Chapter 2
Regulation of T-Cell Functions by Oxidative Stress

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Abstract The principal role of adaptive immunity is to distinguish between "self" and "nonself" and thus provide a highly specific line of immunological defence for the efficient removal of foreign material. It comprises of two arms: the effector B-cell arm and effector T-cell arm which act together to remove nonself. The balance between oxidising and reducing agents within these immune cells governs their redox state. This is important as transient controlled changes in the redox state, such as increased production of reactive oxygen species, are vital for signalling and induction of various biological processes, including cell growth and apoptosis. However, in chronic inflammatory diseases, the prolonged and persistent production of ROS, which overwhelms cellular antioxidant systems leading to oxidative stress, may influence T-cell function. This contributes to a T-cell phenotype which is hyporesponsive to growth and death signals and persists at the site of inflammation, perpetuating the immune response. The regulation of T-cell function by oxidative stress therefore has implications for rheumatoid arthritis.

Abbreviations

APC	Antigen-presenting cell
DC	Dendritic cell
DMARD	Disease-modifying antirheumatic drug
GSH	Reduced glutathione
GSSG	Oxidised glutathione
H_2O_2	Hydrogen peroxide
iGSH	Intracellular glutathione

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IL-1β	Interleukin-1β
IL-2	Interleukin-2
LAT	Linker for activation of T cells
MDA	Malondialdehyde
MTX	Methotrexate
NAC	*N*-acetylcysteine
NFκB	Nuclear factor kappa B
NO	Nitric oxide
NOS	Nitric oxide synthase
$O_2^{\bullet-}$	Superoxide anion radical
ONOO	Peroxynitrite
PBMC	Peripheral blood mononuclear cells
RA	Rheumatoid arthritis
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
TCR	T-cell receptor
TNFα	Tumour necrosis factor-α
Treg	Regulatory T cell
Trx	Thioredoxin
TXRX1	Thioredoxin reductase 1

2.1 Introduction

The immune system can be considered as two functional parts: innate and adaptive immunity. They act together to provide protection against invading foreign pathogens. Innate immunity is a fast acting, non-specific first line of defence towards microbial infection. Adaptive immunity is a highly specific line of defence which provides immunological memory allowing rapid and efficient removal of previously encountered pathogens. The principal role of adaptive immunity is to distinguish between "self" and "nonself". It comprises of two arms: the effector B-cell arm and effector T-cell arm, which together are responsible for removal of the "nonself" (i.e. pathogens). During the very early stages of T-cell development, self-reactive T cells, which recognise "self", are removed by apoptosis, and a subset of specialised regulatory T cells (Tregs) exist which inhibit autoimmune T cells and ensure self-tolerance is maintained [1, 2]. Aberrant T-cell function has been implicated in the development of autoimmune diseases such as rheumatoid arthritis (RA) [3]. RA is associated with intra- and extracellular oxidative stress: the imbalance between pro-oxidant (e.g. reactive oxygen species (ROS)) and antioxidant species in favour of the former [4]. In addition to specialised antioxidant enzymes, the most important intracellular low-molecular-weight antioxidant is glutathione (GSH), which has a thiol moiety and is reactive with pro-oxidant species. The importance of ROS in immune defence is exemplified by their generation and release in the form of an "oxidative burst" by phagocytic cells (e.g. neutrophils and macrophages, part of the innate immune cell network) to effectively destroy pathogens and clear debris. It is also becoming increasingly apparent that altered redox states leading to changes in the oxidative environments at the surface of, and within, T cells are important for their function in health, ageing and disease. Due to their pivotal role in this adaptive immune response, the effect of the oxidative stress on T-cell biochemistry and function and its implications in the autoimmune disease RA will be the focus of this chapter.

