Virchows Archiv A Pathol Anat (1992) 420:213–217

Virchows Archiv A
Pathological Anatomy and Histopathology
© Springer-Verlag 1992

Expression of adult T-cell leukaemia-derived factor, a human thioredoxin homologue, in the human ovary throughout the menstrual cycle

Toshiko Iwai [1], Shingo Fujii [3], Yoshihiko Nanbu [1], Hirofumi Nonogaki [1], Ikuo Konishi [1], Takahide Mori [1], Hiroshi Masutani [2], and Junji Yodoi [2]

[1] Department of Gynaecology and Obstetrics, Faculty of Medicine
[2] Institute for Virus Research, Kyoto University, Kyoto, Japan
[3] Department of Obstetrics and Gynaecology, School of Medicine, Shinshu University, Asahi, Matsumoto 390, Japan

Received June 6, 1991 / Received after revision August 13, 1991 / Accepted August 15, 1991

Summary. An immunohistochemical study of the expression of adult T-cell leukaemia-derived factor (ADF), a human thioredoxin homologue, was performed in the normal human ovary throughout the menstrual cycle. Primordial follicles were negative for ADF. Both granulosa cells and theca interna cells at the stages of preantral and antral follicles contained ADF. The staining intensity of these cells was very strong in the preovulatory dominant follicle. After ovulation, both granulo-lutein and theca-lutein cells were positive for ADF. During pregnancy, the theca-lutein cells revealed very intense ADF staining. The theca interna cells of the atretic follicles showed ADF staining, while the granulosa cells of such follicles did not. These results suggest that ADF localizes in the ovarian steroidogenic cells which have the binding sites of either luteinizing hormone or follicle-stimulating hormone, and that ADF expression is closely associated with the activity of the ovarian steroidogenic cells.

Key words: Adult T-cell leukaemia-derived factor – Thioredoxin – Human ovary – Immunohistochemistry

Introduction

Human T-lymphotrophic virus type 1 (HTLV-1) transformed T-cells not only express high levels of interleukin-2 receptors [IL-2R/p55(Tac)], but also produce an IL-2R/Tac inducer, designated adult T-cell leukaemia(ATL)-derived factor (ADF) (Okada et al. 1985; Yodoi et al. 1985; Tagaya et al. 1987). The human ADF protein has been purified, and we have cloned ADF cDNA (Tagaya et al. 1989). Recombinant ADF produced by *Escherichia coli* is a multi-functional protein with many biological activities besides its IL-2R/Tac-induced activity, activities such as the promotion of lymphocyte proliferation and synergism with IL-1 or IL-2 (Tagaya et al. 1989). A homology search has revealed a close relationship between ADF and a dithiol reducing enzyme, thioredoxin (Holmgren 1985; Tagaya et al. 1989). ADF is thus considered to be a human homologue of thioredoxin with strong dithiol-reducing activity.

We recently synthesized a synthetic peptide incorporating the C-terminal 29mer of ADF (ADF C-peptide), and an antibody against the peptide was raised in rabbits. Immunohistochemical study on human fetal tissues using this antibody has revealed that ADF is widely distributed in different organs and tissues during the fetal period (Fujii et al. 1991). ADF expression in fetal steroidogenic organs in particular, such as its expression in adrenal cortex, ovarian interstitial cells, and Leydig cells of the testis, prompted us to study the immunohistochemical localization of ADF in the steroidogenic cells of the human ovary. Moreover, it has been reported that both luteinizing hormone(LH) and follicle-stimulating hormone(FSH) not only contain a sequence homologous to thioredoxin (ADF) (Cys–Gly–Pro–Cys) but also have thioredoxin-like catalytic activity (Boniface and Reichert 1990). Since the ovary is a target organ of LH and FSH, we attempted to gain some new insights into the thioredoxin system during ovarian steroidogenesis by studying ADF expression in the steroidogenic tissues of the ovary throughout the menstrual cycle.

