non-enzymatic glycation process (Bailey *et al.*, 1998), and is associated with elevated levels of partially degraded collagen. Increased collagen fragments is also a

[1]*Department of Dermatology and Skin Diseases Research Center, University Hospitals Case Medical Center, Case Western Reserve University, Cleveland, Ohio, USA and* [2]*The Procter & Gamble Company, Cincinnati, Ohio, USA*

Correspondence: Dr Kevin D. Cooper, Professor and Chairman, Department of Dermatology, University Hospitals Case Medical Center, Case Western Reserve University, 11100 Euclid Avenue Lakeside 3516, Cleveland, Ohio 44106, USA. E-mail: Kevin.Cooper@uhhospitals.org

Abbreviations: CHS, contact hypersensitivity; DLN, draining lymph node; LC, Langerhans cells; Mph, macrophage; ROS, reactive oxygen species

Received 8 May 2007; revised 8 August 2007; accepted 8 September 2007

prominent feature in photodamaged skin. Procollagen gene and protein expression are significantly reduced in the upper one-third of the dermis, likely reflecting the depth of penetration of UV irradiation (Talwar *et al.*, 1995). Following chronic UV exposure, the composition of collagen changes with the increased levels of type III collagen (Plastow *et al.*, 1987). In the photoaged dermis, large quantities of abnormal, thickened, tangled, and nonfunctional elastic fibers are seen, which eventually degenerate into a nonfibrous, amorphous mass—a finding known histologically as solar elastosis (Kligman and Kligman, 1986).

CHRONOLOGICAL AGING AND PHOTOAGING MECHANISM

Fisher *et al.* (2002) have extensively reviewed the mechanisms of photoaging. They concluded that chronological aging and photoaging share fundamental molecular pathways. Converging cellular machinery that mediates aging and UV damage to human skin connective tissue includes cell-surface receptors, protein kinase signal transduction pathways, transcription factors, and enzymes synthesizing and degrading structural proteins in the dermis, which confer strength and resiliency to skin. UV-induced photochemical generation of ROS causes direct deleterious chemical modifications to cellular components (that is, DNA, proteins, and lipids) and also recruits cellular machinery that further damages skin connective tissue.

Immune aging and photoaging

The immune system of aged animals and humans undergoes alterations that may account for an increased susceptibility to certain infections, autoimmune diseases, and malignancies. With advancing age, human and murine T cells reveal reductions in the proliferative response to activation, in diversity of the T-cell receptor antigen repertoire, and in cytolytic activity. B cells of aging individuals show a restricted diversity of their antibody repertoire, resulting in a reduced response to certain viral infections or vaccinations. In addition to a reduction in LCs, there also appears to be a reduced LC migration in aged mice in response to allergen (that is, fewer Class II + cells leave the ear epidermis 4 hours after challenge), and a reduced draining lymph node (DLN) cellularity (fewer dendritic cells are migrating to the lymph node after skin challenge) (Cumberbatch *et al.*, 2002). These data from murine studies suggest that contact hypersensitivity (CHS) is not necessarily reduced with age. Is the picture any clearer in humans? Recent data have suggested that LCs, in the state of differentiation that they display within the epidermis, may, in fact, play a role in limiting cutaneous inflammation, and not strictly as inducers of hypersensitivity (Romani *et al.*, 2006; van der Aar *et al.*, 2007). Thus, LC dysfunction with age may not result in reduced induction of hypersensitivity responses, and could potentially play a role in increased autoimmunity that occurs with age. Interestingly, data from our laboratory indicate that within the ages of 18–65 years, CHS responses to cutaneous allergen dinitrochlorobenzene are unaffected by age, in a system in which dinitrochlorobenzene sensitization was performed on sun-protected sites (Morrissey *et al.*, 2008). In the same study, no age-related increased susceptibility to immune suppression by UV was seen when simulated solar radiation was administered to the sensitization site before application of the sensitizer. Other studies have shown that a decrease in LC numbers is only detectable after 70 years of age, which may account for these findings (Bhushan *et al.*, 2002, 2004).