2.2 T Cells in the Immune System

T cells originate in the bone marrow as haematopoietic stem cells, where they develop into progenitor cells and then migrate to the thymus via the bloodstream for maturation. Naïve T cells (i.e. mature T cells which have never previously been exposed to antigen) circulate to secondary lymphoid sites (e.g. lymph nodes, Peyer's patches, spleen and skin) where they become activated on recognising antigen-presenting cells (APC) displaying specific antigen–MHC molecule complexes. Dendritic cells (DC) are the principal APC, but macrophages and B cells can also perform this role. DCs originate in the bone marrow as haematopoietic stem cells, are released into the bloodstream where they travel to different sites and mature upon exposure to foreign pathogen. DCs internalise antigens and express them on MHC class molecules at their surface in the form of antigen. In this activated state, DCs travel to peripheral lymphoid organs and interact with T cells creating an immunological synapse and providing the necessary signals for T-cell activation: first, the T-cell receptor (TCR) engages with antigen-bearing MHC molecules; second, the co-stimulatory molecules, especially CD80 and CD86, bind co-receptor CD28 on surface of T-cells; and third, cytokines are produced to orchestrate the adaptive immune response [5, 6]. Once activated, T cells are retained in the lymphoid organ where they proliferate and differentiate into effector T cells under the control of local cytokines. The effector T-cell arm can be grouped into at least four subtypes: Th1, which mediate responses against intracellular pathogens and are involved in some autoimmune diseases; Th2, which are responsible for host defence; Th17, which promote bacterial immune responses and are responsible for autoimmunity; and Tregs, which make up 5–10% of total $CD4^+$ T cells and maintain self-tolerance [2]. With regard to RA, Th1 and Th17, effector T cells in particular have been implicated in the recruitment and activation of inflammatory cells and in mediating bone and cartilage damage associated with the disease [7].

The redox environment at the interface between APC and T cells within the immunological synapse also impacts on T-cell activation. The demonstration that upon activation T cells exhibit increased cell surface thiol levels suggests that a reduced extracellular environment is associated with T-cell activation [8]. Moreover, DCs have been shown to create this reducing environment by releasing cysteine into the extracellular space, thereby facilitating an immune response [9], and the mechanism by which Tregs exert their immunosuppressive effect has been suggested as an interference with this process [10]. It is now therefore becoming increasingly recognised that changes in intracellular levels of ROS or altered redox state, as well as levels at the interface between APC and T cells at the cell surface, can impact on T-cell activation, proliferation and differentiation, thereby modulating their function. This has wider implications for ageing and disease which are associated with oxidative stress.

2.3 ROS and Redox State Are Central to Immunity and T-Cell Function

An imbalance between oxidising (e.g. reactive oxygen species (ROS)) and reducing agents (e.g. antioxidants) towards a pro-oxidant state leads to oxidative stress [11]. Prolonged and persistent oxidative stress can lead to macromolecular damage in the form of protein carbonylation, lipid peroxidation and DNA oxidation. This phenomenon has therefore historically been perceived as harmful and damaging; however, ROS are now more widely recognised as important signalling molecules [12] which exert a wide range of biological effects. With regard to the immune system, high levels of ROS can be beneficial; neutrophils generate ROS and release them intracellularly and extracellularly in the form of an "oxidative burst" to defend against and destroy pathogens, thus providing antimicrobial protection [13]. However, excessive ROS are generated in the presence of immune complexes with auto-antigens where further macromolecular damage is induced. Prolonged exposure to high ROS concentrations can inhibit T-cell proliferation and lead to apoptosis [14], and incubation of T cells with the reactive nitrogen species (RNS) peroxynitrite can also inhibit their proliferation [15]. It has also been observed that oxidative stress-induced modification to selective molecules involved in T-cell receptor (TCR) signalling is sufficient to render T cells hyporesponsive to activating stimuli [16]. Conversely small amounts of ROS have been shown to be important for T-cell function. Los and colleagues [17] reported that T-lymphoma cells exposed to low levels of hydrogen peroxide (H_2O_2) induced transcription of nuclear factor kappa B (NFκB) and gene expression of interleukin-2 (IL-2) and the IL-2 receptor chain-α [17]. The different T-cell responses to ROS production may be due to the extent of change to the cellular redox environment [18] (see Fig. 2.1).

