Review

The epigenetic effects of glucocorticoids, sex hormones and vitamin D as steroidal hormones in rheumatic musculoskeletal diseases

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ABSTRACT

Chronic rheumatological diseases are multifactorial conditions in which both the neuroendocrine hormone pathway, including cortisol, sex hormones and active vitamin D_3 (calcitriol), all deriving from cholesterol, and the epigenetic modifications that they cause play an important role. In fact, epigenetics modulates the function of the DNA of immune cells, through three main mechanisms: DNA methylation, modifications to the histones that make up chromatin and production of non-coding RNAs (microRNA - miRNA).

In this narrative review, the main data regarding the epigenetic modifications induced by cortisol, 17β-oestradiol, progesterone, testosterone and calcitriol on immune cells were collected, discussing how these can interfere in the predisposition and course of chronic rheumatological diseases (i.e. rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis). An ever-increasing number of miRNAs have been identified, which are produced by neuroendocrine hormones and can influence the inflammatory-fibrotic response at various levels. Concerning the involvements of the neuro-endocrine-immunology within the pathophysiology of rheumatic diseases, the epigenetic effects induced by steroid hormones must be taken into consideration to evaluate their impact on the progression of the single condition and even inside the single patient.

Introduction

Neuroendocrine immunology (NEIRD) influences the course of chronic rheumatic diseases through fine regulation exerted by pro- and anti-inflammatory hormones and cytokines (1, 2). Generally, steroid hormones (cortisol, sexual hormones, calcitriol), all deriving from the biochemical structure of cholesterol (cyclopentane-perhydro-phenanthrene ring), play both rapid non-genomic effects (through membrane coupled receptors) and slow genomic effects (through cytosolic receptors and DNA interaction), regulating the activation of the immune system against acute and chronic stressors (1, 2).

Rheumatic diseases have a multifactorial pathogenesis, with a genetic susceptibility deeply influenced by epigenetic factors (3). The study of epigenetics has been promoted in the early 1940s by the biologist Conrad Waddington, who focused on the changes which occur to the gene expression of cells without alterations of DNA (4).

The first epigenetic modification to be found was DNA methylation and, even if though it was known since the 1940s that DNA can be methylated, the connection to epigenetic regulation of gene expression by this DNA modification was only made thirty years later (5-7).

Three major mechanisms of epigenetic regulation have been identified: DNA methylation, histones modifications and synthesis of non-coding RNAs (ncRNAs) (3).

DNA methyltransferases (*i.e.* DNMT1, 3a and 3b) are a family of enzymes that catalyses the transfer of methyl -CH3 groups to cytosine dinucleotides of DNA (8). DNA methylation of gene regulatory regions can silence or activate transcription factors, depending on the biological condition (9).

Histones are basic proteins involved in the packing of the DNA into chromatin (10). Histones can undergo post-translational modifications, as acetylation, methylation, phosphorylation, ubiquit-

