Reactive Oxygen Species: Do They Play a Role in Adaptive Immunity?

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The immune system protects the host from a plethora of microorganisms and toxins through its unique ability to distinguish self from non-self. To perform this delicate but essential task, the immune system relies on two lines of defense. The innate immune system, which is by nature fast acting, represents the first line of defense. It involves anatomical barriers, physiological factors as well as a subset of haematopoietically-derived cells generically call leukocytes. Activation of the innate immune response leads to a state of inflammation that serves to both warn about and combat the ongoing infection and delivers the antigenic information of the invading pathogens to initiate the slower but highly potent and specific second line of defense, the adaptive immune system. The adaptive immune response calls on T lymphocytes as well as the B lymphocytes essential for the elimination of pathogens and the establishment of the immunological memory. Reactive oxygen species (ROS) have been implicated in many aspects of the immune responses to pathogens, mostly in innate immune functions, such as the respiratory burst and inflammasome activation. Here in this mini review, we focus on the role of ROS in adaptive immunity. We examine how ROS contribute to T-cell biology and discuss whether this activity can be extrapolated to B cells.

Keywords: reactive oxygen species, adaptive immunity, T lymphocytes, B lymphocytes, tumor microenvironment

INTRODUCTION

Reactive oxygen species (ROS) include both radical and non-radical species and are formed by the partial reduction of oxygen. The radical species, e.g., superoxide anion (O2·−), hydroxyl radical (·OH), and nitric oxide (NO), have unpaired electrons (1, 2). In contrast, the non-radical products, e.g., hydrogen peroxide (H2O2), hypochlorous acid (HOCl), and peroxynitrite (ONOO−), do not have unpaired electrons but remain powerful oxidizing agents (1). Interestingly, cellular enzymatic systems such as the nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidases, the myeloperoxidases, the nitric oxide synthases (NOS), the monoxygenase activity of cytochrome P450, xanthine oxidase, monoamine oxidase (MAO) and the mitochondrial respiratory chain are sources of the primary radical species (O2·−, NO, and H2O2) (1, 3). At low concentrations, which can be handled by the cellular antioxidant system, O2·−, NO and H2O2 (1, 3). At low concentrations, which can be handled by the cellular antioxidant system, O2·−, NO and H2O2 are necessary for signal transduction, cell migration, cell differentiation, cell proliferation, vasoconstriction, inflammation, senescence and aging (4–14). This can be explained, in part, by the fact that to some extent primary species reactions with biomolecules are reversible and they are easily controlled by enzymatic and non-enzymatic antioxidant molecules of the cell antioxidant machinery (15–18).
Interestingly, although even at high concentrations O$_2^-$, NO and H$_2$O$_2$ are not directly damaging to cells, they react with themselves or with metal ions to produce the extremely toxic secondary reactive species OH, ONOO$^-$ and HOCl. These secondary species are poorly controlled and rapidly and irreversibly react with virtually all classes of biomolecules causing oxidative damage. The accumulation of ROS can lead to a state of oxidative stress when the endogenous antioxidant machinery of the cell is overwhelmed (19–24). Consequently, the cells accumulate oxidative damage within the DNA, lipids and proteins, causing cellular dysfunction and cell death (19–23). Excessive ROS production plays a major role in the initiation and amplification of cell death by modulating many signaling pathways. Consequently, ROS levels are contributing determinants for various forms of cell death, including apoptosis, necrosis/necroptosis, ferroptosis, pyroptosis and autophagic cell death (25–32).