T-cell differentiation is also affected by the redox environment. King et al. [19] stimulated peripheral blood mononuclear cells (PBMC) with the ROS generator 2, 3-dimethoxy-1, 4-naphthoquinone and reported that Th2 and Th1 phenotypes were promoted and inhibited, respectively. Moreover, in the absence of APC, reactive carbonyls including 4-hydroxy-2-nonenal and malondialdehyde (MDA), which are generated on proteins and lipids randomly in the presence of ROS, promote differentiation towards a Th2 phenotype [20]. These data emphasise that the importance of ROS homeostasis and flux in governing cell maturation and that the balance between oxidising and reducing agents is a delicate process which must

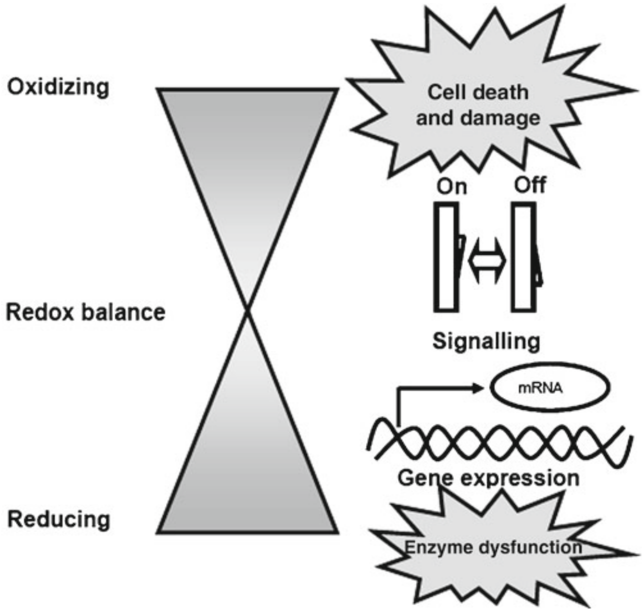

Fig. 2.1 Redox balance and T-cell function. Prolonged and persistent production of reactive oxygen and nitrogen species resulting in oxidative stress induces macromolecular damage, inhibits T-cell proliferation and leads to cell death. In contrast, small amounts of ROS are important for inducing transcription of NFκB and gene expression of cytokines and receptors essential for T-cell proliferation (e.g. IL-2 and IL-2 receptor), together highlighting an important role for cellular redox environment on T-cell function. Changes in levels of oxidative stress and therefore redox state can switch T cells towards a more hyporesponsive or proliferative phenotype

be tightly regulated and well managed, depending on whether the requirement is for protecting against bacteria, in an immune response, or requirements for T-cell signalling, activation and regulation of function.

2.4 Glutathione, Cysteine, Low-Molecular-Weight Thiols and T-Cell Function

GSH is a tripeptide consisting of amino acids glycine, glutamate and cysteine. It is synthesised in the cell by two ATP-dependent reactions: the first reaction, catalysed by γ-glutamyl cysteine ligase, combines glutamate and cysteine to form γ-glutamyl-cysteine; the second reaction, catalysed by GSH synthetase, combines γ-glutamyl-cysteine with glycine to form GSH [21]. The presence of a thiol group (–SH), provided by cysteine affords GSH its antioxidant activity in the form of radical scavenging. It is the major intracellular antioxidant and serves as a redox buffer cycling between its reduced and oxidised (GSSG) forms, and thus the ratio between GSH and GSSG serves as an indicator of intracellular oxidative stress [22].

