Published in final edited form as:

Mol Cell Endocrinol. 2008 January 2; 280(1-2): 6–12.

S179D prolactin diminishes the effects of UV light on epidermal gamma delta T cells

Esther A. Guzmán¹, John L. Langowski¹, Ariel De Guzman², H. Konrad Muller³, Ameae M. Walker^{*2}, and Laurie B. Owen²

¹The University of Texas, Houston, Graduate School of Biomedical Sciences, Houston, Texas 77030

²Division of Biomedical Sciences, University of California, Riverside, CA 92521

³Discipline of Pathology, University of Tasmania, Hobart 7000, Australia

Abstract

Epidermal gamma delta T cells ($\gamma\delta$ T) and Langerhans cells (LC) are immune cells altered by exposure to ultraviolet radiation (UVB), a powerful stressor resulting in immune suppression. Prolactin (PRL) has been characterized as an immunomodulator, particularly during stress. In this study, we have asked whether separate administration of the two major forms of prolactin, unmodified and phosphorylated, to groups of 15 mice (3 experiments, each with 5 mice per treatment group) affected the number and morphology of these epidermal immune cells under control conditions, and following UV irradiation. Under control conditions, both PRLs reduced the number of $\gamma\delta$ T, but a molecular mimic of phosphorylated PRL (S179D PRL) was more effective, resulting in a 30% reduction. In the irradiated group, however, S179D PRL was protective against a UV-induced reduction in $\gamma\delta$ T number and change in morphology (halved the reduction and normalized the morphology). In addition, S179D PRL, but not unmodified (U-PRL), maintained a normal LC: $\gamma\delta$ T ratio and sustained the dendritic morphology of LC after UV exposure. These findings suggest that S179D PRL may have an overall protective effect, countering UV-induced cellular alterations in the epidermis.

Keywords

S179D prolactin; ultraviolet light; gamma delta T cells; Langerhans cells

Introduction

Ultraviolet (UVB) radiation in sunlight is the primary carcinogen for the development of skin cancers. It is absorbed mostly by the epidermis where it creates damage (Everett *et al*, 1966) and triggers immunosuppressive mechanisms known to be critical in cutaneous tumorigenesis (Fisher *et al*, 1977).

Resident immune cells of the skin include Langerhans cells (LC) and gamma delta T lymphocytes ($\gamma\delta$ T cells). LC are potent antigen-presenting cells, which take up antigen and migrate to regional lymph nodes to stimulate T cell responses (Stingl *et al*, 1978). Following

*Corresponding author: Ameae M. Walker, Division of Biomedical Sciences, University of California, Riverside, California 92521. Tel: 951-827-5942; Fax 951-827-5504; E mail: ameae.walker@ucr.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

UV-irradiation, LC round up, lose their dendrites, and have impaired antigen-presenting abilities (Toews *et al*, 1980; Simon *et al*, 1991). Their number in the epidermis declines (Dandle *et al*, 2001), but they are rapidly replaced from circulating precursors (Merad *et al*, 2002). Epidermal $\gamma\delta$ Ts express predominantly one $\gamma\delta$ T cell receptor variety which is produced only during fetal life (Ikuta *et al*, 1990). Although the function of the $\gamma\delta$ Ts is less well understood, they appear to have an important role in controlling inflammation (Girardi *et al*, 2002), and in wound healing (Jameson *et al*, 2002). In addition, an increased number of malignancies in mice deficient in $\gamma\delta$ Ts suggests a protective role against skin carcinogenesis (Girardi *et al*, 2001). The effects of UV on $\gamma\delta$ Ts are controversial. Though their number in the epidermis is decreased following UV, some investigators have subsequently found them in the draining lymph nodes (Dandle *et al*, 2001), while others have not (Sontag, *et al*, 1995). Unlike LCs, $\gamma\delta$ Ts are not rapidly replenished after being depleted from the skin, taking weeks to reappear (Aberer *et al*, 1986; Alcalay *et al*, 1989) since they are derived from preexisting epidermal $\gamma\delta$ Ts and not from circulating bone marrow-derived precursors.

Both psychological and physical stressors increase total circulating PRL (Almeida *et al*, 2000; Halford *et al*, 2003; Zelena *et al*, 2004) and PRL has been shown to be an immunomodulator (reviewed in Matera *et al*, 2000), although its function in this regard is most obvious under conditions of stress (reviewed in Dorshkind *et al*, 2001). PRL receptors have been demonstrated on B and T cells, as well as macrophages (Pellegrini *et al*, 1992; Dardenne *et al*, 1994; Hartmann *et al*, 1989). Moreover, PRL enhances the proliferation of T cells *in vitro* and promotes maturation of dendritic cells by increasing expression of co-stimulatory molecules and major histocompatibility (MHC) class II molecules (Matera *et al*, 1992; Matera *et al*, 2001). In addition, PRL has been shown to affect skin biology by enhancing squamous cell carcinoma formation in response to carcinogen (Lupulescu, 1985), a finding that suggests a possible effect on LC and $\gamma\delta$ T.

