Endocrine (1995) 3, 725-728

Tumor necrosis factor increases interleukin-6 release from adrenal zona glomerulosa cells *in vitro*

Allan M. Judd & Paul K. Ritchie

Department of Zoology, 537 WIDB, Brigham Young University, Provo, Utah 84602, USA

Rat adrenal zone glomerulosa cells secrete tumor necrosis factor (TNF) and interleukin-6 (IL-6). We have extended previous studies to determine if TNFα can modify the release of adrenal IL-6. Primary cultures of rat adrenal zone glomerulosa cells were prepared by enzymatic techniques and cultured for 4–6 days. The cells were then exposed to serum-free RPMI 1640 incubation medium containing vehicle (RPMI-1640 medium alone), TNFα and/or selected agents known to stimulate adrenal IL-6 release. Following a 5 h incubation, the incubation medium was removed from the cells and the IL-6 content of the medium measured with the 7TD1 bioassay. TNFα (0.5–50 ng/mL) increased basal adrenal IL-6 release in a concentration-dependent manner. Furthermore, TNFα potentiated in a more than additive manner the adrenal IL-6 release stimulated by lipopolysaccharide (LPS), interleukin-1β, angiotensin II and ACTH. TNFα potentiated the IL-6 release stimulated by a wide range of concentration of IL-1β (0.01–100 ng/mL) and ACTH (0.1–100 nM). Because IL-6 and TNFα modify the steroid secretion from adrenal cells, these cytokines may interact together to regulate the function of the adrenal cortex.

Keywords: Interleukin-6; tumor necrosis factor; adrenal; angiotensin II; lipopolysaccharide; interleukin-1; ACTH

Introduction

Various cytokines are present in endocrine tissues including the ovaries, testes, anterior pituitary, and posterior pituitary. In the rat adrenal gland, interleukin-1 (IL-1) is present in the medulla (Schultzberg *et al.*, 1989) and interleukin-6 (IL-6) and tumor necrosis factor (TNF) are present in the zona glomerulosa (Judd *et al.*, 1990; Judd & MacLeod, 1991, 1992, 1995). TNFα inhibits aldosterone and glucocorticoid release from adrenal cells (Natarajan *et al.*, 1989; Jäättelä *et al.*, 1990, 1991), whereas IL-6 stimulates glucocorticoid release from adrenal cells (Salas *et al.*, 1990). Therefore, these cytokines may have important paracrine or autocrine roles in the regulation of adrenal function. The release of cytokines by adrenal zona glomerulosa cells is modified by secretagogues that regulate adrenal function. Specifically, the release of IL-6 from the rat adrenal zona glomerulosa is stimulated by ACTH, angiotensin II, IL-1α, IL-β and lipopolysaccharide (LPS). LPS, IL-1α and IL-1β stimulate adrenal TNF release, but ACTH inhibits the release of this cytokine (Judd *et al.*, 1990, Judd & MacLeod, 1991, 1992, 1995).

IL-6 and TNFα affect the release of each other in a complex manner in many tissues. In some tissues, TNFα enhances basal and secretagogue-stimulated IL-6 release (Elias & Lentz, 1990; Kohda *et al.*, 1992; Aderka *et al.*, 1993; Maimone *et al.*, 1993). In contrast, in other tissues TNFα does not affect IL-6 release (Zubiaga *et al.*, 1990; Gorospe & Spangelo, 1993). Because TNF and IL-6 are released from the rat adrenal zona glomerulosa, we investigated the effects of TNFα on the release of IL-6 from this tissue.

Correspondence: Allan M. Judd
Received 10 April 1995, accepted 28 June 1995

Results

As reported previously (Judd & MacLeod, 1992), ACTH increased IL-6 release from primary cultures of rat adrenal glomerulosa cells in a concentration-dependent manner. TNFα (5 ng/ml) increased basal IL-6 release and potentiated the IL-6 release stimulated by each concentration of ACTH. The combined effects of ACTH and TNFα on IL-6 release were more than additive of the effects of ACTH and TNF alone (Figure 1). In the experiment illustrated in Figure 2, TNFα (0.5–50 ng/mL) increased in a concentration-related manner both basal and ACTH-stimulated IL-6 release from zona glomerulosa cells. At all concentrations of TNFα, the effects of TNFα and ACTH on IL-6 release were more than additive of the effects of these secretagogues by themselves. Similarly, TNFα (0.5–50 ng/mL) in a concentration-related fashion potentiated the release of IL-6 stimulated by AII and the effects of AII and TNFα combined were more than additive to the effects of AII and TNFα alone (Figure 3).

