BIOCHEMISTRY
including biophysical chemistry & molecular biology
Current Topic

Biochemistry **2010,** *49,* 1059–1066 1059
DOI: 10.1021/bi902022n

Redox Remodeling as an Immunoregulatory Strategy[†]

Zhonghua Yan and Ruma Banerjee*

Department of Biological Chemistry, University of Michigan Medical Center, University of Michigan, Ann Arbor, Michigan 48109-5606

Received November 25, 2009; Revised Manuscript Received January 13, 2010

ABSTRACT: Activation and proliferation of T cells require a reducing extracellular microenvironment in the immune synapse that is provided by antigen presenting cells, especially dendritic cells. Stimulation of dendritic cells by T cells activates the NF-κB pathway in dendritic cells and induces an antioxidant response. It also enhances system x_c^--dependent cystine uptake, leading to enhanced glutathione synthesis, export, and, finally, degradation to cysteine outside the cell. Accumulation of extracellular cysteine supports glutathione synthesis in T cells while also leading to a more reducing redox potential that is needed for T cell proliferation. Naturally occurring regulatory T cells, a suppressor subpopulation of T cells, prevent autoimmune diseases and maintain peripheral tolerance by suppressing self-reactive effector T cells. They also suppress beneficial immune responses to parasites, viruses, and tumors. However, their mechanism of suppression is still not fully understood. Recently, we have found that inhibition by regulatory T cells of dendritic cell-induced extracellular redox remodeling is a component of the regulatory T cell suppression mechanism. In this review, we describe recent advances in our understanding of redox regulation and signaling in the adaptive immune system with a focus on T cell activation by dendritic cells. The role of regulatory T cells in perturbing redox remodeling by dendritic cells and its implications as a general regulatory T cell suppression mechanism are discussed.

Discriminating self from nonself is a primary function of the immune system, and regulatory T cells play a cardinal role in maintaining self-tolerance and preventing autoimmunity by mechanisms that remain to be fully elucidated (*1*−*3*). T cells derive their name from the thymus, the organ in which they mature, and are distinguished by the presence of T cell receptors (TCRs).[1] The latter recognize antigens bound to the major histocompatibility complex (MHC) molecules on antigen presenting cells (MHC class II) or target cells (MHC class I). Like most immune cells, T cells are initially derived from hematopoietic stem cells in the bone marrow where progenitor T cells are formed. They migrate to the thymus via the bloodstream to mature via positive and negative selection processes (*4*). "Naïve" T cells released from the thymus have yet to encounter an antigen and are in the G0 stage of the cell cycle. They circulate through the vascular system to secondary lymphoid tissues such as lymph nodes where they may encounter antigen−MHC complexes and, in the process, become activated. Activated T cells play important roles in cell-mediated functions in the adaptive immune system, and their dysfunction is manifest in a number of immune diseases (*5*).

Antigen presenting cells such as dendritic cells (DCs), macrophages, and B cells express MHC class II molecules and costimulatory molecules on their membranes and specialize in presenting antigens to naïve $CD4^+$ T cells. DCs are the most potent professional antigen presenting cells (*6*) and originate from hematopoietic stem cells in the bone marrow. Precursor DCs are released from the bone marrow and circulate in the bloodstream to different tissues where they reside as immature DCs until they encounter antigens. Once internalized, antigens are processed and then displayed on MHC class II molecules, and the resulting mature DCs migrate to lymph nodes where they interact with and activate antigen-specific T cells, which subsequently proliferate and differentiate into effector T cell subsets (*4*, *7*, *8*).

Classically, three sets of signals in the immune synapse are recognized to be essential for priming naïve T cells: (i) specific engagement of the TCR by an antigen−MHC class II complex, (ii) interactions between costimulatory molecules, CD28 on T cells and CD80/86 on antigen presenting cells, and (iii) secretion of cytokines (Figure 1A). These signals result in the activation, survival, and differentiation of T cells. In addition to these signals, T cell activation and proliferation require a reducing microenvironment that is achieved by secretion of cysteine from antigen presenting cells (*9*, *10*). The physiological relevance of redox remodeling by antigen presenting cells for T cell activation is demonstrated by the hyporesponsiveness of T cells from normal gut, which results from the inability of mucosal macrophages to provide a reducing microenvironment and contrasts with the presence of this capacity in peripheral blood

[†]This work was supported in part by a grant from the National Institutes of Health (DK64959).

*To whom correspondence should be addressed. Telephone: (734) 615-5238. E-mail: rbanerje@umich.edu.

[1]Abbreviations: TCR, T cell receptor; MHC, major histocompatibility complex; DC, dendritic cell; Foxp3, forkhead box P3; CTLA-4, cytotoxic T lymphocyte antigen 4; Trx, thioredoxin; GSH, glutathione; GSSG, glutathione disulfide; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; TNF, tumor necrosis factor; FACS, fluorescence-activated cell sorting; ROS, reactive oxygen species; NF-κB, nuclear factor κB; NADPH, nicotinamide adenine dinucleotide phosphate; Ncf1, neutrophil cytosolic factor 1; AP1, activator protein 1; Bcl-2, B cell leukemia/lymphoma 2; LAT, linker for activation of T cells; TGFβ, transforming growth factor β; ref-1, redox factor 1; LCK, leukocyte-specific protein tyrosine kinase; ZAP-70, ζ-chain-associated protein kinase 70; GRB2, growth factor receptor-bound protein 2; PLCγ1, phospholipase C γ1.

Published on Web 01/13/2010

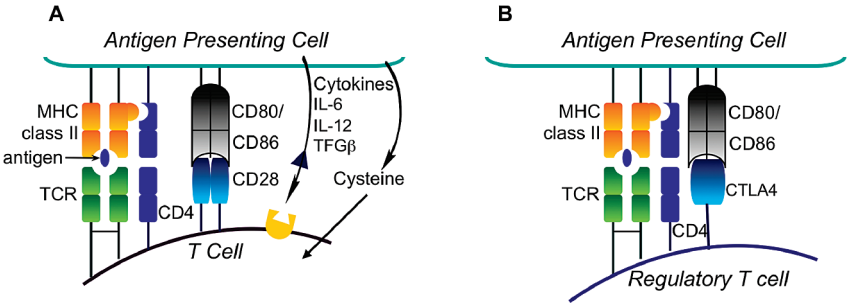

FIGURE 1: Molecular interactions in the immune synapse. (A) Signals required for $CD4^+$ T cell activation and proliferation include (i) TCR-antigen·MHC complex interaction, (ii) interaction of CD28 on T cells and CD80/CD86 on antigen presenting cells, (iii) secreted cytokines such as IL-6, IL-12, and TGFβ, and (iv) a reducing microenvironment shaped mainly by extracellular cysteine accumulation. (B) Interaction of regulatory T cells with antigen presenting cells. Regulatory T cells constitutively express high levels of CTLA-4, which interacts with CD80/CD86 on antigen presenting cells, thus inhibiting their presentation capacity for interactions with naïve T cells.

monocytes in the same organism (*11*, *12*). Under conditions of chronic mucosal inflammation as seen in inflammatory bowel disease, ulcerative colitis, and Crohn's disease, recruitment of peripheral blood monocytes results in sustained antigen-driven responses of T cells in the gut and is believed to be important in the etiology of these diseases (*12*).

