Local cytokine levels associated with delayed-type hypersensitivity responses: modulation by gender, ovariectomy, and estrogen replacement

Lisa J Ma, Esther A Guzmán[1], Ariel DeGuzman, H Konrad Muller[2], Ameae M Walker and **Laurie B Owen**

Division of Biomedical Sciences, The University of California, Riverside, California 92521, USA

[1]Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

[2]Discipline of Pathology, University of Tasmania, Hobart, Tasmania 7000, Australia

(Requests for offprints should be addressed to L J Ma; Email: lma003@student.ucr.edu)

Abstract

It is well established that females mount stronger immune responses than males, but only very little is understood about the underlying mechanisms. We have analyzed local cytokine differences among intact females, those that had been ovariectomized (OVX), those receiving estrogen replacement after OVX, and males, both before and after production of delayed-type hypersensitivity (DTH) responses. We report confirmation of a much larger DTH response in females versus males. However, OVX resulted in an even larger response, while estrogen replacement resulted in a smaller response when compared with intact females. In animals exposed for the first time to an antigen (without a DTH response), OVX increased interleukin-6 (IL-6) and estrogen replacement after OVX suppressed IL-6. Of the cytokines that differed between males and females exposed for the first time to an antigen, only IL-6 was higher in females versus males when exposure to antigen occurred for the second time (when the DTH response occurs). Analysis of cytokines with OVX and estrogen replacement after a second exposure to antigen showed that IL-6 did not significantly change. Levels of IL-4; Regulated upon Activation, Normal T-cell Expressed; and Secreted; and thrombopoietin, however, correlated with the DTH response, suggesting direct or indirect positive regulation by estrogen. These results suggest an important role for both IL-6 and IL-4 in determining the degree of DTH response, with IL-6 (which appears negatively regulated by estrogen) increasing and IL-4 (which appears positively regulated by estrogen) decreasing the response. The results further suggest that IL-6 may play a role in predisposing to a larger DTH response, while IL-4 levels seem more important during an active response.

Journal of Endocrinology (2007) **193,** 291–297

Introduction

It is generally accepted that females have more robust immune responses after immunization and infection when compared with males (reviewed in Verthelyi 2001), but relatively little is known about how this occurs. In this study, we have used two readily quantifiable inflammatory immune responses, the delayed-type hypersensitivity (DTH) response to *Candida albicans* and the contact hypersensitivity (CHS) response to fluorescein isothiocyanate (FITC). DTH is elicited by injecting antigen intradermally, while the CHS response, a form of DTH, is induced by epicutaneous application of a sensitizing antigen or hapten (Reeve 2002). Both of these responses are T-cell derived and mediated by cytokines (Janeway *et al.* 2001), although different effector cells are responsible for eliciting each. The effector cells involved in the DTH response are local T cells, macrophages, and dendritic cells found at the site into which antigen has been injected. The effector cells involved in the CHS response are the Langerhans cells and the dendritic epidermal T cells found within the epidermis. Langerhans cells have until recently been considered the primary antigen-presenting cell in the skin and have generally been considered to be of importance in the induction of a CHS response (Schwarz 2002). A recent paper by Kaplan *et al.* (2005), however, suggests an additional modulatory role for Langerhans cells during CHS responses. Dendritic epidermal T cells, which have the gamma delta form of the T-cell receptor (Koning *et al.* 1987, Steiner *et al.* 1988), have been shown to be important in controlling inflammation (Girardi *et al.* 2002), and to play a role in wound healing (Jameson *et al.* 2002). Cytokines most often associated with down-regulation of inflammatory responses include interleukin-4 (IL-4) and IL-10, while IL-6 and tumor necrosis factor-α (TNF-α) are considered pro-inflammatory (Johnson 1997, Opal & DePalo 2000). In this study, we have examined DTH responses in females that were intact, ovariectomized (OVX), or were OVX with estrogen (E2) replacement, and males to ask which local cytokines are

0022–0795/07/0193–291 © 2007 Society for Endocrinology *Printed in Great Britain*
DOI: 10.1677/JOE-06-0024
Online version via http://www.endocrinology-journals.org

Downloaded from Bioscientifica.com at 10/15/2024 07:31:17PM
via free access

implicated as important players in the hormonal regulation of the DTH response.

Materials and Methods

Mice

Pathogen-free male and female C57BL/6 (B6) mice were obtained from the Jackson Laboratories (Bar Harbor, MD, USA). Five-week-old OVX B6 female mice were obtained from the National Cancer Institute (Frederick, MD, USA). Mice were between 10 and 12 weeks of age at onset of all experiments. In total, 188 mice were used for all experiments and their repeats. Animals were housed in cages with controlled temperature and humidity and alternating 12 h light:12 h darkness cycles. The animals were maintained in facilities approved by the Association for the Assessment and Accreditation of Laboratory Animal Care International and in accordance with the current United States Department of Agriculture, Department of Health and Human Services, and National Institutes of Health Regulations. Commercial diet and water were available *ad libitum*.