Prolactin can exist in a variety of posttranslationally modified forms (reviewed in Lorenson and Walker, 2001). In rodents, greater than 95% of PRL exists as either an unmodified or a phosphorylated form (Ho *et al*, 1993 a,b). The presence of phosphorylated PRL in pituitary extracts or purified pituitary PRL has been directly demonstrated in human (Wu *et al*, 2003), ovine, bovine, rat, mouse and turkey pituitary extracts (Oetting *et al*, 1986; Brooks *et al*, 1990; Aramburo *et al* 1992). The proportion of total PRL that is phosphorylated is physiologically regulated (Ho *et al*, 1993 a,b), with states of high estrogen resulting in both increased PRL and reduced phosphorylation. Most studies to date have used a mixture of both forms of PRL since these are both present in the hormone standards distributed by the NIDDK or those available from commercial sources (e.g. Wu *et al*, 2003, Wang and Walker, 1993). However, studies in which the two major forms of PRL have been individually analyzed have shown them to have very different biological functions. Some of these studies used the naturally phosphorylated material (Krown *et al*, 1992, Wang and Walker, 1993), while others utilized a stable molecular mimic of phosphorylated PRL, S179D PRL (Chen *et al*, 1998; Xu *et al*, 2001; Yang *et al*, 2001; Kuo *et al*, 2002; Yang *et al*, 2002; Schroeder *et al*, 2003; Wu *et al*, 2003; Xu *et al*, 2003; Naylor *et al*, 2005; Guzman *et al*, 2005; Ueda *et al*, 2006a,b; Wu *et al* 2006). In the current study, we assessed the effects of either U-PRL or S179D PRL on LC and $\gamma\delta$ T in mature skin, in the absence or presence of a suberythemal dose of UVB. The mimic of phosphorylated PRL was used in preference to the natural form primarily because it cannot be dephosphorylated during the course of an experiment. Dephosphorylation of the natural form although a slow process (Wang and Walker 1993) would result in the production of U-PRL and hence would negate differences in biological activity between the two forms. The mimic was made by substituting the normally phosphorylated serine (Wang *et al*, 1996; Tuazon *et al*, 2002) with an aspartate residue, thereby producing S179D PRL (Chen *et al*, 1998). Molecular mimicry of a phosphoserine by an aspartate has become commonplace and several studies examining the three-dimensional structure and activity of the phosphorylated *versus*

aspartate versions of molecules have shown them to be very comparable (e.g. Thorsen and Koshland, 1987). In the case of PRL, S179D PRL inhibits the proliferation of Nb2 cells and GH3 cells like naturally phosphorylated PRL (Wang and Walker, 1993; Chen et al., 1998; Krown et al., 1992), it signals like phosphorylated PRL (Coss et al., 1999, Wu et al., 2003) and it promotes β -casein expression (Wu et al., 2003).

Materials and methods

Treatment of animals

Pathogen-free male C3H/HeJ mice were obtained from the Jackson Laboratories (Bar Harbor, MD). Animals were housed in cages with controlled temperature and humidity and alternating 12-h light and dark cycles. The animals were maintained in facilities approved by the Association for the Assessment and Accreditation of Laboratory Animal Care and all procedures were approved by an institutional committee. Commercial diet and water were given *ad libitum*. Unmodified recombinant PRL (U-PRL) and the mimic of phosphorylated PRL, S179D PRL, were produced and characterized as previously described (Chen *et al.*, 1998). In a series of 3 replicate experiments, groups of 5 adult male mice per treatment group (in two instances in the 3 experiments one of the six groups per experiment contained 3 mice) were shaved on their dorsum and one day later Alzet osmotic pumps (Durect; Cupertino CA) containing 200 μ l of either saline, U-PRL, or S179D PRL (latter two at 0.4 μ g/ml saline to deliver 2.4 μ g/24h/mouse) were implanted subcutaneously through a small incision in the shaved skin. Closure was with surgical staples, which were removed five days later. Mice were re-shaved on their dorsum 10 days post pump implantation and irradiated with UV the following day. For irradiation, mice were placed in shallow Plexiglas boxes with multiple dividers and mesh tops. UVB exposure was delivered by a bank of six FS40 sunlamps (280-360 nm, peak at 312nm; Philips). Irradiance was measured with an IL-1400 radiometer and a SEL240 detector with UVB filter (International Light, Inc; Newburyport MA). Average UVB output from FS40 sunlamps was 809 μ W/cm². Mice were given a suberythral dose of 3 KJ/m² UVB. As the term implies, this dose is insufficient to cause sunburn in this strain of mice (the dose required differs with strain). Some cell damage is likely, but a degree of damage just below the threshold needed to induce an inflammatory response. The dose is sufficient to result in immune suppression in this strain of mice and is equivalent to a midsummer midday 15-20 min exposure to the sun (reviewed in Guzman et al., 2003). Multiple exposures at this intensity would normally be required to produce tumors within a 30 week period in mice (Ouhtit et al., 2000) or during the lifetime of an individual.

