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REVIEW

Mitochondria as Oxidative Signaling Organelles in T-cell Activation: Physiological Role and Pathological Implications

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Abstract Early scientific reports limited the cell biological role of reactive oxygen species (ROS) to the cause of pathological damage. However, extensive research performed over the last decade led to a wide recognition of intracellular oxidative/redox signaling as a crucial mechanism of homeostatic regulation. Amongst different cellular processes known to be influenced by redox signaling, T-cell activation is one of the most established. Numerous studies reported an indispensible role for ROS as modulators of T-cell receptor-induced transcription. Nevertheless, mechanistic details regarding signaling pathways triggered by ROS are far from being delineated. The nature and interplay between enzymatic sources involved in the generation of "oxidative signals" are also a matter of ongoing research. In particular, active participation of the mitochondrial respiratory chain as ROS producer constitutes an intriguing issue with various implications for bioenergetics of activated T cells as well as for T-cell-mediated pathologies. The aim of the current review is to address these interesting concepts.

Keywords Reactive oxygen species (ROS) · Complex I (NADH:ubiquinone oxidoreductase) · T-cell receptor (TCR) · NF-kappaB (NF-κB) · Glycerol-3-phosphate dehydrogenase (GPD2) · ADP-dependent glucokinase (ADPGK)

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Introduction

Oxygen is indispensable for multicellular life. At the same time, it is one of the most reactive and life-threatening agents known. For aerobic organisms, oxidation is the main way to generate energy. Thus, to harness the possible deleterious effects of the oxidative chemistry, intracellular homeostasis is maintained by a balance of oxidation and reduction (redox) reactions, the "intracellular redox equilibrium". In an extreme case, when metabolic processes or toxic insults lead to a situation when pro-oxidants outbalance the anti-oxidative system, the state of "oxidative stress" is reached. This breakdown of cellular homeostasis results in oxidation-induced damage of lipids, proteins, carbohydrates and nucleic acids ultimately leading to cell death (Kaminski et al. 2004).

The causative agents are chemical compounds belonging to reactive oxygen species (ROS) or reactive nitrogen species (RNS). ROS/RNS are instable, have a short half-life, and mostly exist in a radical form, i.e., contain unpaired electrons on the outer orbital. The most recognized ROS/RNS include radicals of oxygen [superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals (OH·), and peroxyradicals (ROO·)] or nitrogen [nitric oxide (NO·)] as well as non-radical species, such as hydrogen peroxide (H_2O_2) and singlet oxygen. Nitric oxide, itself less reactive and generally non-damaging, can rapidly react with superoxide anion to form peroxinitrate ($ONOO^-$), one of the most deleterious ROS/RNS known. ROS and RNS have long been implicated in the pathogenesis of a plethora of diseases such as stroke, myocardial infarction, general inflammation, neurodegenerative diseases or cancer. Interestingly, low levels of ROS/RNS produced by the cell in a regulated fashion act as second messengers for signal transduction/amplification and fulfill specific intracellular

functions (Reth 2002). Thus, the term "oxidative signaling" has been coined.

The Nature of the T-cell Activation-Induced Oxidative Signal

Recognition of the physiological role for free radicals came with the discovery that nitric oxide, a RNS, is a regulator of various cellular processes (Ignarro and Kadowitz 1985), e.g., activation of guanylate cyclase. Nitric oxide became a model mediator of an "oxidative signaling", since it fulfills crucial prerequisites for a second messenger: (1) it is a small molecule able to diffuse locally, (2) it is rapidly generated and easily removed, (3) its mode of action is, to a significant extent, substrate-specific. In case of ROS, these requirements are fulfilled by hydrogen peroxide. Both nitric oxide and hydrogen peroxide have been implicated in T-cell activation-induced oxidative signaling (Ibiza et al. 2006; Nagy et al. 2003; Williams et al. 1998). In the present review, nitric oxide will only be mentioned briefly due to its different chemistry and to focus the review on the role of ROS.

Nitric oxide is produced by three isoforms of nitric oxide synthase (NOS): neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). nNOS and eNOS are constitutively expressed, with activity regulated by intracellular Ca^{2+} concentration, while iNOS expression (thus activation) is induced by stimuli, like cytokines or lipopolysaccharide (Gergely et al. 2002b). In T cells, existence of eNOS and nNOS isoforms has been suggested (Gergely et al. 2002b; Nagy et al. 2003; Williams et al. 1998); their particular role, however, is still a matter of debate.

In contrast, hydrogen peroxide is generally not considered to be a primary product of a dedicated enzymatic reaction [some disputable exceptions exist, e.g., p66Shc (Giorgio et al. 2005) or "dual oxidases", DUOX1/2 (De Deken et al. 2002; Geiszt et al. 2003)]. The biosynthesis of hydrogen peroxide begins with a generation of superoxide anion by a number of enzymes and often as a by-product of their basic activities. These are: components of the mitochondrial respiratory chain (Drose and Brandt 2012), cytochrome P450 (endoplasmic reticulum) (Rashba-Step et al. 1993), xanthine oxidase (cytoplasm) (Fridovich 1970) or NADPH oxidases (NOX) (Nathan and Root 1977; Sareila et al. 2011) and phospholipases (Rosen and Freeman 1984) (plasma membrane). Upon contact with protons in water, superoxide anion rapidly converts to hydrogen peroxide and molecular oxygen ($2\ O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$). The reaction could be facilitated by superoxide dismutases, the "first-line" anti-oxidative defence enzymes, which in humans are: SOD1 (cytosolic and mitochondrial inter-membrane space, Zn/CuSOD), SOD2 (mitochondrial matrix, MnSOD) and SOD3 (extracellular, Zn/CuSOD).

Compared to other ROS, hydrogen peroxide is relatively stable (half-life in "msec range"), thus less reactive and dangerous for cellular homeostasis (Reth 2002). Lack of electric charge enables for membrane crossing and, additionally, contributes to significant diffusion range. In addition, aquaporins have been implicated in cellular uptake of hydrogen peroxide, also by T cells (Hara-Chikuma et al. 2012; Miller et al. 2010). Most importantly, at low physiologically relevant concentrations, hydrogen peroxide can influence the activity of proteins by introducing reversible oxidative changes. This "oxidative switch" is thought to relay on the reversible oxidation of reactive cysteines [i.e., the cysteine thiol group (–SH) exists in a de-protonated form ($–S^-$)], to sulfenic acid (–SOH) (Finkel 2011). Sulfenic acid is reduced back to cysteine by the cellular reducing agents, glutathione (GSH) and thioredoxin (Trx), or it can undergo S-glutathionylation and form mixed disulfide (reversed by glutaredoxin). At higher hydrogen peroxide concentrations, sulfenic groups are further oxidized to essentially irreversible sulfinic ($–SO_2H$) or sulfonic acids ($–SO_2H$). In addition, hydrogen peroxide can alter function/conformation of a protein by forming intra-molecular disulfite bonds between structurally adjacent cysteines.

Hydrogen peroxide-mediated oxidation influences activity of a number of proteins, including important transcription factors: p53, AP-1 (c-Jun, c-Fos) and NF-κB (Droge 2002). A prominent example of hydrogen peroxide-influenced proteins are phosphatases (protein tyrosine phosphatases, dual-specific phosphatases or S/T-specific phosphatases), which can undergo an "oxidative switch" on the deprotonated cysteines localized in enzymatic active centres (Finkel 2011; Groeger et al. 2009). Since phosphatases are common negative regulators of kinase-driven signaling pathways, oxidative inactivation of phosphatases constitutes a mechanism for positive regulation and enhancement of early signaling events, i.e., for T-cell receptor (TCR) and B cell receptor (BCR) triggering (Singh et al. 2005).