Estrogen replacement

Mice were anesthetized using isoflurane (Halocarbon Laboratories, River Edge, NJ, USA) and a small patch of hair was shaved from the scruff. The area was disinfected with alcohol and a small incision was made. A 3-week release 17β-estradiol pellet (E_2, 0·25 mg/pellet; Innovative Research of America, Toledo, OH, USA) was inserted subcutaneously, which results in constant levels in the circulation. The E_2 levels in control mice are ∼17·5–22 pg/ml in males (Hilakivi-Clarke *et al.* 1996, 1997) and vary between ∼22 and 55 pg/ml in females, according to the stage of the estrous cycle (Walmer *et al.* 1992). In OVX animals, previous work from this laboratory has shown that levels of E_2 are ∼25 pg/ml at 17 days and ∼30 pg/ml at 24 days post-OVX (Liu *et al.* 1997). The E_2 replacement pellets used result in circulating blood levels of 100–200 pg/ml for a mouse weighing 20–25 g. These levels of E_2 are within the physiological range and comparable with levels found during pregnancy (Jacquet *et al.* 1977, Parkening *et al.* 1978). Placebo pellets and sham surgeries were used in preliminary experiments and shown to have no effect. The incision was closed using a surgical staple and Neosporin (Warner-Lambert, Morris Plains, NJ, USA) was applied to the area. Neosporin was reapplied 24 h after insertion of the E_2 pellet. The surgical staple was removed after 5 days. After 7 days from the time of insertion of the E_2 pellet, the DTH or CHS response was initiated.

DTH response

DTH is a type IV hypersensitivity response. Some mice (46 mice in total) were sensitized with an intradermal injection of fixed *C. albicans* into both flanks. All mice were anesthetized and some received a second injection 7 days later with *C. albicans* protein antigen (Alercheck, Inc., Portland, ME, USA) in the footpad. Footpad thickness, a T-cell-mediated response, was measured 24 h (peak footpad thickness) later using a micrometer (Mitutoyo, Tokyo, Japan). Footpads were measured prior to the second exposure and 24-h post-challenge.

CHS response

Mice were shaved on their dorsum and 24 h later some (46 mice total) were sensitized with 0·5% FITC (Sigma) in 1:1 acetone: dibutyl phthalate. Mice were challenged by a second exposure 5 days later on their ears, and the change in ear thickness was measured 24-h post-challenge, previously described as the time of maximal response (Back & Larsen 1982), using a micrometer (Mitutoyo). Ear thickness was measured prior to the second exposure and 24-h post-challenge. Control animals received FITC on their ears (were challenged) without prior sensitization.

Cytokine array

Mice were killed after measuring footpad thickness and their popliteal lymph nodes isolated. A single cell suspension was obtained and the red blood cells lysed. The resulting cells were used to obtain protein using radio-immunoprecipitation assay (RIPA) cell lysis buffer. Supernatant containing protein were quantified using Bradford assay and 250 μg protein were devoted to cytokine analysis using a RayBio Mouse Cytokine Array (RayBiotech, Inc., Norcross, GA, USA) as directed by their protocol. The cytokines detected by the array were granulocyte colony stimulating factor (CSF), granulocyte macrophage colony stimulating factor (GM-CSF), IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, interferon-γ (IFN-γ), macrophage chemoattractant protein-1 (MCP-1), MCP-5, Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES), stem cell factor, soluble TNF receptor 1 (sTNFR1), TNF-α, thrombopoietin (TPO), and vascular endothelial growth factor. Densitometric analysis was then performed on the blots using MCID Elite 7.0 software (Imaging Research, Ontario, CA, USA). Background was subtracted and data were normalized against positive controls included on each blot. All data directly compared were derived from the same batch of array membranes.

Statistical analysis

Data were analyzed by ANOVA with post-tests and Bonferroni corrections for multiple comparisons against a single control group on occasions when this was appropriate. The value $P<0·05$ was considered statistically significant. All experiments used four animals per treatment group (except OVX with E_2 groups which consisted of three animals per

Downloaded from Bioscientifica.com at 10/15/2024 07:31:17PM
via free access

treatment group). Two individual inflammatory responses were measured on each animal. Therefore, $n=8$ for all groups except OVX with E_2 replacement groups where $n=6$. Each experiment was repeated at least twice and each repetition gave similar results. Cytokine array results are presented as the average of two individual experiments, each consisting of pooled samples from four animals (three animals in OVX with E_2 group) in which duplicate spots on the array were used for each cytokine measured. All results are presented as means ± S.E.M. Correlations between cytokine levels and DTH responses were determined using the Pearson product-moment coefficient of correlation with the level of significance set at $P<0{\cdot}05$.

Results

As expected, both the DTH and CHS responses were 2·5- to 3·0-fold higher in female versus male mice (Fig. 1a and b), thus reproducing a well-documented phenomenon under our experimental conditions. In order to manipulate the endocrine environment, we subjected female animals to OVX or OVX with E_2 replacement. There was an increased DTH response 6–8 weeks after OVX. Upon E_2 replacement in the OVX animals, the DTH response was decreased below that seen in controls (Fig. 2a). Analysis of the CHS response showed no increase with OVX, but still showed a decreased response upon E_2 replacement (Fig. 2b). The inability of OVX to increase the CHS response may be due to maximization of ear thickness, since the maximal change possible appears to be about 0·11 mm (Kawagoe *et al.* 2002) and ear thickness observed in our study was about 0·1 mm, and thus was already at the top of the range.