The immune system has the unique ability to distinguish self from non-self to protect the host organisms from a plethora of microorganisms and toxins (33–35). It eliminates foreign entities (pathogens and toxins) but tolerates the self (host's own tissues) and its associated microbiota (33, 36, 37). The innate immune system, the components of which are already present before any pathogenic intrusion, is fast acting. It relies on anatomical barriers (the skin and the mucosa lining the respiratory, gastrointestinal and urogenital tracts) to prevent foreign entities from entering the organism (33, 34). These anatomical barriers are reinforced by soluble factors (complement system, pentraxins, collectins and the defensins antimicrobial peptides) as well as by leukocytes (macrophages, dendritic cells, mast cells, neutrophils, eosinophils, natural killer [NK] cells) that neutralize pathogens or kill the infected cells (33, 34). The innate immune system is activated by the recognition of antigenic determinants common to a wide spectrum of microbes (the pathogen associated molecular patterns [PAMP]) and leads to a state of inflammation to alert and combat the ongoing infection (33, 34, 38). Importantly, the activated innate immune system delivers the antigenic information of the invading pathogens to activate the slower but highly potent and specific second line of defense known as the adaptive immune system. The adaptive immune response calls on T lymphocytes and B lymphocytes as, respectively, the effectors of the cellular adaptive immune response and as the antibody-producing cells with the essential functions of eliminating pathogens and establishing immunological memory (35, 39, 40).

ROS have been implicated in many aspects of the immune response to pathogens mainly related to innate immunity. Indeed, they have been proposed to be the common determinant of inflammasome activation, which is critical in the inflammatory process and thus necessary for an efficient immune response. ROS are also essential for pathogen killing by phagocytic cells, as illustrated in chronic granulomatous disease (CGD), an inherited disorder of NADPH oxidase characterized by recurrent and severe bacterial and fungal infections as phagocytes from these patients cannot do the respiratory burst. Here in this mini review, we focus on the role of ROS in adaptive immunity. We examine how ROS contribute to T-cell biology and briefly discuss whether these activities can be extrapolated to B cells.

**ROS AND LYMPHOCYTE ACTIVATION**

The engagement of the B-cell receptor (BCR) or T-cell receptor (TCR) provides the specific signal 1, which in association with signal 2 coming from the co-stimulatory receptors, triggers intracellular phosphorylation cascades (Figure 1). This results in activation of the transcription factors activator protein 1 (AP1), nuclear factor (NF)-κB, nuclear factor of activated T cells (NFAT), Oct binding factor (OBF)-1/OCA-B (OCA-B/OBF-1 and Pip/interferon regulatory factor (IRF)-4, which are critical for T and B lymphocyte activation (Figure 1) (35, 41–45). Early research demonstrated that ROS scavengers such as N-acetyl cysteine (NAC) inhibit NF-κB activation following exposure to phorbol 12-myristate 13-acetate, tumor necrosis factor (TNF)-α, or interleukin-1 (IL-1), indicating that ROS are involved in physiological activation pathways (46, 47).

**ROS CONTRIBUTE TO TCR SIGNALING**

Actually, within minutes of TCR stimulation, there is a production of both O$_2^-$ and H$_2$O$_2$, which seems to originate from different TCR signaling pathways (48). Specifically, studies in Jurkat T cells showed that ROS increase the phosphorylation and activity of p56$lck$, ZAP-70, protein kinase C (PKC) and intracellular Ca$^{2+}$ levels (Figure 1) (49–51). This results in a phosphoinositide-3 kinase (PI3K)/AKT/mTOR-, Myc- and ERK-dependent augmentation of the global metabolism (52–56). It was further demonstrated that T cells from p47$^{phox}$-deficient mice do not undergo TCR-induced H$_2$O$_2$ production, whereas TCR-induced O$_2^-$ is unaffected following TCR stimulation in these cells, indicating that H$_2$O$_2$ originates from a lymphocyte-encoded NADPH oxidase (NOX) (57). Additionally, using superoxide scavenger treatments or superoxide deficiency in OT-II.Ncf$^{-/-}$ mice having CD4 T-cell specific superoxide deficiency, it was shown that superoxide is necessary for Th1 responses as well as IL-12R and proinflammatory chemokine ligand expression in CD4 T cells (58, 59). In fact, in T cells, ROS contribute not only to proximal but also to distal signaling pathways and modulate the activities of transcription factors NFAT, AP-1, and NF-κB to induce gene expression (60, 61). Activated T cells take up large amounts of glucose and produce lactate, indicating that they are primarily glycolytic (61, 62). Interestingly, during CD4 T-cell stimulation, mitochondrial oxygen consumption increases as an indication that mitochondrial function is also important for T-cell activation not only to support the glutamine requirement of these cells but also as a source of ROS (Figure 1) (61, 63). It was even shown that mitochondrial ROS specifically from respiratory chain complex III are required for CD4+ and CD8+ T-cell expansion in vivo (61). Deletion of the Rieske iron sulfur protein (RISP), a subunit of mitochondrial complex III in T cells, resulted in a lack of oxidative phosphorylation and complex III-dependent ROS production and no expression of IL-2 upon CD3/CD28 stimulation (61). This phenotype was rescued by the addition of exogenous H$_2$O$_2$, clearly demonstrating the ROS requirement for full activation of the CD4 T cells (61). In this context, mitochondrial ROS were downstream of the TCR-mediated cytosolic and mitochondrial
calcium increase, in agreement with the calcium dependency of mitochondrial TCA cycle dehydrogenases that fuel the electron transport chain (ETC) to increase mitochondrial membrane potential and ROS production (64, 65). Interestingly, mitochondrial ROS are also necessary for CD8 T-cell activation, as inhibition of the respiratory chain complex I decreases activation of naive cells but also decreases interferon (IFN)-γ and TNF-α production as well as degranulation of effectors and memory CD8+ T cells isolated from lymphocytic choriomeningitis virus-infected mice (66).

