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Mitochondrial hyperpolarization: a checkpoint of T-cell life, death and autoimmunity

Andras Perl, **Peter Gergely Jr**, **Gyorgy Nagy**, **Agnes Koncz**, and **Katalin Banki**
Departments of Medicine, Microbiology and Immunology, and Pathology, State University of New York Upstate Medical University, College of Medicine, 750 East Adams St, Syracuse, NY 13210, USA

Abstract

T-cell activation, proliferation and selection of the cell death pathway depend on the production of reactive oxygen intermediates (ROIs) and ATP synthesis, which are tightly regulated by the mitochondrial transmembrane potential ($\Delta\Psi_m$). Mitochondrial hyperpolarization (MHP) and ATP depletion represent early and reversible steps in T-cell activation and apoptosis. By contrast, T cells of patients with systemic lupus erythematosus (SLE) exhibit persistent MHP, cytoplasmic alkalinization, increased ROI production and depleted ATP, which mediate enhanced spontaneous and diminished activation-induced apoptosis and sensitize lupus T cells to necrosis. Necrotic, but not apoptotic, cell lysates activate dendritic cells and might account for increased interferon a production and inflammation in lupus patients. MHP is proposed as a key mechanism of SLE pathogenesis and is therefore a target for pharmacological intervention.

Innate and adaptive immune responses depend on controlled production of ATP and reactive oxygen intermediates (ROIs) in mitochondria. In response to antigenic stimulation, clonal expansion of T and B cells are continuously downsized and potentially autoreactive cells are eliminated by apoptosis. An array of signals through the T-cell receptor (TCR), co-stimulatory molecules, cell death receptors, lymphokines, and other circulating metabolites, such as ATP, NAD, cADPR, glucose, glutathione, nitric oxide (NO) and ROIs, determine the fate of T cells [1]. T-cell activation and death pathway selection depend on the production of ROIs and ATP synthesis, which are tightly regulated by the mitochondrial transmembrane potential ($\Delta\Psi_m$) (Box 1). Disruption of $\Delta\Psi_m$ has been proposed as the point of no return in apoptotic signaling [2]. We recently discovered that an elevation of Dcm [or mitochondrial hyperpolarization (MHP)] occurs before activation of caspases, phosphatidylserine (PS) externalization and disruption of $\Delta\Psi_m$in Fas- [3] and H_2O_2-induced apoptosis of Jurkat human leukemia T cells and normal human peripheral blood lymphocytes (PBLs) [4]. These observations were confirmed and extended to p53 [5], tumor necrosis factor (TNF)-α [6], staurosporin [7], camptothecin [8] and NO-induced apoptosis [9] (Table 1). MHP is also triggered by activation of the CD3–CD28 complex [10] or stimulation with concanavalin A (ConA) [3], interleukin (IL)-10, IL-3, interferon (IFN)-γ or transforming growth factor (TGF)-β [11]. T-cell-activation-induced MHP is associated with

Corresponding author: Andras Perl (perla@upstate.edu).

transient inhibition of F_0F_1-ATPase, enhanced ROI production, ATP depletion and sensitization to necrosis [10], suggesting that $\Delta\Psi_m$ elevation is a crucial checkpoint of T-cell fate decisions.

T-cell activation is regulated by mitochondrial ROI production

ROIs modulate T-cell activation, cytokine production and proliferation at multiple levels [12]. The antigen-binding αβ or γδTCR is associated with a multimeric receptor module comprising the CD3γδε and TCRζ chains. The cytoplasmic domains of the CD3 and ζ chains contain a common motif, termed the immunoreceptor tyrosinebased activation motif (ITAM), which is crucial for the coupling of intracellular tyrosine kinases [13]. Expression of the TCRζ chain is suppressed by ROIs [14]. Binding of $p56^{lck}$ to CD4 or CD8 attracts this kinase to the TCRz–CD3 complex, leading to phosphorylation of ITAM. Phosphorylation of both tyrosines of each ITAMis required for Src-homology 2 (SH2)-mediated binding by ZAP-70 (ζ-chain-associated protein of 70 kDa) or the related SYK. ZAP-70 is activated through phosphorylation by $p56^{lck}$. Activated ZAP-70 and SYK target two key adaptor proteins, LAT and SLP-76 [13]. Oxidative stress reduces phosphorylation and displacement of LAT from the cell membrane, thus causing T-cell hyporesponsiveness [15]. Phosphorylated LAT binds directly to phospholipase Cγ1, which controls hydrolysis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] to inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol (DAG). Phosphorylation of inositol lipid second messengers is mediated by phosphoinositide 3-kinase (PI3-K). The stimulatory effect of the TCR alone on PI3-K activity is small. Concurrent triggering of the CD28 co-stimulatory molecule by its ligands CD80 or CD86 is required for optimal PI3-K activation. Ins(1,4,5)P_3 binds to its receptors in the endoplasmic reticulum, opening Ca^{2+} channels that release Ca^{2+} into the cytosol. Increased cytosolic Ca^{2+} concentration activates the phosphatase calcineurin, which dephosphorylates the transcription factor NFAT. Dephosphorylated NFAT can translocate to the nucleus, where it promotes transcription of IL-2 in concert with AP-1, NF-κB and Oct-1. Whereas activities of AP-1 and NF-κB are increased by oxidative stress [16], both thiol insufficiency and H_2O_2 treatment suppresses calcineurin-mediated activation of NFAT [17]. Thus, expression of cytokines can be selectively regulated by oxidative stress depending on the relative expression level of transcription factors involved (e.g. IL-2 is expressed through a promoter that has AP-1 and NFAT motifs, and IL-4 is expressed through an AP-1-less NFAT enhancer; Figure 1).