Regulatory T cells are critical mediators of self-tolerance and immune homeostasis (*2*, *3*). Mutations in the transcriptional regulator, Foxp3, which is preferentially expressed in regulatory T cells (*13*), result in multiorgan autoimmune diseases and are fatal (*14*). Enrichment of regulatory T cells suppresses autoimmune responses and promotes tolerance to organ grafts and feto-maternal tolerance. On the other hand, depletion of regulatory T cells augments tumor and microbial immunity while provoking autoimmunity and inflammatory bowel disease. The coreceptor, cytotoxic T lymphocyte antigen 4 (CTLA-4) (*15*), expressed preferentially on regulatory T cells, interacts with CD80/CD86 (Figure 1B), i.e., the same ligand that binds CD28 expressed on naïve T cells. However, CTLA-4 interacts with CD80/CD86 with a much higher affinity and suppresses induction of CD80/86 expression by antigen-specific T cells, consequently limiting the capacity for activating naïve T cells (*16*). Given the importance of regulatory T cells in controlling autoimmunity and inflammation and their influence on tumor and microbial immunity, elucidation of the mechanisms by which these cells exert their effects has important implications for therapeutic target identification and development of intervention strategies. In this review, we describe recent insights into the significance of intercellular redox signaling during activation of naïve $CD4^+$ T cells by antigen presenting cells and the perturbation of this circuitry by regulatory T cells (*10*).

REDOX POTENTIALS IN THE INTRA- AND EXTRACELLULAR COMPARTMENTS

The cytoplasmic and extracellular redox potentials are vastly different and influence the structure, stability, and function of the macromolecules that reside in each compartment. Within the intracellular compartments, several redox buffers exist, e.g., thioredoxin (Trx), glutathione (GSH), and cysteine, and the relative concentrations of their oxidized versus reduced species set the ambient redox poise for the system. Interestingly, the individual redox systems appear to be under kinetic control, are not in equilibrium with each other, and independently regulate the redox status of their client redox partners (*17*, *18*). Quantitatively, GSH is the major intracellular redox buffer and is found

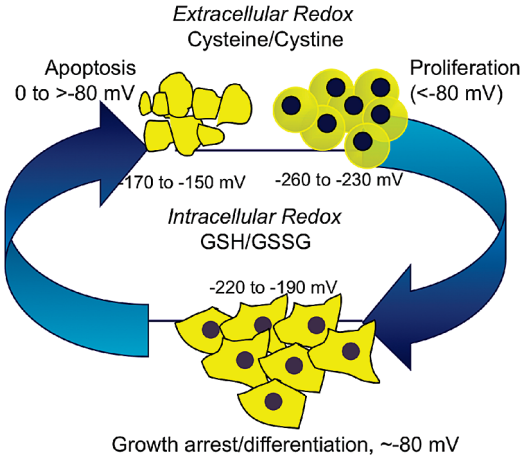

FIGURE 2: Correlated changes between cell cycle progression and the extra- and intracellular redox potentials. The GSH/GSSG couple represents the major intracellular redox buffer. The redox potential of the intracellular GSH/GSSG couple becomes more oxidized when cells progress from proliferation (−260 to −230 mV) to differentiation and/or growth arrest (−220 to −190 mV) to apoptosis (−170 to −150 mV). The cysteine/cystine couple is the main extracellular thiol/disulfide pool. Changes in the extracellular cysteine/cystine redox potential follow the same pattern; i.e., it is most reduced during proliferation (less than −80 mV) and becomes increasingly oxidized during differentiation and/or growth arrest (approximately −80 mV) and apoptosis (0 to −80 mV). This figure was adapted from ref *17*.

at concentrations ranging from 0.5 to 10 mM in mammalian cells (*19*). The intracellular GSH/GSSG (glutathione disulfide) redox potential in dividing cells is estimated to range from −260 to −230 mV and is progressively more oxidized in cells undergoing differentiation and/or growth arrest (−220 to −190 mV) or apoptosis (−170 to −150 mV) (*17*) (Figure 2). The redox potentials for Trx1 in cytoplasm and nuclei are approximately −280 and −300 mV, respectively, while the redox potential of mitochondrial Trx2 is estimated to range from −360 to −340 mV (*18*).

Extracellularly, the cysteine/cystine couple represents the major thiol/disulfide redox buffer. Plasma cystine and cysteine concentrations are reported to be 100−200 and 10−25 μM, respectively, and a redox potential of approximately −80 mV for this couple has been estimated for plasma in healthy humans (*20*). Paralleling the changes in the intracellular GSH/GSSG redox potential, increasing extracellular cysteine/cystine potentials are associated with cells undergoing proliferation (less than −80 mV), differentiation and/or growth arrest (approximately −80 mV), or apoptosis (0 to −80 mV) (Figure 2). An age-dependent increase in

the extracellular redox potential has been reported, which is also influenced by lifestyle choices such as smoking and by diseases such as AIDS (*17*). In contrast to the intracellular compartment, the GSH concentration in the extracellular space is very low (2−4 μM in human plasma). A major fate of secreted GSH is cleavage to its component amino acids, glutamate, cysteine, and glycine. The cysteine thus released is a major source of extracellular cysteine and cystine, which is formed rapidly in the oxidizing milieu of this compartment (*21*).

Although dynamic regulation of the extracellular redox potential, which is linked to intracellular metabolism, has an important bearing on cell function, it is less well-studied and appreciated than intracellular redox control and its perturbations in pathological states. Reactive cysteines on proteins can be reversibly oxidized to sulfenic acids or form disulfide bonds, which can induce changes in their structure and function and elicit downstream effects in redox signaling pathways (*22*, *23*). Disulfide bonds on ectodomains of membrane proteins and in secreted soluble and matrix proteins form a dynamic scaffold that can be reorganized by their shuffling or by their reduction (*24*). It has been proposed that a general loosening of the extracellular disulfide cross-link scaffold might precede cell division (*25*, *26*). Cancer cells typically have higher membrane thiol levels in comparison to nontransformed cells, and there is speculation that this might facilitate higher proliferative rates (*27*). The redox status of specific membrane proteins influences their transport or receptor activity (*17*). For instance, CD4 (cluster of differentiation 4), a glycoprotein found on the surface of helper T cells that is used as a receptor by HIV-1 for gaining entry, has a redox-sensitive disulfide bond in one of its four immunoglobulin-like domains (D2). T cell activation shifts the equilibrium from the disulfide to the dithiol state (*28*). Locking the dithiols in the D2 domain by chemical modification blocks HIV-1 entry, indicating that a redox-linked conformational change in CD4 is critical for penetration of the virus into T cells (*28*).

T CELL-INDUCED EXTRACELLULAR REDOX REMODELING BY DENDRITIC CELLS

The physiological relevance of extracellular reductive modeling during an adaptive immune response is supported by the dramatic increase in the level of free thiols in lymphoid tissue following immunization (*29*). Under these conditions, enhanced nonprotein thiol staining is observed both inside cells and in the extracellular space. In contrast, Peyer's patches from the gut show virtually no staining for nonprotein thiols under these conditions, consistent with the antigenic hyporesponsiveness of this intestinal microenvironment (*11*, *12*).