It is worth noting that some studies suggest mitochondria more than NADPH oxidase are the essential source of ROS involved in the activation process (61–66). This apparent discrepancy could come from the activation status (naïve/primed) or developmental state (CD8/CD4, T helper 1/2/9/17 or regulatory CD4 T cells) considered, which might have different ROS requirements for full activation (67). Nevertheless, collectively these results showed that ROS play a role in the activation and maturation of both CD8 and CD4 T cells (57, 61, 66, 68, 69). Mechanistically, by inhibiting phosphatases, ROS might tilt the balance toward phosphorylation, ultimately potentiating the activation of kinase cascades and transcription factors such as NFAT, which is critical for IL-2 production (61).

Interesting, although they are required, ROS levels must be kept in check by the glutathione-dependent antioxidant machinery (70, 71). Indeed, preventing glutathione (GSH) production impairs...
T-cell activation, as the energy and anabolic demands of these cells can no longer be met (72). GSH deficiency alters mammalian target of rapamycin (mTOR) and Myc activation, preventing the metabolic switch to glycolysis and glutaminolysis in an adenosine monophosphate–activated protein kinase (AMPK)-dependent manner (62, 73). Paradoxically, it was reported that ROS can also downregulate T-cell activation by regulating the degradation of signaling molecules and the activation of cytoskeletal proteins (74, 75). To prevent excessive ROS from triggering the mitochondrial permeability transition pore (PTP) opening and causing cell death, CD4+ T cells upregulate microRNA (miR)-23a, which targets peptidylprolyl isomerase F (PPIF or Cyclophilin D), a key regulator of the PTP. The reduction in PPIF is expected to keep the mitochondrial PTP closed and reduce the escape of ROS, preserving CD4+ T-cell survival during the early hypermetabolic and inflammatory state of the activation process (76).

**ROS CONTRIBUTE TO BCR SIGNALING**

Unlike that of T cells, B-cell metabolism is less well characterized. However, it was recently demonstrated that energy demand is elevated during antigen (Ag)-driven proliferation and differentiation (77, 78). B-cell stimulation with lipopolysaccharide (LPS) or anti-immunoglobulin M (IgM) antibodies drastically increases glucose import, although increased mitochondrial respiration still occurs, suggesting that here again mitochondrial function is important (Figure 1) (79, 80). Interestingly, ROS production in response to BCR stimulation occurs in two waves. An early NADPH oxidase 2-dependent ROS increase take place within minutes of BCR stimulation, and a second wave of increasing ROS levels from the mitochondria occurs at later time point (81). B cells deficient in early Nox2-dependent ROS production have no defects in proximal BCR signaling, cell activation or the ability to mount an antibody response following T cell-dependent Ag stimulation (81). However, preventing the later ROS increase attenuates BCR-dependent signaling, leading to defective activation, proliferation and response to BCR stimulation (81). These results indicate that the continuous production of mitochondrial ROS at later times during the activation process is critical for BCR signaling and optimal Ag-induced B-cell activation and proliferation, in agreement with findings from gene set enrichment analysis showing upregulation of OXPHOS and the TCA cycle in activated B cells (79, 81).