In order for T cells to synthesise intracellular glutathione (iGSH), they require cysteine. However, given that circulating levels of cysteine are low, coupled to the fact that T cells are unable to import the oxidised form of cysteine, cystine, due to the lack of a cystine transporter, they rely completely on APC (e.g. dendritic cells) to provide them with cysteine [23]. DCs, in contrast to T cells, have the appropriate transporter required for cystine uptake, and once inside the cell cystine is reduced to cysteine which can then be secreted into the extracellular space. Cysteine is thus accessible both intra- and extracellularly for T cells and allows for their proliferation. The ability of DCs to control extracellular cysteine allows them to affect intracellular GSH levels and T-cell signalling [24].

Several studies implicate a role for GSH in T-cell function. Depletion of GSH in human and murine T cells, using the α-glutamylcysteine synthetase inhibitor L-buthionine-(S, R) sulfoximine, attenuates proliferative response to mitogenic and antigenic stimulation [25]. In addition, the reintroduction of exogenous GSH can restore a T-cell proliferative response [26] suggesting a direct relationship between iGSH levels and T-cell proliferation. Further investigation by Hadzic et al. [27] into the role of GSH and T-cell proliferation, using murine T cells, suggested that GSH is the rate limiting step for T-cell proliferation but only in the absence of other small molecular weight thiols. In addition depletion of GSH impaired T-cell function, as measured by IL-2 secretion, which was overcome by the addition of endogenous IL-2, suggesting that T-cell proliferation is regulated by a thiol-dependent pathway involving IL-2 [27].

A recent study by Checker et al. [28] supports the notion that thiols play an important role in T-cell function. In murine T cells treated with plumbagin, a naphthoquinone present in plants from Plumbaginaceae species, intracellular oxidative stress as measured by ROS production and GSH levels was increased and decreased, respectively. Proliferative responses to mitogenic stimulation as well as IL-2 production were also inhibited by plumbagin. The anti-proliferative effect and inhibition of IL-2 production was only prevented by thiol-containing antioxidants and not non-thiol antioxidants. The authors concluded that the anti-proliferative effects and reduced IL-2 production were due to the modulation of intracellular thiols, rather than altered ROS levels. Further support for the importance of thiols on T-cell function is demonstrated by studies which investigate the effect of selenium, an essential cofactor in GSH metabolic enzymes, on immune response; T-cell proliferation in response to antigenic stimulation in selenoprotein-deficient T cells isolated from mice is suppressed [29]. In a more recent study, Hoffman et al. [30] isolated $CD4^+$ T cells from mice fed either a diet of high, medium or low selenium for 8 weeks and investigated the dietary effect of selenium on T-cell function. $CD4^+$ T cells isolated from mice fed a diet high in selenium exhibited increased proliferation and expression of IL-2 and IL-2 receptor in response to antigenic stimulation compared to $CD4^+$ T-cells isolated from mice fed a low selenium diet, which was paralleled with a reduction in intracellular thiols and iGSH in diets low in selenium. The proliferative response to antigenic stimulation was rescued in $CD4^+$ T cells with the addition of *N*-acetylcysteine (NAC) suggesting that the effects of selenium on T-cell proliferation involve a pathway involving the modulation of free thiols. Taken together, these data suggest an important role for iGSH and intracellular thiols in regulating T-cell activation and proliferation and highlight the importance of redox environment for T-cell function.

2.5 Oxidative Stress and Regulatory T Cells

Oxidative stress plays an important role in the function of regulatory T cells (Tregs). These specialised immunosuppressive T cells account for 5–10% of the total $CD4^+$ T-cell population [2]. One way in which Tregs exert their suppressive effect is by altering the redox state at the interface between DC and naïve T cells at the immune synapse, leading to reduced cysteine availability for naïve T cells [24]. In a recent study, Yan et al. [10] demonstrated that Tregs reduce extracellular cysteine concentration in an antigen-dependent but not antigen-specific manner, which requires cell to cell contact through the interaction between CLTA-4 on Tregs and CD80/CD86 on dendritic cells; this initiates an intracellular signalling response which inhibits DC iGSH synthesis and thus reduces extracellular cysteine generation [10]. In addition to this, Tregs compete with effector T cells at the immune synapse for extracellular cysteine, which they preferentially catabolise to sulphate, which therefore limits the amount of available cysteine for effector T cells [10]. As a consequence, iGSH levels in effector T cells are reduced and T-cell activation and proliferation is inhibited [10], and thus by altering the redox status at the immunological synapse, Tregs can have a profound impact on the functional response of effector T cells.