Of note, in the presence of reduced ions of transition metals (e.g., Fe^{2+} or Cu^+), hydrogen peroxide can be converted via the Fenton reaction into highly aggressive and toxic hydroxyl radicals (Fe^{2+} or $Cu^+ + H_2O_2 \rightarrow OH\cdot + OH^- + Fe^{3+}$ or Cu^{2+}). Therefore, concentration and accessibility of Fe^{2+} or Cu^{2+} are strictly controlled by proteins such as ferritin, transferrin or ceruloplasmin.

The half-life of hydrogen peroxide critically depends on the cellular redox balance, since its spontaneous decomposition into water is accelerated by the strong reducing capacity of the cytosol. Enzymes like catalase, GSH peroxidase or peroxiredoxins act as a "second-line" of

anti-oxidative defence by actively preventing excessive accumulation of hydrogen peroxide, which could lead to irreversible oxidative changes as well as perpetuate release of Fe^{2+}/Cu^{2+} ions from damaged proteins.

The Function of the TCR-Induced Oxidative Signal

TCR triggering is necessary for rapid proliferation and differentiation of an activated T-cell. At the early signaling stage, PLCγ1 induction splits the TCR response into two pathways which depend on inositol 3,4,5-triphosphate (IP_3) or diacylglycerol (DAG), respectively (Fig. 1). IP_3 generation leads to a rise in cytosolic Ca^{2+} and activation of Ca^{2+}-dependent transcription factors, such as NF-AT. DAG activates protein kinase C θ (PKCθ) and RAS guanyl nucleotide-releasing protein (RasGRP) leading to triggering of the transcription factors NF-κB and AP-1. Essentially, NF-AT, NF-κB and AP-1 control T-cell activation-induced gene expression. Thus, simultaneous treatment with a Ca^{2+} ionophore, e.g., ionomycin (Iono), and a DAG mimetic, phorbol 12-myristate 13-acetate (PMA), results in a TCR-driven transcriptional response (Truneh et al. 1985) (Fig. 1).

Initial observations that antioxidants inhibit TCR-induced transcription suggested a regulatory role of ROS in T-cell activation (Chaudhri et al. 1986, 1988; Novogrodsky et al. 1982). Later, TCR-triggered ROS production was directly demonstrated (Devadas et al. 2002; Gulow et al. 2005; Gutscher et al. 2008; Hildeman et al. 1999; Sekkat et al. 1988; Tatla et al. 1999; Williams and Henkart 1996). Many studies, including ours, have shown that a transient generation of low physiologically relevant levels of ROS, i.e., a hydrogen peroxide-mediated oxidative signal, facilitates triggering of oxidation-dependent transcription factors, NF-κB and AP-1 (Devadas et al. 2002; Kaminski et al. 2010, 2012a; Tatla et al. 1999). Full transcription of interleukin (IL)-2, IL-4 and CD95 ligand (L) genes was only possible when the Iono-introduced Ca^{2+} signal was simultaneously supplemented with constant, moderate hydrogen peroxide influx via activity of medium-added glucose oxidase (Gulow et al. 2005; Kaminski et al. 2010).

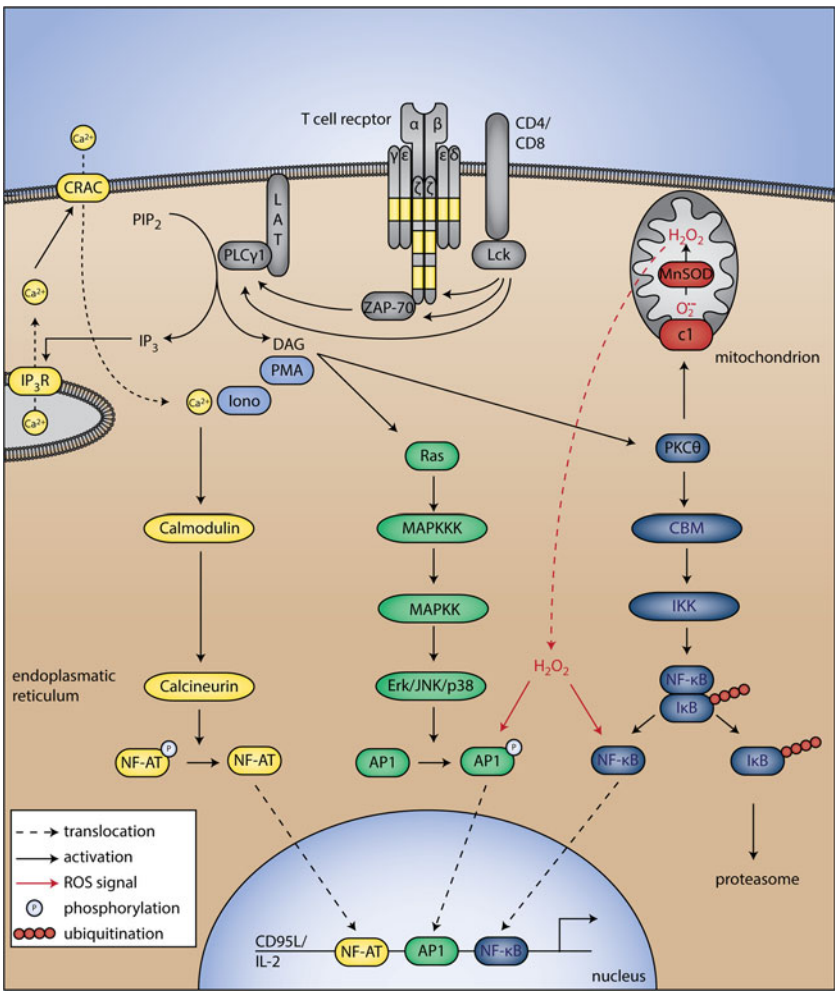

Fig. 1 TCR signaling pathways (see text for details). *IKK complex* IκB kinase complex, *CBM complex* a protein complex comprising Carma1, Bcl10 and MALT1, *c1* respiratory complex I, *MAPKKK/MAPKK* mitogen-activated protein kinase kinase kinase/MAP kinase kinase

Therefore, simultaneous action of the oxidative signal and Ca^{2+} influx is indispensible for T-cell activation-induced gene expression (Fig. 1). Molecular mechanisms governing hydrogen peroxide-triggered activation of NF-κB and AP-1 pathways are complex and still unclear. Below several are listed, however, readers are encouraged to refer to excellent subject-oriented reviews (Gloire et al. 2006b; Gloire and Piette 2009; Groeger et al. 2009; Oliveira-Marques et al. 2009).

NF-κB

Already, Schreck et al. (1991) demonstrated that DNA-binding of NF-κB is directly stimulated by hydrogen peroxide treatment. Numerous follow-up studies showed inhibition of NF-κB activation by antioxidants (see references in Droge 2002). Although hydrogen peroxide does not induce NF-κB triggering in all cell types, in activated T cells, NF-κB triggering is generally recognized to be dependent on ROS (Israel et al. 1992). Described molecular mechanisms of hydrogen peroxide-dependent NF-κB induction are diverse and dose-dependent. They can be divided into two main groups. The first mechanism encompasses influence on transcription factor itself or its interactors: for instance, hydrogen peroxide- or pro-oxidative GSH status-induced enhancement of NF-κB activation via degradation of IκB (a NF-κB inhibitor) (Hehner et al. 2000; Kretz-Remy et al. 1996; Schoonbroodt and Piette 2000; Traenckner et al. 1994), negative regulation of NF-κB via direct oxidation and inactivation (by excessive amounts of ROS) (Matthews et al. 1992; Toledano and Leonard 1991), or modulatory chromatin remodeling via oxidized epigenetic factors (Moodie et al. 2004). The second mechanism involves the oxidative enhancement of upstream signaling cascades. For example, ROS are essential for immunological synapse formation and TCR clustering via lipid raft generation (Lu et al. 2007). Moreover, in T cells, ROS-induced NF-κB activation via phosphorylation of IκBα was reported to involve an atypical mechanism encompassing phosphorylation of Y42 as well as classical IKK complex-mediated phosphorylation of S32/36. Phosphorylation of Y42 could be induced by exposure to hydrogen peroxide (Livolsi et al. 2001; Schoonbroodt et al. 2000; Takada et al. 2003) and results in IκBα degradation or sequestration (Beraud et al. 1999; Gloire et al. 2006a). PI3K, Lck or Syk kinases were proposed to be responsible for Y42 phosphorylation of IκBα (Beraud et al. 1999; Livolsi et al. 2001; Takada et al. 2003). Gloire et al. (2006a) demonstrated that hydrogen peroxide treatment of T-cell lines expressing SHIP-1 phosphatase resulted in classical IKK-dependent IκBα phosphorylation on S32/36. Interestingly, studies by Kwon et al. (2005, 2010) demonstrated that a TCR-induced hydrogen peroxide signal oxidatively inactivates SHIP-2 but not SHIP-1, and, thus, enhances proximal TCR signaling (via Vav-1 and ZAP-70 Y-phosphorylation). However, a report by Michalek et al. (2007) indicated oxidative modification of both SHIP-1 and SHIP-2.