Since we obtained opposing effects of OVX and E_2 replacement on the DTH response, and the footpad draining lymph nodes are readily identifiable, we focused cytokine analysis on this response. This allowed us to look for cytokines that followed changes in the response. These changes in cytokines could be the result of altered expression or changes in cytokine-producing cell number. First, we analyzed differences between cytokine levels in males and females that had only received a single injection (i.e. under circumstances where immunization, but no inflammation

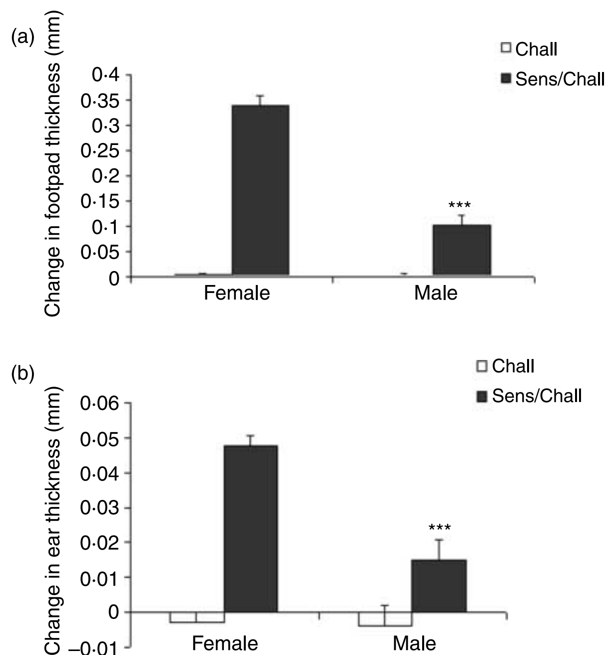

Figure 1 Female mice produce a more robust immune inflammatory response compared with male mice. (a) Mice were sensitized with an intradermal injection of fixed *Candida albicans* and 7 days later were challenged in the footpad with *C. albicans* protein antigen. Footpad thickness was measured prior to challenge and 24-h post-challenge. $n=8$; $^{***}P<0{\cdot}001$ compared with female mice. (b) Mice were sensitized with FITC on their dorsum and 5 days later challenged with FITC on their ears. Ear thickness was measured prior to challenge and 24-h post-challenge. $n=8$; $^{***}P<0{\cdot}001$ compared with female mice. Chall, challenge only (injected only once with *C. albicans* protein antigen); sens/chall, sensitized and challenged (injected with fixed *C. albicans* and later with *C. albicans* protein antigen).

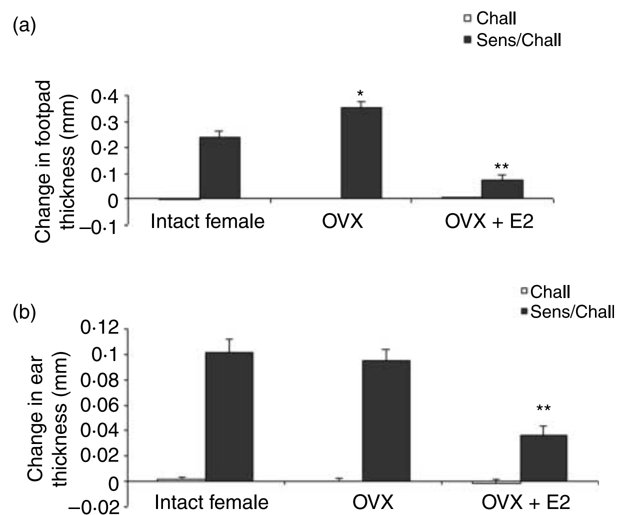

Figure 2 Ovariectomy causes an increase in the immune inflammatory response to *Candida* but not FITC, while E_2 replacement significantly diminishes both responses. (a) E_2 pellets were inserted subcutaneously into OVX mice 1 week prior to sensitization. Mice were then sensitized with an intradermal injection of fixed *C. albicans* and 7 days later were challenged in the footpad with *C. albicans* protein antigen. Footpad thickness was measured prior to challenge and 24-h post-challenge. $n=8$ for all groups except OVX+E2 where $n=6$; $^{*}P<0{\cdot}05$ compared with intact female mice. $^{**}P<0{\cdot}01$ compared with intact female and OVX mice. (b) E_2 pellets were inserted subcutaneously into OVX mice at a point distant to subsequent sensitization. Mice were sensitized with FITC on their dorsum and 5 days later were challenged with FITC on their ears. Ear thickness was measured prior to challenge and 24-h post-challenge. $n=8$ for all groups except OVX+E2 where $n=6$; $^{**}P<0{\cdot}01$ compared with intact female and OVX mice. Chall, challenge only; sens/chall, sensitized and challenged.

Downloaded from Bioscientifica.com at 10/15/2024 07:31:17PM
via free access

had occurred). Of the 22 cytokines measured (see Materials and Methods for complete listing), only IL-6 and MCP-5 were different between the genders (Fig. 3). OVX and OVX with E_2 replacement had no effect on MCP-5, whereas OVX produced threefold the level of IL-6 and E_2 replacement reduced this, a finding similar in pattern to the overall DTH response.