Tregs can actually withstand greater levels of oxidative stress than other $CD4^+$ T-cell subsets [31], which likely aids their ability to regulate the immune response. One factor which contributes to their increased tolerance to oxidative stress compared to other T-cell subsets is that they express and secrete greater levels of thioredoxin (Trx), a 12 kDa oxidoreductase enzyme substrate which contains a dithiol–disulfide, providing it with potential to scavenge ROS and metabolise H_2O_2 [32]. By blocking total and secreted Trx, Mougiakakos et al. [32] demonstrated that cell surface thiols on Tregs could be decreased and that their treatment with the thiol-depleting agent *N*-ethyl maleimide increased Treg susceptibility to H_2O_2-induced cell death. Moreover, they reported that treatment of Tregs with the inflammatory mediator tumour necrosis factor-α (TNFα) resulted in Trx release, with a paralleled increase of cell surface thiols and increased resistance to H_2O_2.

2.6 Ageing, Oxidative Stress and T-Cell Function

It is widely accepted that oxidative stress increases with age as evidenced by an increase in several stress markers, including protein carbonylation, lipid peroxidation and thiol to disulphide oxidation in plasma [22]. Studies of T-cell ageing reveal an association between increased oxidative stress and altered function. Early studies by Murasko et al. [33] and Franklin et al. [34] demonstrated that with age, T cells exhibit a poor proliferative response to mitogenic stimuli. Moreover, GSH increases the proliferative response to mitogenic stimuli in older rats compared to young rats. Additionally, it has been reported that human lymphocytes exhibit reduced levels of iGSH and antioxidant enzymes in parallel with increased levels of protein carbonylation and lipid peroxidation, which correlates with age [35, 36]. Together, these data suggest that with age lymphocytes are under a state of increased oxidative stress which may lead to a poor response to mitogenic stimulus and thus affect T-cell function which may be attributed to either thiol loss or increased oxidants.

2.7 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a progressive, chronic inflammatory and autoimmune disease of joints which typically affects older adult. Histopathologically, RA is characterised by expansion and inflammation of the synovial membrane. In this state, chemokines are secreted from the synovial membrane leading to the recruitment and infiltration of diverse immune (e.g. $CD4^+$ and $CD8^+$ T cells and B cells) and inflammatory cells (e.g. neutrophils, monocytes and macrophages) [3]. $CD4^+$ T cells responsible for cell-mediated immunity activate monocytes, macrophages and synovial-like fibroblast cells to produce, amongst others, proinflammatory cytokines TNFα and interleukin-1β (IL-1β). TNFα and IL-1β, as well as T-cell-dependent cytokines, activate osteoclasts and chondrocytes to release proteolytic enzymes, ROS and RNS resulting in cartilage and bone destruction, characteristic of RA. Investigation of synovial tissue from RA patients confirms the presence of proinflammatory cytokines and metalloproteinases [37]. Moreover, synovial fluid from RA patients exhibits hallmarks of oxidative damage [38] suggesting an important role for oxidative stress in the pathogenesis of the disease.