AP-1

Many different oxidative stress-inducing stimuli, e.g., hydrogen peroxide, UV light or γ-irradiation, lead to AP-1 activation (Droge 2002). On the one hand, in T-cell lines, low amounts of hydrogen peroxide trigger expression of c-Fos and c-Jun, thus, enhance AP-1-mediated transcription (Beiqing et al. 1996; Los et al. 1995a). On the other hand, activation of AP-1 could be achieved by potentiation of the extracellular signal-regulated kinase (ERK)-, Jun N-terminal kinase (JNK)- or p38-dependent upstream signaling cascades, e.g., due to a shift in the intracellular GSH redox state (JNK, p38) or treatment with hydrogen peroxide (ERK) (Galter et al. 1994; Griffith et al. 1998; Hehner et al. 2000). Dependence of TCR-induced ERK activation on the hydrogen peroxide signal has been amply documented. At first, ERK activation was shown to be positively regulated by the oxidative signal (Devadas et al. 2002). However, later reports subdividing the oxidative signal into NOX-, DUOX1- or FasL-dependent events (see below) postulated the negative influence of the oxidative signal on early (Kwon et al. 2003) or late (Jackson et al. 2004, via NOX2) ERK activation as well as positive regulation of early ERK signaling (Kwon et al. 2010, via DUOX1). A well-established mechanism of activation of JNK and p38 pathways is a hydrogen peroxide-dependent triggering of the upstream kinase ASK1 (apoptosis signaling kinase 1) via oxidation and dissociation of inhibitory Trx (Saitoh et al. 1998). The oxidative upregulation of AP-1-mediated transcription can also be induced by activation of c-Jun via JNK-dependent phosphorylation (Karin 1995). In NF-κB-deficient cells, tumor necrosis factor-α (TNF-α) or TCR-induced hydrogen peroxide oxidatively inactivates MAP kinase phosphatases (suppressors of JNK activity) resulting in potentiation of JNK pathway, and, thus, of c-Jun-mediated transcription (Kamata et al. 2005; Kiessling et al. 2010). Like in the case of NF-κB, transcriptional activation of AP-1 can also be modulated by oxidation-triggered chromatin remodeling (Rahman et al. 2002). Redox regulation of JNK and p38 pathways was recently extensively reviewed (Trachootham et al. 2008).

It is also important to mention that under conditions of extracellular oxidative stress associated with abrupt pro-inflammatory conditions, e.g., during rheumatoid arthritis or sepsis, T cells are often hypo-activated and the TCR-induced response is blunted (for further reading, please refer to Kesarwani et al. 2013).

The Enzymatic Sources of the Oxidative Signal

Quantitatively, NOX and mitochondria are regarded the most significant cellular contributors to superoxide anion/hydrogen peroxide generation (Finkel 2011). The NOX family consists of inducible plasma membrane-associated NOX complexes 1–5 and DUOX1 or 2. The signaling function of NOX is recognized for many cellular systems, e.g., the NOX-dependent oxidative signal is important for the homeostasis of vascular endothelial cells or B cells (Brandes and Schroder 2008; Singh et al. 2005).

NADPH Oxidases: Regulatory ROS Generators

NOX2 (respiratory burst NOX), the best studied isoform, is present in phagocytes [e.g., at high levels—neutrophils, macrophages, dendritic cells; low levels—B cells, mast cells, eosinophils, natural killer (NK) cells], where it mediates ROS-dependent pathogen killing (Sareila et al. 2011). It is also present in some non-phagocytic cell types, e.g., cardiomyocytes, endothelial and muscle cells. NOX2 consists of several components: the transmembrane flavocytochrome b558 (a heterodimer containing gp91phox and p22phox), the four cytosolic proteins p47phox, p67phox, p40phox, and the small G protein Rac1/2 (Reth 2002). Upon activation, the enzymatic complex produces membrane-impermeant superoxide anions towards the extracellular side of the plasma or phagosome membranes. Thus, generation of the hydrogen peroxide-derived intracellular oxidative signal is indirect, i.e., the superoxide anion is converted into hydrogen peroxide and crosses the membrane to re-enter the cell (Reth 2002).

The presence and molecular characteristics of NOX enzymes in human T cells are still debated. At first, the Ca^{2+}-dependent NOX5 isoform was identified in the T cell-rich regions of spleen and lymph nodes (Banfi et al. 2001; Cheng et al. 2001). The presence of a NOX2-like Ca^{2+}-independent isoform in T cells was questioned (Reth 2002). Subsequently, Jackson et al. (2004) reported functional NOX2 in T cells suggesting its role in TCR-induced oxidative signal generation. The experimental evidence was based on T cells of p47phox- and p91phox-deficient mice. Of note, the levels of NOX2 components in human T cells were very low, thus, in agreement with other reports stating low T-cell-specific NOX activity (Gelderman et al. 2007; van Reyk et al. 2001). In mouse T cells, NOX2 is not an exclusive source of T-cell activation-induced ROS, since three distinct events of ROS generation were observed upon TCR triggering: (1) a rapid hydrogen peroxide generation, independent of pre-formed CD95L (FasL/APO-1L)/CD95 (Fas/APO-1) complex and NOX2 activity; (2) a sustained hydrogen peroxide production, CD95L/CD95 complex- and NOX2-dependent; and (3) a delayed superoxide anions production, CD95L/CD95 complex-dependent and NOX2-independent. TCR-induced NOX activation was implicated in negative regulation of ERK signaling (Jackson et al. 2004). In T cells from NOX2-deficient mice, it was also reported to positively regulate STAT-5 phosphorylation and $CD4^+$ Th2 cell differentiation (Shatynski et al. 2012). Interestingly, in murine models of NOD (non-obese diabetes) (Thayer et al. 2011) or neglect-induced apoptosis (Purushothaman and Sarin 2009), T-cell-specific NOX2 was shown to be crucial for autoimmunity and $CD4^+$ Th1 function or cytokine deprivation-induced apoptosis of activated T cells.

"Dual oxidases" (DUOX) 1 and 2 are NADPH oxidase enzymes addressed to directly produce hydrogen peroxide and characterized by an additional peroxidase-like domain as well as two EF-hand Ca^{2+}-binding domains (Fischer 2009). DUOX1, a typical thyroid enzyme, has been reported to be responsible for the TCR-induced NOX2-independent and rapid hydrogen peroxide generation [first phase (1) mentioned above (Jackson et al. 2004; Kwon et al. 2010]. IP_3/Ca^{2+}-dependent triggering of DUOX1 was proposed to be crucial for subsequent amplification of the Ca^{2+} signal by store-operated Ca^{2+} influx, enhancement of proximal TCR signaling due to oxidative inactivation of SHIP-2 (Kwon et al. 2005) and positive (Kwon et al. 2010) or negative (Kwon et al. 2003) influence on ERK-mediated signaling.