The classical secretagogues that regulate the function of the adrenal zona glomerulosa (ACTH and AII) moderately simulate IL-6 release. However, endotoxin (lipopolysaccharide or LPS) and interleukin-1β greatly increase the release of IL-6 from rat adrenal zona glomerulosa cells (Judd *et al.*, 1990; Judd & MacLeod, 1991, 1992). Therefore, we determined if TNFα could potentiate the effect of these powerful IL-6 secretagogues. TNFα (0.5–50 ng/mL) in a concentration-dependent manner potentiated the release of IL-6 stimulated by LPS (Figure 4). In Figure 5, IL-1β (0.01–100 ng/mL) stimulated IL-6 release in a concentration-related manner. TNFα (10 ng/mL) enhanced basal IL-6 release and potentiated the IL-6 release stimulated by each

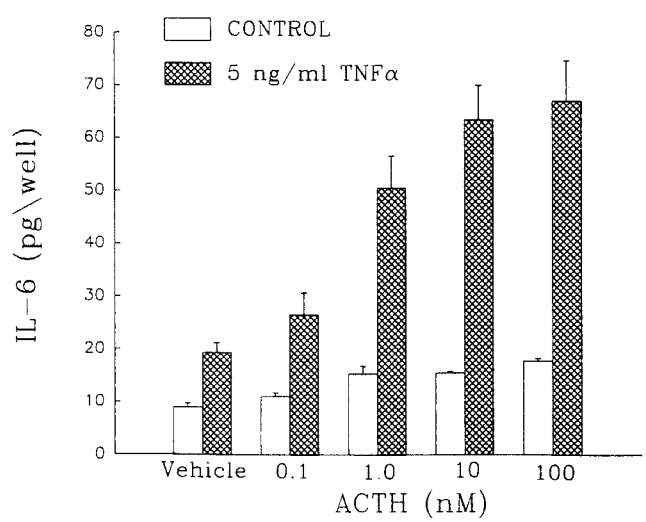

Figure 1 The effect of TNFα on ACTH-stimulated IL-6 release from primary cultures of rat adrenal zona glomerulosa cells. ACTH at concentrations of 1 nM and greater significantly ($P<0.01$) increased IL-6 release. TNFα (5 ng/ml) increased ($P<0.01$) basal IL-6 release and potentiated ($P<0.01$) ACTH-stimulated IL-6 release at each concentration of ACTH. All incubations were of 5 h duration

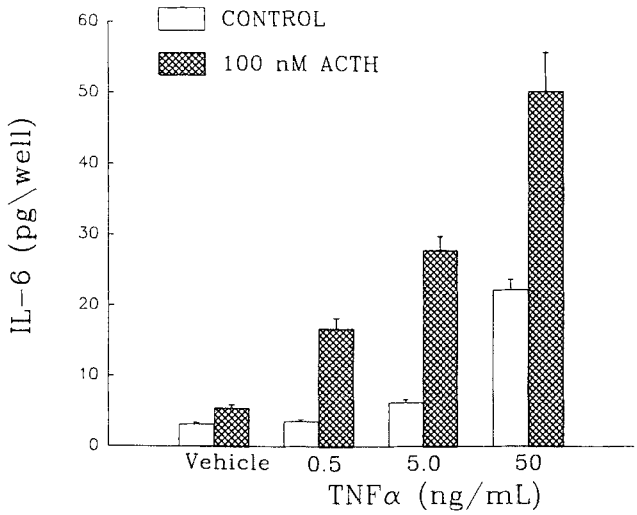

Figure 2 TNFα potentiated ACTH-stimulated IL-6 release from primary cultures of rat adrenal zona glomerulosa cells in a concentration-dependent manner. ACTH (100 nM) increased ($P<0.01$) IL-6 release. TNFα at 5.0 and 50 ng/ml increased ($P<0.01$) basal IL-6 release. TNFα at concentrations of 0.5 ng/ml and greater potentiated ($P<0.01$) ACTH-stimulated IL-6 release. All incubations were of 6 h duration