Immunohistochemistry

Skin samples, at a minimal distance of 2 cm from the site of pump implantation, were obtained 20 days post-implantation; 11 days post UV-irradiation. This time point was selected to concentrate analysis on longer term effects more relevant to carcinogenesis. At this time point, effects on $\gamma\delta$ T cells should still be evident since they exhibit a long recovery time after UV exposure. By contrast, effects on LC should be negated since this is sufficient time for repopulation of the epidermis from circulating precursors (Merad *et al.*, 2002). After removal of subcutaneous tissue, skins were placed in a 20mM buffered EDTA solution. Following a 1½-h incubation at 37°C for non-irradiated skin and a 1-h incubation for UV-irradiated skin, the epidermal layer was mechanically separated and fixed in ice-cold acetone. Staining of epidermal sheets was with cell-surface marker anti-CD205 (Serotec; Oxford UK) for LC or anti-Thy1.2 (BD Pharmingen; San Diego CA) for $\gamma\delta$ T followed by goat-anti-rat horseradish peroxidase (Jackson ImmunoResearch Laboratories; West Grove PA) and diaminobenzidine as substrate (Research Genetics, Invitrogen; Carlsbad CA). Controls for non-specific staining and peroxidase activity utilized isotype matched immunoglobulins and the same second antibody. Epidermal sheets were mounted on slides using Immu-mount (Shandon; Pittsburgh PA). For

each epidermal sheet, analysis was blinded, 6 random fields were photographed at 400x magnification, positively stained cells were counted, and the number per high power field averaged. During this process, cells were classified as “normal,” “intermediate,” or “rounded” according to the number of dendrites. Rounded cells were those with no dendrites, intermediate cells were those with one dendrite or two very foreshortened dendrites, and normal cells were those with more than one normal length dendrite. Since these were epidermal sheets and not sections, all dendrites were seen and could therefore be appropriately quantified. Numerical data are presented in the figures as the mean of three successive experiments $\pm$ standard error. Statistical significance was determined by two-way analysis of variance and least squares analysis using SYSTAT software, version 10.2 in collaboration with Dr Edward Proffitt (Harbor Branch Oceanographic Institution, Ft Pierce, FL).

Results

In non-irradiated animals, the two forms of PRL reduced the number of $\gamma\delta$ T (figures 1A and B and table 1), but had no significant effect on the overall morphology of those remaining. Of the two PRLs, S179D PRL was more effective at reducing cell number, with U-PRL reducing by 16% and S179D PRL reducing by 30%. As expected, UV exposure decreased the number of $\gamma\delta$ T in the control animals (by about 42%), but contrary to what one might have expected given the result in non-irradiated animals, S179D PRL ameliorated this effect (reduced the effect by about half such that the reduction in response to UV was $\sim$ 23%). There was some indication of a lesser, but similar effect of U-PRL, but this was not statistically significant.

In non-irradiated animals, neither PRL had any effect on the morphology or number of LC (figures 2A and B and table 1). In irradiated animals, one would normally expect a decrease in LC number. However in this study, the experiment was designed to allow repopulation of the skin with LC after UV irradiation. The lack of effect of UV on LC number in the control animals was therefore anticipated. U-PRL did not change this outcome, whereas S179D PRL reduced the number of LC (by about 18%). When LC are exposed to UV, they round up and migrate to a local lymphnode. One might therefore expect an increased number of rounded LC in response to S179D PRL. However, the exposure to UV was 11 days prior to sampling and so this phase would have occurred long before. Moreover, the most striking result was the reduced number of rounded LC in the S179D PRL-treated UV-irradiated group (reduced by about 50%). As a consequence of this reduction in LC, the ratio of LC to $\gamma\delta$ T was returned to that seen in control non-irradiated animals (1.13 for S179D PRL versus 1.07 for the controls). This is in contrast to the UV-exposed saline treated animals where the ratio rose to 1.7.