2.7.1 Rheumatoid Arthritis: ROS and T-Cell Function

Several lines of evidence suggest that oxidative stress exists in rheumatoid arthritis (RA), these include reduced total antioxidant capacity and increased levels of lipid peroxidation and oxidative stress markers in plasma from RA compared to healthy control subjects [39, 40]; increased serum MDA levels in RA compared to osteoarthritis and healthy control subjects [41]; reduced glutathione peroxidase and catalase activity reported in erythrocytes isolated from RA patients [39]; increased oxidative DNA damage in PBMC and urine from RA subjects [42, 43]; increased plasma Trx levels [43]; and increased 3-nitrotyrosine in synovial fluid from RA joints compared to other noninflammatory joints [38]. More specifically in RA SF T cells, intracellular free-radical production and intracellular GSH are increased and decreased, respectively [44, 45]. In peripheral blood T cells, intracellular GSH levels are similar between RA and healthy controls [44] and steady-state ROS levels in response to mitogenic stimulation are not altered compared to healthy controls [3]. These studies are independent and not undertaken in paired samples, but suggest the role of local niche environment in regulating T-cell oxidative stress.

Although an important role for low level ROS has been implied during the initial events for T-cell activation, prolonged and elevated levels such as those that are evident in the synovium from RA patients may counteract this benefit and lead to a hyporesponsive T-cell phenotype. Indeed, the elevated levels of ROS within the synovial T cells located at site of inflammation (RA joint) are produced intracellularly possibly through a mechanism involving the activation and inactivation of Ras and Rap1, respectively [45, 46]. Gringhuis et al. [47] suggested under conditions of oxidative stress, such as those reported at the site of inflammation in RA, phosphorylation of the adaptor protein linker for activation of T cells (LAT), responsible for TCR engagement, and T-cell activation becomes impaired and leads to hyporesponsiveness. This subsequently leads to its displacement from the plasma membrane, thus providing a molecular mechanism for impaired intracellular signalling and showing the importance of redox status. In the periphery, T cells show no increase in ROS when cultured in vivo in the presence or absence of a mitogenic stimulus compared to controls; one possible consequence of this observation is that intracellular ROS production required for TCR signalling coupled with the failure to elicit intracellular ROS leads to an unresponsive phenotype [3].

2.7.2 Rheumatoid Arthritis: RNS and T-Cell Function

Few studies have investigated the effect of the nitrogen radical nitric oxide (NO) on T-cell function in RA. The inflamed joint in RA is a major NO-producing site [48], as several cells capable of producing NO via nitric oxide synthases (NOS) including osteoblasts, macrophages, fibroblasts and endothelial cells are present [49]. Furthermore, inhibition of NOS in rats with experimental arthritis results in the reduction of synovial inflammation and tissue damage [50]. Recently, RA human peripheral blood T cells were reported to exhibit a twofold increase in NO production compared to healthy human T cells [51], which is of particular interest given that NO promotes a proinflammatory Th1 effector cell phenotype [52] which may perpetuate an autoimmune response. The presence of NO in synovial fluid may also lead to the production of peroxynitrite ($ONOO^-$) through reaction with the superoxide anion ($O_2^{\bullet-}$) generated in the respiratory burst. Indeed, 3-nitrotyrosine, a hallmark for the presence of peroxynitrite, is elevated in synovial fluid of RA subjects compared to individuals with noninflammatory arthritic disease [38]. A recent study by Kavic and colleagues [15] demonstrated that primary human T cells pretreated with peroxynitrite showed inhibition of T-cell activation and inhibition of migration directed by chemokines. Together, these studies implicate a role for NO in RA pathogenesis and infer that increased production of NO in the inflamed joint, coupled with $O_2^{\bullet-}$, leading to $ONOO^-$ production, may contribute to the hyporesponsive nature of T cells.