When a DTH response was induced in males or females, there were significant differences between the genders in the levels of IL-6, IL-9, IL-10, IL-12p40, and IL-12p70, all of which were higher in females (Fig. 4). However, there was no difference in the levels of MCP-5 between the genders (data not shown). Thus, although the levels of MCP-5 were different between males and females before the DTH response, the male mice were capable of equal production once the response was ongoing.

When a DTH response was induced in OVX animals and OVX animals with E_2 replacement, we looked for significant changes that followed the pattern or inverse pattern of the DTH response when compared with the intact females. The same pattern (i.e. higher with OVX and reduced in OVX+ E_2) was observed with sTNFR1, although there was no correlation with the DTH response ($R=0{\cdot}097$). An inverse correlation (i.e. reduced with OVX and higher with OVX+ E_2) was observed for IL-4 ($R=-0{\cdot}915$, $P<0{\cdot}01$), RANTES ($R=-0{\cdot}673$, $P<0{\cdot}05$), and TPO ($R=-0{\cdot}992$, $P<0{\cdot}01$; Fig. 5). Although IL-12p70 also had an inverse pattern, there was no correlation with the DTH response ($R=-0{\cdot}648$). No significant differences were found in the other analytes measured.

Discussion

It is generally accepted that females have more robust immune responses after immunization and infection when compared with males (reviewed in Verthelyi 2001), but relatively little is known about how this occurs. There are many endocrine differences between males and females, which may be responsible for direct or indirect modulation of the immune response including levels of E_2, progesterone, testosterone, and their metabolites. Although E_2 is higher in females, most articles in the literature have reported an immunosuppressive effect of E_2. Thus, for example, E_2 has been shown to inhibit the development of experimental autoimmune encephalomyelitis, collagen-induced arthritis, and inflammatory bowel disease (Jansson *et al.* 1994, Bebo *et al.* 2001, Ito *et al.* 2001, Harnish *et al.* 2004), and to suppress the inflammatory response in castrated mice (Josefsson *et al.* 1992). Pregnancy, a state of elevated E_2 and progesterone, has also been reported to be immunosuppressive (Purtilo *et al.* 1972, Thong *et al.* 1973, Kovacs *et al.* 2002). Findings in the current study are in agreement with these previous reports since we showed an increased DTH response upon OVX and a decreased response upon E_2 replacement. Thus, while one can conclude that E_2 modulates some immune responses, including those examined in the present study, it is not the higher levels of E_2 in females that produces a more robust DTH response.

Others have approached an investigation of the gender difference in immune response by the administration or blockade of various candidate hormones and their metabolites and have uncovered important pro- and anti-inflammatory actions for some of them. E_2, for example, is

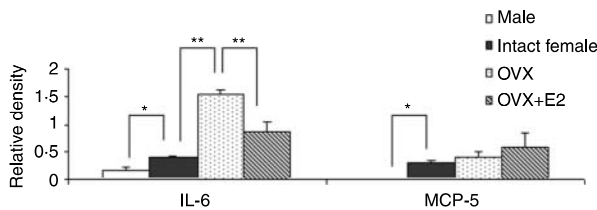

Figure 3 IL-6 and MCP-5 expression in the challenge only group. Popliteal nodes were obtained from mice that had not been sensitized but only challenged with *C. albicans* protein. Protein extracted from the nodes was used to perform a cytokine array to measure the levels of cytokines produced. In this graphical representation, data from two separate experiments were combined to allow for ease of comparison. Data are expressed as relative density ± S.E.M. *$P<0{\cdot}05$ compared with male; **$P<0{\cdot}01$ compared with intact female and OVX+ E_2.

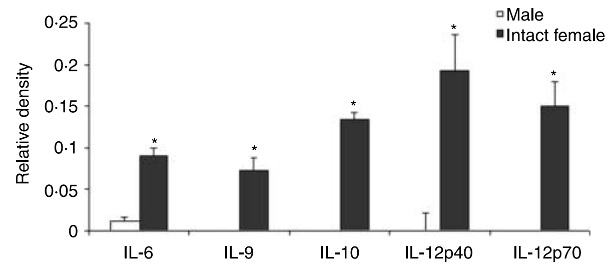

Figure 4 Cytokine expression differs in males and females after sensitization and challenge. Popliteal nodes were obtained from mice sensitized and challenged with *C. albicans*. Data are expressed as relative density ± S.E.M. *$P<0{\cdot}05$ compared with males.

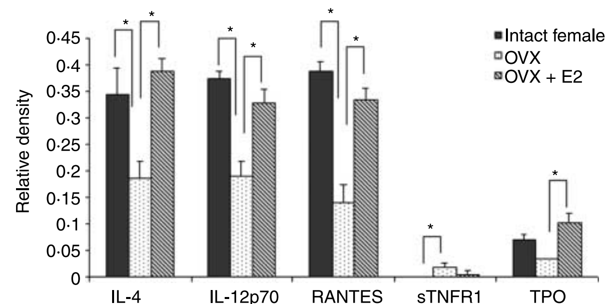

Figure 5 Cytokine levels significantly altered after sensitization and challenge in OVX and OVX with E_2 replacement animals. E_2 pellets were inserted into OVX mice 1 week prior to the start of the DTH response experiment. Popliteal nodes were obtained from mice both sensitized and challenged with *C. albicans*. Data are expressed as relative density ± S.E.M. *$P<0{\cdot}05$ for all cytokines listed.