Materials and methods

Human ovarian specimens were obtained from 84 women, with their informed consent, at laparotomy for medical indications. The specimens were either biopsies or totally removed ovaries. The donors (32–49 years of age) had regular menstrual cycles (28–30 days) at surgery, except for 4 women who were pregnant. None of the women had received exogenous hormones for at least two cycles prior to surgery. The stage of their menstrual cycle at laparotomy was determined by a combination of the following: (1) days since the onset of the last menstrual period; (2) plasma levels of LH, oestradiol and progesterone; and (3) histological examination of the endometrium. Endometrial tissues were obtained either

Offprint requests to: S. Fujii

from extirpated uteri or from endometrial biopsies, and the day of the menstrual cycle was estimated histologically according to the method of Noyes et al. (1950). On histological examination, individual follicles were categorized as primordial, preantral, antral, preovulatory, and atretic. Corpora lutea were grouped as of the luteal phase, of the previous cycle, of pregnancy, and corpus albicans.

A synthetic peptide incorporating the C-terminal 29mer of ADF (ADF C-peptide) was synthesized using an automatic peptide synthesizer (Applied Biosystems, Foster Calif), and an anti-ADF antibody was then raised against this peptide conjugated with bovine serum albumin (BSA). The conjugate was injected subcutaneously into rabbits, together with Freund's complete adjuvant. After three immunizations, serum from the rabbits was purified by saturated ammonium sulphate precipitation and this was followed by application to a BSA-sepharose column to remove anti-BSA components. The serum was further purified using an immobilized ADF column. The specific antibody solution was dialysed against phosphate-buffered saline and stored frozen at −20° C. The specificity of the antibodies was tested by the Western blotting method using recombinant ADF, ADF C-peptide, and purified ADF obtained from the conditioned medium of ATL-2 cells (Maeda et al. 1985).

Tissues were fixed in either formalin or Bouin's solution and embedded in paraffin. Thin sections were deparaffinized in toluene and immunohistochemical staining was then performed as follows. Deparaffinized sections were treated with 3% hydrogen peroxide in methanol to inhibit endogenous peroxidase activity and were then incubated with normal goat serum to block non-specific antibody binding. The sections were then incubated with the primary antibody (rabbit anti-ADF IgG, 2.0 μg/ml) at 37° C for 60 min or with normal rabbit serum for the negative controls. The sections were then treated with anti-rabbit IgG-biotin complex and stained with 3-amino-9-ethylcarbazole solution plus 0.15% hydrogen peroxide. Reagents, except for the primary antibody, were obtained from the Universal Rabbit Kit System (Biomeda Corp., Foster, Calif). Counterstaining was performed with haematoxylin. ATL-2 cells served as the positive controls (Maeda et al. 1985). The intensity of staining was evaluated by staining the same specimens several times and observations were performed by more than two observers. Intensity was graded as (−) for no staining, (+) for weak staining, (++) for moderate staining, and (+++) for strong staining. Histological observation of the specimens was performed using routinely processed sections stained with haematoxylin- and eosin.

Results

A large number of ATL-2 cells (HTLV-1-transformed cells) had the cytoplasm stained red, indicating positivity for ADF. Tables 1 and 2 show the immunohistochemical localization of ADF in the human ovary.

In the primordial follicles, neither follicular cells nor oocytes showed specific staining for ADF. Both the granulosa and the theca interna cells of the preantral follicle showed weak staining for ADF. In the antral follicle, both granulosa and theca interna cells showed positivity for ADF (Fig. 1A, B). The granulosa cells and the theca interna cells of the preovulatory dominant follicle showed very strong staining for ADF (Fig. 2A, B). ADF staining was usually localized in the cytoplasm, but the granulosa cells often showed immunostaining in the cell membranes and/or intercellular spaces (Fig. 2A). ADF staining was also detected in non-dominant follicles of the preovulatory and luteal phase, but the staining in the granulosa cells was weaker than that in the dominant follicle. The granulosa cells of the atretic follicle showed no staining for ADF, but the theca interna cells were positive (Fig. 3).