Redox control of apoptosis signal processing

Programmed cell death (PCD) or apoptosis is a physiological mechanism for elimination of autoreactive lymphocytes during development. Signaling through the Fas or structurally related TNF family of cell-surface death receptors has emerged as a major pathway in the elimination of unwanted cells under physiological and disease conditions [18]. Fas and TNF receptors mediate cell death through cytoplasmic death domains (DDs) shared by both receptors [19]. They trigger sequential activation of caspases, resulting in cleavage of cellular substrates and the characteristic morphological and biochemical changes of apoptosis [20].

Disruption of the mitochondrial membrane potential ($\Delta\Psi_m$) has been proposed as the point of no return in apoptotic signaling, leading to caspase activation and disassembly of the cell [2]. Interestingly, MHP and ROI production precede disruption of $\Delta\Psi_m$, activation of caspases and PS externalization in Fas- [3], TNF-α- [6] and H_2O_2-induced apoptosis of Jurkat human leukemia T cells and normal human PBLs [4]. Elevation of $\Delta\Psi_m$ is independent from activation of caspases and represents an early event in apoptosis [3]. Pretreatment with caspase inhibitors completely abrogated Fas-induced PS externalization, indicating that activation of caspase-3, caspase-8, and related cysteine proteases were absolutely required for cell death [3]. ROI levels were partially inhibited in Jurkat cells treated with caspase inhibitor, suggesting that caspase-3 activation, perhaps through damage of mitochondrial membrane integrity, contributes to ROI production and serves as a positive-feedback loop at later stages of the apoptotic process. Cleavage of cytosolic Bid by caspase-8 generates a p15 carboxy-terminal fragment that translocates to mitochondria. This might increase mitochondrial membrane permeability and lead to secondary elevation of ROI levels in the Fas and TNF pathway [21].

Mitochondrial checkpoints of cell death pathway selection

MHP appears to be the earliest change associated with several apoptosis pathways (Table 1). Elevation of $\Delta\Psi_m$ is also triggered by activation of the CD3–CD28 complex [10] or stimulation with ConA [3], IL-10, IL-3, IFN-γ or TGF-β [11]. Therefore, MHP represents an early but reversible switch not exclusively associated with apoptosis. MHP is a probable cause of increased ROI production [22] and might be ultimately responsible for increased susceptibility to apoptosis following T-cell activation [10].

MHP in T cells is associated with a dramatic increase, more than sixfold, of NO production lasting 24 hours after CD3–CD28 co-stimulation. Molecular ordering of T-cell-activation-induced NO production revealed crucial roles for ROI production and cytoplasmic and mitochondrial Ca^{2+} influx [23]. CD3–CD28 co-stimulation-induced ROI production, similar to H_2O_2, enhances expression of nitric oxide synthase (NOS) isoforms eNOS and nNOS, which require elevated Ca^{2+} levels for enzymatic activity. These results suggest that T-cell-activation-induced ROI and Ca^{2+} signals contribute to NO production, with the latter representing a final and dominant step in MHP (Figure 1).

Proteins of the Bcl-2 family are localized to membranes of distinct organelles including mitochondria [24]. Both the pro-apoptotic (Bax, Bad) and anti-apoptotic (Bcl-2, Bcl-XL) members of the family can form ion-conducting channels in lipid membranes [21]. Bax can create a channel in the outer mitochondrial membrane, thus releasing cytochrome *c* and other caspase-activating moieties into the cytosol. Bcl-2 and Bcl-X_L inhibit this process through dimerization with Bad or Bax. Bcl-2 expression appears to be unaltered in lupus PBLs [25].