Downloaded from Bioscientifica.com at 10/15/2024 07:31:17PM
via free access

metabolized to several hydroxyestrogens (reviewed in Mueck *et al.* 2002), and 4- and 16α-hydroxyestrogens are considered to be pro-inflammatory (Janele *et al.* 2006), while 2-hydroxyestrogens are considered to be anti-inflammatory (Janele *et al.* 2006). Progesterone is also anti-inflammatory under some circumstances, contributing to tolerance of the embryo during pregnancy (Zhao *et al.* 2002), and likewise testosterone has been shown to reduce some immune responsivity (Angele *et al.* 1997, Wichmann *et al.* 1997). A previous study in our laboratory demonstrated that E_2 replacement in OVX animals resulted in physiological levels of circulating progesterone (Liu *et al.* 1997). Kinetic analyses conducted by Shimizu *et al.* (1993) demonstrated that natural estrogens, like estrone and estradiol, inhibit the process of androgen aromatization. In addition, glucocorticoids are anti-inflammatory and OVX has been shown to decrease glucocorticoid secretion and E_2 replacement to increase it (Lo *et al.* 2000, Seale *et al.* 2004). Remembering that OVX increased and E_2 replacement decreased the DTH response, one might suggest therefore that the conversion of E_2 to 2-hydroxyestrogens, the stimulatory effect of E_2 on progesterone production by the adrenal gland (Liu *et al.* 1997), E_2 inhibition of aromatase activity in extra-ovarian tissues (Shimizu *et al.* 1993), or E_2 stimulation of glucocorticoid secretion (Lo *et al.* 2000, Seale *et al.* 2004) may contribute in some measure to the effects observed. To begin to unravel these complex possibilities, the current study has used the observed different degrees of DTH response in males and females, and manipulation of the latter by OVX and E_2 replacement, to uncover important hormonally regulated cytokines, which by their pattern may indicate a significant regulatory role.

First, we duplicated the findings of others concerning gender differences (reviewed in Verthelyi 2001) and showed very clearly that for two different inflammatory responses, females had a far more robust response. In addition, we demonstrated that OVX increased one response and produced no change in the other, the latter most likely due to maximal tissue thickness in the intact animals, which could not be increased further. Consistent with this result and interpretation, E_2 replacement in OVX animals decreased both responses to a level below those of intact females. The increased DTH response to OVX and the decreased response upon E_2 replacement allowed us to correlate cytokines with the magnitude of these responses as well as with responses in intact females and males.

Analysis of cytokine differences between control males and females after one antigen exposure (i.e. in the absence of a DTH response) suggested a potentially important role for both MCP-5 and IL-6 in determining the magnitude of the DTH response. However, after a second exposure to antigen, MCP-5 levels were no different between males and females, thereby suggesting that MCP-5 levels were not a governing aspect of the degree of response. This conclusion was substantiated by the failure of OVX (which increases the DTH response) or OVX with E_2 replacement (which decreases the DTH response) to influence the level of MCP-5. IL-6, on the other hand, showed higher levels in females both before and after the second exposure to antigen (compare Figs 3 and 4), thereby suggesting a more important role for this cytokine. In addition, OVX increased levels of this cytokine, and E_2 replacement in OVX animals decreased them, although not lower than those in intact animals. Thus, changes in the level of this cytokine follow expectations for an important positive regulator of the DTH response. Higher levels of IL-6 would be expected to result in a faster onset of the acute phase reaction (Heinrich *et al.* 2003) and hence a larger DTH response.

Of the cytokines whose levels changed in a similar or inverse pattern to the degree of the DTH response in the OVX and OVX with E_2 replacement animals, only IL-12p70 was also significantly different between males and females post-DTH response. IL-12p70 is considered a pro-inflammatory cytokine (Sypek *et al.* 1993, Romani *et al.* 1994, Heinzel *et al.* 1995), but levels were decreased by OVX when the DTH response was increased, and increased upon E_2 replacement when the DTH response was decreased. Thus, levels of this cytokine suggest either direct or indirect regulation by E_2. However, Pearson's analysis showed no correlation between the levels of this cytokine and the DTH response. The results therefore do not suggest that IL-12p70 is a cytokine which plays a major local role in governing the degree of the DTH response.

Other cytokines not different between males and females, but which nevertheless show patterns after the DTH response consistent with regulation by E_2, include IL-4, sTNFR1, RANTES, and TPO.

IL-4 is an anti-inflammatory cytokine found to be essential for immunosuppression (Souza *et al.* 2004). Levels of this cytokine were decreased after OVX and increased after E_2 replacement, just as one would expect for an anti-inflammatory cytokine, should it be playing an important role in modulating the DTH response; moreover, levels of IL-4 correlated with the DTH response. However, although E_2 replacement was able to restore levels of IL-4 to those observed in intact female mice, they did not go higher than intact females and hence restoration of IL-4 cannot completely explain the degree to which the OVX immune responses were suppressed by E_2 replacement. This suggests that IL-4 alone is not the sole cytokine responsible for E_2 suppression of the DTH response, but the results certainly suggest an important contributory role.