2.7.3 Rheumatoid Arthritis: Cell Surface Thiols

Cell surface thiols (–SH) and intracellular SH are redox buffers and protect T cells from oxidative stress. In mutant neutrophil cytosolic factor 1 (Ncf1) rats, deficient in p47phox, a component of the NADPH oxidase complex, which produce low levels of ROS compared to native Ncf1 rats, an increased susceptibility to pristane-induced arthritis is evident [53]. In addition to the observed decrease in ROS production, T cells possessed greater levels of cell surface SH; increasing cell surface SH increased the proliferative response to an immunodominant CII peptide in a mouse hybridoma T-cell line. Furthermore, naïve rats exposed to $CD4^+$ T cells, which were artificially treated with GSH to increase cell surface thiols to a comparable level to that of ncf1 mutant rats susceptible to arthritis, also developed arthritis. Reciprocal experiments in mutant rats, using GSSG to reduce SH levels, resulted in less severe arthritis [53]. Together these experiments suggest that T-cell surface thiol groups influence T-cell activation, proliferation and the development of arthritis. Intriguingly, in contrast, cell surface thiols and iGSH have been investigated in peripheral human T cells; $CD4^+$ T cells and $CD8^+$ T cells show lower cell surface SH and iGSH in RA, with the $CD4^+$ T-cell surface SH negatively correlated to age [54], implying that these T cells would generally be less sensitive to activation.

2.7.4 Rheumatoid Arthritis: Oxidative Stress and Apoptosis

Apoptosis or programmed cell death is a controlled, energy-requiring process which removes damaged or infected cells in a noninflammatory manner. Synovial RA T cells do not undergo apoptosis despite exhibiting proapoptotic characteristics [55], and inhibition of apoptosis in RA leukocytes appears to occur during the earliest stage of the disease [56]. The inability of T cells to undergo apoptosis in RA, resulting in their persistence and accumulation within the synovial joint, may provide one possible reason for disease progression. Oxidative stress and the subsequent changes to cellular redox environment have been implicated in apoptosis [57]. The association of increased ROS in RA synovium [45], coupled to unresponsive nature of synovial fluid (SF) T cells and their persistence in the joint due to their inability to undergo apoptosis [55], suggests a link between cellular redox state and apoptosis in RA. A recent study by Kabuyama and colleagues [58] reported enhanced oxidative stress and an up-regulation of antioxidant genes in RA synovial cells. More specifically, thioredoxin reductase 1 (TRXR1), an enzyme involved in antioxidant defence, was identified as up-regulated at the gene and protein levels. Treatment of RA synovial cells with 1-chloro-2, 4-dinitrobenzene, an inhibitor of TRXR1, led to a dose-dependent increase in cellular H_2O_2 and cell apoptosis. These data suggest an important role for TRXR1 in preventing ROS-driven apoptosis and enhancing survival of SF T cells [58]. It is feasible that increased levels of ROS at the earliest stages of RA could result in SF T cells which exhibit enhanced antioxidant levels and are more tolerant to oxidative stress. This may provide a reason for why these synovial cells are less able to undergo apoptosis and accumulate and persist within the RA synovial joint. The demonstration that the RA synovial fluid is enriched with regulatory $CD25^{bright}CD4^{+}$ T cells [59], coupled to the report that regulatory T cells express and secrete greater levels of Trx-1 compared to $CD4^{+}$ T cells and exhibit greater tolerance to oxidative stress [32], further supports this notion. Collectively, these data highlight an important role for oxidative stress and apoptosis in RA which may be harnessed therapeutically.