Of note, although the above-mentioned studies on the role of NOX2 and DUOX1 for T-cell activation-induced oxidative signal generation significantly contributed to our understanding of this phenomenon, many questions remain still open. For example, the ability of DUOX1 to produce hydrogen peroxide is a matter of debate (the enzyme could possess a peroxidase activity) (Edens et al. 2001; Harper et al. 2006). The conclusion on Ca^{2+}-dependence of the oxidative signal generation was based on the inhibitory effect of the intracellular Ca^{2+} chelator BAPTA-AM, which is known to have adverse effects on cellular physiology (e.g., cytoskeleton, and importantly, mitochondrial respiration) (Furuta et al. 2009; Saoudi et al. 2004) and dichlorodihydrofluorescein diacetate (DCF-DA)-mediated detection (unpublished observations). In general, according to the present knowledge, fluorescent detectors used in these studies (DCF-DA for hydrogen peroxide and dihydroethidium, DHE, for superoxide anion) are suitable to assay the intracellular redox status but do not have exclusive specificity for investigated ROS and are prone to artifacts (Eruslanov and Kusmartsev 2010; Kalyanaraman et al. 2012), thus, limit conclusions. Differences in uptake/sensitivity of the indicators and the inter-conversion of ROS were also not considered.

Mitochondria: Oxidative Signaling Organelles

Aerobic respiration leads to a significant generation of ROS (Finkel 2011). Depending on the experimental conditions used for respiring isolated mitochondria, superoxide anions are released predominantly at two sites of the electron transport chain: complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome c reductase; Fig. 2) (Drose and Brandt 2012; Lambert and Brand 2009). Mitochondrial ROS are gradually gaining recognition as important signals for cellular processes, such as adaptation to hypoxia, regulation of innate or adaptive immunity, differentiation or autophagy (Finkel 2012; Sena and Chandel 2012). Moreover, a crucial role of mitochondria and metabolic pathways for T-cell physiology is becoming increasingly evident.

The initial phase of activation-triggered proliferation of naive T cells is intimately associated with an increase in mitochondrial mass, mitochondrial DNA (mtDNA) content and oxidative phosphorylation (Darzynkiewicz et al. 1981; D'Souza et al. 2007; Frauwirth and Thompson 2004). Interestingly, as discussed below, at the later stage of an immune response, activated T cells rely predominantly on aerobic glycolysis for ATP production (Fox et al. 2005). Nevertheless, mitochondrial activity is essential for correct T-cell function, e.g., the anti-viral response of cytotoxic $CD8^+$ T cells (Case et al. 2011; Grayson et al. 2003), the $CD4^+$ T-cell effector response (Christie et al. 2012), thymocyte differentiation (Case et al. 2011), graft-versus-host disease (Gatza et al. 2011) or progression of experimental autoimmune encephalomyelitis (EAE) (Michalek et al. 2011). Importantly, mitochondria and the cellular metabolic state are decisive for differentiation of T-cell subsets and development of memory and regulatory phenotypes (for further reading, please refer to (Gerriets and Rathmell 2012; Wang and Green 2012). Mitochondria are an important part of the immunological synapse (Baixauli et al. 2011; Quintana et al. 2006, 2007) where they determine amplitude and duration of the Ca^{2+} signal via Ca^{2+} buffering (Hoth et al. 1997) and provide a local ATP gradient for TCR-triggered phosphorylation events (Baixauli et al. 2011). Importantly, mtDNA depletion or mitochondrial uncoupling results in inhibition of the TCR-induced Ca^{2+} signal due to diminished mitochondrial membrane potential, and thus reduced ability to buffer and prolong Ca^2 influx (Hoth et al. 2000; Koziel et al. 2006). Moreover, T-cell activation-induced mitochondrial Ca^{2+} uptake stimulates mitochondrial function, e.g., by activating enzymes of the Krebs cycle (Carafoli 2012).

Foremost, mitochondrial activity is indispensible for generation of T-cell activation-induced ROS (Kaminski

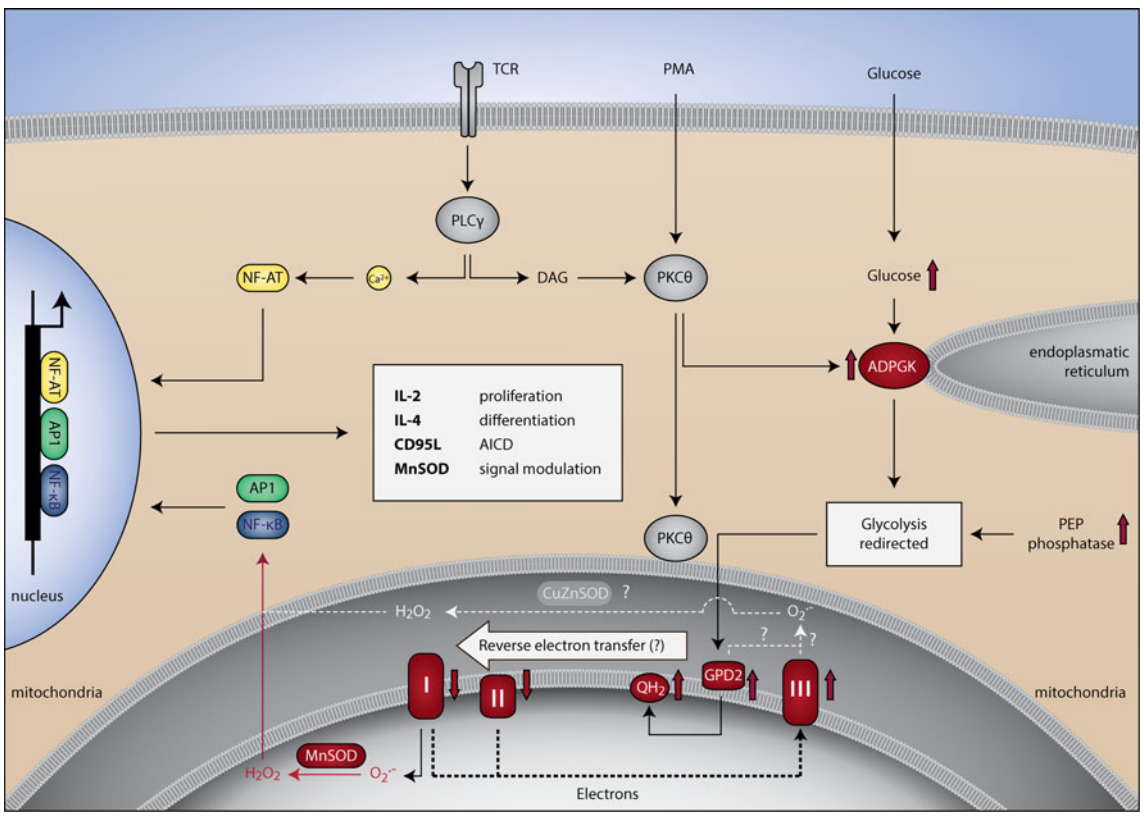

Fig. 2 T-cell activation-induced mitochondrial oxidative signal (see text for details). *red block arrows* enzymatic activity, transport (glucose) or ubiquinol content, *I, II, III* respiratory complexes

et al. 2007, 2010; Nagy et al. 2003; Yi et al. 2006). Nagy et al. (2003) reported that TCR/CD28 co-stimulation leads to a rapid (4 h) mitochondrial hyperpolarisation as well as nitric oxide and ROS generation. Our experimental results clearly demonstrate a crucial role for a mitochondria-originated oxidative signal in T-cell activation-induced NF-κB- and AP-1-dependent transcription (IL-2, IL-4, IL-8, IκBα, CD95L and MnSOD genes) as well as in a CD95L-dependent activation-induced T-cell death (AICD) (Gulow et al. 2005; Kaminski et al. 2007, 2010, 2012a, b).