The magnitude of extracellular cyteine accumulation during activation of T cells increases with time and with the DC to T cell ratio and requires sustained contact between DCs and T cells. An increased level of cell surface thiols on T cells is correlated with increased production of the cytokine, IL-2, in vitro and enhanced proliferation in vivo (*30*). Naïve T cells require cysteine for GSH synthesis. However, cysteine is the least abundant of all amino acids in circulation (*31*), and naïve T cells are unable to import cystine efficiently because of the absence of the cystine transporter, x_c^- (*32*), thus creating a metabolic dependence on antigen presenting cells to meet their cysteine needs. Antigen presenting cells possess the x_c^- antiporter that uses the glutamate gradient to drive import of cystine, which is subsequently converted to cysteine in the reducing intracellular milieu and is ultimately

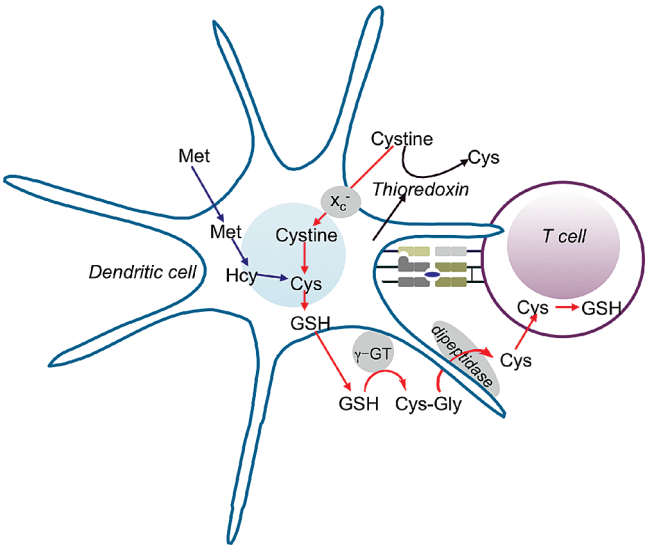

FIGURE 3: Mechanism of redox remodeling by DCs. The possible sources of extracellular cysteine that accumulates during DC and T cell coculture include (i) increased flux through the transsulfuration pathway leading to enhanced synthesis of cysteine from methionine, (ii) direct reduction from cystine catalyzed by extracellular thioredoxin, and (iii) x_c^--dependent import of cystine and its subsequent intracellular conversion to GSH, which is exported and degraded by the ectoenzymes γ-glutamyltranspeptidase and a dipeptidase, to furnish cysteine. The extracellular accumulation of cysteine results in a reducing microenvironment for T cell activation and proliferation and also provides T cells with cysteine needed for the synthesis of GSH.

secreted into the extracellular space. In addition to stimulating cysteine secretion, the interaction between antigen presenting cells and T cells results in the appearance of extracellular Trx1 (*9*). Trx1 is secreted by several cell types via a nonclassical leaderless secretory pathway under conditions of oxidative stress and inflammation (*33*). Secreted Trx1 does not appear to play a direct role in the reduction of extracellular cystine, leading to cysteine accumulation during T cell activation (*10*). Extracellular Trx1 interacts in a redox-sensitive manner with the TNF receptor superfamily member 8 (*34*) and exhibits proinflammatory effects by stimulating cytokine release and proliferation of lymphocytes (*33*, *35*).

The pathway for extracellular cysteine accumulation during coculture of DCs and naïve T cells has been mapped recently (*10*). In principle, two metabolic routes could be considered to lead to enhanced cysteine accumulation outside the cell (Figure 3): (i) the transsulfuration pathway (blue), which provides an avenue for conversion of methionine to cysteine, and (ii) import of cystine into the cell where it is rapidly reduced to cysteine and converted to GSH, which is subsequently secreted and degraded by the ectoenzymes γ-glutamyltranspeptidase and a dipeptidase. Expression of the catalytic subunit of the system x_c^- transporter is induced in DCs during cocultivation with naïve T cells and is correlated with increased extracellular cystine clearance (*10*). Metabolic labeling and pharmacological inhibition studies have established the involvement of the convoluted metabolic pathway originating in cystine and culminating in GSH-derived cysteine as the source of extracellular cysteine provided by DCs (*10*). This pathway demonstrates the dynamic interplay between the intra- and extracellular compartments for redox homeostasis via interconnected but independent redox nodes, i.e., GSH and cysteine.

The extracellular cysteine/cystine redox potential for DCs in culture is approximately −80 mV, a value that is consistent for

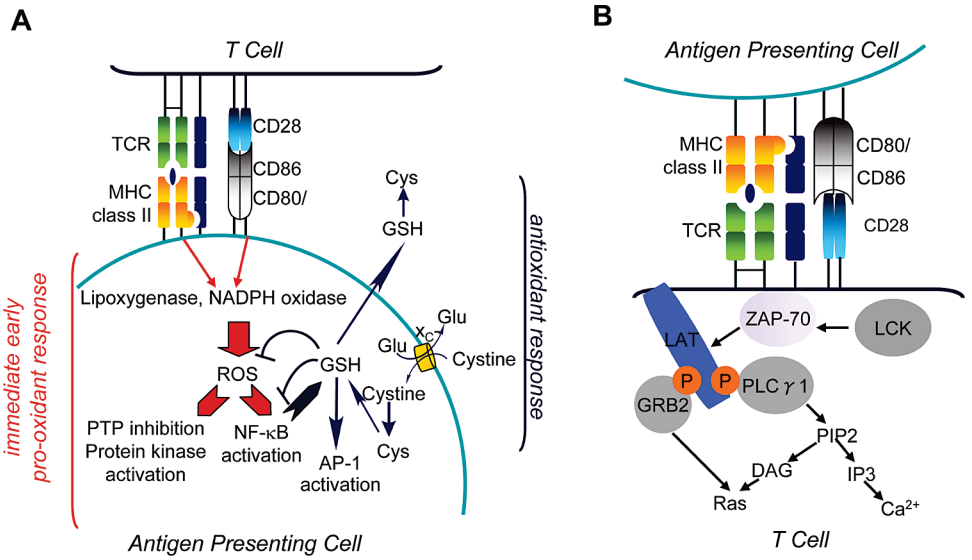

FIGURE 4: Redox signaling responses in DCs and T cells during T cell activation. (A) Redox signaling in DCs. The TCR-antigen·MHC complex interaction and the costimulatory signal result in an immediate early pro-oxidant response in DCs with ROS production, e.g., by lipoxygenase and NADPH oxidase. Low levels of ROS act as signaling molecules to inhibit protein tyrosine phosphatases (PTPs) and activate protein kinases. ROS also activate the NF-κB pathway, which stimulates the expression of γ-glutamylcysteine ligase, thus increasing the extent of GSH synthesis. GSH activates the AP1 signaling pathway and initiates an antioxidant response. We postulate that system x_c^--dependent cystine uptake, GSH export, and degradation into extracellular cysteine are stimulated as part of this response. (B) TCR signaling in T cells. Stimulation of T cells by DCs via the TCR results in phosphorylation and activation of ZAP-70 by leukocyte-specific protein tyrosine kinase (LCK). ZAP-70 directly phosphorylates the adaptor protein LAT and causes the assembly of multiprotein signaling complexes. Recruitment of the growth factor receptor-bound protein 2 (GRB2) and phospholipase C γ1 (PLCγ1) to LAT leads to activation of downstream Ras and calcium signaling pathways.

cells experiencing growth arrest (*10*). Naïve T cells in culture that have not received activation signals are compelled to undergo apoptosis and exhibit an extracellular cysteine/cystine redox potential of approximately −45 mV. In contrast, when naïve T cells receive activation signals during coculture with DCs, a more reducing extracellular environment reflected in a redox potential of −110 mV (at 36 h) results. This redox potential change is consistent with conditions that are conducive for T cell proliferation (*10*).