Discussion

Since UV is a stressor and PRL, which is elevated in response to stress, is an immunomodulator under stress, we determined whether either of the two major forms of PRL influenced skin immune cell number and morphology and response to UV. Either U-PRL or S179D PRL was administered to adult male mice as a chronic infusion to produce levels around 150 ng/ml in the circulation (Yang *et al*, 2001), thereby mimicking the elevated levels associated with significant chronic stress (Almeida *et al*, 2000).

The results show that both forms of PRL, but particularly S179D PRL, have the potential to affect skin immunity. In the non-irradiated state, the major effect of both PRLs was a reduction in the number of $\gamma\delta$ T. Since $\gamma\delta$ T have been shown to have cytotoxic effects on skin carcinoma cells (Ebert *et al* 2006, Girardi *et al* 2001), hyperprolactinemia may increase susceptibility to carcinogen-induced skin cancer, a suggestion in keeping with the work of Lupulescu (1985) who found enhanced squamous cell carcinoma formation in response to a carcinogen in the presence of increased PRL.

Under the stress of UV damage, however, a different picture emerges in which treatment with S179D PRL maintained $\gamma\delta$ T numbers. These cells are therefore available for cytotoxic removal of irreparably damaged epithelial cells. Since T cell receptor $\delta^{-/-}$ mice develop chronic dermatitis (Girardi *et al*, 2002), maintenance of $\gamma\delta$ T numbers may also keep inflammatory responses in check, thereby improving elimination of cancerous cells. In this regard, previous analysis has shown S179D PRL to reduce inflammatory cytokines during a delayed type hypersensitivity response to *Candida albicans* (Guzman *et al*, 2005).

Treatment with S179D PRL also reduced LC number after UV exposure. Others have suggested that it is not the absolute numbers of either LC or $\gamma\delta$ T that are important to normal skin immunity, but rather the ratio of LC to $\gamma\delta$ T, with as little as a 0.2 difference in the ratio affecting immune responses (Bigby *et al*). By reducing LC number, treatment with S179D PRL in the UV exposed group resulted in a ratio of 1.13 *versus* 1.07 in the non-irradiated saline controls. By contrast, UV exposure resulted in a ratio of 1.7 in the saline treated animals, a difference from non-irradiated skin that would be expected to result in abnormal immune responses. Since the experiment was designed such that LC had time to repopulate the skin after UV exposure, the reduced total number of LC after UV in this group could reflect a reduced repopulation of the skin by LC precursors. Since it is the rounded cells that are missing post UV, S179D PRL may have promoted the specific loss, and inhibited the repopulation of, a subset of LC. In this regard, there is some evidence for a specific subpopulation of rounded LC which are more mature in terms of their ability to migrate and present antigen (Morelli *et al*, 1996).

Horseman *et al* (1997) and Bouchard *et al* (1999) examined the role of PRL in immune function by assessing immune status in PRL or PRL receptor null mice, respectively. In both of these models no substantive immune dysfunction was observed in non-stressed animals. Under conditions of stress, however, the PRL null mouse showed significant immunodeficiencies compared to normal mice (Dugan *et al*, 2002). This demonstrates an important role for some form of PRL in stress-related immune modulation and provides a rationale for the elevation in PRL observed during stress (Almeida *et al*, 2000; Halford *et al*, 2003; Zelena *et al*, 2004). The particular stressor chosen for study by the Horseman laboratory was thermal injury to the skin (Dugan *et al*, 2002,2004a,b) and therefore involved both LC and $\gamma\delta$ T. A crucial role for $\gamma\delta$ T in the survivability of thermal injury to the skin has been described by Schwacha *et al* (2000). The current results demonstrating improved skin numbers and morphology of $\gamma\delta$ T after UV in the presence of S179D PRL therefore fit well together with these previous observations. What factors change the response of $\gamma\delta$ T to S179D PRL after UV exposure are unclear at present, but likely involve changes in local cytokine production by damaged keratinocytes.

It is clear that both rodents and humans produce phosphorylated PRL in the pituitary (e.g. Oetting *et al*, 1986; Wu *et al*, 2003) and so this is one potential source of this form of the hormone, with delivery to the skin *via* the circulation. However, PRL has also been demonstrated to be produced in the skin where it can act as an autocrine/paracrine factor (Foitzik *et al*, 2006). Unfortunately, the quantities produced in the skin make it currently impossible to determine whether any proportion of this PRL becomes phosphorylated. The relative contribution of each source of PRL to skin immunity therefore remains to be established. Also unknown at present is the ratio of unmodified to phosphorylated PRL found under conditions of hyperprolactinemia or chronic stress in the human population. What is known in rodents is that hyperprolactinemia due to prolactinomas is characterized by a lack of phosphorylation of the released PRL (Johnson *et al*, 2003; Krown *et al*, 1992).