The mitochondrion is the site of ATP synthesis through oxidative phosphorylation. The synthesis of ATP is driven by an electrochemical gradient across the inner mitochondrial membrane maintained by an electron transport chain and the membrane potential. Caspase activity requires ATP to the extent that depletion of ATP by inhibition of F_0F_1-ATPase with

oligomycin [26] or exhaustion of intracellular ATP stores by prior apoptosis signals, Fas stimulation [26] or H_2O_2 pretreatment leads to necrosis [27]. Thus, intracellular ATP concentration is a key switch in the decision of the cell to die by apoptosis or necrosis [26].

MHP, increased ROI production, cytoplasmic alkalinization and ATP depletion in lupus T cells

Current evidence suggests that the regulation of PCD is impaired in both human and murine systemic lupus erythematosus (SLE), and could contribute to disease pathogenesis [28]. In *lpr* and *gld* mice, defects in PCD signaling through the Fas pathway appear to predispose to autoimmunity [29]. Whereas mutations of the Fas receptor (FasR) or Fas ligand (FasL) have been associated with a lupus-like autoimmune syndrome in mice with the *lpr* or *gld* genetic background [29], Fas-mediated signaling appears to be intact in human SLE [30]. Lupus T cells demonstrate defective activation-induced cell death (AICD) [31]. By contrast, increased spontaneous apoptosis of PBLs has also been observed in SLE [32]. Thus, paradoxically, SLE T cells exhibit both defective AICD and enhanced spontaneous apoptosis (Table 2).

Coordinate MHP and ATP depletion play key roles in abnormal T-cell death in lupus patients [10]. $\Delta\Psi_m$ and ROI levels, as well as cytoplamic pH, are elevated in patients with SLE in comparison with healthy or rheumatoid arthritis controls [10,11]. Baseline MHP and ROI levels correlated with diminished levels of reduced glutathione (GSH), suggesting increased utilization of reducing equivalents in patients with SLE. It is presently unclear whether synthesis of GSH or its regeneration from its oxidized form (GSSG) is deficient in lupus patients. GSH is also required for IL-2-dependent T-cell proliferation [33], as well as CD2- and CD3-mediated T-cell activation [12]. Thus, low GSH content might also inhibit CD3-induced H_2O_2 production. Nevertheless, GSH deficiency predisposes for ROI-induced cell death [4,34]. Diminished H_2O_2-induced apoptosis of cells with low baseline GSH levels indicates a severe dysfunction of redox signaling in patients with SLE [10].

Increased ROI production might lead to skewed expression of redox-sensitive surface receptors and lymphokines in SLE (Table 2). As examples, ROIs regulate gene transcription and release of TNF-α and IL-10 [35], both of which are elevated in sera [36] and freshly isolated PBLs of SLE patients [37]. Expression of the TCRζ chain is sensitive to oxidative stress [14] and thus increased ROI levels could explain, at least in part, the low expression of the TCRz chain in lupus T cells [38]. Cell-surface expression of FasR [39] and FasL is also redox sensitive [40]. Increased ROI levels might be related to increased IL-10 production, release of FasL and overexpression of FasR in SLE [30]. Elevated NO production might also contribute to increased spontaneous apoptosis [41]. Increased ROI levels confer sensitivity to H_2O_2, NO, TNF-α or Fas-induced cell death [34]. Therefore, persistent MHP, causing increased ROI production (a trigger of apoptosis) and depletion of ATP (required for AICD), might be responsible for the paradox of increased spontaneous apoptosis and diminished AICD in SLE.

MHP and ATP depletion predispose lupus T cells to necrosis

In response to treatment with exogenous H_2O_2, a precursor of ROIs, lupus T cells failed to undergo apoptosis, and cell death preferentially occurred by necrosis. Endogenous H_2O_2 is generated by superoxide dismutase from the ROIs O_2^- or OH^- in mitochondria [42]. In turn, H2O2 is scavenged by catalase and glutathione peroxidase [43]. Whereas H_2O_2 is freely diffusible, it has no unpaired electrons and, by itself, is not a ROI [42]. Induction of apoptosis by H_2O_2 requires mitochondrial transformation into an ROI (e.g. OH^-) through the Fenton reaction. [22,42]. As previously noted [4], H_2O_2 triggered a rapid increase of $\Delta\Psi_m$ and ROI production that was followed by apoptosis of PBLs in healthy subjects. By contrast, H_2O_2 failed to elevate $\Delta\Psi_m$, ROI production and apoptosis, but rather elicited necrosis of lupus T cells. Both CD3–CD28-induced H_2O_2 production and H_2O_2-induced apoptosis require mitochondrial ROI production. Therefore, diminished CD3–CD28-induced H_2O_2 production and H_2O_2-induced apoptosis, together with deficient elevation of $\Delta\Psi_m$ and ROI levels, reveal deviations of key biochemical checkpoints in mitochondria of patients with SLE.