In addition to triggering intracellular signaling pathways, engagement of DCs and T cells during activation leads to dynamic changes in the redox status of exofacial proteins in both cell types. A 30 mV potential shift is expected to lead to a 10-fold change in the ratio of reduced to oxidized cysteines in proteins. Indeed, enhanced cell surface labeling of protein thiols with the fluorescent dye Alexa maleimide is seen during coculture of DCs and T cells (by ~4- and 8-fold, respectively) as visualized by confocal microscopy and quantified by FACS analysis (*10*).

REDOX SIGNALING DURING T CELL ACTIVATION

Paralleling reductive remodeling of the extracellular redox poise with consequent effects on the exofacial protein thiol status and intracellular redox metabolism is the initiation of a flurry of redox active signaling across the immune synapse. The timing and balance between oxidative and reductive responses to engagement of antigen presenting cells and T cells are important for modulating activation, proliferation, and apoptosis of T cells. At low levels, ROS (reactive oxygen species), e.g., H_2O_2 and $O_2^{\bullet -}$, are considered to be mitogenic, and their downstream effects are commonly mediated via changes in protein phosphorylation and/or activation or inhibition of transcription factors (*36*). Cross-linking of the TCR and the costimulatory molecule, CD28, results in enhanced intracellular H_2O_2 production that is needed for NF-κB activation and IL-2 and IL-2 receptor α chain gene transcription (*37*) and is consistent with an important role for ROS in the immediate early events during activation. Significant sources of ROS include membrane-bound NADPH-dependent oxidase, lipoxygenase, and the mitochondrial respiratory chain (Figure 4A, red arrows). However, sustained pro-oxidant conditions inhibit T cell proliferation and promote apoptosis (*38*).

During activation, increased ROS levels launch an early pro-oxidant response that is relayed via signaling pathways in antigen presenting cells and in T cells and result in activation of protein tyrosine kinases [e.g., Fyn, Src, and Lck in T cells (Figure 4B)], oxidative inhibition of protein tyrosine phosphatases, e.g., SHP1, and activation of transcription factors, e.g., NF-κB (*39*). The NF-κB pathway regulates the expression of various inflammatory genes, including cytokines, chemokines, and costimulatory molecules. We speculate that as a consequence of an initial increase in ROS levels by mechanisms that are not clear, NF-κB is activated in DCs and stimulates GSH biogenesis [via activation of γ-glutamylcysteine ligase (*40*)] (Figure 4A, red arrows). An increased level of GSH synthesis is an autocorrective reaction to oxidizing conditions and initiates the next response phase, i.e., an antioxidant wave (Figure 4A, blue arrows). We hypothesize that the NF-κB signaling pathway is important for stimulating extracellular cysteine accumulation (*10*). The combined effect of these cellular responses would be the initiation of an antioxidant response leading to a reductive milieu in both the intra- and extracellular space that is conducive to T cell proliferation. The importance of plasticity in redox remodeling during T cell activation is supported by the observation that deficiency of Ncf1 encoding neutrophil cytosolic factor 1 (or P47phox), the activating protein in the NADPH oxidase complex, results in a reduced capacity for ROS genesis, an increased number of cell surface thiols, and enhanced T cell autoreactivity in an arthritis model (*30*).

GSH serves as an important proliferative signal in T lymphocytes (*41*) and is required for cell cycle progression from the G1 to S phase (*42*). It is needed for the activity of ribonucleotide reductase and, therefore, for DNA synthesis (*43*). Furthermore, the activities of telomerase (*44*) and of key transcriptional factors, e.g., NF-κB and AP1 (*45*), and cell cycle proteins, e.g., Id2 and E2F4 (*44*), are redox-regulated. GSH is concentrated in the nucleus during the early phase of cell proliferation and becomes more evenly distributed in confluent cells (*46*). GSH regulates nuclear protein function via glutathionylation and protects DNA from oxidative damage during the key stage of replication (*46*). GSH affects ROS levels in cells, which can either activate or inactivate specific redox-sensitive targets at cell cycle checkpoints, thereby influencing cell fate (*47*). Interestingly, an increased level of synthesis and nuclear sequestration of GSH and decreased sensitivity to apoptosis were observed in response to overexpression of the B cell leukemia/lymphoma 2 (Bcl-2) protein in HeLa cells (*48*).

Redox-sensitive signaling cascades are also elicited in T cells upon activation. For instance, a 10−30% decrease in the intracellular level of GSH in peripheral T lymphocytes completely abrogates T cell receptor-stimulated calcium signaling (*49*). The adaptor protein linker for activation of T cells (LAT) (Figure 4B), a membrane protein that plays a central role in signal transduction during T cell activation, is also influenced by the intracellular redox status (*50*). Marked diminution in the intracellular GSH level as seen under chronic oxidative stress conditions causes a conformational change in LAT, apparently via formation of an intramolecular disulfide bond, and results in its displacement from the membrane (*50*). This cytoplasmic relocalization results in failure to phosphorylate in response to T cell activation and derails the signal transduction cascade that leads eventually to expression of IL-2 and other genes. This redox-sensitive conformational displacement is associated with the hyporesponsive phenotype of synovial T cells in rheumatoid arthritis because of their depleted antioxidant capacity resulting from the chronic inflammation associated with this disease of the joints (*50*).

In summary, redox responsive signaling networks during T cell priming involve dynamic and spatially regulated changes in the intra- and extracellular compartments and comprise both small molecules (e.g., ROS and redox active metabolites) and proteins. Redox signaling has several important implications for T cell biology (*36*). Hypoxic conditions as encountered in poorly oxygenated tumors might limit the efficiency of T cell priming and contribute to their anergic phenotype in this environment. Alternatively, a pro-oxidant environment resulting from ROS production by active neutrophils might facilitate priming of T cells but, if overwhelming, impair signaling via inhibitory signals such as tyrosine phosphatases or the NF-κB inhibitor, IkB. Additionally, redox signaling appears to influence T cell commitment to the Th1, Th2, and regulatory T cell phenotypes (*51*, *52*).