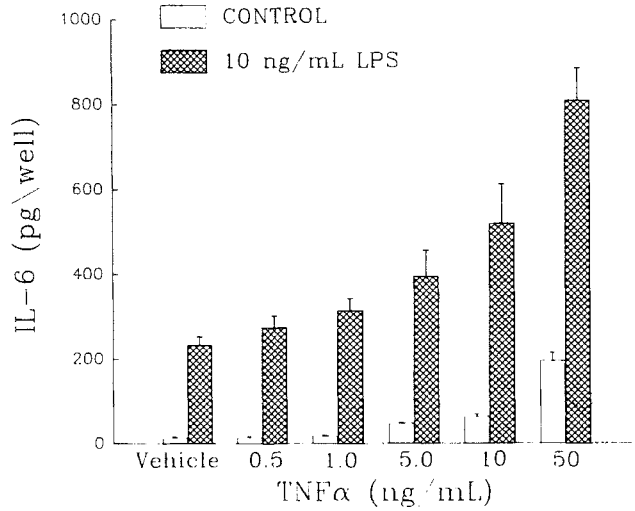

Figure 4 TNFα is a concentration-related fashion increased LPS-stimulated IL-6 release from primary cultures of adrenal zona glomerulosa cells. LPS (10 ng/mL) increased ($P<0.01$) IL-6 release. TNFα at concentrations of 5.0 ng/ml and greater increased ($P<0.01$) basal IL-6 release and potentiated LPS-stimulated IL-6 release. All incubations were of 6 h duration

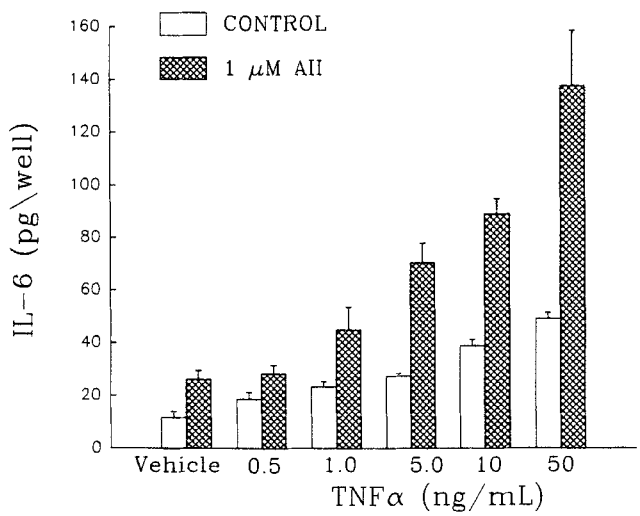

Figure 3 Exposure of primary cultures of rat adrenal zona glomerulosa cells to TNFα resulted in a concentration-related increase of basal and angiotensin II (AII)-stimulated IL-6 release. AII (1 μM) increased ($P<0.01$) IL-6 release. TNFα at 5.0 ng/ml and greater stimulated ($P<0.01$) basal IL-6 release. TNFα at concentrations of 1.0 ng/ml and greater increased ($P<0.01$) the IL-6 release stimulated by AII. The incubations interval was 5 h

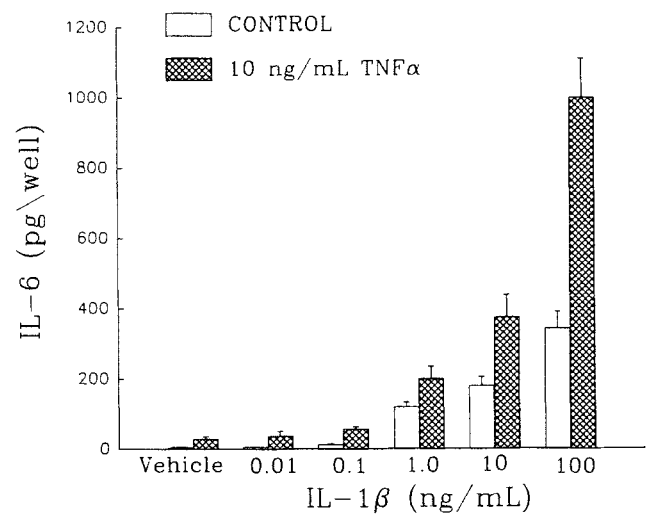

Figure 5 TNFα augmented IL-1β-stimulated IL-6 release from rat adrenal zona glomerulosa cells. IL-1β at concentrations of 0.1 nM and greater increased ($P<0.01$) IL-6 release in a concentration-dependent manner. TNFα (10 ng/mL) increased ($P<0.01$) basal IL-6 release and enhanced the IL-6 release stimulated by each concentrations of IL-1β. The incubation interval was 5 h

concentration of IL-1β. Similar to the effects of TNFα on the weaker secretagogues, the effects of TNFα on LPS- and IL-1β stimulated IL-6 release were more than additive of the effects of these secretagogues when administered alone.