The pattern in sTNFR1 roughly followed the DTH response since amounts were higher after OVX and appeared reduced by E_2 replacement, but Pearson's analysis showed no correlation. Soluble TNFR1 has been shown to induce the cell adhesion molecules ICAM-1 (intercellular adhesion molecule-1), E-selectin, and VCAM-1 (vascular cell adhesion molecule-1) (Amrani *et al.* 2000, Kitakata *et al.* 2002). Increased levels of sTNFR1 upon OVX may thus result in increased levels of these cell adhesion molecules, thereby

Downloaded from Bioscientifica.com at 10/15/2024 07:31:17PM
via free access

increasing the migration of cells into the region of inflammation.

The other cytokines showing patterns consistent with positive regulation by E_2, and correlating with the DTH response in the post-DTH group were RANTES and TPO. RANTES has been shown to play a role in DTH responses, but would normally be considered pro-inflammatory (Devergne *et al.* 1994). The reduction in local levels of RANTES in response to OVX (i.e. when the DTH response was increased) therefore is opposite to what one would predict should it be playing an important role in the response. Similiarly, TPO is associated with inflammatory reactions (Burmester *et al.* 2005) and yet is reduced after OVX. Given the current knowledge, this suggests that RANTES and TPO have a less important role in governing the degree of a DTH response.

The ability of estrogen (either directly or indirectly) to regulate the levels of IL-4, the IL-12p35 and IL-12p40 subunits of IL-12p70, RANTES, and TPO led us to examine the promoter and intronic regions of the genes for these cytokines for consensus response elements (NCBI BLAST). Also examined was the *IL-6* gene. The genes for IL-4, both subunits of IL-12p70 and IL-6, each contained likely consensus sequences for estrogen receptor binding. The gene for the p40 subunit of IL-12p70 contained a potential androgen response element, a glucocorticoid response element and a partial progesterone response element. The RANTES gene contained an androgen response element and a glucocorticoid response element. The genes for the p35 subunit of IL-12p70, IL-4, IL-6, and TNFR1 contained potential glucocorticoid response elements. None of the response elements examined were found in the gene for TPO. This suggests that the expression of IL-4, IL-12p35, and IL-12p40 may be directly stimulated by estrogens, while regulation of expression of IL-6, which is higher in females than in males is apparently negatively regulated by E_2. This is in agreement with work *in vitro* showing direct repression of the IL-6 promoter by E_2 (Pottratz *et al.* 1994, Ray *et al.* 1994, Stein & Yang 1995, Galien *et al.* 1996). Regulation of RANTES, TPO, and sTNFR1 may be more complex.

Study of the endocrinology of estrogen and testosterone has been aided by the development of mice lacking estrogen receptors or having mutations in the androgen receptor. Interpretation of changes in DTH responses in these mice, however, would be complex since estrogen receptor knockout mice have been shown to have altered immune development as well as function (Staples *et al.* 1999, Thurmond *et al.* 2000). No known studies on the immunity of androgen knockout mice have been published so far, but one might expect similar complications to interpretation. Future analyses of the role of androgens could benefit from examination of the effect of dihydrotestosterone in orchidectomized animals.

In conclusion, our results have demonstrated that the DTH response in particular is a readily quantifiable *in vivo* assay that can be used to further analyze at least some aspects of hormonal regulation. The data derived from the current study suggest an important role for IL-6 and IL-4 in regulating the degree of the DTH response. Although IL-6 is higher in females than males, it appears to be negatively regulated by E_2. Results obtained for IL-4 on the other hand are consistent with positive regulation of this cytokine by E_2. The results further suggest that IL-6 levels may play a role in predisposing to a larger DTH response, while IL-4 levels seem to be more important during an active DTH response.

Funding

This work was supported by the NIH grant R01CA52457 to L B O and a grant from the Cancer Federation to A M W. L J M was partially supported by a Burden fellowship. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

Amrani Y, Lazaar AL, Hoffman R, Amin K, Ousmer S & Panettieri RA Jr 2000 Activation of p55 tumor necrosis factor-alpha receptor-1 coupled to tumor necrosis factor receptor-associated factor 2 stimulates intercellular adhesion molecule-1 expression by modulating a thapsigargin-sensitive pathway in human tracheal smooth muscle cells. *Molecular Pharmacology* **58** 237–245.

Angele MK, Wichmann MW, Ayala A, Cioffi WG & Chaudry IH 1997 Testosterone receptor blockade after hemorrhage in males. Restoration of the depressed immune functions and improved survival following subsequent sepsis. *Archives of Surgery* **132** 1207–1214.

Back O & Larsen A 1982 Contact sensitivity in mice evaluated by means of ear thickness and a radiometric test. *Journal of Investigative Dermatology* **78** 309–312.

Bebo BF Jr, Fyfe-Johnson A, Adlard K, Beam AG, Vandenbark AA & Offner H 2001 Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. *Journal of Immunology* **166** 2080–2089.