Application of human peripheral blood T cells (resting or in vitro expanded) or Jurkat J16/145 human T-cell line may partially explain discrepancies observed between our and other studies, e.g., NOX2 levels are very low in human T cells. In addition, a use of a particular stimulation setting (plate-bound anti-CD3 antibody, OKT3, 30 μg/ml) and staining protocol could contribute to different kinetics of ROS generation observed (Devadas et al. 2002; Jackson et al. 2004; Kaminski et al. 2012a; Kwon et al. 2003, 2010; Michalek et al. 2007; Remans et al. 2004). In in vitro expanded human T cells, the TCR-induced oxidative signal peaks approx. between 1 and 2 h after activation (in Jurkat T cells 30 min–1 h) (Kaminski et al. 2012a). Importantly, in our hands, mitochondria-dependent oxidation of fluorescent ROS detectors, DCF-DA (measure of an increase in overall intracellular redox status, i.e., oxidative signal) or MitoSOX (mito-targeted DHE, mitochondrial superoxide indicator) was induced not only by TCR but also by PMA (DAG mimetic, 10 ng/ml) alone, thus was Ca^{2+}-independent (Gulow et al. 2005; Kaminski et al. 2007, 2010, 2012a). The oxidative signal could not be induced by exclusive Iono (10 μM)-treatment of Jurkat T cells (Gulow et al. 2005; Kwon et al. 2010), while expanded human T cells were perfectly capable of TCR-induced oxidative signal generation under the Ca^{2+}-free conditions (phosphate buffered saline) (Kaminski et al. 2012b).

Alternative sources reported for the TCR-induced oxidative signal could all regulate a complex enzymatic and non-enzymatic network governing the intracellular redox equilibrium. Thus, our preliminary results on NOX inhibitors (Kaminski et al. 2007) suggest a ROS-mediated mitochondria-NOX cross-talk (Daiber 2010). In addition, PMA(DAG)-mediated PKC triggering results in activation of Ca^{2+}-independent NOX isoforms, such as NOX2 (Bedard and Krause 2007) and BCR-induced ROS generation was recently reported to involve consecutive activation of NOX2 and mitochondrial respiratory chain (Wheeler and Defranco 2012).

Experiments based on extracellular catalase strongly suggested a predominantly intra-cellular origin of the T-cell activation-induced oxidative signal/hydrogen peroxide [in human neutrophils catalase clearly blocked PMA-induced and NOX2-mediated DCF-DA oxidation but was inefficient for PMA-triggered DCF-DA oxidation in Jurkat T-cell line (Kaminski 2008)]. The requirement of intact mitochondria for activation-induced ROS production was demonstrated using mtDNA-depleted Jurkat T cells (ps-ρ0 phenotype, (Kaminski et al. 2007) or human T cells (mtDNA depleted by prolonged ciprofloxacin exposure, Kaminski et al. 2010). mtDNA-less cells showed diminished levels of activation-induced ROS, and thus, decreased expression of IL-2, IL-4 and CD95L genes and inhibition of CD95L-dependent AICD. The proximal TCR signaling machinery consisting of ZAP70, LAT, SLP-76 and PLCγ1 proteins is necessary for ROS production (Kaminski et al. 2007; Kwon et al. 2010). The next signaling stage involves PMA(DAG)-dependent triggering of PKCθ, where a pool of this protein associates with mitochondria upon stimulation (Kaminski et al. 2007). Since the oxidative signal generation depends on the active Ras protein (Remans et al. 2004), and TCR induction leads to PKC-dependent mitochondrial recruitment of K-Ras (Bivona et al. 2006), a functional interaction between PKCθ and Ras is probable [directly or via PMA(DAG)-activated RasGRP]. Intriguingly, mitochondrial K-Ras interacts with Bcl-XL (Bivona et al. 2006), and Bcl-XL over-expression as well as Bax/Bak depletion blocks TCR-induced ROS release (Devadas et al. 2002; Jones et al. 2007). Thus, a non-apoptotic role of Bcl-2 family members in regulation of the oxidative signal seems possible.

The mitochondrial respiratory complexes are multimers consisting of nuclear- and mtDNA-encoded subunits. We and others have shown that rotenone, an inhibitor of complex I, blocks T-cell activation-induced ROS generation (Kaminski et al. 2007; 2010, 2012b; Yi et al. 2006) and, consequently, IL-2, IL-4 and CD95L gene expression (Bauer et al. 1998; Kaminski et al. 2007, 2010). Since rotenone interferes with centrosomal function and tubulin assembly (Brinkley et al. 1974; Diaz-Corrales et al. 2005; Marshall and Himes 1978; Ren et al. 2005;), specificity of observed effects was confirmed by additional complex I inhibitors, piericidin A and metformin (an anti-diabetic and mild complex I blocker) (El-Mir et al. 2000; Horgan et al. 1968; Owen et al. 2000). A crucial role for complex I was shown by knocking-down NDUFAF1, an essential complex I assembly factor (Kaminski et al. 2007, 2010; Vogel et al. 2005), resulting in abrogation of T-cell activation-induced ROS generation, and consequently, CD95L, IL-2, IL-4 gene expression and AICD. Thus, intact, functional respiratory complex I is indispensible for the oxidative signal generation. Importantly, PMA(DAG)-induced oxidative signal followed the TCR-induced one regarding responsiveness towards mtDNA depletion, all respiratory chain inhibitors tested and NDUFAF1 knock-down (Kaminski et al. 2007, 2010, 2012b).

Complex I inhibitors block mitochondrial ROS release if superoxide anion is generated at complex I via the reverse

electron transfer (RET) (Drose and Brandt 2012) (Fig. 2). In particular, metformin abrogates mitochondrial ROS release only in case of RET-induced ROS generation (Batandier et al. 2006; Kaminski et al. 2007). The assumption that RET contributes to the TCR/PMA(DAG)-induced oxidative signal is further supported by the moderate blocking effect of complex II inhibitors, atpenin a5 and TTFA (higher concentrations) (Drose et al. 2011; Drose and Brandt 2012; Kaminski et al. 2012b). Moreover, T-cell activation leads to a hyper-reduced status of ubiquinone, an electron carrier between complex I/II and complex III. Since a hyper-reduced ubiquinone pool is a pre-requisite for RET (Drose and Brandt 2012; Lambert and Brand 2009; Miwa et al. 2003), occurrence of this phenomenon upon TCR triggering is very probable (Fig. 2).

Complex I releases ROS towards the matrix side of mitochondria (Drose and Brandt 2012; Lambert and Brand 2009). TCR triggering leads to an increased superoxide anion content in the mitochondrial matrix (Kaminski et al. 2012b; Sena et al. 2013). It is, therefore, expectable that MnSOD, a major matrix anti-oxidant, participates in activation-induced ROS release (Kaminski et al. 2007, 2012a; Kiessling et al. 2010). Indeed, MnSOD content and activity are upregulated during a late phase of a TCR-triggered response (Kaminski et al. 2012a). This temporally coincides with a decrease in the activation-induced oxidative signal and, in turn, in inhibition of NF-κB/AP-1-dependent gene expression (Kaminski et al. 2012a). MnSOD overexpression abrogates oxidative signal generation and, thus, oxidation-driven NF-κB-dependent IL-2/CD95L transcription and AICD. Since T-cell activation-triggered MnSOD up-regulation itself depends on NF-κB induction, this feed-back loop constitutes an important control switch for the generation of the mitochondrial oxidative signal. Initially, residual MnSOD-driven dismutation partially contributes to hydrogen peroxide release from mitochondria. However, at a later stage (4–8 h after TCR stimulation), increased MnSOD levels diminish the oxidative signal (probably due to an out-titration effect) (Kaminski et al. 2012a). Reports by Case et al. (2011) and Maric et al. (2009) support a crucial role of MnSOD for T-cell homeostasis. T-cell-specific MnSOD knock-out results in increased mitochondrial superoxide anion levels, enhanced thymocyte apoptosis, decreased number of peripheral T cells and impaired clearance of an influenza virus. Both thymocytes and peripheral T cells appear hyperactivated (Case et al. 2011). Decreased MnSOD activity and content in mouse T cells deficient in the interferon (IFN)-γ-inducible lysosomal thiol reductase (*Gilt*$^{-/-}$) lead to enhanced TCR-triggered ERK activation and higher intracellular superoxide levels (Maric et al. 2009). Of note, T lymphocyte activation up-regulates expression and content of the uncoupling protein 2 (UCP2), an exclusively mitochondrial matrix protein of possible anti-oxidative/uncoupling function, yet another negative regulator of mitochondrial ROS release (Degasperi et al. 2006; Rupprecht et al. 2012).