UV ALTERATION OF IMMUNITY AND INFLAMMATION

Triggering of innate immunity

Defensins, small cationic proteins active against bacteria, fungi, and enveloped viruses, are produced and released by keratinocytes in response to UV (Seo *et al.*, 2001). They are a component of the rapid innate immune response, important not only for skin resistance to infection, but also for regulation of subsequent adaptive immune responses (Bowdish *et al.*, 2006). Urocanic acid, a component of the stratum corneum, is isomerized by UV into an immunosuppressive compound (Walterscheid *et al.*, 2006). Complement, notably C3, which is produced in the skin, has been implicated to be crucial to the generation of UV immunosuppression and antigenic tolerance (Hammerberg *et al.*, 1998), and can recruit inflammatory leukocytes. Neutrophils quickly infiltrate sunburned skin (McGregor and Hawk, 1999), and it has been suggested that they participate in the process of photoaging through the release of enzymatically active elastase, collagenase (MMP-1), and gelatinase (MMP-9) (Rijken *et al.*, 2005). Monocyte/macrophages (Mphs) dominate the infiltrate after a few hours and participate in additional generation of ROS as well as proteolysis, cytokines/growth factors, oxidized cell phagocytosis, and immunosuppression (Cooper *et al.*, 1986; Hammerberg *et al.*, 1994; Kang *et al.*, 1998).

UV immunosuppression of adaptive immunity

The two most common experimental models employed to study integrated *in vivo* immune responses after UV exposure in humans and animals are CHS and delayed-type hypersensitivity (Fourtanier *et al.*, 2005). UV is known to suppress CHS and delayed-type hypersensitivity responses very effectively, and epicutaneous application of normally highly immunogenic haptens to UV-exposed skin can induce hapten-specific tolerance, rather than hypersensitivity. UV-impaired cell-mediated adaptive immune responses are thought to depress tumor surveillance, resulting in decreased ability to detect and reject incipient cells expressing UV-mutated proteins (tumor antigens), thus permitting tumor progression to cancer. Studies using the CHS model have shown that suppression can be mediated through antigen-specific T regulatory (Treg) cells that are stimulated through antigen-presentation by UV-altered antigen-presenting cells present in the lymph nodes draining irradiated skin (Schwarz *et al.*, 2004). The DLNs contain macrophagic cells that migrate from the UV-exposed skin upon antigen challenge, with altered IL-12/IL-10 profiles characteristic of the skin monocytic cells (Toichi *et al.*, 2002). Treg cells have been shown to be induced by UV irradiation (Schwarz *et al.*, 2004). It is, however, uncertain whether Treg cells are generated in the DLNs of UV-irradiated skin or migrate there from the skin. rIL-12 can prevent both local and systemic UV-induced immunosuppression (Muller *et al.*, 1995; Schmitt *et al.*, 1995). Anti-IL-10 antibody treatment before UVB

exposure has been reported to prevent UV-induced tolerance (Niizeki and Streilein, 1997). DLN IL-10 is upregulated and IL-12 is downregulated with exposure to a hapten after UV irradiation, and the responsible cell type appears to be the DLN macrophages that have migrated from the skin (Toichi *et al.*, *J Invest Dermatol* (in press) (MS# JID-2007-0607.R1)).

IMMUNOCELLULAR LINKAGE TO INFLAMMATORY DAMAGE AND ROS

Infiltration and activation of myeloid monocytic cells and macrophages

Histologically, chronically sun-exposed skin contains more infiltrating mononuclear cells than the sun-protected skin (Bosset *et al.*, 2003). Immunohistochemical analysis showed an increased number of mast cells, Mphs, and CD4+ CD45RO+ T cells in chronically sun-exposed dermis and a higher number of CD1a+ dendritic cells in sun-exposed epidermis (Bosset *et al.*, 2003), a finding which is in contrast to the changes in skin shortly after acute UV. Chronic solar damage is the result of repeated acute injuries, and one can propose that the chronic infiltrates derive from repeated cycles of leukocytic infiltration after each exposure. The population of cells that influx into skin 6–72 hours post UV irradiation of human skin are myeloid monocytic cells, many of which undergo differentiation into activated Mphs (Hammerberg *et al.*, 1994; Meunier *et al.*, 1995). These cells are a major source of immunosuppressive IL-10, but they fail to secrete Th1-driving IL-12 (Kang *et al.*, 1998), and play a critical role in the UV-induced immune suppression and tolerance (Hammerberg *et al.*, 1994). IL-12, a cytokine produced by dendritic cells (depleted by UV), critical for the maturation of TH1 cells and induction of CHS, can restore immune function in UV-exposed mice rendered antigen-tolerant (Schwarz *et al.*, 1996). Activated Mphs may be needed to phagocytose UV-induced apoptotic cells, and to kill skin cells with oxidized surface lipids (CD36 recognition) that may harbor UV-DNA damage. Their production of IL-10 may help to downmodulate inflammation and prevent autoimmunity. These cells likely represent a blood-derived population of newly infiltrating monocytic cells that undergo transient arrest from dendritic cell differentiation with concomitant promotion of differentiation toward phagocytic, activated Mphs (Yoshida *et al.*, 1998; Takahara *et al.*, 2003).