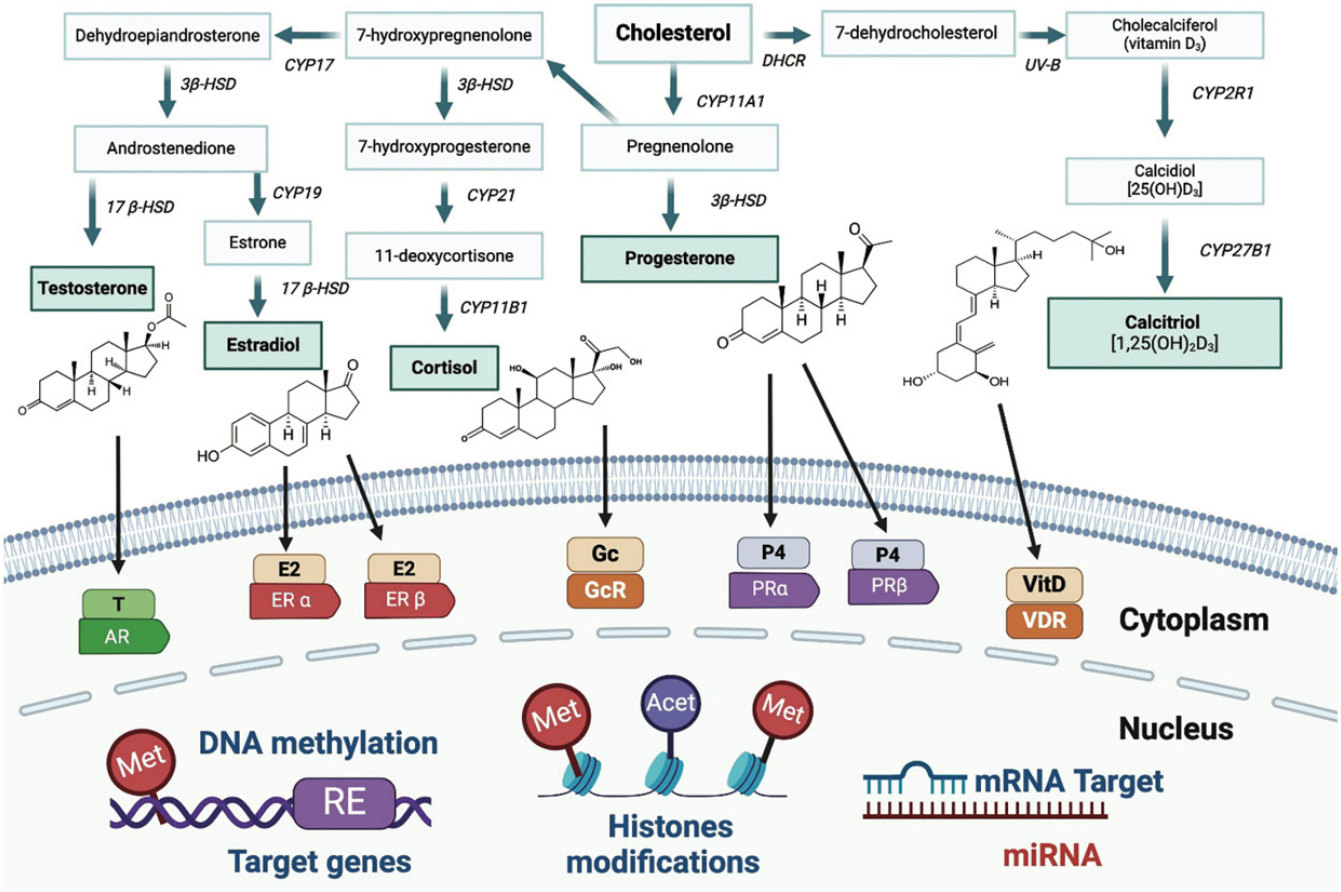

Fig. 1. Cortisol, sex hormones (17β-estradiol, progesterone, testosterone) and calcitriol biosynthesis, cytoplasmic receptors and nuclear epigenetic effects. Acet: acetylation; AR: androgen receptor; E2: 17β-oestradiol; ER: oestrogen receptor; Gc: glucocorticoid; GcR: glucocorticoid receptor; mRNA: messenger RNA; Met: methylation; miRNA: microRNA; P4: progesterone; PR: progesterone receptor; RE: responsive elements; VitD: vitamin D_3; VDR: vitamin D receptor; T: testosterone.
3β-HSD: 3β-hydroxysteroid dehydrogenase; 17β-HSD: 17β-hydroxysteroid dehydrogenase; CYP2R1: vitamin D_3 25-hydroxylase; CYP11A1: cholesterol side chain cleavage enzyme; CYP11B1: steroid 11β-hydroxylase; CYP19: aromatase; CYP21: steroid 21-hydroxylase; CYP27B1: 25-hydroxyvitamin D_3 1α-hydroxylase; DHCR: 7-dehydrocholesterol reductase; UV-B: ultraviolet b radiation.

ination and sumoylation. These modifications lead to an alteration of the chromatin structure and can modify protein transcription and cell replication (10).
At last, ncRNAs include microRNAs (miRNAs) and long ncRNAs, which do not code for any protein but regulate mRNA translation (11).
Epigenetic modifications induced by cortisol, sex hormones and vitamin D signalling are implicated in the pathogenesis of many chronic rheumatological diseases and have been the subject of several research. Interestingly, a growing body of evidence has demonstrated that these neuroendocrine hormones are in turn capable of epigenetically modifying the cells of the immune system, beyond their classic genomic and non-genomic effects (8).
Therefore, the purpose of this narrative review is to resume the direct epigenetic effects induced by steroid hormones (cortisol, sex hormones and calcitriol) on immune cells, highlighting their implication on chronic rheumatic diseases.

Hypothalamic-pituitary-adrenal axis and epigenetic effects of cortisol on immune cells

The hypothalamic-pituitary-adrenal (HPA) axis is rapidly activated during acute stress (12). In response to pro-inflammatory cytokines, *i.e.* interleukin (IL)-1, IL-6, tumour necrosis factor (TNF) α, hypothalamus secretes corticotropin releasing hormones (CRH), which stimulates adenohypophysis to release adrenocorticotropic hormone (ACTH) (13). ACTH stimulates steroidogenesis in the zona fasciculata of adrenal glands, promoting the conversion of cholesterol into pregnenolone (14). Pregnenolone is further hydroxylated into 7-hydroxypregnenolone, that is common precursor of cortisol and sex hormones (oestradiol, progesterone and testosterone) (Fig. 1) (14).
Cortisol binds to glucocorticoid receptors (GcRs) that are transcription factors ubiquitously expressed in the cytoplasm of immune cells (Fig. 2).
Inactive GcRs are complexed with chaperon proteins, particularly FK506-binding protein 51 (FKBP5). When activated, GcRs undergo conformational changes and translocate into cell nucleus, where bind to glucocorticoid response elements of DNA sequences, influencing the transcription of thousands of genes, involved in stress response (15).
Of note, activated GcRs downregulate the transcription of nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) and consequently the synthes-