Interestingly, inhibition of respiratory complexes III and IV (by antimycin A and NaN_3, respectively) potentiates generation of TCR/PMA(DAG)-induced oxidative signal (Kaminski et al. 2007, 2012b). This could indicate ROS release at complex I (via RET) but also at complex III (Chowdhury et al. 2005; Drose and Brandt 2012; Tretter et al. 2007). Of note, upon TCR triggering, complex III seems to be activated by a stable modification in a PKC-dependent manner (Kaminski et al. 2012b). Transient over-expression of Zn/CuSOD (SOD1, a mitochondrial inter-membrane space and cytoplasmic enzyme) exerts some inhibitory effects on PMA(DAG)-induced oxidative signal (preliminary, unpublished data). Thus, additional contribution of complex III-originated ROS release (predominantly towards inter-membrane space) could not be excluded (Fig. 2). Importantly, shortly before submission of this manuscript, a study by Sena et al. (2013) reported necessity of intact respiratory complex III for antigen-specific (TCR- and CD28-mediated) T cell activation-induced mitochondrial ROS generation. Using T-cell restricted knock-out of complex III subunit RISP, the authors demonstrated impaired $CD4^+$ and $CD8^+$ T-cell responses in murine models of asthma and listeriosis, respectively. On the one hand, complex III-originated mitochondrial ROS release was suggested to depend on T-cell activation-triggered mitochondrial Ca^{2+} uptake and upregulation of the Krebs cycle. On the other hand, the complex III-released ROS were addressed to oxidatively enhance the activation of NF-AT and Ca^+-dependent transcription. However, impaired function of complex III might influence entire respiratory chain (e.g., complex I) and be accompanied by decreased mitochondrial membrane potential. In consequence, lowering of mitochondrial capacity to buffer and prolong TCR-induced Ca^{2+} signal could inhibit NF-AT-mediated transcription. Interestingly, the observed TCR/CD28-triggered oxidative signal occurred solely due to the TCR-induced generation of mitochondrial matrix ROS, although CD28-triggering was previously reported to lead to lipoxygenase-dependent ROS generation (Los et al. 1995b). Thus, as suggested by the accompanying "preview" article (Murphy and Siegel 2013), the T-cell activation-induced mitochondrial ROS generation could result from a more complex mechanism involving several enzymatic sources.

Upon activation, T cells change from being dependent on mitochondria-produced ATP into reliance on aerobic glycolysis. In other words, they undergo the Warburg effect (Wang et al. 1976; Warburg 1956), a phenomenon described also for fast proliferating cancer cells (Vander

Heiden et al. 2009; Warburg 1956) where up-regulated glycolytic flux provides cells with essential anabolic intermediates. Of note, glucose supply is critical for TCR-triggered transcription (Jacobs et al. 2008; Stentz and Kitabchi 2005). Cancer cells often display constitutive up-regulation of ROS levels and NF-κB activity (Baud and Karin 2009; Wellen and Thompson 2010). Up-regulation of glucose metabolism due to hyperglycemic and hypoxic conditions induces mitochondrial ROS release (Bell et al. 2007; Nishikawa et al. 2000; Wellen and Thompson 2010). Thus, the T-cell activation-associated Warburg effect could allow mitochondria to function as TCR-triggered oxidative signaling organelles.

Indeed, in expanded human T cells, TCR/PMA(DAG)-induced generation of the mitochondrial oxidative signal is accompanied by a metabolic switch closely resembling the Warburg effect (Kaminski et al. 2012b). Mitochondrial ATP production-coupled respiration decreases, while glucose uptake and cellular ATP concentration rise (Kaminski et al. 2012b). Mitochondria display cristae rearrangements closely reminiscent of ROS release and low respiratory activity (Arismendi-Morillo 2011; Hackenbrock 1966; Mannella 2006). Interestingly, this immediate metabolic change is associated with decreased production of lactate (in vitro expanded T cells are already of a predominantly glycolytic phenotype, (Bental and Deutsch 1993; Wang et al. 1976)) and redirection of glycolytic flow due to the induction of ATP-independent phosphoenol pyruvate phosphatase activity (probably triggered by pyruvate kinase M2) (Kaminski et al. 2012b; Vander Heiden et al. 2010). This, in turn, leads to activation of the mitochondrial glycerol-3-phosphate shuttle due to enhanced activity of the respiratory chain-localized glycerol-3-phosphate dehydrogenase 2 (GPD2) (Kaminski et al. 2012b) (Fig. 2). Knock-down of GPD2 gene expression results in abrogation of the oxidative signal, NF-κB activation and NF-κB-dependent transcription (Kaminski et al. 2012b). Thus, the metabolic switch towards aerobic glycolysis participates in mitochondrial ROS generation. Activated GPD2 directly reduces ubiquinone and releases ROS either at the complex I via RET or itself. Moreover, since complex III and IV inhibitors enhance GPD2-dependent ROS production (Chowdhury et al. 2005; Lambert and Brand 2009; Miwa et al. 2003; Tretter et al. 2007), complex III involvement is also possible. Therefore, GPD2 plays a major role for T-cell activation-induced ROS release by connecting enhanced glycolysis with hyper-reduction of the mitochondrial respiratory chain (Fig. 2). Of note, thymocyte mitochondria were demonstrated to produce considerable amounts of ROS at complex I via GPD2-sustained RET (Clarke and Porter 2013).

Unexpectedly, TCR-triggered metabolic switch encompasses activation of an alternative glycolytic enzyme, ADP-dependent glucokinase (ADPGK) (Kaminski et al. 2012b). ADPGK mediates the first step of glycolysis by phosphorylating glucose using ADP (Richter et al. 2012; Ronimus and Morgan 2004). The enzyme with a previously unknown role for eukaryotic cells is typical for thermophylic *Archaea* (Guixe and Merino 2009). The ADPGK activity is triggered shortly after TCR- or PMA(DAG)-mediated stimulation in a PKC-dependent manner (Kaminski et al. 2012b). Knock-down and over-expression demonstrate that ADPGK is crucial for activation-induced oxidative signal generation and subsequent up-regulation of NF-κB-dependent transcription. ADPGK lacks an end-product inhibition by glucose-6-phosphate (Ronimus and Morgan 2004). This endoplasmic reticulum-associated protein seems to have its active site facing the cytoplasm (Kaminski et al. 2012b). Thus, it is probable that TCR-induced ADPGK activation enhances glycolytic flux, and thus, contributes to mitochondrial ROS generation (Fig. 2). Moreover, since secondary structure prediction indicates close similarity to thermostable archaeal ADPGK, and ADPGK activity in human T cells rises in the pro-inflammatory temperature range of 37–42 °C, ADPGK may play a crucial role in maintaining T-cell activation at the inflammatory site (Kaminski et al. 2012b).