REGULATORY T CELLS INTERFERE WITH REDOX REMODELING BY DENDRITIC CELLS

The immune system balances the host's needs for microbial and tumor immunity with keeping autoimmunity in check (*2*). To achieve self-tolerance, T cells are "educated" in the thymus and autoreactive T cells are destroyed. However, a small fraction of self-reactive T cells escape from the thymus into the periphery and, if left unchecked, can cause autoimmune diseases (*53*).

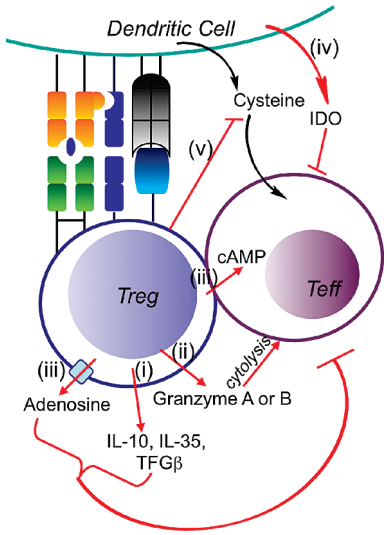

FIGURE 5: Mechanisms used by regulatory T cells for suppressing autoreactive effector T cells. Regulatory T cells suppress the function of effector T cells via the following mechanisms: (i) secretion of inhibitory cytokines such as TGFβ, IL-10, and IL-35, (ii) cytolysis by granzyme-A or granzyme-B, (iii) metabolite disruption, e.g., cAMP and adenosine, (iv) inhibition of DC function via the CTLA4-dependent induction of indoleamine 2,3-dioxygenase (IDO), and (v) modulation of the extracellular redox microenvironment. The red arrows denote the actions of regulatory T cells.

Naturally occurring $CD4^+CD25^+Foxp3^+$ regulatory T cells, which comprise ~5−10% of the total $CD4^+$ T cell population, suppress autoreactive T cells to maintain immune tolerance (*54*). Sakaguchi and co-workers made the groundbreaking discovery of this distinct T cell subpopulation in 1995 and demonstrated that depletion of the $CD25^+$ population from the $CD4^+$ T cells induced autoimmunity when T cells were transferred to immunodeficient nude mice (*1*). In contrast, transfer of the $CD4^+CD25^+$ T cells together with the $CD4^+CD25^-$ T cells prevented autoimmune diseases. Besides the role of regulatory T cells in controlling autoimmunity, they also play important roles in controlling antimicrobial and antitumor responses and transplantation immunity (*54*). Regulatory T cells mature in the thymus, migrate to lymph nodes, and are activated by self or nonself antigen presenting cells. The homing receptors on regulatory T cells enable them to move to sites of infection to control immune responses (*2*). Regulatory T cells also suppress the activation and proliferation of B cells, DCs, and natural killer cells by mechanisms that remain to be fully elucidated (*55*).

Some of the strategies used by regulatory T cells for mediating their suppressive effects (*2*, *3*) are shown in Figure 5 and include (i) secretion of inhibitory cytokines, viz., TGFβ, IL-35, and IL-10 (*56*−*58*), (ii) cytolytic suppression by secretion of the proteases granzyme-A and granzyme-B (*59*, *60*), (iii) metabolic disruption, e.g., by direct transfer of cAMP to effector T cells (*61*) or by secretion of pericellular adenosine (*62*), which inhibits effector T cell functions and enhances induced regulatory T cell generation, (iv) suppression of DC maturation and/or function (*63*) by induction of indoleamine 2,3-dioxygenase, which catalyzes the rate-limiting step in tryptophan catabolism and creates, in turn, a shortage of this essential amino acid for effector T cells (*64*), and (v) interference with extracellular reductive redox remodeling by DCs during T cell activation (*10*). The panoply of suppressive strategies identified to date for regulatory T cells raises questions about their relative importance and how they are integrated in

vivo. In the proposed "hierarchical" model, one or more master mechanisms govern regulatory T cell suppressive functions in various physiological settings (*3*). Alternatively, in the "contextual" model, the microenvironment and tissue compartment govern the suppressive strategy that is deployed, resulting in the differential contribution of a given mechanism in different disease models (*3*).

In contrast to naïve T cells, coculture of regulatory T cells with DCs does not affect the extracellular cysteine concentration. However, regulatory T cells suppress cysteine accumulation in the extracellular compartment when added to cocultures of DCs and naïve T cells. As a consequence, both intracellular (diminished GSH levels in T cells) and extracellular (diminished cell surface thiol labeling on T cells and on DCs) perturbations in the redox status result (*10*). Remarkably, although regulatory T cells are known to mediate their suppressive functions by multiple strategies, provision of a single reagent, i.e., exogenous cysteine at concentrations seen under DC−T cell coculture conditions, alleviates inhibition of T cell proliferation (*10*). This observation begs the question of whether redox regulation serves as a master switch in the multipronged suppressive action of regulatory T cells.

We posit that the redox changes in the intra- and extracellular compartments influence one or more of the well-known regulatory T cell suppressive mechanisms. For instance, the anti-inflammatory cytokine IL-10 has antioxidant properties (*65*), and TGFβ, a multifunctional cytokine, is redox regulated (*66*). Activation of latent TGFβ requires reductive cleavage of a disulfide bond that links it to the latency-associated peptide, but over-reduction leads to formation of inactive TGFβ monomers. Thus, activation and inactivation of TGFβ are subject to redox control, and dynamic changes in the extracellular redox milieu might be important for the regulation of TGFβ activity (*67*). Furthermore, granzyme-A, the cytolytic T cell protease, cleaves redox factor 1 (ref-1), which in turn enhances cell death (*68*). Thus, redox control may be integral to regulatory T cell-mediated suppression mechanisms and could be more pervasive than previously recognized.

IN VIVO STUDIES AND THERAPEUTIC IMPLICATIONS

The secondary lymphoid organs such as lymph nodes and spleens are more reduced than nonlymphoid organs (*29*). The nonprotein thiol content in lymphoid tissues is reported to increase in response to immunization with DCs, B cells, and macrophages, contributing to the reductive remodeling (*29*, *69*). There is speculation that the reducing microenvironment might protect lymphoid organs from oxidative stress during T cell activation and antibody production (*70*, *71*). However, low levels of ROS are essential for the onset of the immune response. In vivo treatment of mouse models with catalytic antioxidants (manganese porphyrin derivatives) causes inefficient $CD4^+$ T cell activation and proliferation by inhibiting ROS generation in antigen presenting cells (*72*). The catalytic antioxidants inhibit DNA binding by NF-κB and subsequent production of proinflammatory cytokines (*73*). Redox modulation by catalytic antioxidants also suppresses $CD8^+$ T cell functions such as proliferation and lysis of target cells (*74*).

The x_c^- cystine transporter, which transports cystine using the glutamate gradient, plays an important role in redox-based immunoregulation. Under normal conditions, lamina propria macrophages are unable to transport cystine and secrete cysteine because they lack the x_c^- transporter (*12*). In inflammatory bowel disease, local recruitment of peripheral blood monocytes which exhibit a high level of expression of the x_c^- transporter leads to extracellular cysteine accumulation and hyperreactivity of lamina propria T cells (*12*). Furthermore, lymphoma cells, which cannot import cystine like naïve T cells, depend on tumor-associated somatic cells such as activated macrophages and DCs for their cysteine supply. Inhibition of the x_c^- transporter by sulfasalazine inhibits growth of lymphoma cells and tumor progression (*75*). Overexpression of the x_c^- transporter in lymphoma cells greatly increases intracellular and extracellular cysteine levels, protecting cells from oxidative stress-induced cell death (*76*).