Table 1. Expression of adult T-cell leukaemia-derived factor (ADF) in follicular cells

Follicular state	Granulosa cells	Theca interna cells
Primordial	−	−
Preantral	+	+
Antral	++	+
Preovulatory (dominant follicle)	+++	++
Preovulatory (non-ovulatory follicle)	+	++
Atretic	−	++

Grading by intensity: −, not detectable; +, weak staining; ++, moderate staining; +++, strong staining

Table 2. ADF expression in luteal cells

Luteal state	Granulo-lutein cells	Theca-lutein cells
Corpus luteum (luteal phase) ($n=31$)	++	++
Corpus luteum (pregnancy) ($n=4$)	++	+++
Corpus luteum (previous cycle) ($n=4$)	+/++	++
Corpus albicans	−	−

Footnotes as in Table 1

ADF staining was observed in the corpus luteum throughout the luteal phase (Fig. 4A, B). Both granulo-lutein and theca-lutein cells showed homologous staining for ADF in the cytoplasm. The corpus luteum of the previous cycle also showed ADF staining; however, the staining intensity was weaker than that of the current corpus luteum. The theca-lutein cells which surrounded the degenerated corpus luteum also showed moderate ADF staining. The corpus albicans showed no ADF staining. The corpus luteum of pregnancy was also positive for ADF, but the theca-lutein cells and/or gonadotropin-stimulated stromal cells showed more intense ADF staining than that in the granulo-lutein cells (Fig. 5).

Discussion

This study describes the immunohistochemical localization of ADF, a human thioredoxin homologue, in the human ovary at different stages of the menstrual cycle, using an antibody against the C-terminal peptides of ADF protein. ADF was not expressed in the primordial

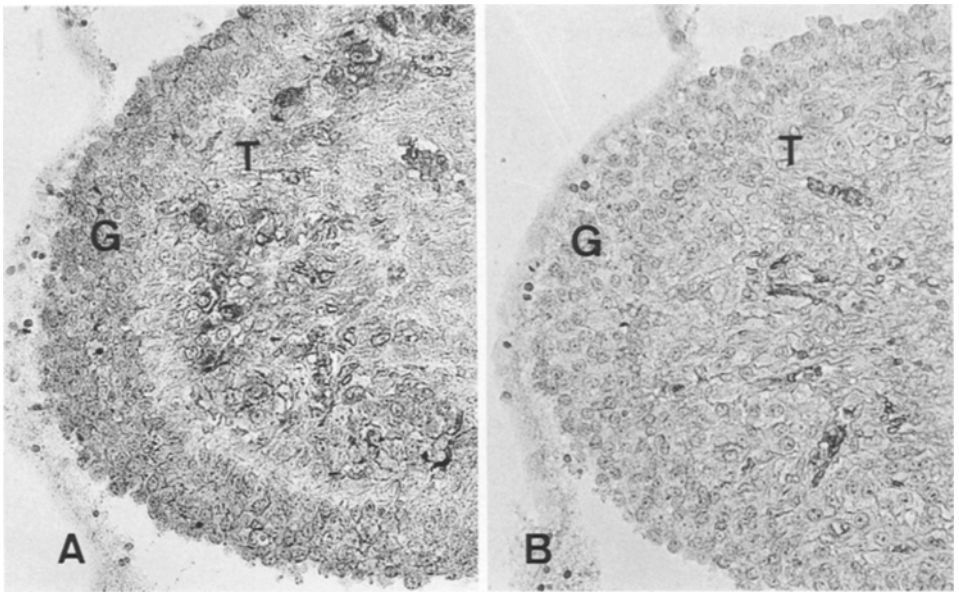

Fig. 1A, B. Sections of antral follicles. Both granulosa cells (*G*) and theca interna cells (*T*) showed adult T-cell leukaemia-derived factor (ADF) staining **A**. **B** Negative control, ×200