Burmester H, Wolber EM, Freitag P, Fandrey J & Jelkmann W 2005 Thrombopoietin production in wild-type and interleukin-6 knockout mice with acute inflammation. *Journal of Interferon and Cytokine Research* **25** 407–413.

Devergne O, Marfaing-Koka A, Schall TJ, Leger-Ravet MB, Sadick M, Peuchmaur M, Crevon MC, Kim KJ, Schall TT, Kim T *et al.* 1994 Production of the RANTES chemokine in delayed-type hypersensitivity reactions: involvement of macrophages and endothelial cells. *Journal of Experimental Medicine* **179** 1689–1694.

Galien R, Evans HF & Garcia T 1996 Involvement of CCAAT/enhancer-binding protein and nuclear factor-kappa B binding sites in interleukin-6 promoter inhibition by estrogens. *Molecular Endocrinology* **10** 713–722.

Girardi M, Lewis J, Glusac E, Filler RB, Geng L, Hayday AC & Tigelaar RE 2002 Resident skin-specific gammadelta T cells provide local, non-redundant regulation of cutaneous inflammation. *Journal of Experimental Medicine* **195** 855–867.

Harnish DC, Albert LM, Leathurby Y, Eckert AM, Ciarletta A, Kasaian M & Keith JC Jr 2004 Beneficial effects of estrogen treatment in the HLA-B27 transgenic rat model of inflammatory bowel disease. *American Journal of Physiology, Gastrointestinal and Liver Physiology* **286** G118–G125.

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G & Schaper F 2003 Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochemical Journal* **374** 1–20.

Downloaded from Bioscientifica.com at 10/15/2024 07:31:17PM
via free access

Heinzel FP, Rerko RM, Ahmed F & Pearlman E 1995 Endogenous IL-12 is required for control of Th2 cytokine responses capable of exacerbating leishmaniasis in normally resistant mice. *Journal of Immunology* **155** 730–739.

Hilakivi-Clarke L, Cho E & Onojafe I 1996 High-fat diet induces aggressive behavior in male mice and rats. *Life Sciences* **58** 1653–1660.

Hilakivi-Clarke L, Raygada M & Cho E 1997 Serum estradiol levels and ethanol-induced aggression. *Pharmacology, Biochemistry, and Behavior* **58** 785–791.

Ito A, Bebo BF Jr, Matejuk A, Zamora A, Silverman M, Fyfe-Johnson A & Offner H 2001 Estrogen treatment down-regulates TNF-alpha production and reduces the severity of experimental autoimmune encephalomyelitis in cytokine knockout mice. *Journal of Immunology* **167** 542–552.

Jacquet P, Gerber GB, Leonard A & Maes J 1977 Plasma hormone levels in normal and lead-treated pregnant mice. *Experientia* **33** 1375–1377.

Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R & Havran WL 2002 A role for skin gammadelta T cells in wound repair. *Science* **296** 747–749.

Janele D, Lang T, Capellino S, Cutolo M, Da Silva JA & Straub RH 2006 Effects of testosterone, 17beta-estradiol, and downstream estrogens on cytokine secretion from human leukocytes in the presence and absence of cortisol. *Annals of the New York Academy of Sciences* **1069** 168–182.

Janeway CA, Travers P, Walport M & Shlomchik MJ 2001 *Immunobiology.* 5th edn, Garland Publishing: New York, NY.

Jansson L, Olsson T & Holmdahl R 1994 Estrogen induces a potent suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice. *Journal of Neuroimmunology* **53** 203–207.

Johnson RW 1997 Inhibition of growth by pro-inflammatory cytokines: an integrated view. *Journal of Animal Science* **75** 1244–1255.

Josefsson E, Tarkowski A & Carlsten H 1992 Anti-inflammatory properties of estrogen. I. *In vivo* suppression of leukocyte production in bone marrow and redistribution of peripheral blood neutrophils. *Cellular Immunology* **142** 67–78.

Kaplan DH, Jenison MC, Saeland S, Shlomchik WD & Shlomchik MJ 2005 Epidermal Langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity* **23** 611–620.

Kawagoe J, Takizawa T, Matsumoto J, Tamiya M, Meek SE, Smith AJ, Hunter GD, Plevin R, Saito N, Kanke T *et al.* 2002 Effect of protease-activated receptor-2 deficiency on allergic dermatitis in the mouse ear. *Japanese Journal of Pharmacology* **88** 77–84.

Kitakata H, Nemoto-Sasaki Y, Takahashi Y, Kondo T, Mai M & Mukaida N 2002 Essential roles of tumor necrosis factor receptor p55 in liver metastasis of intrasplenic administration of colon 26 cells. *Cancer Research* **62** 6682–6687.

Koning F, Stingl G, Yokoyama WM, Yamada H, Maloy WL, Tschachler E, Shevach EM & Coligan JE 1987 Identification of a T3-associated gamma delta T cell receptor on Thy-1+ dendritic epidermal cell lines. *Science* **236** 834–837.

Kovacs EJ, Messingham KA & Gregory MS 2002 Estrogen regulation of immune responses after injury. *Molecular and Cellular Endocrinology* **193** 129–135.