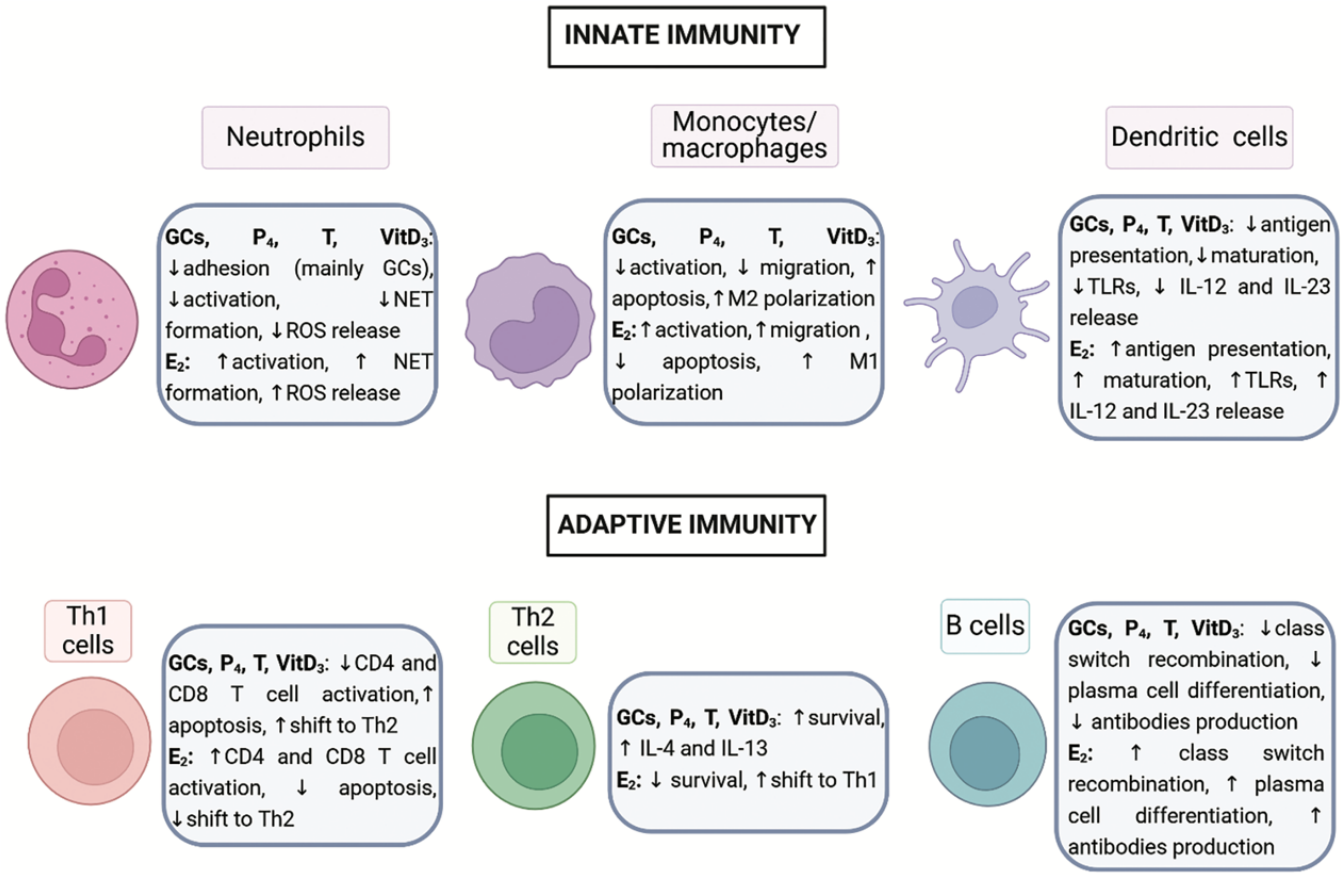

Fig. 2. Schematisation of the biologic effects of glucocorticoids (GCs), 17β-oestradiol (E_2), progesterone (P_4), testosterone (T) and active vitamin D_3 ($VitD_3$) on main cells of innate and adaptive immunity. Biologic effects of 17β-oestradiol refer to normal serum concentrations during follicular and luteal phases of woman menstrual cycle.
IL: interleukin; M1: pro-inflammatory macrophages; M2: pro-fibrotic macrophages; NET: neutrophils extracellular traps; ROS: reactive oxygen species; TLRs: Toll-like receptors.

is of pro-inflammatory cytokines (15). Beyond these genomic mechanisms, epigenetic effects of cortisol have also been studied, especially under conditions of its exogenous administration (16).

In a cohort of 21 healthy volunteers, blood samples were taken 24 hours after ACTH stimulation to investigate the relationship between DNA methylation status and cortisol serum concentrations (17). A significant demethylation of FKBP5 has been observed after 2-4 weeks in human white blood cells (17). Similarly, betamethasone administration during routine clinical pregnancy care (52 cases *vs*. 84 gestational age-matched controls) has been significantly associated with lower placental DNA methylation of FKBP5 (18).