In conclusion, it is evident that mitochondria play a major role in the generation of the T-cell activation-induced oxidative signal. Functional respiratory complex I (and complex III), PKCθ and MnSOD triggering as well as an interplay with aerobic glycolysis via GPD2 and ADPGK induction constitute signaling mediators delineated so far. Thus, it is interesting to consider their possible participation in the etiology of T cell-mediated diseases.

Disrupted Mitochondrial Oxidative Signal: a Reason for T-cell Dysfunction?

Mitochondrial Disorders

These rare and clinically heterogeneous genetic diseases emerge from mtDNA or nuclear DNA (nDNA) mutations impairing mitochondrial functions and may manifest at any age. In general terms, nDNA mutations present shortly after birth or at infancy, while mtDNA mutations (primary or secondary to a nDNA abnormality) present in late childhood or adult life. They are manifested by a plethora of symptoms, involving mainly tissues of high energetic demand, thus leading to severe muscular, neurological and cardiac failures often classified into multi-organ syndromes. However, they can also result in single organ-disease, as in case of Leber's hereditary optic neuropathy (LHON) (Chinnery and Turnbull 2001) .

Commonly, in affected mitochondria, multiple functions are impaired. Nevertheless, isolated complex I deficiency

(mostly due to nDNA mutations) is relatively common (Triepels et al. 2001). The disease is fatal and results primarily from brain and/or spinal cord dysfunction followed by lactic acidosis and encephalomyopathies appearing early in life. The Leigh syndrome is the most common picture among extremely variable disease phenotypes (Bugiani et al. 2004; Triepels et al. 2001). Point mutations in the mtDNA may also lead to isolated complex I deficits, as in the case of LHON disease which is probably the best-characterized pathological manifestation of complex I defects (mutations in complex I subunits: ND1/3460, ND4/11778 and ND6/14484). LHON leads to an adult-onset painless blindness due to the optic nerve atrophy.

Dysfunctions of respiratory chain emerging from mtDNA/nDNA mutations or mtDNA deletions generally result in a higher basal ROS release as it was found in cells of patients with defective complex I (Distelmaier et al. 2009; Raha and Robinson 2000). Nevertheless, since regulatory mechanisms of mitochondrial ROS production, and, specifically, generation of the T-cell activation-induced oxidative signal, are far from being delineated, it is difficult to predict the outcome of specific genetic aberrations on the T-cell function.

In many cases, mitochondrial disorders display divergent hematological manifestations and result in recurrent infections (Distelmaier et al. 2009; Finsterer 2007). In particular, complex I-deficient patients present with a severely impaired immune response. They are prone to coincidental infections that inevitably lead to worsening of symptoms or even are a triggering factor for primary occurrence of the disease (Distelmaier et al. 2009; Fassone et al. 2011). Recently, two cases of neonatal cardiomyopathy caused by NDUFAF1 mutations were reported (Dunning et al. 2007; Fassone et al. 2011). In each case, the disease was triggered by a viral illness, which could be indicative of a T-cell activation defect.

In general, reports on mitochondrial disorder-associated impairment of the function and differentiation of T-cell subsets are rare. Nevertheless, severe T-cell immunodeficiency phenotypes have been described. Reichenbach et al. (2006) reported a case of fatal neonatal-onset respiratory chain disease with T-cell immunodeficiency. The child suffered from severe recurrent infections, anemia and thrombocytopenia as well as defects in myelinization and brain development. Extremely low numbers of $CD8^+$ T cells and NK cells together with a progressive loss of $CD4^+$ T cells were observed. T-cell activation-induced expression of the memory marker CD45RO and triggering of IL-2 receptor were impaired. Skeletal muscle biopsy revealed combined defects in respiratory complexes II + III and IV. Karačić et al. (2008) reported a case of a 20-month-old girl with a combined respiratory chain defect (muscle biopsy demonstrated decreased activity of complexes I and IV), T-cell immunodeficiency and autoimmunity. Clinical findings included generalized hypotonia, failure to thrive, nystagmus and respiratory deficiency due to persistent lung infections. In the blood, hypergammaglobulinemia as well as autoantibodies to cardiolipin and platelets were found. The CD4/CD8 T-cell ratio was increased, the total CD8 T-cell number decreased and CD8-specific IFN-γ secretion was impaired.

Progression of LHON is sporadically accompanied by hematological malignancies, such as leukemia or lymphoma (Lewis et al. 2010; Zanssen and Buse 2003). LHON-related mtDNA mutations were also described for the myelodysplastic syndrome (Linnartz et al. 2004). Strikingly, LHON progression is often associated with an autoimmune multiple sclerosis (MS)-like syndrome (Kalman and Alder 1998). MS is a T cell-mediated autoimmune disorder of the brain and spinal cord, manifested by inflammation and demyelination of nerve tissue. Interestingly, genetic screening demonstrated that about 20 % of MS patients bear mutations in mtDNA (Kalman et al. 1999). In addition, mitochondrial complex I gene variants are associated with MS (Vyshkina et al. 2005). More recently, Ban et al. (2008) confirmed that mtDNA haplogroup U as well as genetic variation of complex I subunit NDUFS2 is indicative for MS susceptibility. In EAE, a mouse model of MS, T-cell-specific PKCθ activation is necessary for disease progression (Salek-Ardakani et al. 2005). A crucial role of PKCθ for T-cell-specific CD95L expression was also documented (Kaminski et al. 2007; Manicassamy and Sun 2007). Underlining the role of complex I-derived ROS for IL-2 expression, T cells of MS patients were reported to be defective in activation-induced IL-2 secretion (Wandinger et al. 1997). In addition, UCP2-deficient mice are known to be more prone to EAE development (Vogler et al. 2006).

Oxidative signal/NF-κB-dependent transcription of genes, such as CD95L, IL-2 and IL-4, is crucial for T-cell development, differentiation, activation and AICD-mediated regulation of autoimmunity. Thus, our findings support the role of mitochondria-, and, specifically, the complex I-originated oxidative signal for activation-induced gene expression. Therefore, they may shed new light on the pathophysiology of mitochondrial diseases and MS.

Autoimmunity and Immunodeficiency

Apart from MS, disturbed mitochondrial ROS generation could be substantial for aberrant T-cell function in several other disorders. T cells isolated from patients suffering from the autoimmune disease, systemic lupus erythematosus, are endowed with higher mitochondrial mass, persistently hyperpolarized mitochondria and elevated ROS levels (Gergely et al. 2002a, b). This results in

disturbed activation-induced Ca^{2+} signaling and susceptibility to pro-inflammatory necrotic T-cell death instead of apoptotic AICD (Fernandez and Perl 2009).

In atopic dermatitis, a chronic, heterogeneous inflammatory skin disease, T cells are often found to spontaneously express high levels of IL-4, a major mediator of hypersensitivity reaction (Bieber 2008; Tang and Kemp 1994). We have demonstrated that blocking complex I activity decreases and normalizes basal IL-4 transcript levels in atopic dermatitis patients' T cells. Treatment of such T cells with a low dose of a complex I inhibitor or depleting mtDNA by ciprofloxacin also efficiently reduced hyperactivation-induced IL-4 expression (Kaminski et al. 2010).

A mitochondrial basis for defective T-cell-mediated immunity was demonstrated in case of nucleoside phosphorylase deficiency, a rare metabolic disorder leading to severe combined immunodeficiency (Arpaia et al. 2000). Inhibition of mtDNA repair due to the accumulation of dGTP in mitochondria results in an impairment of T-cell number and function.