$\Delta\Psi_m$ is dependent upon the electron transport chain transferring electrons from NADH to molecular oxygen and proton transport mediated by the F_0F_1-ATPase complex [22]. During oxidative phosphorylation, the F_0F_1-ATPase converts ADP to ATP by utilizing the energy stored in the electrochemical gradient. Alternatively, using the energy of ATP hydrolysis, F_0F_1-ATPase can pump protons out of the mitochondrial matrix into the intermembrane space, causing $\Delta\Psi_m$ elevation. Thus, MHP can occur in several ways. First, deficiency of cellular ADP could cause diminished utilization of the electrochemical gradient, ATP depletion and MHP. However, ADP levels were not diminished but slightly elevated in lupus PBLs [10]. This suggested that ATP depletion and $\Delta\Psi_m$ hyperpolarization were not caused by a lack of ADP in patients with SLE. Second, MHP might occur through calcium-activated dephosphorylation of cytochrome *c* oxidase [44]. Phosphorylation of cytochrome *c* oxidase is mediated by protein kinase A (PKA); thus, deficiency of PKA could also contribute to MHP in SLE [45]. Third, inhibition of the enzymatic activity of F_0F_1-ATPase would decrease utilization of the electrochemical gradient and cause $\Delta\Psi_m$ hyperpolarization, ATP depletion and ADP accumulation. A similar mechanism might also be operational in patients with SLE given that blocking of F_0F_1-ATPase by oligomycin led to $\Delta\Psi_m$ hyperpolarization and elevated ROI production, prevented H_2O_2- or CD3–CD28-induced elevation of $\Delta\Psi_m$ in normal PBLs, and sensitized to H_2O_2-induced necrosis [10]. With $\Delta\Psi_m$ hyperpolarization and extrusion of H^+ ions from the mitochondrial matrix, the cytochromes within the electron transport chain become more reduced, which favors generation of ROIs [22]. Thus, MHP is a likely cause of increased ROI production and might be ultimately responsible for increased spontaneous apoptosis in patients with SLE.

A 28–32% increase of the 2200 mV $\Delta\Psi_m$ might have a tremendous impact on mitochondrial energy coupling and ATP synthesis [22]. Both T-cell activation and apoptosis require the energy provided by ATP [46]. Intracellular ATP concentration is a key switch in the decision of the cell to die by apoptosis or necrosis [26] and, therefore, depletion of ATP might be responsible for defective H_2O_2-induced apoptosis and a shift to necrosis in patients with SLE. Apoptosis is a physiological process that results in nuclear condensation and

break-up of the cell into membrane-enclosed apoptotic bodies suitable for phagocytosis by macrophages, thus preventing inflammation. By contrast, necrosis is a pathological process that results in cellular swelling, followed by lysis and release of proteases, oxidizing molecules, and other proinflammatory and chemotactic factors, resulting in inflammation and tissue damage [46]. Indeed, lymphocyte necrosis occurs in the bone marrow [47] and lymph nodes of lupus patients, and could significantly contribute to the inflammatory process [48].

Increased necrosis might initiate a proinflammatory state, activation of DCs and IFN production in SLE

Swollen lymph nodes of patients with SLE harbor increased numbers of necrotic T cells and dendritic cells (DCs) [49]. Necrotic, but not apoptotic, cell death generates inflammatory signals that are necessary for the activation and maturation of DCs, which are the most potent antigen-presenting cells [50–52]. High-mobility group B1 (HMGB1) protein, an abundant DNA-binding protein, remains immobilized on chromatin of apoptotic bodies, but is released from necrotic cells [53]. HMGB1 stimulates human monocytes to release TNF-α, IL-1α, IL-1β, IL-1 receptor antagonist (IL-1RA), IL-6, IL-8, macrophage inflammatory protein (MIP)-1α, and MIP-1β, but not IL-10 or IL-12 [54], and induces arthritis [55]. Necrotic, but not apoptotic, cells also release heat shock proteins (HSPs) HSPgp96, HSP90, HSP70 and calreticulin. HSPs stimulate macrophages to secrete cytokines, and induce expression of antigen-presenting and co-stimulatory molecules on the DC [51]. Mature DCs express high levels of the DC-restricted markers CD83 and lysosome-associated membrane glycoprotein (DC-LAMP), and the co-stimulatory molecules CD40 and CD86 [52], which might contribute to the altered intercellular signaling in SLE (Figure 2). $CD14^+$ monocytes isolated from the blood of lupus patients, but not those from healthy individuals, act as DCs [56]. Their activation is driven by circulating IFN-α, which might be generated by one of the DC subsets [e.g. plasmacytoid dendritic cells (PDCs)] that infiltrate lupus skin lesions. Tissue lesions [57,58] and blood of patients with SLE harbor activated PDCs that might be responsible for increased production of IFN-α in SLE[56,59].