TNFα over the same concentration ranges that stimulated adrenal IL-6 release did not increase either basal or IL-1β-stimulated IL-6 release from primary cultures of rat anterior pituitary cells (data not presented).

Discussion

The cytokines IL-6 and TNFα play a significant role in the body's response to stress. In general, TNFα promotes a state of inflammation (Tracey *et al.*, 1989). Because of the powerful inflammatory action of TNFα, it is not surprising that TNFα production is closely regulated. TNF promotes the release of both CRH from the hypothalamus and ACTH from the pituitary, and thus indirectly promoted adrenal glucocorticoid release. Adrenal glucocorticoids in turn inhibit the production of TNFα (Tracey *et al.*, 1989). Although IL-6 has some pro-inflammatory properties, IL-6 protects the various tissues of the body from the effects of TNFα (Tilig *et al.*, 1994). Therefore, the effects of IL-6 on the whole organism may be anti-inflammatory (Aderka *et al.*, 1989; Barton & Jackson, 1993; Tilig *et al.*, 1994). In many tissues, IL-6 plays an important role in either mediating the effects of TNFα (Iho *et al.*, 1993), attenuating the effects of TNFα by

decreasing its production (Aderka *et al.*, 1989; Schindler *et al.*, 1990), or inhibiting its action (Bermudez *et al.*, 1992; Aderka *et al.*, 1993; Hatzigeorgiou *et al.*, 1993). Therefore, TNF often increases basal and secretagogue-stimulated IL-6 production (Elias & Lentz, 1990; Kohda *et al.*, 1992; Aderka *et al.*, 1993; Maimone *et al.*, 1993). However, this effect of TNF is not found universally in all tissues because TNFα has no effect on IL-6 release in the rat anterior pituitary (data not presented), rat ovary (Gorospe & Spangelo, 1993) and murine T helper type 2 cells (Zubiaga *et al.*, 1990).

The rat adrenal zona glomerulosa produces both TNF and IL-6. The cell type that produces IL-6 and TNF in the rat adrenal zona glomerulosa has not been identified. However, in preliminary experiments we have determined that over 80% of the cells in our adrenal zona glomerulosa cultures are labeled with antibodies against rat IL-6 (unpublished observation). Furthermore, these adrenal cell cultures secrete aldosterone in a normal manner and respond to secretagogues that normally control the function of this tissue (Judd & MacLeod, 1992). In addition, rat adrenal zona fasciculata/reticularis cells do not secrete IL-6. Therefore, it is improbable that in the zona glomerulus cultures the IL-6 is secreted by zona fasciculata cells that are contaminating the cultures or by zona glomerulosa cells that have taken on characteristics of zona fasciculata cells. Similarly, it is improbable that fibroblasts are solely responsible for the IL-6 released from the zona glomerulosa cultures because it is doubtful that these cells make up 80% of the cells in our culture. Furthermore, both the zona glomerulosa and zona fasciculata/reticularis cultures probably contain some fibroblasts and only the zona glomerulosa cultures release IL-6. Therefore, it is probable that the steroid-secreting cells of the zona glomerulosa are the primary source of the adrenal IL-6. In support of this hypothesis, human adrenal IL-6 is produced by the steroid-secreting cells of the gland (Gonzalez-Hernandez *et al.*, 1994).

In this study, it was found that TNFα in a concentration-dependent manner increases both basal and secretagogue-stimulated IL-6 release from rat adrenal zona glomerulosa cells. TNFα inhibits the release of aldosterone from the zona glomerulosa. In contrast, in preliminary experiments we have learned that IL-6 may stimulate steroid release from rat adrenal zona glomerulosa cells. Therefore, IL-6 in the zona glomerulosa may antagonize the effects of TNFα on this adrenal tissue. It is of interest that some secretagogues stimulate the release of both IL-6 and TNFα from the adrenal zona glomerulosa whereas other secretagogues stimulate IL-6 release, but inhibit TNFα release (Judd & MacLeod, 1995).