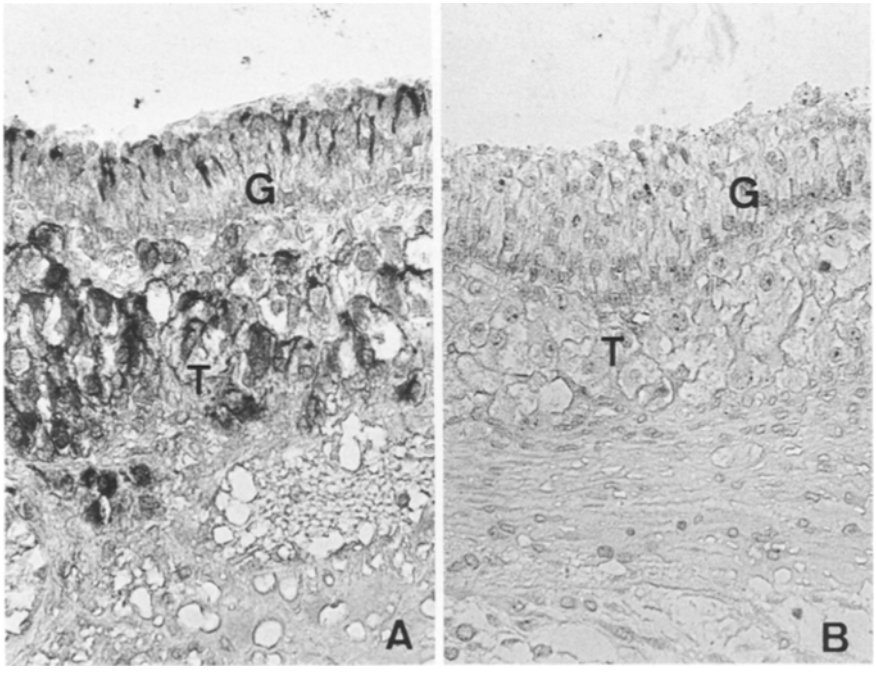

Fig. 2A, B. Sections of preovulatory dominant follicles. Both granulosa cells (*G*) and theca interna cells (*T*) showed very strong ADF staining **A**. In the follicle of this stage, ADF staining was detected not only in cytoplasm but also in cell membrane and/or intercellular spaces. **B** Negative control, ×200

follicles. Depending on the growth stage of the follicles, ADF was detected in both granulosa and theca interna cells. Preovulatory dominant follicles showed strong positivity for ADF in both types of follicular cells which developed into steroid hormone-producing cells (Ryan et al. 1968; McNatty et al. 1976). During the preovulatory phase, follicles other than the dominant follicle, which can also produce steroids (Goodman et al. 1977), showed ADF staining in the granulosa and theca interna cells. However, the staining intensity was weaker than that in the dominant follicle.

After ovulation, the granulo-lutein and theca-lutein cells, both of which produce amounts of progesterone, oestrogen and other steroid hormones, showed moderate to strong positivity for ADF. In the atretic follicles, the granulosa cells, which are believed to produce no oestrogen, stained negatively for ADF, but the theca interna cells, which are believed to produce small amounts of androgen, showed positive ADF staining as long as the cell structures were light microscopically preserved. Thus, it can be seen that ADF expression is very closely associated with the activity of steroid-producing cells in the human ovary. In addition, ADF localization in the ovary corresponds to the distribution of gonadotropin-binding sites (Tsang et al. 1979, 1980; Yamoto et al. 1986; Ohara et al. 1987; Shima et al. 1987) and with the distribution of oestrogen receptors and progesterone receptors (Iwai et al. 1990). Moreover, the localization