The current findings suggest that S179D PRL may have an overall protective effect, countering UV-induced cellular alterations in the epidermis.

Acknowledgements

This work was supported in part by NIH grants CA 52457 (LBO) and DK 61005 (AMW) and by grants from the American Cancer Society (CIM 88929 to LBO), the California Breast Cancer Research program (10PB 127 to AMW) and Cancer Federation (AMW). The authors gratefully acknowledge expert animal husbandry by Ms Sally Scott.

References

- Aberer W, Romani N, Elbe A, Stingl G. Effects of physicochemical agents on murine epidermal Langerhans cells and Thy-1-positive dendritic epidermal cells. *J Immunol* 1986;136:1210–1216. [PubMed: 2868057]
- Alcalay J, Craig JN, Kripke ML. Alterations in Langerhans cells and Thy-1+ dendritic epidermal cells in murine epidermis during the evolution of ultraviolet radiation-induced skin cancers. *Cancer Res* 1989;49:4591–4596. [PubMed: 2568173]
- Almeida SA, Petenussi SO, Franci JA, Rosa e Silva AA, Carvalho TL. Chronic immobilization-induced stress increases plasma testosterone and delays testicular maturation in pubertal rats. *Andrologia* 2000;32:7–11. [PubMed: 10702860]
- Aramburo C, Montiel JL, Proudman JA, Berghman LR, Scanes CG. Phosphorylation of prolactin and growth hormone. *J Mol Endocrinol* 1992;8:183–191. [PubMed: 1632894]
- Bigby M, Kwan T, Sy MS. Ratio of Langerhans cells to Thy-1+ dendritic epidermal cells in murine epidermis influences the intensity of contact hypersensitivity. *J Invest Dermatol* 1987;89:495–499. [PubMed: 2889783]
- Bouchard B, Ormandy CJ, Di Santo JP, Kelly PA. Immune system development and function in prolactin receptor-deficient mice. *J Immunol* 1999;163:576–582. [PubMed: 10395643]
- Brooks CL, Kim BG, Aphale P, Kleeman BE, Johnson GC. Phosphorylated variant of bovine prolactin. *Mol Cell Endocrinol* 1990;71:117–123. [PubMed: 2376284]
- Chen TJ, Kuo CB, Tsai KF, Liu JW, Chen DW, Walker AM. Development of recombinant human prolactin receptor antagonists by molecular mimicry of the phosphorylated hormone. *Endocrinology* 1998;139:609–616. [PubMed: 9449632]
- Coss D, Kuo C-YB, Yang L, Ingleton P, Luben R, Walker AM. Dissociation of Janus kinase 2 and signal transducer and activator of transcription 5 activation after treatment of Nb2 cells with a molecular mimic of phosphorylated prolactin. *Endocrinology* 1999;140:5087–5094. [PubMed: 10537136]
- Dandle GW, Clydesdale GJ, Radcliff FJ, Muller HK. Migration of Langerhans cells and gamma delta dendritic cells from UV-B-irradiated sheep skin. *Immunol Cell Biol* 2001;79:41–48. [PubMed: 11168622]
- Dardenne M, de Moraes Mdo C, Kelly PA, Gagnerault MC. Prolactin receptor expression in human hematopoietic tissues analyzed by flow cytofluorometry. *Endocrinology* 1994;134:2108–2114. [PubMed: 8156910]
- Dorshkind K, Horseman ND. Anterior pituitary hormones, stress, and immune system homeostasis. *Bioessays* 2001;23:288–294. [PubMed: 11223886]
- Dugan AL, Thellin O, Buckley DJ, Buckley AR, Ogle CK, Horseman ND. Effects of prolactin deficiency on myelopoiesis and splenic T lymphocyte proliferation in thermally injured mice. *Endocrinology* 2002;143:4147–4151. [PubMed: 12239129]
- Dugan AL, Malarkey WB, Schwemberger S, Jauch EC, Ogle CK, Horseman ND. Serum levels of prolactin, growth hormone, and cortisol in burn patients: correlations with severity of burn, serum cytokine levels, and fatality. *J Burn Care Rehabil* 2004a;25:306–313. [PubMed: 15273472]
- Dugan AL, Schwemberger S, Babcock GF, Buckley D, Buckley AR, Ogle CK, Horseman ND. Effects of prolactin level on burn-induced aberrations in myelopoiesis. *Shock* 2004b;21:151–159. [PubMed: 14752289]
- Ebert LM, Meuter S, Moser B. Homing and function of human skin gamma delta T cells and NK cells: relevance for tumor surveillance. *J Immunol* 2006;176:4331–4336. [PubMed: 16547270]
- Everett MA, Yeagers E, Sayre RM, Olson RL. Penetration of epidermis by ultraviolet rays. *Photochem Photobiol* 1966;5:533–542. [PubMed: 5981960]