Redox modulation as a strategy for immunoregulation has been used in treating several diseases. HIV infects and kills $CD4^+$ T cells, leading to a significant decrease in the level of functional $CD4^+$ T cells in AIDS. HIV-infected individuals have lower cellular and plasma GSH levels compared with healthy controls, which correlates with low T cell numbers and deficient function. Administration of *N*-acetylcysteine, a cysteine precursor, restores intracellular GSH levels and has shown benefits for HIV-infected individuals (*77*). Sulfasalazine is used in the treatment of T cell-mediated autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis. It decreases the level of proliferation of autoreactive T cells by inhibiting the x_c^- cystine transporter on antigen presenting cells, thereby perturbing the redox environment (*78*).

Since regulatory T cells play a central role in suppression of various immune responses, manipulation of their function is an important strategy for immune intervention. Enhancing regulatory T cell function in autoimmunity, allergy, transplantation, and pregnancy disorders can weaken unwanted immune responses. On the other hand, attenuating regulatory T cell function in cancer and microbial infection may be desirable (*79*). The recent identification of a novel immunosuppressive strategy deployed by regulatory T cells, which has an impact on the intra- and extracellular redox environments during T cell activation (*10*), illuminates a new therapeutic target.

CONCLUDING REMARKS

Redox modulation has emerged as a key regulatory strategy in the adaptive immune system. It has long been known that T cell activation and proliferation require a reducing milieu. This reducing microenvironment is shaped primarily by the metabolic activity of antigen presenting cells, especially DCs. Interaction of DCs with naïve T cells stimulates cystine consumption and cysteine accumulation in the extracellular space, which produces an extracellular redox potential suitable for T cell proliferation (*9*, *10*). A more reducing extracellular redox potential is reflected in the increased T cell surface thiol status (*10*). The specific membrane targets of redox remodeling and their effects on T cell biology, i.e., activation and proliferation, remain to be elucidated. The greater availability of extracellular cysteine also influences the intracellular antioxidant capacity within T cells since cysteine limits GSH biosynthesis. Consequently, intracellular GSH levels rise and, in turn, influence T cell signal transduction pathways and gene expression. The choreography of GSH localization and the GSH/GSSG redox potential changes during T cell activation, and their correlation with the onset and operation of signaling pathways and cell cycle progression, await elucidation.

Modulation by regulatory T cells of the extracellular redox microenvironment during T cell activation could be mediated by one or more mechanisms. For instance, by limiting cysteine availability, regulatory T cells deprive effector T cells of a building block needed for protein and GSH synthesis. Alternatively, by perturbing the redox environment, regulatory T cells can have both indirect effects by enhancing other suppressive mechanisms used by them (as discussed above) and direct effects on T cell activation and proliferation targets, which are sensitive to the redox potential and the redox status of key signaling proteins. Many questions remain to be addressed regarding how regulatory T cells inhibit reductive remodeling by DCs. For instance, do they interfere with cystine uptake, inhibit the cysteine secretion pathway, or simply compete with effector T cells for the extracellular cysteine pool? Is there a connection between the mechanism for perturbing redox remodeling and Foxp3 expression, and what is the extent of cross-talk between the other suppressive mechanisms and redox remodeling? And, finally, what is the physiological relevance of the redox remodeling mechanism in normal and disease states? The answers to these questions will help illuminate the biology of regulatory T cell suppressive mechanisms and identify potential therapeutic targets.