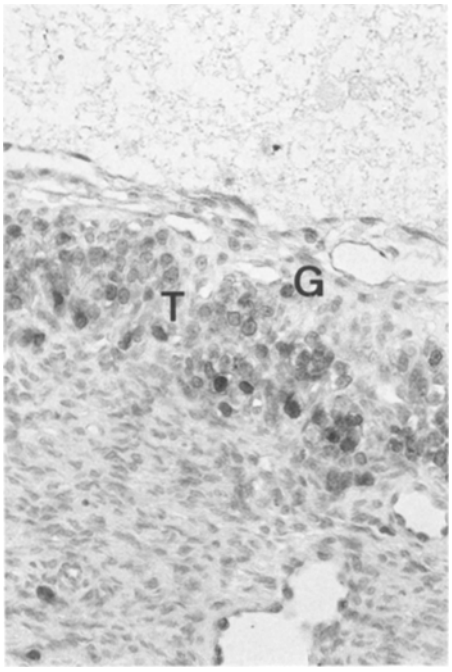

Fig. 3. Sections of atretic follicles. Granulosa cells (*G*) showed no ADF staining, but theca interna cells (*T*) were positive for ADF. ×200

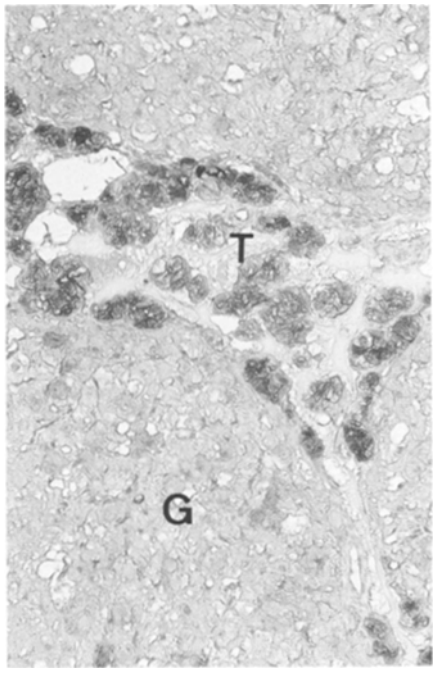

Fig. 5. Sections of the corpus luteum of pregnancy (8 weeks' gestation). Theca-lutein cells (*T*) and/or gonadotropin-stimulated stromal cells showed more intense ADF staining than that seen in granulo-lutein cells (*G*), ×200

of ADF in the human ovary resembles the localization of thioredoxin and thioredoxin reductase in rat ovary (Hannson et al. 1986).

In general, it has been reported that growing or proliferating cells contain thioredoxin at some level and that the variations which exist in the immunoreactive thioredoxin content of non-proliferating, differentiated cells are related to their metabolic activity, secretory activity and their accumulation of cell products (Holmgren 1985; Rozell et al. 1985). In the human fetus, some cellular function(s) acquired by differentiation during the fetal period seemed to be necessary for the expression of ADF (Fujii et al. 1991). Of the hormone-producing cells in the human fetus, steroid-producing cells in the adrenal cortex, the ovary (interstitial cells) and the testis (Leydig cells), all of which are functionally active during the fetal period, showed strong reactivity for ADF (Fujii et al. 1991).

It is well known that aromatase cytochrome P-450, 17-hydroxysteroidodehydrogenase cytochrome P-450,