- Fisher MS, Kripke ML. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. *Proc Natl Acad Sci USA* 1977;74:1688–1692. [PubMed: 300876]
- Foitzik K, Krause K, Conrad F, Nakamura M, Funk W, Paus R. Human scalp hair follicles are both a target and a source of prolactin, which serves as an autocrine and/or paracrine promoter of apoptosis-driven hair follicle regression. *Am J Pathol* 2006;168:748–756. [PubMed: 16507890]
- Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, Hobby P, Sutton B, Tigelaar RE, Hayday AC. Regulation of cutaneous malignancy by gamma delta T cells. *Science* 2001;294:605–609. [PubMed: 11567106]
- Girardi M, Lewis J, Glusac E, Filler RB, Geng L, Hayday AC, Tigelaar RE. Resident skin-specific gamma delta T cells provide local, nonredundant regulation of cutaneous inflammation. *J Exp Med* 2002;195:855–867. [PubMed: 11927630]
- Guzman E, Langowski JL, Owen-Schaub L. Mad dogs, Englishmen and apoptosis: the role of cell death in UV-induced skin cancer. *Apoptosis* 2003;315–325. [PubMed: 12815274]
- Guzman EA, Chen Y-H, Langowski JL, De Guzman A, Lo H-L, Walter B, Muller HK, Walker AM, Owen LB. Abrogation of delayed type hypersensitivity response to *Candida albicans* produced by a molecular mimic of phosphorylated prolactin. *J Neuroimmunol* 2005;170:31–40. [PubMed: 16169603]
- Halford C, Anderzen I, Arnetz B. Endocrine measures of stress and self-rated health: a longitudinal study. *J Psychosom Res* 2003;55:317–320. [PubMed: 14507542]
- Hartmann DP, Holaday JW, Bernton EW. Inhibition of lymphocyte proliferation by antibodies to prolactin. *Faseb J* 1989;3:2194–2202. [PubMed: 2787766]
- Ho TWC, Kawaminami M, Walker AM. Secretion of phosphorylated and non-phosphorylated monomer prolactin isoforms during rat pregnancy and pseudopregnancy. *Endocrine* 1993;1:435–439.
- Ho TWC, Leong FS, Olaso CH, Walker AM. Secretion of specific nonphosphorylated and phosphorylated rat prolactin isoforms at different stages of the estrous cycle. *Neuroendocrinology* 1993;58:160–165. [PubMed: 8264862]
- Horseman ND, Zhao W, Montecino-Rodriguez E, Tanaka M, Nakashima K, Engle SJ, Smith F, Markoff E, Dorshkind K. Defective hematopoiesis, but normal hematopoiesis, in mice with a targeted distribution of the prolactin gene. *EMBO J* 1997;16:6926–6935. [PubMed: 9384572]
- Ikuta K, Kina T, MacNeil I, Uchida N, Peault B, Chien YH, Weissman IL. A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. *Cell* 1990;62:863–874. [PubMed: 1975515]
- Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, Havran WL. A role for skin gamma delta T cells in wound repair. *Science* 2002;296:747–749. [PubMed: 11976459]
- Johnson TE, Vue M, Brekhuis S, Khong A, Ho TWC, Walker AM. Unmodified prolactin (PRL) promotes PRL secretion and acidophil hypertrophy and is associated with pituitary hyperplasia in female rats. *Endocrine (special edition on prolactin)* 2003;20:101–110.
- Krown KA, Wang YF, Ho TW, Kelly PA, Walker AM. Prolactin isoform 2 as an autocrine growth factor for GH3 cells. *Endocrinology* 1992;131:595–602. [PubMed: 1639009]
- Kuo CB, Wu W, Xu X, Yang L, Chen C, Coss D, Birdsall B, Nasser D, Walker AM. Pseudophosphorylated prolactin (S179D PRL) inhibits growth and promotes beta-casein gene expression in the rat mammary gland. *Cell Tissue Res* 2002;309:429–437. [PubMed: 12195299]
- Lorenson, MY.; Walker, AM. Structure-function relationships in prolactin. In: Horseman, N., editor. *Prolactin*. Kluwer Academic Publishers; Norwell: 2001. p. 189–217.
- Lupulescu A. Enhancement of epidermal carcinoma formation by prolactin in mice. *J Natl Cancer Inst* 1985;74:1335–1346. [PubMed: 3858600]
- Matera L, Cesano A, Bellone G, Oberholtzer E. Modulatory effect of prolactin on the resting and mitogen-induced activity of T, B, and NK lymphocytes. *Brian Behav Immun* 1992;6:409–417.
- Matera L, Mori M, Geuna M, Buttiglieri S, Palestro G. Prolactin in autoimmunity and antitumor defense. *J Neuroimmunol* 2000;109:47–55. [PubMed: 10969181]
- Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, Weissman IL, Cyster JG, Engleman EG. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol* 2002;3:1135–1141. [PubMed: 12415265]