Interestingly, the final biologic effect of FKBP5 demethylation due to an excess of exogenous glucocorticoids in non-inflammatory conditions seems the activation of the pro-inflammatory pathway (NF-κB) (19).

Glucocorticoids stimulate histone deacetylases (HDACs), a group of enzymes that are divided into three classes (I, IIa/IIb and III or sirtuins) (20). Activation of HDACs has an overall anti-inflammatory effect, downregulating the activation of genes involved in the synthesis of pro-inflammatory cytokines (*i.e.* IL-1β, IL-6, IL-17) in chronic diseases (21).

At last, glucocorticoids can regulate the biosynthesis of several miRNAs (Table I), while an effect on lncRNAs has not been yet clearly identified (22, 23).

In lipopolysaccharide (LPS)-injected mice, glucocorticoids (dexamethasone) inhibit the biosynthesis of miR-155, that is highly expressed in peripheral monocytes, M1 macrophages and fibroblasts of rheumatoid arthritis (RA) synovium (24-26).

As matter of fact, miR-155 enhances the production from RA monocytes genes of pro-inflammatory chemokines, as CCL3, CCL4, CCL5 and CCL8, up-regulating the expression of CCR7, that promote homing of dendritic cells and lymphocytes to lymph nodes (25). In addition, miR-155 downregulates also CCR2, that promotes monocyte migration in inflamed tissues (25). Moreover, the expression of miR-155 in CD14+ cells (LPS receptor and markers of monocytes/macrophages) in the synovial fluid of RA patients has been associated with a downregulation of anti-inflammatory Src homology 2 domain containing inositol polyphosphate 5-phosphatase (SHIP-1) (26).

Interestingly dexamethasone can also inhibit the synthesis of miR-101, enhancing expression of mitogen-activated protein kinase phosphatase-1 (MKP-1), that downregulates the release of pro-inflammatory cytokines by LPS treated M1 macrophages (27).

On the other hand, glucocorticoids favor the polarisation of M1 macrophages to anti-inflammatory M2 macrophages, enhancing the synthesis of miR-511 that downregulate gene expression of p55 TNF receptor (TNFR) (28).
Finally, methylprednisolone can upregulate the biosynthesis of miR-98 by activated human CD4+ T lymphocytes *in vitro* (29). Of note, miR98 suppresses pro-apoptotic genes Fas and Fas ligand and pro-inflammatory gene of TNFR superfamily member 1B (29).
Therefore, glucocorticoids act at least as anti-inflammatory compounds through epigenetic mechanisms that should be evaluated also based on the circadian rhythms of their endogenous production and night-release formulations contrasted by night melatonin rise (30-33).

Key messages:
Glucocorticoids and epigenetics

- Activation of glucocorticoids anti-inflammatory signalling requires methylation (inactivation) of receptor regulatory protein FKBP5: on the other hand, high dosages glucocorticoids in the long term stimulate demethylation of FKBP5 and consequently inactivate glucocorticoid receptor signalling (19).
- Glucocorticoids activate histone deacetylases in immune cells, downregulating the activation of the genes involved in the synthesis of pro-inflammatory cytokines (*i.e.* IL-1β, IL-6, IL-17) (21).
- Glucocorticoids regulate the biosynthesis of different micro-RNAs (*i.e.* miR-155, miR-101, miR-511, mir-98) that overall mediates the anti-inflammatory response (25-29).

Hypothalamic-pituitary-gonadal axis and epigenetic effects of sex hormones on immune cells

The hypothalamic-pituitary-gonadal (HPG) axis is traditionally involved in the development of human sexual characteristics and in regulating reproductive processes (34). Hypothalamus secretes gonadotropin releasing hormone (GnRH) to stimulate adenohypophysis to release luteinising hormone (LH) and follicle stimulating hormone (FSH). LH and FSH target female ovaries to produce 17β-oestradiol (E2) and progesterone and male testes to produce testosterone (34).
A little amount of sex hormones is produced also in adrenal glands from the conversion of 7-hydroxypregnenolone into dehydroepiandrosterone by CYP17. Dehydroepiandrosterone is then converted into androstenedione by 3β-hydroxysteroid dehydrogenase. Depending on further catalysation processes, androstenedione can be the precursor of both E2 (CYP 19 and 17β-hydroxysteroid dehydrogenase) and testosterone (only 17β-hydroxysteroid dehydrogenase) (Fig. 1) (12).