Lymphocyte activation clearly requires a metabolic reprogramming for a diversification of the source of energy and biosynthetic building blocks (62, 82, 83). Therefore, one could wonder whether the observed increase in ROS during cell activation could be a consequence of this metabolic reprogramming and reciprocally any genetic manipulation of the ROS input could also alter the metabolism of these cells, questioning the real significance of the ROS in the activation process? This latter possibility is readily excluded by the fact that exogenous H2O2 can rescue lymphocyte activation in the context of genetic ablation of complex III (61, 66, 79, 81). Taken together, these results clearly demonstrate that cell intrinsic ROS signaling participates in the activation processes of both B and T lymphocytes.

**ROS AND LYMPHOCYTE VIABILITY**

We have seen that one of the proximal events following TCR signaling is an increase in ROS production both in the form of O2- and H2O2. One direct consequence of the increase in these ROS in the context of T cell blasts is the initiation of activation-induced cell death (AICD) following the induction of FasL expression (84, 85). In fact, downstream of the TCR engagement, activated ZAP70 phosphorylates liker of activated T cells (LAT), which docks phospholipase Cγ1 that generates inositol 3 phosphate (IP3) and diacylglycerol (DAG) (84). DAG activates protein kinase Cδ (PKCδ) and its translocation into the mitochondria to enhance the production of ROS in a mitochondrial complex I-dependent manner, which is necessary for the expression of the ligand of the death receptor Fas (FasL) (84). FasL engages Fas receptor and triggers apoptotic cell death, a process where mitochondrial ROS further play a role, as it was later shown that caspase 3 can induced a ROS-dependent cell death by cleaving the respiratory chain complex I subunit NDUPS1 (30). We have also shown that ROS potentiate the apoptotic cascade by amplifying the release of apoptogenic factor from the mitochondria and increasing oligonucleosomal DNA fragmentation (86). Moreover, exposure to exogenous H2O2 differentially affects T-cell viability, according to their subset and maturation status. Central memory and effector memory T cells are more sensitive to H2O2 followed by naïve T cells, among which the CD8+ effector memory T-cell compartment is more sensitive to even low doses of H2O2 (Figure 2) (87, 88). In this context, exogenous H2O2 exposure triggers cell death in a mitochondrial pathway-dependent manner (87, 89). T cells treated with H2O2 experience the opening of the mitochondrial permeability transition pore (PTP), a rapid decrease in the mitochondrial transmembrane potential ΔΨm, and the release of cytochrome C (89). Blocking the mitochondrial PTP opening or interference with the respiratory electron transport chain with rotenone or antimycin A, a respiratory chain complex III inhibitor that increases the release of mitochondrial ROS, enhanced apoptosis, while overexpression of Bcl-2 and the viral anti-apoptotic proteins BHFRF-1 and E1B 19K counteracted H2O2-induced T-cell apoptosis (89). Furthermore, inhibition of the transcription factor NF-κB protected cells from H2O2-induced cell death in a process that likely relies on the expression of a death effector gene such as p53 (89). Paradoxically, T regulatory cells, which have lower intracellular ROS levels, are particularly protected from H2O2-dependent inhibition of suppressive function and H2O2-induced death (90). Taken together, the higher sensitivity of effector memory CD8 T cells combined with the reduced susceptibility of T regulatory cells to H2O2-induced death suggest that the oxidized tumor microenvironment (TME) may be a particularly inhospitable site for CD8 T cells and detrimental to T-cell-based adoptive cell transfer therapies. This is even more critical as effector memory T cells are the primary phenotype of cells administered during such therapeutic protocols. Thus, research is needed to determine the effect of the TME of chimeric antigen receptor
(CAR)-T cell therapies. Beyond apoptosis, ROS critically regulate T-cell viability through the induction of ferroptosis. Both Ag-specific CD8+ and CD4+ T cells deficient for glutathione peroxidase 4 (Gpx4) are unable to expand or protect against viral and parasitic infection (91). This phenotype can be rescued by dietary vitamin E supplementation, indicating that lipid peroxidation-dependent ferroptosis plays a critical role in the T-cell depletion during these antigenic challenges (91).