- Morelli A, Larregina A, Chuluyan I, Kolkowski E, Fainboim L. Expression and modulation of C5a receptor (CD88) on skin dendritic cells. Chemotactic effect of C5a on skin migratory dendritic cells. *Immunology* 1996;89:126–134. [PubMed: 8911150]
- Naylor MJ, Oakes SR, Gardiner-Garden M, Harris J, Blazek K, Ho TW, Li FC, Wynick D, Walker AM, Ormandy CJ. Transcriptional changes underlying the secretory activation phase of mammary gland development. *Mol Endocrinol* 2005;19:1868–1883. [PubMed: 15705664]
- Oetting WS, Tuazon PT, Traugh JA, Walker AM. Phosphorylation of prolactin. *J Biol Chem* 1986;261:1649–1652. [PubMed: 3003080]
- Ouhtit A, Gorny A, Muller HK, Owen-Schaub L, Ananthaswamy HN. Loss of Fas-ligand expression in mouse keratinocytes during UV carcinogenesis. *Am J Pathol* 2000;157:1975–1981. [PubMed: 11106570]
- Pellegrini L, Lebrun JJ, Ali S, Kelly PA. Expression of prolactin and its receptor in human lymphoid cells. *Mol Endocrinol* 1992;6:1023–1031. [PubMed: 1508218]
- Schroeder MD, Brockman JL, Walker AM, Schuler LA. Inhibition of prolactin (PRL)-induced proliferative signals in breast cancer cells by a molecular mimic of phosphorylated PRL, S179D-PRL. *Endocrinology* 2003;144:5300–5307. [PubMed: 12970160]
- Schwacha MG, Ayala A, Chaudry IH. Insights into the role of gamma delta T lymphocytes in the immunopathogenic response to thermal injury. *J Leukocyte Biol* 2000;67:644–650. [PubMed: 10811004]
- Simon JC, Tigelaar RE, Bergstresser PR, Edelbaum D, Cruz PD Jr. Ultraviolet B radiation converts Langerhans cells from immunogenic to tolerogenic antigen-presenting cells. Induction of specific clonal anergy in CD4+ T helper 1 cells. *J Immunol* 1991;146:485–491. [PubMed: 1670944]
- Sontag Y, Guikers CL, Vink AA, de Gruijl FR, van Loveren H, Garssen J, Roza L, Kripke ML, van der Leun JC, van Vloten WA. Cells with UV-specific DNA damage are present in murine lymph nodes after in vivo UV irradiation. *J Invest Dermatol* 1995;104:734–738. [PubMed: 7738349]
- Stingl G, Katz SI, Clement L, Green Shevach EM. Immunologic functions of Ia-bearing epidermal Langerhans cells. *J Immunol* 1978;121:2005–2013. [PubMed: 81860]
- Thorsness PE, Koshland DE Jr. Inactivation of isocitrate dehydrogenase by phosphorylation is mediated by the negative charge of the phosphate. *J Biol Chem* 1987;262:422–425.
- Toews GB, Bergstresser PR, Streilein JW. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J Immunol* 1980;124:445–453. [PubMed: 6153101]
- Tuazon PT, Lorensen MY, Walker AM, Traugh JA. p21-activated protein kinase gamma-PAK in pituitary secretory granules phosphorylates prolactin. *FEBS Lett* 2002;515:84–88. [PubMed: 11943200]
- Ueda EK, Lo HL, Batrolini P, Walker AM. S179D prolactin primarily uses the extrinsic pathway and mitogen-activated protein kinase signaling to induce apoptosis in human endothelial cells. *Endocrinology* 2006;147:4627–4637. [PubMed: 16840547]
- Ueda E, Ozerdem U, Chen YH, Yaom M, Huang KT, Sun H, Martins-Green M, Bartolini P, Walker AM. A molecular mimic demonstrates that phosphorylated human prolactin is a potent anti-angiogenic hormone. *Endocr Relat Cancer* 2006;13:95–111. [PubMed: 16601282]
- Walker AM. Therapeutic potential of S179D prolactin – from prostate cancer to angioproliferative disorders: the first selective prolactin receptor modulator. *Expert Opin Invest Drugs* 2006;15:1257–1267.
- Wang YF, Walker AM. Dephosphorylation of standard prolactin produces a more biologically active molecule: evidence for antagonism between nonphosphorylated and phosphorylated prolactin in the stimulation of Nb2 cell proliferation. *Endocrinology* 1993;133:2156–2160. [PubMed: 8404666]
- Wang YF, Liu JW, Mamidi M, Walker AM. Identification of the major site of rat prolactin phosphorylation as serine 177. *J Biol Chem* 1996;271:2462–2469. [PubMed: 8576208]
- Wu W, Coss D, Lorensen MY, Kuo CB, Xu X, Walker AM. Different biological effects of unmodified prolactin and a molecular mimic of phosphorylated prolactin involve different signaling pathways. *Biochemistry* 2003;42:7561–7570. [PubMed: 12809512]
- Wu W, Chen Y-H, Ueda E, Tan D, Bartolini P, Walker AM. Different forms of prolactin have opposing effects on the expression of cell cycle regulatory proteins in differentiated mammary epithelial cells. *Oncol Res* 2006;16:75–84. [PubMed: 16898268]