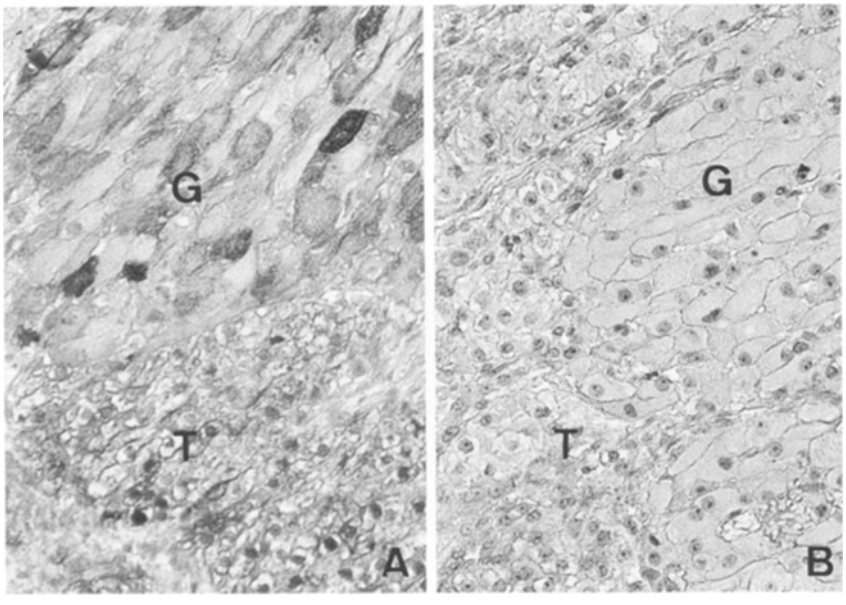

Fig. 4 A, B. Sections of corpus luteum. Both granulo-lutein (*G*) and theca-lutein (*T*) cells showed homologous ADF staining in the cytoplasm (**A**). **B** Negative control, ×200

and cholesterol side-chain cleavage cytochrome P-450 are all important regulatory enzymes of steroidogenesis in the overy, and the localization of P-450 corresponds to the site of steroidogenesis (Sasano et al. 1989). The catalytic action of cytochrome P-450, as well as that of thioredoxin, is one kind of NADPH-dependent oxidation-reduction reaction. It has been proposed that dithiol-disulphide interchange and oxidation-reduction reactions may play a role in hormone-induced receptor activation (Grippo et al. 1983). Recently, inspection of the sequences of the gonadotropic hormones has revealed a homologous tetrapeptide (Cys—Gly—Pro—Gys) between the beta-subunit of LH and the active site of thioredoxin (Boniface and Reichert 1990). In addition, bovine LH preparation was found to be more than 300 times as active as thioredoxin, as a catalytic agent, on a molar basis, and it is suggested that this catalytic property of LH may be important in understanding the mechanism of receptor activation and signal transduction. Therfore, the ADF expression in gonadotropin target cells which also have catalytic properties is interesting to note.

In this communication, we have described the distribution of ADF in the follicular cells of the human ovary and have suggested a close relationship between ADF expression and the activity of steroidogenic cells. Work is now in progress to clarify whether or not ADF has a critical regulatory role in steroid-producing cells.

Acknowledgements. We greatly appreciate the kind advice of Dr. Michiyuki Maeda at the Chest Research Institute of Kyoto University. We thank Dr. Akira Mitsui at Aji-no-moto Central Laboratory for technical help in the cloning and expression of recombinant ADF. This work was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education (No. 02454381), Japan.

References

Boniface JJ, Reichert LE jr. (1990) Evidence for a novel thioredoxin-like catalytic property of gonadotropic hormones. Science 247:61–64

Fujii S, Nanbu Y, Nonogaki H, Konishi I, Mori T, Masutani H, Yodoi J (1991) Immunohistochemical localization of adult T-cell leukaemia-derived factor, a human thioredoxin homologue, in human fetal tissues. Virchows Arch [A] 419:317–326

Goodman AL, Nixon WE, Hodgen DK (1977) Regulation of folliculogenesis in the cycling rhesus monkey: selection of the dominant follicle. Endocrinology 100:155–161

Grippo JF, Tienrungroi W, Dahmer MK, Housley PR, Pratt WB (1983) Evidence that the endogenous heat-stable glucocorticoid receptor activating factor is thioredoxin. J Biol Chem 258:13658–13664

Hannson HA, Holmgren A, Rozell B, Taljedal IB (1986) Immunohistochemical localization of thioredoxin and thioredoxin reductase in mouse exocrine and endocrine pancreas. Cell Tissue Res 245:189–195