A key connection between inflammatory damage and innate and adaptive immune modulation is that the above-mentioned activation of complement C3 into iC3b ligates the monocytes' β2-integrin receptors, thus, transforming $CD11b^+$ monocytic cells into activated Mphs with induced matrix metalloproteinases and ROS-generating activity (Takahara *et al.*, 2002). Initially, UV-damaged LCs move to the DLNs, but within a few hours, the main antigen-presenting cell carrying antigen to DLNs is the tolerance-inducing, IL-10 high, IL-12 low, monocytic/macrophagic population activated through CD11b and other UV-induced skin cytokines critical for immunosuppression, such as IL-6 (Toichi *et al.*, 2002; Lu *et al.*, 2005).

ROS

Generation of ROS (that is, superoxide anion, peroxide, and singlet oxygen) is a well-documented effect of UV exposure in skin (Fisher *et al.*, 2002). UVA and, to a lesser extent, UVB can damage DNA indirectly through the generation of ROS (Cadet *et al.*, 2000). UVA induces a base-pair deletion in mitochondrial DNA found in fibroblasts as a consequence of ROS (Berneburg *et al.*, 1999). Activated inflammatory cells, as described above, are also potent producers of ROS and further contribute to oxidative damage, as evidenced by the finding that these cells produce ROS (Katiyar and Mukhtar, 2001) and that epidermal histological damage post UV irradiation is attenuated when CD11b signaling to the monocytic cells is blocked (Hammerberg *et al.*, 1994).

There is increasing evidence that UV-induced ROS mimic the actions of receptor ligands (Rabe *et al.*, 2006). Receptors for IL-1, EGF, and tumor necrosis factor α are activated in keratinocytes and fibroblasts (Fisher *et al.*, 2002). It is postulated that ROS through oxidation inhibits protein-tyrosine phosphatases, which function to downregulate these receptors (Gross *et al.*, 1999; Rabe *et al.*, 2006). Various transcription factors are thus brought into play including activator protein-1 and nuclear factor-kappa B (Fisher *et al.*, 1998; Reelfs *et al.*, 2004). Activator protein-1 controls the transcription of matrix metalloproteinases. Nuclear factor-kappa B activation results in the production of a range of immunomodulatory cytokines. Activated $CD11b^+$ Mph ROS may inflict damage on keratinocytes already strained to the limit of their ROS defense systems.

APPLICATION OF PROTEOMICS, GENOMICS, AND BIOINFORMATICS

Because of the complexity of the UV-injury/photoaging cascade, systems biology approaches, such as proteomics and genomics, allow a more comprehensive assessment of these processes. The standard method of evaluating a sunscreen's effectiveness is to measure the sun protection factor, whose value reflects protection from developing erythema only. Immune protection factor should be considered as an important adjunctive measure of a sunscreen's effectiveness, as it is known that immunosuppression occurs at suberythemogenic doses. Studies of global genetic and proteomic expression will help to identify potential biomarkers of UV-induced immunosuppression that can be used for the measurement of immune protection factor.