Oestradiol

Oestrogens, and particularly the most active form E2, bind to cytosolic oestrogen receptor α (ERα) and oestrogen receptor β (ERβ) (35). ERα is mainly expressed in female reproductive tissues (breasts, ovary, uterus), while ERβ is expressed ubiquitously (35). Immune cells express both ERα and ERβ, with opposite functions (ERα pro-inflammatory and ERβ anti-inflammatory) (35).
Activated ERs translocate into the nucleus and bind to oestrogen response elements of DNA sequences, regulating the transcription of NF-κB, signal transducer and activator of transcription 3 (STAT3), forkhead box protein O3 (FOXO3) and interferon regulatory factor 1 (IRF1), therefore modulating the inflammatory response (Fig. 1) (35).
Low serum concentrations of E2 (*i.e.* follicular and luteal phases of woman menstrual cycle) stimulate a pro-inflammatory response, while high serum concentrations (*i.e.* ovulatory phase of woman menstrual cycle or pregnancy) reduce the activation of innate immunity and T cells, however promoting a B cell response (Fig. 2) (36).
E2 exerts epigenetic effects on different cell types (37). In systemic lupus erythematosus (SLE) women, E2 significantly downregulates DNMT1 expression in CD4+ T cells, through overexpression of ERα promoting DNA hypomethylation that is associated with exacerbation of SLE (38).
Moreover, in oestrogen-treated mice, E2 downregulates the synthesis of miR-146a and miR-125a, negative regulators of interleukin 1 receptor associated kinase 1 (IRAK1) gene. Therefore, Toll-like receptors are activated and produce interferon (IFN)-α and pro-inflammatory CCL5 (39-41).
Furthermore, in oestrogen-treated mice, there is an up-regulation of miR-148a, that promotes B cell tolerance, survival and maturation, suppressing autoimmune suppressor Gadd45α, tumor suppressor PTEN and pro-apoptotic protein Bim (42). E2 downregulates also mir-26a, that usually limits AID (Activation Induced Deaminase) gene, that expresses a key enzyme for class switch DNA recombination and somatic hypermutation, further promoting B cell response (43).
Of note, beyond autoantibodies production, B cells show an up-regulation of IFN-signalling, through downregulation of let-7e-5p, miR-98-5p and miR-145a-5p, that are negative regulators of IKBKE gene (44).
On the other hand, TNF-α driven inflammatory response seems attenuated by E2. In fact, human macrophages treated with E2 *in vitro* show a lower expression of let-7a and a higher expression of miR-125b, inducing kB-Ras2, that inhibits pro-inflammatory NF-κB (45). Furthermore, in LPS-treated macrophages, E2 upregulates *in vitro* the synthesis of miR-29a-5p, that can repress the expression of NLRP3 gene inflammasome by macrophages (46).
At last, in another experimental model, osteoblasts (MC3T3-E1 cells) have been oxidative stressed with hydrogen peroxide and subsequently treated with E2 (47). Interestingly, E2, downregulating the synthesis of miR-320-3p, attenuated inflammation through inhibition of RUNX2 (Table I) (47).
In conclusion, oestrogens at least E2, exert strong and complex immunomodulatory activities epigenetically mediated on immune cells, that are reflected in clinical setting by the gender and age well known epidemiological differences in presence of autoimmune rheumatic diseases (48-49).

Key messages:
Oestrogens and epigenetics

- Oestrogens play opposite effects on inflammation depending on serum

Table I. Steroid hormones (glucocorticoids, sex hormones and vitamin D) and regulation of microRNAs in immune cells.

	Gene targets	Biological function	Effect of hormone	Reference
Glucocorticoids				
miR-155	CCL3, CCL4, CCL5, CCL8	Stimulation of release of pro-inflammatory chemokines	Downregulation	(25)
	CCR7	Expression of a receptor for homing of lymphocytes and dendritic cells	Downregulation	(25)
	SHIP-1	Inhibition of anti-inflammatory proteins	Downregulation	(26)
miR-101	MKP-1	Stimulation of pro-inflammatory polarisation of macrophages	Downregulation	(27)
miR-511	p55 subunit of TNFR	Inhibition of expression of subunit receptor of TNF	Upregulation	(28)
miR-98	Fas, FasL, TNFR	Inhibition of pro-inflammatory proteins	Upregulation (at high dosages)	(29)
17-β oestradiol				
miR-146a	IRAK1	Inhibition of activation of Toll-like receptors	Downregulation	(39,40)
miR-125a	IRAK1	Inhibition of activation of Toll-like receptors	Downregulation	(41)
miR-148a	Gadd45α, PTEN, Bim	Stimulation of B cells survival and maturation	Upregulation	(42)
miR-26a	AID	Inhibition of DNA recombination and somatic hypermutation	Downregulation	(43)
let-7e-5p	IKBKE	Inhibition of IFN signalling	Downregulation	(44)
miR-98-5p	IKBKE	Inhibition of IFN signalling	Downregulation	(44)
miR-145a-5p	IKBKE	Inhibition of IFN signalling	Downregulation	(44)
let-7a	kB-Ras2	Inhibition of NF-κB signalling	Downregulation	(45)
miR-125b	kB-Ras2	Activation of NF-κB signalling	Upregulation	(45)
miR-29a-5p	NLRP3	Downregulation of innate response	Upregulation	(46)
miR-320-3p	RUNX2	Inhibition of osteoblastogenesis	Downregulation	(47)
Testosterone				
miR-125b	TGFβ	Activation of fibrotic pathway	Upregulation	(67)
Calcitriol				
miR-155	SOCS1	Inhibition of negative regulators of STAT1-STAT3	Downregulation	(79,81)
miR-98-5p	ROR γ-t	Inhibition of Th17 response	Upregulation	(80)
let-7a	ROR γ-t	Inhibition of Th17 response	Upregulation	(80)
miR-149-5p	ATF6	Inhibition of endoplasmic reticulum stress-induced inflammation	Upregulation	(82)