Outstanding questions and future directions

MHP represents an early but reversible checkpoint associated with activation and apoptosis of human T cells. Although Ca^{2+}-, ROI- and NADPH-dependent production of NO appears to be a dominant factor in T-cell-activation-induced MHP, the relative impact and hierarchy of the metabolic and redox signaling pathways involved require further studies. The chemical composition of ROIs (i.e. OH^-, O_2^-, $ONOO^-$ and ONOOH), and their compartmentalization during T-cell activation and cell death, are unexplored. Selective targeting of ROIs might prove valuable in regulating T helper (Th)-cell differentiation and cytokine production, activation of cytotoxic T cells, and selection of the cell death pathway. Although MHP was not affected, IL-10 antibody or IL-12 was found to normalize ROI production and intracellular alkalinization in lupus PBLs [11]. Therefore, IL-10 antagonists might partially correct the redox signaling dysfunction in lupus. Bz-423, an experimental drug that binds to the benzodiazepine receptor in mitochondria, was found to reduce $\Delta\Psi_m$, induce selective death of autoreactive lymphocytes, and improve clinical outcome of lupus

in two different murine models [60,61]. Precise delineation of the mechanism of MHP and ATP depletion could identify novel targets for pharmacological intervention in patients with SLE.

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Box 1. Regulation of the mitochondrial transmembrane potential ($\Delta\Psi_m$)

The mitochondrial transmembrane potential ($\Delta\Psi_m$; negative inside and positive outside) is the result of an electrochemical gradient maintained by two transport systems – the electron transport chain and the F_0F_1-ATPase complex [44]. The electron transport chain catalyzes the flow of electrons from NADH to molecular oxygen and the translocation of protons across the inner mitochondrial membrane, thus creating a voltage gradient with negative charges inside the mitochondrial matrix [22]. A small fraction of electrons react directly with oxygen and form reactive oxygen intermediates (ROIs). Activity of the F_0F_1-ATPase complex has crucial roles in oxidative phosphorylation (i.e. conversion of ADP to ATP at the expense of the electrochemical gradient during oxidative phosphorylation) [22]. Mitochondrial membrane integrity is dependent on the oxidation–reduction equilibrium of ROI, pyridine nucleotides (NADH/NAD + NADPH/NADP) and reduced glutathione (GSH) levels [68]. Regeneration of GSH by glutathione reductase from its oxidized form, GSSG, depends on NADPH produced by the pentose phosphate pathway (PPP) [43]. ROI levels and $\Delta\Psi_m$ are regulated by transaldolase through the supply of reducing equivalents from PPP [3,34], Ca^{2+} fluxing and NO production [23]. Whereas ROIs have been considered as toxic byproducts of aerobic existence, evidence is now accumulating that controlled levels of ROIs modulate various aspects of cellular function and are necessary for signal transduction pathways, including those mediating T-cell activation and apoptosis [1]. Mitochondrial hyperpolarization (MHP), an early event of T-cell activation and death, appears to be mediated through inhibition of F_0F_1-ATPase or dephosphorylation of cytochrome *c* oxidase [44]. Nitric oxide (NO), acting as a competitive antagonist of oxygen, can also reversibly inhibit cytochrome *c* oxidase and cause MHP [69]. Using the energy of ATP, F_0F_1-ATPase can pump protons out of the mitochondrial matrix into the intermembrane space, thus causing $\Delta\Psi_m$ elevation. MHP leads to uncoupling of oxidative phosphorylation (i.e. continued ROI production in the absence of ATP synthesis), which disrupts $\Delta\Psi_m$ and damages integrity of the inner mitochondrial membrane. Disruption of $\Delta\Psi_m$ has been proposed as the point of no return in cell death signaling [2]. This releases cytochrome *c* and other cell-death-inducing factors from mitochondria into the cytosol. Intracellular ATP concentration is a key switch in the decision of the cell to die by apoptosis or necrosis [26]. Whereas apoptosis is energy dependent and persistent, MHP has been associated with ATP depletion and sensitization to necrosis in lupus T cells [10]. Thus, regulation of $\Delta\Psi_m$ is a crucial checkpoint of T-cell fate decisions.