In addition, T-cell mitochondria and presumably activation-induced mitochondrial ROS release play an important, although divergent role in the pathology of the acquired-immunodeficiency syndrome (AIDS). On the one hand, mtDNA depletion in HIV-infected patients treated with highly active antiretroviral therapy (HAART) is a well-known complication leading to lactic acidosis and lypodistrophy (Miro et al. 2003). In this respect, HAART leads to a depletion of mtDNA in peripheral blood lymphocytes and, consequently, impairment of their function (Einsiedel et al. 2010; Garrabou et al. 2009; Miro et al. 2006). This corresponds with our findings regarding inhibition of oxidative signal-mediated transcription in mtDNA-less T cells (Kaminski et al. 2007, 2010). On the other hand, we have demonstrated that oxidative stress induced by HIV-1 protein Tat contributes to the so-called "by-stander" apoptotic cell death of non-infected $CD4^+$ T cells. Tat-triggered increase in a pro-oxidative status enhances the T-cell activation-induced mitochondrial oxidative signal, and thus, NF-κB-mediated IL-2/CD95L expression resulting in AICD (Gulow et al. 2005; Li-Weber et al. 2000).

Last but not least, the described pathway of T-cell activation-induced mitochondrial ROS generation might play a crucial role in a development of a chronic pro-inflammatory status and cytokine profile of T cells of type 2 diabetic (T2D) patients (Jagannathan-Bogdan et al. 2011; Zeng et al. 2012). The Warburg-like metabolic shift towards aerobic glycolysis is intimately associated with mitochondrial ROS release via GPD2 and ADPGK activation. It may provide a causative link between hyperglycemia-induced intrinsic oxidative stress and hyper-activated status of peripheral blood $CD8^+$ and $CD4^+$ T lymphocytes of T2D patients (Stentz and Kitabchi 2003, 2005).

Cancer

Due to a rapid development of research on cancer metabolism, understanding the role of mitochondrial physiology and mtDNA mutations for tumorigenesis is gaining on importance. It is clear that the original hypothesis by Otto Warburg explaining tumor-specific increase in aerobic glycolysis by damaged respiration is untenable in its simplicity (Scatena 2012; Wallace 2012). In the vast majority of cancer cells displaying a pre-dominantly glycolytic phenotype as well as in activated healthy T cells, the Warburg effect is a result of metabolic reprogramming, which does not preclude functional mitochondrial respiration (Ward and Thompson 2012). It is, however, possible that oxidative respiration, although not deficient, may not be a main ATP provider. Instead, up-regulated glycolytic flow responds to cellular energetic demands (especially in the case of hematologic malignances with the bloodstream as an unlimited glucose supply) and generates glycolytic intermediates used as anabolic precursors necessary for rapid cell division (Fox et al. 2005). At the same time, mitochondrial ROS release might create a pro-oxidative intracellular environment and, thus, enable triggering of a pro-proliferative and pro-inflammatory NF-κB/AP-1-dependent transcription program (Kaminski et al. 2010, 2012a, b).

In this respect, it is currently debated whether mtDNA mutations revealed in numerous malignances, such as breast, bladder, esophageal, lung, head and neck, or colorectal cancers (Abnet et al. 2004; Bi et al. 2011; Fliss et al. 2000; Polyak et al. 1998; Wang et al. 2007) are of primary importance for tumorigenesis or are secondary consequences of transformation. Clearly, a defective respiratory chain results in mitochondrial ROS release, which not only contributes to triggering of a NF-κB/AP-1-mediated response but may also lead to a vicious cycle of oxidation-induced mutations of mtDNA/nDNA and chromosomal instability (Li and Hong 2012). To this end, it is striking that a high statistical association was observed between mitochondrial disorders and lymphoid malignancies (Mende et al. 2007) and that leukemias are among cancers in which mitochondrial dysfunctions and mtDNA mutations are most common (e.g., He et al. 2003; Linnartz et al. 2004; Piccoli et al. 2008; Schildgen et al. 2011; Yao et al. 2007). In particular, defects in complex I mtDNA-encoded genes were often described (Linnartz et al. 2004; Piccoli et al. 2008; Schildgen et al. 2011). Moreover, a recent report by Hashizume et al. (2012) demonstrated that mice carrying a mtDNA point mutation in complex I subunit

ND6 have an exceptionally high incidence of spontaneous B cell lymphoma.

Thus, it is possible that defective T-cell activation resulting from mitochondrial disorders may accelerate tumor development due to impaired immunosurveillance. In addition, in our opinion, there could be a close parallel between the Warburg phenotype of cancer cells displaying high intrinsic ROS production and constitutive activation of the NF-κB pathway (Baud and Karin 2009; Wellen and Thompson 2010) and a T-cell activation-induced Warburg-like metabolic shift resulting in the generation of the mitochondrial oxidative signal leading to NF-κB/AP-1 induction (Kaminski et al. 2010, 2012a, b).

For example, exceptionally high activity of mitochondrial GPD2 was reported for insulinomas and carcinoid tumors (MacDonald et al. 1990) as well as for prostate cancer, where it was indicative for high ROS generation (Chowdhury et al. 2005, 2007). K-Ras-induced transformation results in up-regulation of ADPGK expression, increase in aerobic glycolytic rate and high mitochondrial ROS release (Gaglio et al. 2011; Hu et al. 2012; Weinberg et al. 2010). Elevated ROS levels found in BCR-ABL-transformed cells depend on activity of mitochondrial respiratory complex I as well as on up-regulated glucose metabolism (Kim et al. 2005). In chronic myeloid leukemia, oncogenic *JAK2V617F* mutation results in increased expression of the inducible rate-limiting glycolytic enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), and, thus, in elevated mitochondrial ROS production at complex III and ROS-induced genomic instability (Nieborowska-Skorska et al. 2012; Reddy et al. 2012).

Regarding human T-cell tumors, a recent report by Giambra et al. (2012) on T cells of acute lymphoblastic leukemia showed a close correlation between high PKCθ content/activity and high intrinsic ROS levels being of crucial importance for aggressiveness of the disease. In addition, T cells of the Sézary syndrome, a leukemic form of cutaneous T-cell lymphoma, are endowed with constitutively active NF-κB and are on the edge of intracellular oxidative balance. They are extremely prone to oxidative stress-induced cell death. The abrupt lowering of expression of the free-iron controlling protein, ferritin heavy chain, leads to the Fenton reaction and oxidative stress-induced cell death (Kiessling et al. 2009).

T-cell tumors are relatively rare in humans but common in mice. In $p53^{-/-}$ mice, spontaneously developed T-cell lymphomas fundamentally alter their bioenergetic profile simultaneously up-regulating mitochondrial respiration and glycolysis as well as shifting to a more pro-oxidative status (Samper et al. 2009). Interestingly, $MnSOD^{-/+}$ mice are susceptible to spontaneous lymphoma development (Van Remmen et al. 2003). In general, a function of MnSOD as tumor suppressor is supported by a numerous studies. On the one hand, reduced MnSOD content, inactivating mutations, genetic polymorphism or gene silencing have all been associated with increased oxidative stress leading to enhanced tumorigenesis (Koistinen et al. 2006; Samper et al. 2003; Wang et al. 2006, 2009). On the other hand, higher ectopic MnSOD expression has anti-tumorigenic effects (Church et al. 1993; Venkataraman et al. 2005; Zhao et al. 2001). For example, MnSOD over-expression reduces tumor incidence in the two-stage 7,12-dimethylbenz(a)-anthracene (DMBA)/PMA skin carcinogenesis mouse model (Zhao et al. 2001). Moreover, in the Lck-Bax 38/1 mouse model of T-cell lymphoma, an increased MnSOD gene dosage abrogated the incidence of chromosomal abnormalities and delayed the onset of a disease (van de Wetering et al. 2008). Thus, the interplay of signaling and metabolic pathways described for T-cell activation and resulting in mitochondrial ROS release may have potential meaning for tumorigenesis.

Without doubt, future research will delineate molecular mechanisms of T-cell activation-induced mitochondrial ROS release. However, we can conclude that this novel signaling phenomenon will definitely facilitate our understanding of a number of T cell-related pathologies. Thus, it will be exciting to further follow the field of mitochondrial physiology of T cells.

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