Exposure of B cells to relatively massive doses (100–250 µM) of H2O2 has little effect on cell viability in the short term (30 minutes). However, 5 days later, half of the cells have died, even in the presence of CD40 stimulation (92). These doses of H2O2 although massive are still in the physiological range, as it was estimated that in the proximity of activated neutrophils and macrophages the H2O2 concentration can reach the 100s of µM (93–95). Moreover, this exogenous H2O2 exposure completely suppresses the ability to CD40 stimulation to trigger antibody production (Figure 2) (92). In fact, exogenous H2O2 exposure dissociates TRAF2 from CD40, leading to inefficient IKK phosphorylation, 1kBα degradation, and NF-κB activation, which altogether severely compromises B-cell activation (92).

**ROS-MEDIATED LYMPHOCYTE DYSFUNCTION**

As serial killers, cytotoxic T lymphocytes and NK cells must recognize, engage, kill, and detach from the first target cell before moving to the next (96–98). Detachment of the effector cell from its target requires its repolarization through the reorganization of its cytoskeleton for the disassembly of the first immunological synapse (99). Effector killer cells are quite sensitive to the redox status of their immediate environment (100–102). Oxidizing reagents curb killer cell degranulation, consequently inhibiting their cytotoxicity (102, 103). Interestingly, it was reported that oxidized low-density lipoprotein (ox-LDL), used as oxidizing agent, inhibits killer cell degranulation (102). Pretreatment of NK cells or co-incubation of NK/target cell conjugates with non-cytotoxic doses of ox-LDL markedly and significantly reduces the NK cytotoxic activity against U937 tumor cells (102). This reduced NK cell cytotoxicity is not the consequence of their inability to engage the target cells, because the number of NK:target cell conjugates was not affected nor were the expression levels of CD11a, CD11b, CD18, CD2, and CD62L, key adhesion molecules involved in the effector–target cell interaction (102). Mechanistically, ox-LDL triggers a partial depolarization of the microtubule network that is critical for the polarization of the cytotoxic granules toward the immunological synapse formed between the effector and target cells. Similarly, exposure of mitogen-stimulated peripheral blood mononuclear cells (PBMCs) to ox-LDL reduces their production of TNF-α, IFN-γ and IL-12 (102). Likewise, exogenous and endogenous nitric oxide (NO) inhibits degranulation of lymphokine activated killer (LAK) cells (103). NO inhibits LAK cell exocytosis in part by decreasing the expression of RAS, a critical component of the exocytic signaling cascade, following destabilization of RAS mRNA (103). NO acts by interfering with the mRNA-stabilizing factor HuR, which binds and stabilizes AU-rich elements of the mRNA 3’-untranslated region (104). It was further demonstrated that ROS induced oxidation of the C-terminal portion of the TCRζ chain, and the membrane proximal domain of p56(lck) and coflin promote their degradation or inactivation, suggesting that ROS can also curb the TCR signaling cascade (74, 75). Similarly, increasing evidence suggests that the dynamics of the immunological synapse can be regulated by ROS through their direct or indirect effects via plasma membrane polarization on calcium signaling and effector cell cytoskeletal reorganization (99, 102, 105–107). As stated earlier, activation of the lymphocytes after engagement of their receptor initiates a phosphorylation cascade, resulting in, among other things, the mobilization of intracellular Ca2+ stores, which is essential for the gene expression crucial for lymphocyte activation and the development of adaptive immunity (99, 108–111). Depletion of Ca2+ stored in the endoplasmic reticulum triggers store-operated Ca2+ entry (SOCE). Compared to Orai1, the Ca2+ channel involved in SOCE, Orai3 lacks the redox-sensitive cysteine 195 and therefore is redox-insensitive. Co-expression of Orai3 with Orai1 reduces SOCE sensitivity to ROS inhibition. Consequently, it is not surprising that T lymphocytes display upregulated Orai3 expression during their differentiation into effector T cells. This means that the modulation of the Orai1:Orai3 ratio could be a possible mechanism by which effector T lymphocytes preserve some responsiveness in oxidized environments, such as the hypoxic TME or inflamed tissues (106, 112).
ROS also regulate the effector function of B cells. Overexpression of a phosphorylation-defective mutant of succinate dehydrogenase A to model excessive mitochondrial ROS production suppresses Ig production, germinal center (GC) formation, and GC B-cell proliferation following an encounter with T-cell-dependent Ag. Excessive mitochondrial ROS production also suppresses Ig production against T-cell-independent Ag (113) as well as BCR-dependent Lyn, Btk, and PLCγ2 phosphorylation and CD19 expression. From these collective results, it was hypothesized that excessive mitochondrial ROS dampen B-cell activation most likely by reducing CD19 expression (113). Overall, it seems that mild to moderate exposure to exogenous ROS affects the lymphocyte effector functions, while acute exposure affects their viability (Figure 2). The situation is complicated by the fact that the threshold between mild, moderate and acute exposure strongly depends upon the lymphocyte subset and maturation status.