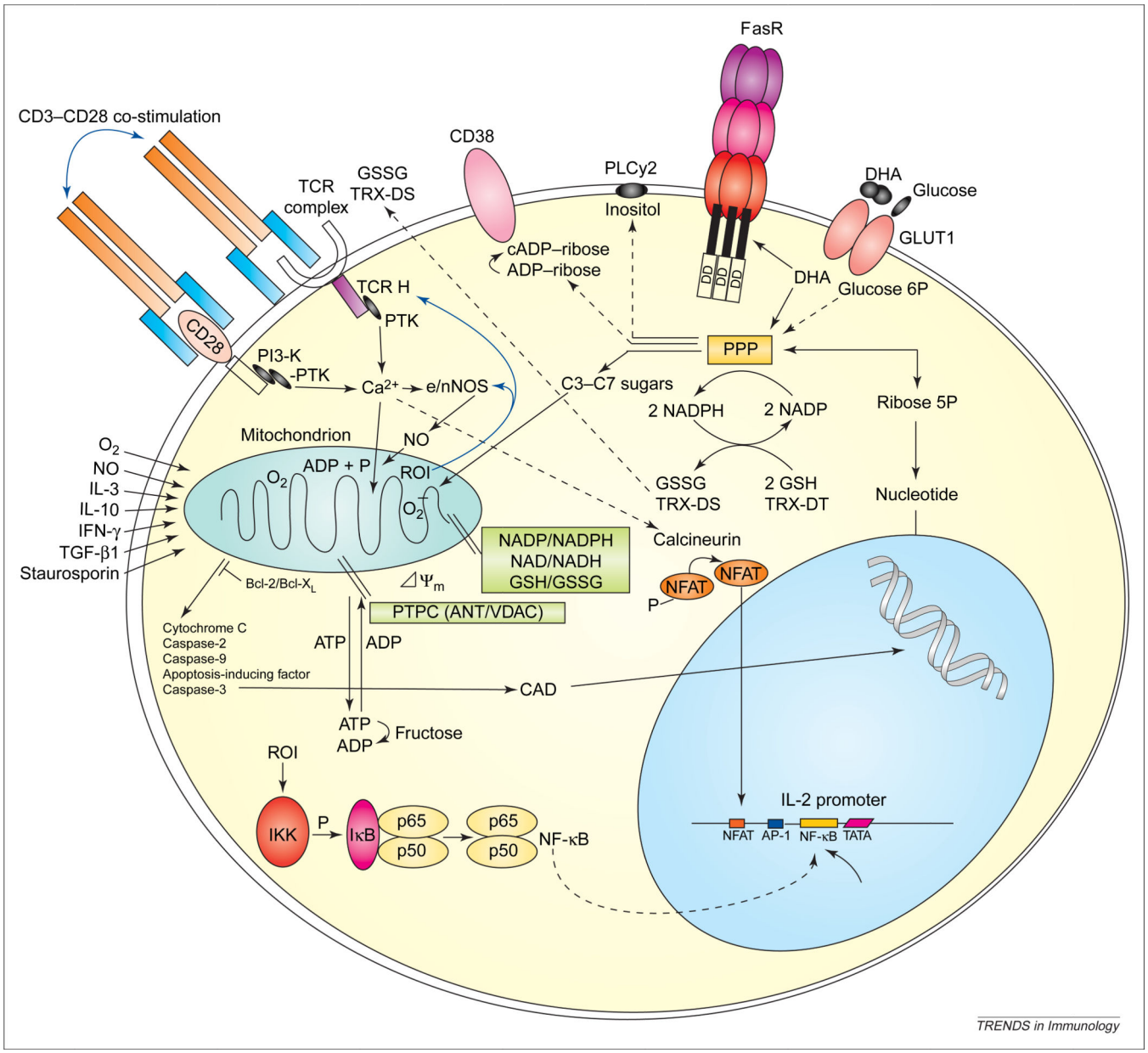

Figure 1.

Mitochondrial and metabolic checkpoints of T-cell activation and apoptosis signals. Antigen binding-initiated signaling through the T-cell receptor (TCR)–CD3–TCRζ-chain complex and the CD28 co-stimulatory molecule activate phosphoinositide 3-kinase (PI3-K) and protein tyrosine kinase (PTK). Increased cytosolic Ca^{2+}; concentration activates the serine/threonine phosphatase calcineurin, which dephosphorylates the NFAT. Dephosphorylated NFAT can translocate to the nucleus, where it promotes transcription of interleukin-2 (IL-2) in concert with AP-1 and NF-κB. Ca^{2+} flux into mitochondria increases production of reactive oxygen intermediates (ROIs) and NF-κB activation [22]. Excess ROI production and disruption of $\Delta\Psi_m$ lead to activation-induced cell death executed by caspase-3 (digesting vitally important proteins PARP, 70K U1RNP, lamin and actin) and caspase-