REFERENCES

1. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155*, 1151–1164.
2. Sakaguchi, S., Yamaguchi, T., Nomura, T., and Ono, M. (2008) Regulatory T cells and immune tolerance. *Cell 133*, 775–787.
3. Vignali, D. A., Collison, L. W., and Workman, C. J. (2008) How regulatory T cells work. *Nat. Rev. Immunol. 8*, 523–532.
4. Tseng, S. Y., and Dustin, M. L. (2002) T-cell activation: A multidimensional signaling network. *Curr. Opin. Cell Biol. 14*, 575–580.
5. Abbas, A. K. (2003) The control of T cell activation vs. tolerance. *Autoimmun. Rev. 2*, 115–118.
6. Banchereau, J., and Steinman, R. M. (1998) Dendritic cells and the control of immunity. *Nature 392*, 245–252.
7. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., and Palucka, K. (2000) Immunobiology of dendritic cells. *Annu. Rev. Immunol. 18*, 767–811.
8. Lanzavecchia, A., and Sallusto, F. (2001) Regulation of T cell immunity by dendritic cells. *Cell 106*, 263–266.
9. Angelini, G., Gardella, S., Ardy, M., Ciriolo, M. R., Filomeni, G., Di Trapani, G., Clarke, F., Sitia, R., and Rubartelli, A. (2002) Antigen-presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. *Proc. Natl. Acad. Sci. U.S.A. 99*, 1491–1496.
10. Yan, Z., Garg, S. K., Kipnis, J., and Banerjee, R. (2009) Extracellular redox modulation by regulatory T cells. *Nat. Chem. Biol. 5*, 721–723.
11. Sido, B., Braunstein, J., Breitkreutz, R., Herfarth, C., and Meuer, S. C. (2000) Thiol-mediated redox regulation of intestinal lamina propria T lymphocytes. *J. Exp. Med. 192*, 907–912.
12. Sido, B., Lasitschka, F., Giese, T., Gassler, N., Funke, B., Schroder-Braunstein, J., Brunnemer, U., Meuer, S. C., and Autschbach, F. (2008) A prominent role for mucosal cystine/cysteine metabolism in intestinal immunoregulation. *Gastroenterology 134*, 179–191.
13. Hori, S., Nomura, T., and Sakaguchi, S. (2003) Control of regulatory T cell development by the transcription factor Foxp3. *Science 299*, 1057–1061.
14. Bennett, C. L., Christie, J., Ramsdell, F., Brunkow, M. E., Ferguson, P. J., Whitesell, L., Kelly, T. E., Saulsbury, F. T., Chance, P. F., and Ochs, H. D. (2001) The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet. 27*, 20–21.
15. Chambers, C. A., Kuhns, M. S., Egen, J. G., and Allison, J. P. (2001) CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. *Annu. Rev. Immunol. 19*, 565–594.
16. Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., Nomura, T., and Sakaguchi, S. (2008) CTLA-4 control over Foxp3+ regulatory T cell function. *Science 322*, 271–275.
17. Moriarty-Craige, S. E., and Jones, D. P. (2004) Extracellular thiols and thiol/disulfide redox in metabolism. *Annu. Rev. Nutr. 24*, 481–509.
18. Kemp, M., Go, Y. M., and Jones, D. P. (2008) Nonequilibrium thermodynamics of thiol/disulfide redox systems: A perspective on redox systems biology. *Free Radical Biol. Med. 44*, 921–937.
19. Meister, A., and Anderson, M. E. (1983) Glutathione. *Annu. Rev. Biochem. 52*, 711–760.
20. Eck, H. P., Gmunder, H., Hartmann, M., Petzoldt, D., Daniel, V., and Droge, W. (1989) Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. *Biol. Chem. Hoppe-Seyler 370*, 101–108.
21. Karp, D. R., Shimooku, K., and Lipsky, P. E. (2001) Expression of γ-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. *J. Biol. Chem. 276*, 3798–3804.
22. Ghezzi, P., Bonetto, V., and Fratelli, M. (2005) Thiol-disulfide balance: From the concept of oxidative stress to that of redox regulation. *Antioxid. Redox Signaling 7*, 964–972.
23. Jones, D. P. (2008) Radical-free biology of oxidative stress. *Am. J. Physiol. 295*, C849–C868.
24. Jordan, P. A., and Gibbins, J. M. (2006) Extracellular disulfide exchange and the regulation of cellular function. *Antioxid. Redox Signaling 8*, 312–324.
25. Hynes, R. O., and Destree, A. (1977) Extensive disulfide bonding at the mammalian cell surface. *Proc. Natl. Acad. Sci. U.S.A. 74*, 2855–2859.
26. Ali, I. U., and Hynes, R. O. (1978) Role of disulfide bonds in the attachment and function of large, external, transformation-sensitive glycoprotein at the cell surface. *Biochim. Biophys. Acta 510*, 140–150.
27. Lawrence, D. A., Song, R., and Weber, P. (1996) Surface thiols of human lymphocytes and their changes after in vitro and in vivo activation. *J. Leukocyte Biol. 60*, 611–618.
28. Matthias, L. J., Yam, P. T., Jiang, X. M., Vandegraaff, N., Li, P., Poumbourios, P., Donoghue, N., and Hogg, P. J. (2002) Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. *Nat. Immunol. 3*, 727–732.
29. Castellani, P., Angelini, G., Delfino, L., Matucci, A., and Rubartelli, A. (2008) The thiol redox state of lymphoid organs is modified by immunization: Role of different immune cell populations. *Eur. J. Immunol. 38*, 2419–2425.
30. Gelderman, K. A., Hultqvist, M., Holmberg, J., Olofsson, P., and Holmdahl, R. (2006) T cell surface redox levels determine T cell reactivity and arthritis susceptibility. *Proc. Natl. Acad. Sci. U.S.A. 103*, 12831–12836.
31. Droge, W., Eck, H. P., Gmunder, H., and Mihm, S. (1991) Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives. *Am. J. Med. 91*, 140S–144S.
32. Ishii, T., Sugita, Y., and Bannai, S. (1987) Regulation of glutathione levels in mouse spleen lymphocytes by transport of cysteine. *J. Cell. Physiol. 133*, 330–336.
33. Nakamura, H., Masutani, H., and Yodoi, J. (2006) Extracellular thioredoxin and thioredoxin-binding protein 2 in control of cancer. *Semin. Cancer Biol. 16*, 444–451.
34. Schwertassek, U., Balmer, Y., Gutscher, M., Weingarten, L., Preuss, M., Engelhard, J., Winkler, M., and Dick, T. P. (2007) Selective redox regulation of cytokine receptor signaling by extracellular thioredoxin-1. *EMBO J. 26*, 3086–3097.
35. Arner, E. S., and Holmgren, A. (2000) Physiological functions of thioredoxin and thioredoxin reductase. *Eur. J. Biochem. 267*, 6102–6109.
36. Pani, G., Colavitti, R., Borrello, S., and Galeotti, T. (2000) Redox regulation of lymphocyte signaling. *IUBMB Life 49*, 381–389.
37. Los, M., Schenk, H., Hexel, K., Baeuerle, P. A., Droge, W., and Schulze-Osthoff, K. (1995) IL-2 gene expression and NF-κB activation through CD28 requires reactive oxygen production by 5-lipoxygenase. *EMBO J. 14*, 3731–3740.
38. Thoren, F. B., Betten, A., Romero, A. I., and Hellstrand, K. (2007) Cutting edge: Antioxidative properties of myeloid dendritic cells: Protection of T cells and NK cells from oxygen radical-induced inactivation and apoptosis. *J. Immunol. 179*, 21–25.
39. Secrist, J. P., Burns, L. A., Karnitz, L., Koretzky, G. A., and Abraham, R. T. (1993) Stimulatory effects of the protein tyrosine phosphatase inhibitor, pervanadate, on T-cell activation events. *J. Biol. Chem. 268*, 5886–5893.
40. Yang, H., Magilnick, N., Lee, C., Kalmaz, D., Ou, X., Chan, J. Y., and Lu, S. C. (2005) Nrf1 and Nrf2 regulate rat glutamate-cysteine