concentrations and through epigenetic modifications: low serum concentrations of 17β-oestradiol (E2) (*i.e.* follicular and luteal phases of woman menstrual cycle) stimulate a pro-inflammatory response, while high serum concentrations (*i.e.* ovulatory phase of woman menstrual cycle or pregnancy) reduce the activation of innate immunity and T cells, however promoting a B cell response (36).

- E2 downregulates DNA methyltransferase-1 in CD4+ T cells of SLE patients, promoting disease flares (38).
- E2 regulates the biosynthesis of different micro-RNAs (*i.e.* miR-146a, miR-125a, miR-148a, miR-26a, let-7e-5p, miR-98-5p, miR-145a-5p, let-7a, miR-125b, miR-29a-5p, miR-320-3p) that mediates pro- or anti-inflammatory responses (39-47).

Progesterone

Progesterone (androgen-like steroid hormone) is a steroid hormone, mainly synthesised in female ovaries, but also in little amount in adrenal glands (35). The main function of progesterone is the regulation of the endometrium during the menstrual cycle, creating favorable conditions for the implantation of a fertilised oocyte. Moreover, in the case of a pregnancy, progesterone is produced in large amount by the placenta and plays, among other functions, a role in regulating the maternal immune system, creating tolerance towards the foetus (35).

Innate and adaptive immune cells (*i.e.* monocytes, dendritic cells, natural killer cells, T and B lymphocytes) exhibit cytoplasmic receptors for progesterone, progesterone receptor α (PRα)

and β (PRβ) (50, 51). After the binding with progesterone, receptors translocate into the nucleus, downregulating pro-inflammatory transcription factors STAT1 and STAT3 (Fig. 1) (52). Moreover, progesterone promotes the production of anti-inflammatory IL-10 and the activity of T regulatory cells, shifting also T cells polarisation towards Th2 response (Fig. 2) (53, 54).

Progesterone exerts immunotolerant effects also via epigenetic mechanisms. Of note, high progesterone serum concentrations, as occurs during pregnancy, have been associated with DNA hypermethylation of IFN-γ gene promoter region in $CD8^+$ T memory cells, so reducing the production of IFN-γ (55).

Progesterone-induced miRNA synthesis has instead been identified during gynecological pathologies, such as endometriosis (upregulation of miR-133a, miR-145/miR-143, miR-199 which are variously involved in the cell proliferation of smooth muscle tissue), but the effects on immune cells via histone modification and miRNA have not yet been clearly established (56-58).

The epigenetic effects of progesterone are the most recently recognised and will deserve important advancements in the gender oriented immune response in female patients as well as concerning the relationships with circadian rhythms of the immune response.

Key messages:

Progesterone and epigenetics

- Progesterone plays anti-inflammatory effects through epigenetic mechanisms, as DNA hypermethylation of IFN-γ gene promoter region in $CD8^+$ T memory cells, so reducing the production of IFN-γ (55).
- Progesterone regulates the biosynthesis of different micro-RNAs that influences non-immune cells (*i.e.* smooth muscle cells), but their effects on immune cells have not been clarified yet (56-58).

Testosterone

Testosterone and the more potent biological active metabolite dihydrotestosterone are the main activators of cytoplasmatic androgen receptors, that have been detected in almost all the cells of immune system (both innate and adaptive immunity, except for mature B cells) (35). Androgen receptors have been identified in the synovial macrophages of healthy subjects and RA patients of both gender for the first time in 1992 by Cutolo *et al*. (59, 60).

Activated cytosolic androgen receptors are transcription factors that migrate into nucleus cells and bind to androgen response elements of DNA sequences, influencing the transcription of genes, involved in immune response (Fig. 1) (35). Overall, male sex hormones promote anti-inflammatory effects, through downregulation of NF-κB (and consequently of IL-1, IL-6, TNF-α), and mainly reduce $CD8^+$ T cells inflammatory response, promoting also M2 monocytes/macrophages polarisation (Fig. 2) (61, 62).

The epigenetic effects of male sex hormones on immune system cells have not yet been well characterised. There is a large literature regarding DNA methylation, histone modifications, disorganisation of chromatin structure of genes expressing androgen receptors and miRNAs in prostate cancer, but their discussion goes beyond the objectives of this review (63-66).

Interestingly, in an experimental model of autoimmune myocarditis, activation of androgen receptors favours the biosynthesis of miR-125b, that is significantly associated with transforming growth factor β-mediated cardiac fibrosis (Table I) (67).