activated DNase (CAD). Cleavage by caspase-3 is thought to expose cryptic epitopes and cause autoantigenicity of self-antigens [70]. Dehydroascorbate (DHA) is imported through glucose transporter 1 (GLUT1) and is metabolized through the pentose phosphate pathway (PPP), thereby enhancing reduced glutathione (GSH) levels. DHA also increases surface expression of Fas receptor (FasR) [64]. Glutathione reductase and TRX reductase synthesize GSH and TRX-DT at the expense of NADPH. Formulation of the PPP and its efficiency to provide NADPH is dependent on the expression of G6PD (glucose 6-phosphate dehydrogenase) and transaldose (TAL) [34,43]. Dcm is controlled by intracellular GSH/NADH/NADPH levels, integrity of the permeability transition pore complex (PTPC), largely composed of the adenine nucleotie translocator (ANT) (inner membrane) and voltage-dependent anion channel (VDAC) (outer membrane), and translocation and dimerization of proand anti-apoptotic Bcl-2 family members in the intermembrane space [21]. TCR-activation-induced mitochondrial hyperpolarization is mediated through nitric oxide (NO) production by endothelial/neuronal NO synthase (e/nNOS), which, in turn, is regulated by ROIs and Ca^{2+}; [23]. Abbreviations: GSSG, oxidized glutathione; IFN-γ, interferon γ; P, phosphate; PLCγ2, phospholipase Cγ2; TGF-β1, transforming growth factor β1; TRX-DT, thioredoxin.