Liu JW, Dawson DD, Peters CE, Baker MA & Walker AM 1997 Estrogen replacement in ovariectomized rats results in physiologically significant levels of circulating progesterone, and co-administration of progesterone markedly reduces the circulating estrogen. *Endocrine* **6** 125–131.

Lo MJ, Chang LL & Wang PS 2000 Effects of estradiol on corticosterone secretion in ovariectomized rats. *Journal of Cellular Biochemistry* **77** 560–568.

Mueck AO, Seeger H & Lippert TH 2002 Estradiol metabolism and malignant disease. *Maturitas* **43** 1–10.

Opal SM & DePalo VA 2000 Anti-inflammatory cytokines. *Chest* **117** 1162–1172.

Parkening TA, Lau IF, Saksena SK & Chang MC 1978 Circulating plasma levels of pregnenolone, progesterone, estrogen, luteinizing hormone, and follicle stimulating hormone in young and aged C57BL/6 mice during various stages of pregnancy. *Journals of Gerontology* **33** 191–196.

Pottratz ST, Bellido T, Mocharla H, Crabb D & Manolagas SC 1994 17 β-Estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. *Journal of Clinical Investigation* **93** 944–950.

Purtilo DT, Hallgren HM & Yunis EJ 1972 Depressed maternal lymphocyte response to phytohaemagglutinin in human pregnancy. *Lancet* **1** 769–771.

Ray A, Prefontaine KE & Ray P 1994 Down-modulation of interleukin-6 gene expression by 17 beta-estradiol in the absence of high affinity DNA binding by the estrogen receptor. *Journal of Biological Chemistry* **269** 12940–12946.

Reeve VE 2002 Ultraviolet radiation and the contact hypersensitivity reaction in mice. *Methods* **28** 20–24.

Romani L, Mencacci A, Tonnetti L, Spaccapelo R, Cenci E, Puccetti P, Wolf SF & Bistoni F 1994 IL-12 is both required and prognostic *in vivo* for T helper type 1 differentiation in murine candidiasis. *Journal of Immunology* **153** 5167–5175.

Schwarz T 2002 Photoimmunosuppression. *Photodermatology, Photoimmunology and Photomedicine* **18** 141–145.

Seale JV, Wood SA, Atkinson HC, Bate E, Lightman SL, Ingram CD, Jessop DS & Harbuz MS 2004 Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. *Journal of Neuroendocrinology* **16** 516–524.

Shimizu Y, Yarborough C & Osawa Y 1993 Competitive product inhibition of aromatase by natural estrogens. *Journal of Steroid Biochemistry and Molecular Biology* **44** 651–656.

Souza VM, Jacysyn JF & Macedo MS 2004 IL-4 and IL-10 are essential for immunosuppression induced by high molecular weight proteins from *Ascaris suum*. *Cytokine* **28** 92–100.

Staples JE, Gasiewicz TA, Fiore NC, Lubahn DB, Korach KS & Silverstone AE 1999 Estrogen receptor alpha is necessary in thymic development and estradiol-induced thymic alterations. *Journal of Immunology* **163** 4168–4174.

Stein B & Yang MX 1995 Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-kappa B and C/EBP beta. *Molecular and Cellular Biology* **15** 4971–4979.

Steiner G, Koning F, Elbe A, Tschachler E, Yokoyama WM, Shevach EM, Stingl G & Coligan JE 1988 Characterization of T cell receptors on resident murine dendritic epidermal T cells. *European Journal of Immunology* **18** 1323–1328.

Sypek JP, Chung CL, Mayor SE, Subramanyam JM, Goldman SJ, Sieburth DS, Wolf SF & Schaub RG 1993 Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *Journal of Experimental Medicine* **177** 1797–1802.

Thong YH, Steele RW, Vincent MM, Hensen SA & Bellanti JA 1973 Impaired *in vitro* cell-mediated immunity to rubella virus during pregnancy. *New England Journal of Medicine* **289** 604–606.

Thurmond TS, Murante FG, Staples JE, Silverstone AE, Korach KS & Gasiewicz TA 2000 Role of estrogen receptor alpha in hematopoietic stem cell development and B lymphocyte maturation in the male mouse. *Endocrinology* **141** 2309–2318.

Verthelyi D 2001 Sex hormones as immunomodulators in health and disease. *International Immunopharmacology* **1** 983–993.

Walmer DK, Wrona MA, Hughes CL & Nelson KG 1992 Lactoferrin expression in the mouse reproductive tract during the natural estrous cycle: correlation with circulating estradiol and progesterone. *Endocrinology* **131** 1458–1466.

Wichmann MW, Angele MK, Ayala A, Cioffi WG & Chaudry IH 1997 Flutamide: a novel agent for restoring the depressed cell-mediated immunity following soft-tissue trauma and hemorrhagic shock. *Shock* **8** 242–248.

Zhao D, Lebovic DI & Taylor RN 2002 Long-term progestin treatment inhibits RANTES (regulated on activation, normal T cell expressed and secreted) gene expression in human endometrial stromal cells. *Journal of Clinical Endocrinology and Metabolism* **87** 2514–2519.

Received in final form 26 February 2007
Accepted 28 February 2007
Made available online as an Accepted Preprint
6 March 2007

Downloaded from Bioscientifica.com at 10/15/2024 07:31:17PM
via free access