ligase catalytic subunit transcription indirectly via NF-κB and AP-1. *Mol. Cell. Biol. 25*, 5933–5946.
41. Suthanthiran, M., Anderson, M. E., Sharma, V. K., and Meister, A. (1990) Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. *Proc. Natl. Acad. Sci. U.S.A. 87*, 3343–3347.
42. Messina, J. P., and Lawrence, D. A. (1989) Cell cycle progression of glutathione-depleted human peripheral blood mononuclear cells is inhibited at S phase. *J. Immunol. 143*, 1974–1981.
43. Thelander, L., and Reichard, P. (1979) Reduction of ribonucleotides. *Annu. Rev. Biochem. 48*, 133–158.
44. Borras, C., Esteve, J. M., Vina, J. R., Sastre, J., Vina, J., and Pallardo, F. V. (2004) Glutathione regulates telomerase activity in 3T3 fibroblasts. *J. Biol. Chem. 279*, 34332–34335.
45. Schulze-Osthoff, K., Los, M., and Baeuerle, P. A. (1995) Redox signalling by transcription factors NF-κB and AP-1 in lymphocytes. *Biochem. Pharmacol. 50*, 735–741.
46. Markovic, J., Borras, C., Ortega, A., Sastre, J., Vina, J., and Pallardo, F. V. (2007) Glutathione is recruited into the nucleus in early phases of cell proliferation. *J. Biol. Chem. 282*, 20416–20424.
47. Menon, S. G., and Goswami, P. C. (2007) A redox cycle within the cell cycle: Ring in the old with the new. *Oncogene 26*, 1101–1109.
48. Voehringer, D. W., McConkey, D. J., McDonnell, T. J., Brisbay, S., and Meyn, R. E. (1998) Bcl-2 expression causes redistribution of glutathione to the nucleus. *Proc. Natl. Acad. Sci. U.S.A. 95*, 2956–2960.
49. Staal, F. J., Anderson, M. T., Staal, G. E., Herzenberg, L. A., and Gitler, C. (1994) Redox regulation of signal transduction: Tyrosine phosphorylation and calcium influx. *Proc. Natl. Acad. Sci. U.S.A. 91*, 3619–3622.
50. Gringhuis, S. I., Papendrecht-van der Voort, E. A., Leow, A., Nivine Levarht, E. W., Breedveld, F. C., and Verweij, C. L. (2002) Effect of redox balance alterations on cellular localization of LAT and downstream T-cell receptor signaling pathways. *Mol. Cell. Biol. 22*, 400–411.
51. Hasan, A. A., Ghaemmaghami, A. M., Fairclough, L., Robins, A., Sewell, H. F., and Shakib, F. (2009) Allergen-driven suppression of thiol production by human dendritic cells and the effect of thiols on T cell function. *Immunobiology 214*, 2–16.
52. Peterson, J. D., Herzenberg, L. A., Vasquez, K., and Waltenbaugh, C. (1998) Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc. Natl. Acad. Sci. U.S.A. 95*, 3071–3076.
53. Fehervari, Z., and Sakaguchi, S. (2006) Peacekeepers of the immune system. *Sci. Am. 295*, 56–63.
54. Sakaguchi, S. (2004) Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol. 22*, 531–562.
55. Sakaguchi, S., and Powrie, F. (2007) Emerging challenges in regulatory T cell function and biology. *Science 317*, 627–629.
56. Nakamura, K., Kitani, A., and Strober, W. (2001) Cell contact-dependent immunosuppression by $CD4^+CD25^+$ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J. Exp. Med. 194*, 629–644.
57. Asseman, C., Mauze, S., Leach, M. W., Coffman, R. L., and Powrie, F. (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med. 190*, 995–1004.
58. Collison, L. W., Workman, C. J., Kuo, T. T., Boyd, K., Wang, Y., Vignali, K. M., Cross, R., Sehy, D., Blumberg, R. S., and Vignali, D. A. (2007) The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature 450*, 566–569.
59. Gondek, D. C., Lu, L. F., Quezada, S. A., Sakaguchi, S., and Noelle, R. J. (2005) Cutting edge: Contact-mediated suppression by $CD4^+CD25^+$ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J. Immunol. 174*, 1783–1786.
60. Cao, X., Cai, S. F., Fehniger, T. A., Song, J., Collins, L. I., Piwnica-Worms, D. R., and Ley, T. J. (2007) Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. *Immunity 27*, 635–646.
61. Bopp, T., Becker, C., Klein, M., Klein-Hessling, S., Palmetshofer, A., Serfling, E., Heib, V., Becker, M., Kubach, J., Schmitt, S., Stoll, S., Schild, H., Staege, M. S., Stassen, M., Jonuleit, H., and Schmitt, E. (2007) Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. *J. Exp. Med. 204*, 1303–1310.
62. Kobie, J. J., Shah, P. R., Yang, L., Rebhahn, J. A., Fowell, D. J., and Mosmann, T. R. (2006) T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5′-adenosine monophosphate to adenosine. *J. Immunol. 177*, 6780–6786.
63. Tadokoro, C. E., Shakhar, G., Shen, S., Ding, Y., Lino, A. C., Maraver, A., Lafaille, J. J., and Dustin, M. L. (2006) Regulatory T cells inhibit stable contacts between CD4+ T cells and dendritic cells in vivo. *J. Exp. Med. 203*, 505–511.
64. Fallarino, F., Grohmann, U., Hwang, K. W., Orabona, C., Vacca, C., Bianchi, R., Belladonna, M. L., Fioretti, M. C., Alegre, M. L., and Puccetti, P. (2003) Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol. 4*, 1206–1212.
65. Haddad, J. J., and Fahlman, C. S. (2002) Redox- and oxidant-mediated regulation of interleukin-10: An anti-inflammatory, antioxidant cytokine? *Biochem. Biophys. Res. Commun. 297*, 163–176.
66. Barcellos-Hoff, M. H., and Dix, T. A. (1996) Redox-mediated activation of latent transforming growth factor-β1. *Mol. Endocrinol. 10*, 1077–1083.
67. Blakytny, R., Erkell, L. J., and Brunner, G. (2006) Inactivation of active and latent transforming growth factor β by free thiols: Potential redox regulation of biological action. *Int. J. Biochem. Cell Biol. 38*, 1363–1373.
68. Fan, Z., Beresford, P. J., Zhang, D., Xu, Z., Novina, C. D., Yoshida, A., Pommier, Y., and Lieberman, J. (2003) Cleaving the oxidative repair protein Ape1 enhances cell death mediated by granzyme A. *Nat. Immunol. 4*, 145–153.
69. Carta, S., Castellani, P., Delfino, L., Tassi, S., Vene, R., and Rubartelli, A. (2009) DAMPs and inflammatory processes: The role of redox in the different outcomes. *J. Leukocyte Biol. 86*, 549–555.
70. Matsue, H., Edelbaum, D., Shalhevet, D., Mizumoto, N., Yang, C., Mummert, M. E., Oeda, J., Masayasu, H., and Takashima, A. (2003) Generation and function of reactive oxygen species in dendritic cells during antigen presentation. *J. Immunol. 171*, 3010–3018.
71. Masciarelli, S., and Sitia, R. (2008) Building and operating an antibody factory: Redox control during B to plasma cell terminal differentiation. *Biochim. Biophys. Acta 1783*, 578–588.
72. Tse, H. M., Milton, M. J., Schreiner, S., Profozich, J. L., Trucco, M., and Piganelli, J. D. (2007) Disruption of innate-mediated proinflammatory cytokine and reactive oxygen species third signal leads to antigen-specific hyporesponsiveness. *J. Immunol. 178*, 908–917.
73. Tse, H. M., Milton, M. J., and Piganelli, J. D. (2004) Mechanistic analysis of the immunomodulatory effects of a catalytic antioxidant on antigen-presenting cells: implication for their use in targeting oxidation-reduction reactions in innate immunity. *Free Radical Biol. Med. 36*, 233–247.
74. Sklavos, M. M., Tse, H. M., and Piganelli, J. D. (2008) Redox modulation inhibits CD8 T cell effector function. *Free Radical Biol. Med. 45*, 1477–1486.
75. Gout, P. W., Simms, C. R., and Robertson, M. C. (2003) In vitro studies on the lymphoma growth-inhibitory activity of sulfasalazine. *Anticancer Drugs 14*, 21–29.
76. Banjac, A., Perisic, T., Sato, H., Seiler, A., Bannai, S., Weiss, N., Kolle, P., Tschoep, K., Issels, R. D., Daniel, P. T., Conrad, M., and Bornkamm, G. W. (2008) The cystine/cysteine cycle: A redox cycle regulating susceptibility versus resistance to cell death. *Oncogene 27*, 1618–1628.
77. Herzenberg, L. A., De Rosa, S. C., Dubs, J. G., Roederer, M., Anderson, M. T., Ela, S. W., and Deresinski, S. C. (1997) Glutathione deficiency is associated with impaired survival in HIV disease. *Proc. Natl. Acad. Sci. U.S.A. 94*, 1967–1972.
78. Edinger, A. L., and Thompson, C. B. (2002) Antigen-presenting cells control T cell proliferation by regulating amino acid availability. *Proc. Natl. Acad. Sci. U.S.A. 99*, 1107–1109.
79. Becker, C., Stoll, S., Bopp, T., Schmitt, E., and Jonuleit, H. (2006) Regulatory T cells: Present facts and future hopes. *Med. Microbiol. Immunol. 195*, 113–124.