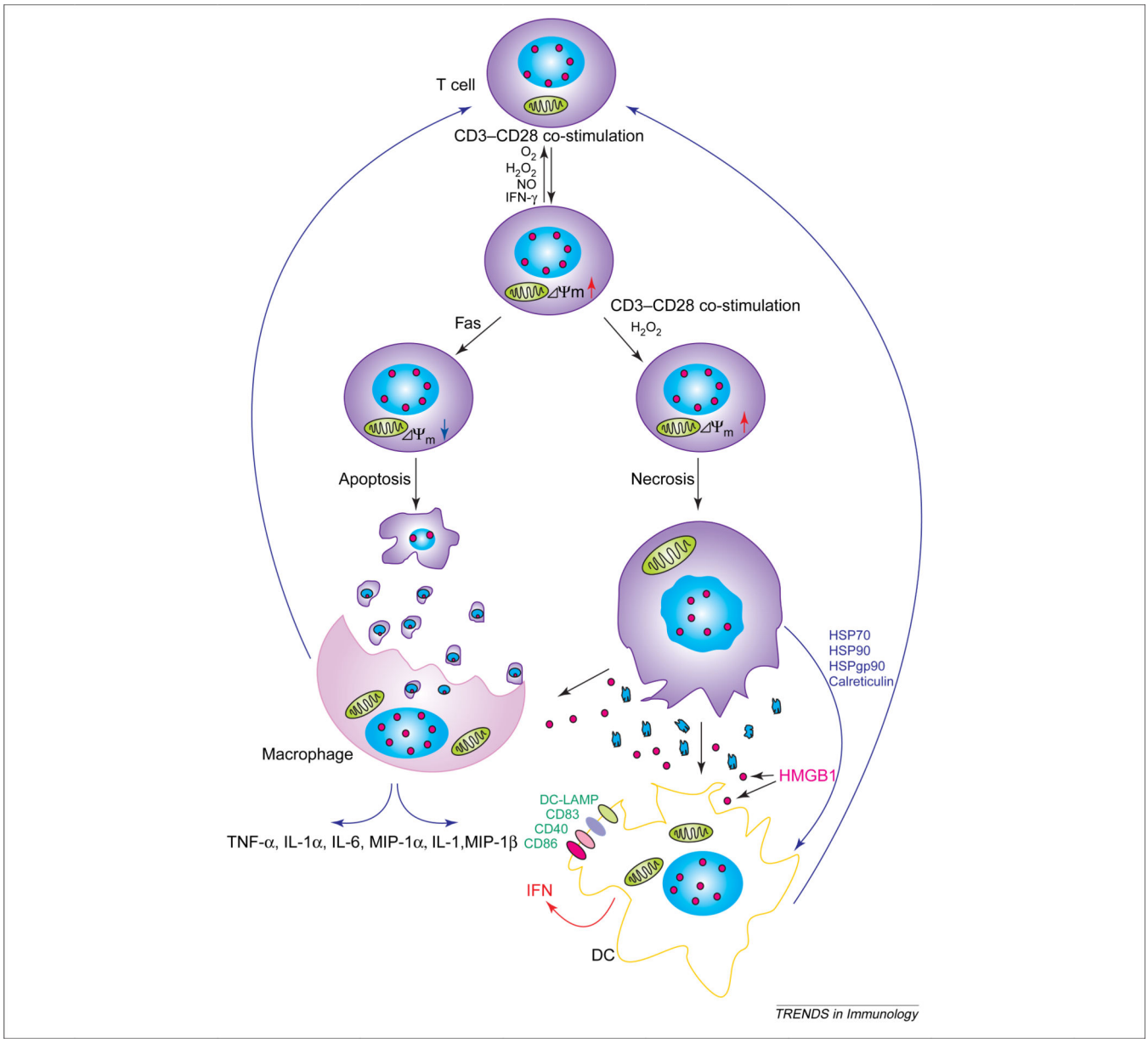

Figure 2.

Impact of $\Delta\Psi_m$ on cell death pathway selection and activation of T cells by macrophages and dendritic cells (DCs). CD3–CD28 co-stimulation, reactive oxygen intermediate (ROI), H_2O_2, nitric oxide (NO) and cytokines, such as interferon-γ (IFN-γ), might elicit reversible elevation of $\Delta\Psi_m$ [i.e. mitochondrial hyperpolarization (MHP)]. Transient MHP is an early event in Fas-mediated apoptosis, resulting in cellular shrinkage and formation of membrane-enclosed apoptotic bodies that are phagocytosed by macrophages. Repeated T-cell receptor (TCR) activation or exposure to ROIs induces necrosis instead of MHP, which, in turn, leads to cellular swelling, membrane breakdown and release of proinflammatory cell lysate. While high-mobility group B1 (HMGB1) protein remains immobilized on chromatin of apoptotic bodies, it is released from necrotic cells. HMGB1, heat-shock protein 70 (HSP70), HSP90, HSPgp95 and calreticulin induce differentiation of DCs and activate macrophages. DCs

produce IFN-γ, whereas macrophages produce a series of proinflammatory cytokines. Both cell types increase expression of co-stimulation molecules, thus contributing to T-cell activation. These positive-feedback loops might play key roles in aberrant T-cell activation in patients with systemic lupus erythematosus (SLE). Abbreviations: DC-LAMP, lysosome-associated membrane glycoprotein; MIP-1γ, macrophage inflammatory protein 1γ; TNF-γ, tumor necrosis factor γ.

Table 1

Signals eliciting MHP and ROI production[a]

Signal	Refs
Fas	[3]
TNF-α	[6]
ConA	[3]
HIV	[62,63]
H_2O_2	[4,10,64]
Activation of the CD3–CD28 complex	[10,11]
IL-10	[11]
IL-3	[11]
TGF-β	[11]
IFN-γ	[11]
Staurosporin	[7]
Camptothecin	[8]
NO	[9,23]

[a]Abbreviations: ConA, concanavalin A; IFN-γ, interferon γ; IL, interleukin; MHP, mitochondrial hyperpolarization; NO, nitric oxide; ROI, reactive oxygen intermediate; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α

Table 2

Signaling abnormalities of T-cell death in patients with SLE[a]

Signal	Effect	Refs
$\Delta\Psi_m\uparrow$	ROI ↑, ATP ↓	[10]
ROI↑	Spontaneous apoptosis ↑, IL-10 production↑	[10,11]
GSH ↓	ROI ↑, spontaneous apoptosis ↑	[10,34]
Spontaneous apoptosis ↑	Compartmentalized autoantigen release, disease activity↑	[32,65]
H_2O_2	Apoptosis ↓, necrosis ↑	[10]
Activation of the CD3–CD28 complex	AICD ↓, necrosis ↑	[11]
ATP ↓	Predisposes for necrosis	[10,26]
Necrosis ↑	Inflammation ↑	[10,48]
AICD ↓	Persistence of autoreactive cells	[11,31]
FasR ↑	Spontaneous apoptosis ↑	[30]
FasL ↑	Spontaneous apoptosis ↑	[30]
IL-10 ↑	Selective induction of apoptosis in SLE	[11,30,66]
NO ↑	Disease activity ↑	[67]
IL-10 blockade	Spontaneous apoptosis ↓, ROI ↓	[11,30]
IL-12	Spontaneous apoptosis ↓, ROI ↓	[11]

[a] Abbreviations: AICD, activation-induced cell death; FasL, Fas ligand; FasR, Fas receptor; GSH, reduced glutathione; IL, interleukin; NO, nitric oxide; ROI, reactive oxygen intermediate; SLE, systemic lupus erythematosus; ↑, increase; ↓, decrease.