TNFα and IL-6 also affect the adrenal zona fasciculata/reticularis. IL-6 stimulates corticosterone release from rat adrenal zona fasciculata/reticularis (Salas *et al.*, 1990). TNFα inhibits cortisol release from the fetal human adrenal (Jäättelä *et al.*, 1990, 1991), but may stimulate the release of cortisol in the adult human adrenal (Darling *et al.*, 1989). The effects of this cytokine on the release of corticosterone from the rat adrenal is not known. Although TNF and IL-6 are produced in very low amounts in the rat adrenal zona fasciculata/reticularis (Judd *et al.*, 1990; Judd & MacLeod, 1995), the blood flow of the adrenal is from the zona glomerulosa to the zona fasciculata/reticularis (Yeasting, 1985). Therefore, cytokines produced in the zone glomerulosa may be carried by the blood to the zona fasciculata/reticularis and thereby affect the function of this adrenal tissue.

Materials and methods

Materials

Recombinant TNFα (Calbiochemical, La Jolla, CA) was reconstituted in sterile culture medium (10 μg/mL) and stored at −20°C until diluted with incubation medium immediately before an experiment. Recombinant human IL-1β (Biological Modifiers Program, National Cancer Institute, Frederick, MD) was dissolved in sterile culture medium, diluted to 100 μg/ml, and stored at 4°C until used. LPS from Salmonella typhosa was dissolved (100 μg/ml) in sterile serum-free RPMI 1640 incubated medium and stored at 4°C until diluted with sterile incubation medium prior to an experiment. ACTH 1-24 (Organon, West Orange, NJ) and angiotensin II (Sigma, St. Louis) were dissolved in sterile water, diluted to 10 mM, and stored in aliquots at −20°C until diluted with incubation medium immediately preceding an experiment. Recombinant mouse IL-6 and the 7TD1 cell line were generously provided by Dr J Van Snick (Ludwig Institute, Brussels, Belgium).

Isolation of adrenal zona glomerulosa and preparation of cultured adrenal zona glomerulosa cells

Female Sprague–Dawley rats (Sasco Inc., Omaha, NE) weighing 220–250 g were housed in a temperature-controlled environment with a 12 h light/12 h dark cycle and were provided with unlimited food and water. Decapitation of the rats took place in the morning. This method of euthanasia was approved by the Institutional Animal Care and Use Committee (IACUC) of Brigham Young University. Adrenal glands were removed rapidly and in all experiments the adrenal zona glomerulosa was separated from the adrenal zona fasciculata/reticularis. The adrenal core and capsule were separated according to the method of Giroud *et al.* (1956). The adrenal capsule is composed primarily of the zona glomerulosa with a small fragment of the zona fasciculata. In contrast, the central core contains most of the zona fasciculata, all of the zona reticularis, and the adrenal medulla (Haning *et al.*, 1970). A shallow incision in the adrenal capsule was made with a razor blade and the adrenal squeezed gently with forceps until the decapsulated core was forced out of the incision. The fragments of adrenal zona glomerulosa were then dispersed into a single cell suspension with collagenase by previously published methods (Judd & McLeod, 1995). Following their dispersion, 25 000 adrenal cells were added to each well of a 48-well culture plate (Costar, Cambridge, MA) containing 750 μl complete RPMI/well (Judd & MacLeod, 1995). The cells were cultured for 4–6 days at 37°C in an atmosphere of 5% CO_2:95% air.

IL-6 release

On the day of an experiment, the complete RPMI was removed from the cultured adrenal cells and replaced with 0.25 ml sterile serum-free RPMI. The serum-free RPMI was immediately removed and replaced with 0.25 ml sterile serum-free RPMI containing the various pharmacological test agents. The adrenal cells were incubated with these agents for 5–7 h and the medium removed from the cells and stored at 4°C until assayed for IL-6.

IL-6 assay

The concentration of IL-6 in the adrenal cell incubation medium was detected with the 7TD1 bioassay for IL-6 as described previously (Judd *et al.*, 1990). The minimal detectable amount of IL-6 was 0.05 pg: the intraassay variability was less than 10% and the interassay variability less than 15%.

Statistical analysis

Each experiment was repeated at least three times. Each figure illustrates the results from a typical experiment. Statistical analyses were performed with a one-way analysis of variance and the Bonferroni test for multiple comparisons (Wallenstein *et al.*, 1980).

Acknowledgements

We wish to acknowledge William McBee, Michael S. Kennedy, Jon Lloyd, Heather Knight, Michael Gunson and John Kennedy for their excellent technical support and Dr J. Van Snick for supplying the 7TD1 cell line and recombinant murine IL-6 standard. This research was supported by grants from the American Heart Association, Utah Affiliate (UT-92-GB-7, 9306257S, 9406270S).

References

Aderka, A., Le, J. & Vilček, J. (1989). *J. Immunol.*, **143,** 3517–3523.