In conclusion, a sexual dimorphism at the epigenetic level (transcriptome and methylome) have been identified in both sexes, that is independent of genetic X-chromosome inactivation and that influence immune cell's function (37). Epigenetic modifications are driven by hormonal changes, that occur in women (*i.e.* puberty, pregnancy, menopause) and men (*i.e.* elderly, exogenous hormone therapy) (36, 37).

Key messages:

Testosterone and epigenetics

- Testosterone plays anti-inflammatory effects, but epigenetic mechanisms, as DNA methylation or histone modifications have not been elucidated in immune cells (63-66).
- Testosterone upregulates the biosynthesis of miR-125b that promotes pro-fibrotic pathways in experimental model of autoimmune myocarditis (67).

The endocrine system of vitamin D_3 and epigenetic effects on immune cells

Vitamin D_3 is a steroid hormone (secosteroid) which derives mostly (around 80%) from the photoconversion of skin cholesterol into 7-dehydrocholesterol by UVB solar rays (wavelength 280-315 nm) and to a small extent from food (around 20%) (68). A thermal reaction converts 7-dehydrocholesterol to cholecalciferol, which is subsequently hydroxylated by the enzyme CYP2R1 in the liver to calcidiol (25-hydroxyvitamin D_3) and then again hydroxylated by CYP27B1 in the kidneys to calcitriol (1,25-hydroxyvitamin D_3). Calcitriol is the active hormonal form of vitamin D_3 and binds nuclear vitamin D_3 receptor on target cells (immune and non-immune cells) (68).

Immune cells can activate circulating calcidiol into calcitriol, displaying CYP27B1, that will bind to VDR expressed by immune cells themselves in an autocrine/intracrine manner. Then, calcitriol exerts anti-inflammatory functions on innate and adaptive immune cells (monocytes, natural killer cells, dendritic cells, T and B lymphocytes), downregulating the transcription of MAP kinases and NF-κB (Fig. 1-2) (69). Of note, the prevalent and best characterised immune cells expressing VDR are monocytes, dendritic cells and T lymphocytes (Fig. 2) (70-72).

Calcitriol plays also epigenetic effects on target genes. Vitamin D_3 regulation of DNA methylation has been recently and extensively reviewed (73). Of note, vitamin D_3 deficiency has been associated with lower DNA methylation and higher expression of pro-inflammatory adipokines, BCL6, CXCL8, HDAC5 (histone deacetylase 5), IL-12A and NF-κB in the adipose tissue of a cohort of obese people (74).

Calcitriol regulates Th9 cell differentiation, through the recruitment of HDAC1 at IL-9 gene promoter (75). Similarly, calcitriol stimulates the activation of

HDAC3 in fibroblast growth factor receptor 1 promoter region, reducing its expression and ameliorating fibrotic effects on cardiac myocytes (76).

Moreover, active vitamin D_3 influences bone morphogenetic protein 2 (BMP2), that is crucial for bone metabolism and formation (77). In genetic hypercalciuric stone-forming rats, calcitriol downregulates BMP2, reducing acetylation of histone H3 at BMP2 promoter region (77).

At last, in an *in vitro* study, calcitriol inhibited histone acetylation of RelB promoter region, induced by DNA-containing immune complex in myeloid dendritic cells of SLE patients (78). RelB is an anti-inflammatory gene, which transcription is inhibited in course of SLE (78).

Calcitriol also influences the biosynthesis of several miRNAs (Table I). In a culture medium of 100 nM of active vitamin D_3, T lymphocytes of multiple sclerosis patients showed after 5 days a down-regulation of miR-155 (pro-inflammatory miRNA already discusses in glucocorticoid paragraph) (79).

Moreover, in a rat with induced autoimmune encephalitis, used as model for multiple sclerosis, treatment with vitamin D_3 together with vitamin A significant upregulated miR-98-5p and Let-7a-5p (80). These miRNAs reduce the activation of Ror γ-t, transcription factor that promote the shift of T cells towards Th17 phenotype (80).

The effects of calcitriol on miR-155 have been tested also in LPS-treated mice (81). Of note, active vitamin D_3 downregulates miR-155 and re-activates suppressed SOCS1, with an overall downregulation of pro-inflammatory STAT1 and STAT3 (81). Finally, in a further rat model of acute lung injury, calcitriol enhanced the synthesis of miR-149-5p, mitigating the inflammatory damage, targeting ATF6 gene (82).

Key messages:

Active vitamin D_3 (calcitriol) and epigenetics

- Calcitriol regulates DNA methylation and histone modifications (acetylation) of several inflammatory genes, with an overall anti-inflammatory effect (74-78).
- Calcitriol promotes the biosynthesis of different miRNAs (miR-155, miR-98-5p, let-7a, miR-149-5p), that downregulate pro-inflammatory pathways (79-82).

Consequences of epigenetic neuroendocrine effects on chronic rheumatic diseases: current and future perspectives

The epigenetic effects played by neuroendocrine steroid hormones help to understand a piece into the complex puzzle of pathophysiology and course of chronic rheumatological diseases.