- Xu X, Kreye E, Kuo CB, Walker AM. A molecular mimic of phosphorylated prolactin markedly reduced tumor incidence and size when DU145 human prostate cancer cells were grown in nude mice. *Cancer Res* 2001;61:6098–6104. [PubMed: 11507059]
- Xu X, Wu W, Williams V, Khong A, Chen YH, Deng C, Walker AM. Opposite effects of unmodified prolactin and a molecular mimic of phosphorylated prolactin on morphology and the expression of prostate specific genes in the normal rat prostate. *Prostate* 2003;54:25–33. [PubMed: 12481252]
- Yang L, Kuo CB, Liu Y, Coss D, Xu X, Chen C, Oster-Granite ML, Walker AM. Administration of unmodified prolactin (U-PRL) and a molecular mimic of phosphorylated prolactin (PP-PRL) during rat pregnancy provides evidence that the U-PRL:PP-PRL ratio is crucial to the normal development of pup tissues. *J Endocrinol* 2001;168:227–238. [PubMed: 11182760]
- Yang LS, Lii S, Kuo B, Buckley A, Buckley D, Chen C, Xu X, Coss D, Walker AM. Maternal prolactin composition can permanently affect epidermal gamma delta T cell function in the offspring. *Dev Comp Immunol* 2002;26:849–860. [PubMed: 12377224]
- Zelena D, Foldes A, Mergl Z, Barna I, Kovacs KJ, Makara GB. Effects of repeated restraint stress on hypothalamo-pituitary-adrenocortical function in vasopressin deficient Brattleboro rats. *Brain Res Bull* 2004;63:521–530. [PubMed: 15249118]

Figure 1. Effects of U-PRL and S179D PRL on $\gamma\delta$ T cells in normal and UV-exposed skin
Mice were treated with one of the forms of PRL for a total of 20 days. Half the animals were exposed to a single suberythemal dose of UVB at day 9 of PRL treatment. Epidermal sheets were harvested at 20 days of PRL treatment and therefore 11 days after UV exposure. Staining for $\gamma\delta$ T was with anti-thy 1.2. Control staining showed no dendritic cells (not shown). Representative images are shown in panel A and quantitative analysis of multiple images is shown in panel B (see materials and methods). U-PRL, unmodified prolactin; S179D PRL, the

mimic of phosphorylated prolactin; NR, non-irradiated; UV, exposed to ultraviolet light; N, normal dendritic appearance; I, intermediate between dendritic and rounded; R, rounded. *, $p < 0.05$ compared to respective saline controls; **, $p < 0.05$ compared to NR.

Figure 2. Effects of U-PRL and S179D PRL on Langerhans cells in normal and UV-exposed skin Panel A shows representative images and panel B quantitative analysis of multiple images (see material and methods). Experimental details and letter abbreviations are as for figure 1. *, $p < 0.05$ compared to NR.

Table 1

Langerhans and $\gamma\delta$ T Cells per mm² in response to treatments. Numbers are the mean $\pm$ SEM

<i>Langerhans (cells/mm²)</i>		
	NR	UV
Saline	275 $\pm$ 24	259 $\pm$ 21
Unmodified Prolactin	237 $\pm$ 15	274 $\pm$ 18
S179D Prolactin	279 $\pm$ 16	227 $\pm$ 13*
<i>$\gamma\delta$T Cells (cells/mm²)</i>		
	NR	UV
Saline	257 $\pm$ 12	155 $\pm$ 15 [§]
Unmodified Prolactin	220 $\pm$ 11*	188 $\pm$ 12
S179D Prolactin	180 $\pm$ 13**	200 $\pm$ 14*

* $p \leq 0.05$ compared to respective saline controls

** $p \leq 0.005$ compared to respective saline controls

[§] $p \leq 0.005$ compared to NR