2.7.5 *Rheumatoid Arthritis: Therapies, ROS and T Cells*

2.7.5.1 Methotrexate

Methotrexate (MTX), a folate antagonist originally developed for treatment of malignant disease, is an effective and widely used drug in the treatment of RA and other inflammatory disorders [60, 61]. It is one of a few known disease-modifying antirheumatic drugs (DMARDs) currently available for the treatment of RA. Several underlying mechanisms including production and release of the anti-inflammatory agent adenosine, inhibition of T-cell proliferation through altered purine and pyrimidine metabolism and prevention of T-cell cytotoxicity have been described for the anti-inflammatory and anti-suppressive actions of MTX [62]. The anti-inflammatory and anti-suppressive effects have also been studied in the context of cell apoptosis and cellular oxidative stress. Apoptosis allows for the controlled removal of damaged cells without causing inflammation, and studies have reported the induction of T-cell-dependent apoptosis by low-dose MTX in vitro and in primary $CD4^{+}$ T cells from active RA compared with nonactive patients [63–65]. A link between apoptosis and production of ROS has been reported. Phillips et al. [66] proposed that the immunosuppressive action of MTX is dependent on the generation of ROS and reported increased cytosolic peroxide levels, reduced iGSH concentrations and induction of growth arrest in Jurkat T cells exposed to MTX [66]. Later work by Herman et al. [64] showed that low-dose MTX induces apoptosis with the production of ROS in T cells, which is abrogated by pretreatment with the thiol antioxidant NAC. These observations suggest that the induction of apoptosis by MTX probably acts through oxidative stress and may underlie the anti-inflammatory and immunosuppressive action of MTX [64].

2.7.5.2 Novel Biologics

Additional DMARDs currently used to treat RA include the biologics infliximab and etanercept. These DMARDs target and block TNFα activity. Altered redox activity and ROS production within the RA synovial T cells [44, 45] which infiltrate the joint, coupled with the demonstration that RA synovial cells stimulated with TNFα enhance $O_2^{\bullet-}$ production [67], suggest a potential indirect mode of action for DMARDs on oxidative stress in RA. Recent studies have indeed demonstrated beneficial effects of infliximab and etanercept on serum redox status in RA patients. Lemarechal et al. [68] reported that increased protein oxidation and decreased thiol levels evident in RA patients compared to healthy control patients were reduced and increased, respectively, after the short-term administration of infliximab. Furthermore, levels of the advanced glycation end product pentosidine, a product produced under conditions of oxidative stress, are reduced in serum from patients treated with infliximab and etanercept [69, 70]. Taken together, these data suggest that DMARD therapies which target TNFα may exert their beneficial effects indirectly through modulation of the redox environment.

2.8 Conclusion

T cells represent an important part of the adaptive immune response and are principally responsible for distinguishing between "self" and "nonself". Upon activation at the immunological synapse with DC, they proliferate and differentiate into various subsets (e.g. Th1, Th2, Th17 and Tregs) under the influence of the local cytokine environment and thus enable a competent immune response. ROS are additional molecules which are important in immune defence as demonstrated by their generation and release in the form of an "oxidative burst" by phagocytic cells to neutralise, destroy and clear pathogens [13] and in signalling which is required for normal T-cell function [17]. However, increased levels of oxidising agents, due either to their increased production or reduced removal by antioxidants, which is prolonged and persistent, can lead to oxidative stress [11]; in this state macromolecular damage can ensue. In the context of T cells, their activation leads to an increase in cell surface thiols, changes to intracellular GSH can affect their function [71] and exposure to ROS and RNS (e.g. peroxynitrite) inhibits their activation and proliferation [15] and thus highlights an important role for the cellular redox environment on their function. Autoimmune diseases such as RA are associated with altered levels of redox activity in the periphery [39, 40] but also in the synovium at the site of inflammation [3]. The latter represents a highly oxidative environment where reduced intracellular GSH and increased ROS production in synovial T cells and increased nitration in synovial fluid are evident [38, 44, 45]. Additionally, RA synovial T cells are also less responsive to antigenic stimulation and are unable to undergo apoptosis [47, 55] leading to a hyporesponsive T-cell phenotype which persists within the rheumatoid joint. These observations suggest a strong link between aberrant redox activity towards a more oxidative environment and a hyporesponsive RA T-cell phenotype. Effective treatments which target TNFα may also act indirectly through modulating the redox environment, and further work into the effect of these therapies on redox state of peripheral and synovial RA T cells may provide further insight into their clinical efficacy in different cases.

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