Chronic inflammatory arthritis, such as RA, begins with a relative deficiency in endogenous cortisol production in response to the inflammatory damage (83). Glucocorticoids treatment is therefore indicated in RA patients, at least in the initial stages, with a role of replacement therapy and must be managed at the lowest doses necessary to obtain satisfactory disease control together with disease-modifying anti-rheumatic drugs (conventional, biological or targeted synthetic), avoiding adrenal cortex insufficiency (84-86). The epigenetic modifications induced by cortisol (endogenous or exogenous) help to understand not only the anti-inflammatory effects (when used at the correct dosages), but also the detrimental effects, due to FKBP5 demethylation (17-19).

In fact, it is recommended to de-escalate chronic therapy with glucocorticoids to less than 5 mg prednisone equivalent per day to reach an acceptable benefit/risk ratio for patients (87).

It has been known for years that gender differences are relevant factors in the impact of chronic rheumatological diseases (88).

The epigenetic effects of oestrogen partially explain the disproportion of women affected by B cell mediated diseases, *i.e.* SLE, compared to men (89). In fact, oestrogens regulate the biosynthesis of a plethora of miRNAs, involved in the IFN response and in the production of autoantibodies by plasma cells, all key features in the pathogenesis of SLE (90).

Interestingly, pregnancy is a paraphysiological period in the woman life span where the higher oestrogen serum concentrations can exacerbate B-driven diseases, as SLE (36). The consequence of pregnancy on RA are less predictable, as RA seropositive forms B-driven (high serum concentrations of rheumatoid factor and anti-citrullinated protein antibodies) can exacerbate, while RA seronegative forms T-driven can ameliorate (36). Of note, the anti-inflammatory effects of progesterone produced by placenta is involved in RA disease activity improvement, even if specific epigenetic mechanisms have to be elucidated and will be matter of our further research (36).

At last, epigenetic regulation of osteoblasts by oestrogens (anabolic activation) seems implicated in a protective role against osteopenia/osteoporosis during fertile years of the woman (47).

On the other hand, also androgens are implicated in the pathogenesis of RA, and their serum concentrations are often reduced in men suffering from RA (91). Furthermore, androgens are converted at the level of the inflamed synovium into oestrogens, thus exerting a local pro-inflammatory effect (92). However, epigenetic effects of androgens on chronic rheumatic diseases are still unclear, even if the demonstrated role of worsening fibrosis in autoimmune myocarditis could partially explain the role of male sex as risk factors, among others, for unfavorable progression of fibrotic diseases, as systemic sclerosis (SSc) (67, 93, 94).

Of note the role of exogenous administration of sex hormones was recently analysed in a case series of 11 transgender individuals who developed chronic rheumatic diseases, and possibly acting as epigenetic modifiers (95). In fact, there was only a case in a transman (developed anti-synthetase syndrome), but ten cases in transwomen, five of which developed SLE due to loss of androgens and treatment with oestrogens (95).

Calcitriol is another pivotal player in chronic inflammatory diseases, as it interferes with almost all the immune cells. Vitamin D_3 deficient serum concentrations (<30 ng/ml) have been associated with worse disease manifestations of RA, SLE and SSc (68). It is important to underline that the epigenetic modification of calcitriol can increase

or potentially antagonise the miRNA synthesis of glucocorticoids and sex hormones. In fact, calcitriol down-regulates the synthesis of pro-inflammatory miR-155, in a similar manner to that carried out by glucocorticoids, therefore suggesting complementary efficacy (24-26, 79, 81). On the other side, calcitriol up-regulates let-7a and miR-149-5p, opposing the downregulation exerted by oestrogens (44, 80).

Furthermore, it is interesting to note the inhibitory role that calcitriol plays on the IL-9 axis through histone deacetylation, also downregulating the transcription factor PU.1, recently identified as a promoter of inflammatory joint damage in mouse models of RA (75, 96).

Moreover, active vitamin D_3 can act on fibroblasts and chondrocytes, that participate in joint inflammatory processes (97).

In general, therefore, it is worth highlighting that studies on the epigenetic mechanisms of neuroendocrine hormones are of great importance in the research field, although their impact in a system of complex biological interactions has yet to be verified.

In this manuscript, available data on dozens of miRNAs have been collected, but, as there are several hundreds of them, the real role of each within the human organism has yet to be discovered.

Finally, the use of omics techniques remains crucial to develop real precision medicine, taking into consideration the neuroendocrine hormonal status of the rheumatic patient also in relation to optimise the hormonal therapies and the predictive models of rheumatic diseases (98-100).

Conclusions

The study of the epigenetic effects exerted by neuroendocrine hormones is crucial to clarify numerous essential aspects in the management of rheumatic patients (glucocorticoid therapy, gender differences in clinical expressions and disease course, role of sun exposure and vitamin D supplementation). The use of increasingly modern omics techniques is, however, required to clarify the relevance in humans of the cellular control mechanisms exerted by neuroendocrine hormones.

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