Aderka, D., Maor, Y., Novick, D., Engelmann, H., Kahn, Y., Levo, Y., Wallach, D. & Revel, M. (1993). *Blood*, **81,** 2076–2084.

Barton, B.E. & Jackson, J.V. (1993). *Infect. Immun.*, **61,** 1496–1499.

Bermudez, L.E., Wu, M., Petrofsky, M. & Young, L.S. (1992). *Infect. Immun.*, **60,** 4245–4252.

Darling, G., Goldstein, D.S., Stull, R., Gorschboth, C.M. & Norton, J.A. (1989). *Surgery*, **106,** 1155–1160.

Elias, J.A. & Lenz, V. (1990). *J. Immunol.*, **145,** 161–166.

Giroud, C.J.P., Stachenko, J. & Venning, E.H. (1956). *Proc. Soc. Exp. Bio. Med.*, **92,** 154–158.

Gonzalez-Hernandez, J.A., Bornstein, S.R., Ehrhart-Bornstein, M., Späith-Schwalbe, E., Jirikowski, G. & Scherbaum, W.A. (1994). *J. Clin. Endocrinol. Metab.*, **79,** 1492–1497.

Gorospe, W.C. & Spangelo, B.L. (1993). *Biol. Reprod.*, **48,** 538–543.

Haning, R., Tait, S.A.S. & Tait, J.F. (1970). *Endocrinology*, **87,** 1147–1167.

Hatzigeorgiou, D.E., He, S., Sobel, J., Grandstein, K.H., Hafner, A. & Ho, J.L. (1993). *J. Immunol.*, **151,** 3682–3692.

Iho, S., Golub, S.H. & Shau, H. (1993). *Scand. J. Immunol.*, **38,** 137–141.

Jäättelä, M., Carpén, O., Stenman, U.-H. & Saksela, E. (1990). *Mol. Cell. Endocrinol.*, **68,** R31–R36.

Jäättelä, M., Ilvesmäki, V., Voutilainen, R., Stenman, U.-H. & Saksela, E. (1991). *Endocrinology*, **128,** 623–629.

Judd, A.M. & MacLeod, R.M. (1991). *Prog. NeuroEndocrinImmunol.*, **4,** 240–247.

Judd, A.M. & MacLeod, R.M. (1992). *Endocrinology*, **130,** 1245–1254.

Judd, A.M. & MacLeod, R.M. (1995). *Am. J. Physiol.*, **268** (*Endocrinol Metab.*, **31**). E114–E120.

Judd, A.M., Spangelo, B.L. & MacLeod, R.M. (1990). *Prog. NeuroEndocrinImmunol.*, **3,** 282–292.

Kohda, H., Ono, M., Torimoto, Y., Ohira, M., Mizuno, M., Tanaka, T., Sekiya, C. & Namiki, M. (1992). *Gastroenterol. Jpn.*, **27,** 685–688.

Maimone, D., Cioni, C., Rosa, S., Macchia, G., Aloisi, F. & Annunziata, P. (1993). *J. Neuroimmunol.*, **47,** 73–81.

Natarajan, R., Ploszaj, S., Horton, R. & Nadler, J. (1989). *Endocrinology*, **125,** 3084–3089.

Salas, M.A., Evans, S.W., Levell, M.J. & Whicher, J.T. (1990). *Clin. Exp. Immunol.*, **79,** 470–473.

Schindler, R., Mancilla, J., Endres, S., Ghorbani, R., Clark, S.C. & Dinarello, C.A. (1990). *Blood*, **75,** 40–47.

Schultzberg, M., Andersson, C., Undén, A., Troye-Blomberg, M., Svenson, S.B. & Bartfai, T. (1989). *Neuroscience*, **30,** 805–810.

Tilig, H., Trehu, E., Atkins, M.B., Dinarello, C.A. & Mier, J.W. (1994). *Blood*, **83,** 113–118.

Tracey, K.J., Vlassara, H. & Cerami, A. (1989). *Lancet*, **1,** 1122–1126.

Wallenstein, S., Zucker, C.L. & Fleiss, J.L. (1980). *Circulation Res.*, **47,** 1–9.

Yeasting, R.A. (1985). In: *The Adrenal Gland.* Mulrow, J. (eds.). Elsevier: New York. pp. 45–63.

Zubiaga, A.M., Munoz, E., Merrow, M. & Huber, B.T. (1990). *Int. Immunol.*, **2,** 1047–1054.