Holmgren A (1985) Thioredoxin. Annu Rev Biochem 54:237–271

Iwai T, Nanbu Y, Iwai M, Taii S, Fujii S, Mori T (1990) The immunohistochemical localization of estrogen receptor and progesterone receptor in the human ovary throughout the menstrual cycle. Virchows Arch [A] 417:369–375

Maeda M, Shimizu A, Ikuta K, Okamoto H, Kashihara M, Uchiyama T, Honjo T, Yodoi J (1985) Origin of HTLV-1(+) T-cell lines in adult T-cell leukemia: analysis on T-cell receptor gene rearrangement. J Exp Med 162:2169–2174

McNatty KP, Baird DT, Bolton A, Chambers P, Corker CS, McLean H (1976) Concentration of oestrogen and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J Endocrinol 71:77–85

Noyes RW, Hertig AT, Rock J (1950) Dating the endometrial biopsy. Fertil Steril 1:3–25

Ohara A, Mori T, Taii S, Ban C, Narimoto K (1987) Functional differentiation in steroidogenesis of two types of luteal cells isolated from mature human corpora lutea of menstrual cycle. J Clin Endocrinol Metab 65:1192–1200

Okada M, Maeda M, Tagaya Y, Taniguchi Y, Teshigawara K, Yoshiki T, Diamantstein T, Smith KA, Uchiyama T, Honjo T, Yodoi J (1985) TCGF(IL-2)-receptor inducing factor(s). II. Possible role of ATL-derived factor(ADF) on constitutive IL-2 receptor expression of HTLV-1(+) T cell lines. J Immunol 135:3995–4003

Rozell B, Hasson HA, Luthman M, Holmgren A (1985) Immunohistochemical localization of thioredoxin and thioredoxin reductase in adult rats. Eur J Cell Biol 38:79–86

Ryan KJ, Petro F, Kaiser J (1968) Steroid formation by isolated and recombined ovarian granulosa and theca cells. J Clin Endocrinol Metab 28:355–358

Sasano H, Okamoto M, Mason JI, Simpson ER, Mendelson CR, Sasano N, Silverberg SG (1989) Immunolocalization of aromatase, 17 alpha-hydroxylase and side-chain cleavage cytochromes P-450 in the human ovary. J Reprod Fertil 85:163–169

Shima K, Kitayama S, Nakano R (1987) Gonadotropin binding sites in human ovarian follicles and corpora lutea during the menstrual cycle. Obstet Gynecol 69:800–806

Tagaya Y, Taniguchi Y, Naramura M, Okada M, Suzuki N, Kanamori H, Nikaido T, Honjo T, Yodoi J (1987) Transcription of IL-2 receptor gene is stimulated by ATL-derived factor produced by HTLV-1(+) T cell lines. Immunol Lett 15:221–228

Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J, Brown N, Arai K, Yokota T, Wakasugi H, Yodoi J (1989) ATL-derived factor(ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. EMBO J 8:757–764

Tsang BK, Moon YS, Simpson CW, Armstrong DT (1979) Androgen biosynthesis in human ovarian follicles: cellular source, gonadotropic control, and adenosine 3′5′ monophosphate mediation. J Clin Endocrinol Metab 48:153–158

Tsang BK, Armstrong DT, Whitfield JF (1980) Steroid biosynthesis by isolated human ovarian follicular cells in vitro. J Clin Endocrinol Metab 51:1407–1411

Yamoto M, Nakano R, Iwasaki M, Ikoma H, Furukawa K (1986) Luteinizing hormone receptors in human ovarian follicles and corpora lutea during the menstrual cycle. Obstet Gynecol 68:200–203

Yodoi J, Teshigawara K, Nikaido T, Fukui K, Noma T, Honjo T, Takigawa M, Sasaki M, Minato N, Tsudo M, Uchiyama T, Maeda M (1985) TCGF (IL-2)-receptor inducing factor(s). I. Regulation of IL-2 receptor on a natural killer-like cell line (YT cells). J Immunol 134:1343–1623