UV-induced generation of ROS and subsequent inflammatory reactions create a milieu in which proteins and lipids are altered through oxidation, a process more amenable to study by proteomics than gene expression. Proteomic profiles of immortalized keratinocytes in response to UV alone, dinitrobenzenesulfonic acid alone, and combined exposures showed a marked upregulation of membrane NOX5 (a potent producer of ROS), redox proteins, and cytosolic calmodulin (which functions as a switch in response to oxidative stress) when cells were treated with the immunosuppressive sequence of UV followed by antigen (dinitrobenzenesulfonic acid), but not with either agent alone (Liu *et al.*, 2005). These data are indicative of overwhelmed oxidative defenses. Thus, we envision at least two sources of various ROS that skin cells are exposed to after UV irradiation: direct photon-induced ROS from lipid and other macromolecule

photophysical interactions, and keratinocyte or fibroblast production of ROS.

Protein signaling and mediators from oxidation necessarily result in gene expression changes to modify the tissue after repeated UV injury. In a gene expression analysis (Robinson *et al.*, 2007), biopsies were taken from sun-protected (buttocks) and sun-exposed (outer forearm) body sites from 10 young women (aged 18–20 years) and 10 older women (aged 60–67 years). The older women had moderate-to-severe forearm photodamage. All biopsies were processed for gene expression analysis using Affymetrix U133 plus 2.0 microarrays (Affymetrix Inc., Santa Clara, CA), covering transcripts from the entire human genome. The numbers of significant gene changes between age group/body site comparisons relevant to chronological (intrinsic) aging and various photoaging comparisons were as follows:

1. Chronological Aging: Young Buttock versus Old Buttock: 7,215 (854)
2. Photo-Aging: Comparison 1: Young Arm versus Old Arm: 13,640 (3,730)
3. Comparison 2: Young Buttock versus Old Arm: 9,930 (1,995)
4. Comparison 3: Old Buttock versus Old Arm: 11,216 (1,966)

The number of significant genes across the entire microarray expected by chance is 2,713 ($54{,}613 \times 0.05 = 2{,}731$). The numbers in parentheses indicate number of significant gene expression changes after additional filtration to eliminate poorly expressed genes and to select those genes differentially expressed by at least 50% (up or down) in each of the comparisons.

To identify overarching themes associated with the two aging processes, those gene ontology terms statistically over-represented among genes associated with chronological aging and various photoaging comparisons were determined. Many themes were common across all comparisons, although to differing extents. Immune and inflammatory (defense) response was the dominant theme with most of the genes upregulated in old versus young or sun-exposed versus sun-protected skin. This effect was more significant in old sun-exposed skin, and more immune- and inflammation-related terms were associated with this aging process, although specific gene expression changes suggested some commonality in the immune and inflammatory pathways underlying both aging processes. Extracellular matrix genes were also upregulated in both, although photoaging was more commonly associated with collagen gene changes and intrinsic aging with keratin gene changes. Both processes were also associated with upregulation of protease/peptidase activity. Deeper analysis of biological pathways interconnecting immune and inflammatory responses, matrix structural elements, proteolytic, and oxidative processes, among others, may provide new insights into the processes of skin aging and suggest novel ways to combat it.

CONCLUSIONS

Both cell biology and systems biology approaches emphasize that inflammatory mechanisms may accentuate the effect of solar radiation UV photons to amplify direct damaging effects on molecules and cells, including DNA, proteins, and lipids, which causes immunosuppression, cancer, and photoaging. These new insights will lead to the development of therapies and preventative measures directed towards immunologic mechanisms. For example, it is clear that traditional measurement of a sunscreen's effectiveness, to prevent erythema, the sun protection factor, is not an adequate indicator of immune protection (that is, different action spectra). Immune suppression occurs at suberythemogenic doses; therefore, sun protection factor may overestimate protection against UV-induced immune suppression. Thus, this measure of immune protection is likely to be inadequate. Determination of the immune protection factor has been proposed as an alternative or adjunctive measure to sun protection factor and current *in vitro* ''broad-spectrum'' or UVA methods. Antioxidants and immunomodulators will have an ever-increasing role in the prevention and treatment of photoaging and photocarcinogenesis as knowledge in this area expands. Although the immune system is poised to defend our skin from environmental hazards, perhaps cumulative damage occurs due to the oxidative effects of, not only UV, but also as a by-product of our host immunity.

CONFLICT OF INTEREST

K.D. Cooper received an honorarium for consultant's services from Procter & Gamble towards the preparation of this article. M.K. Robinson is an employee of Procter & Gamble.

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