**ROS IN IMMUNE CELL DYSFUNCTION: THE CASE OF AUTOIMMUNITY**

As we have discussed earlier, endogenous ROS contribute to lymphocyte activation; however, depending of the lymphocyte activation and/or differentiation status, exogenous ROS can affect their effector function and viability. Thus, we also would like to discuss whether ROS could play a role in the pathogenesis of immune-related disorders such as autoimmunity where the wrath of the immune response mistargets self-antigens (autoantigens). For instance, abnormal functions of T helper (Th)-17 cells, which in a normal setting are essential to fight against extracellular bacteria (114–116), are involved in multiple chronic inflammatory disorders such as psoriasis, multiple sclerosis (MS), inflammatory bowel disease (IBD), Sjögren’s syndrome and rheumatoid arthritis (114).

Interestingly, using sublethal doses of oligomycin A, an inhibitor of the respiratory chain ATP synthase/complex V, it was shown that mitochondrial oxidative phosphorylation (OXPHOS) plays a pivotal role in for Th17-mediated autoimmunity (117). Oligomycin treatment abolished Th17 pathogenicity by altering the expression of Th17 pathogenic signature genes, such as transforming growth factor beta 3 (TGFβ3), interleukin 23 receptor (IL-23R), signal transducer and activator of transcription 4 (Stat4), and G protein-co coupled receptor 65 (Gpr65), while genes inversely associated with Th17 pathogenicity such as suppressor of cytokine signaling 3 (Socs3) and IL-10R subunit alpha (IL-10Ra) were upregulated (117). Although the authors did not directly test this possibility, it is very likely that mitochondrial ROS could be involved in this process, as in their experimental condition the oligomycin treatment severely suppresses the basal mitochondrial oxygen consumption rate, which could result in a severe reduction in mitochondrial ROS, indicating that ROS could protect against the pathogenicity of Th17 cells (117). This agrees with previously work by Tse and coworkers showing that prevention of O2 production by macrophages and T cells skews T-cell polarization toward Th17 (118). Although they used a model of NOX-deficiency, collectively these results agree that the absence of ROS alters T-cell lineage commitment, pointing to a role for superoxide in the modulation of Th17 versus Th1 T cell responses (118). From a more clinical standpoint, it was shown that the hypomorphic allele of the Ncf1 gene encoding for p47phox, a subunit of NOX2, is one of the strongest genetic predispositions for autoimmune arthritis, autoimmune encephalomyelitis and systemic lupus erythematosus (SLE), which are associated with increased numbers of autoreactive T cells (119–122). Interestingly, results from two clinical trials, one using N acetyl cysteine the other Sirolimus to modulate respectively cellular GSH content and mTOR activity resulted in the improvement of SLE condition suggesting that modulation of the mitochondrial ROS output could also contribute to regulate pro inflammatory T cell development (123, 124). Furthermore, by downmodulating the efficacy of antigen processing, ROS may further contribute to limiting the activation of autoreactive lymphocytes. In this regard, in the early stage of the processes, ROS should not simply be considered as effectors to eliminate invading pathogens, but also as modulators to fine-tune the inflammatory response depending on the timing, the site and the level of their production (125, 126). By contrast, in the context of dysregulated and prolonged chronic inflammation, the local microenvironment is characterized by low nutrient levels, increased lactate production, decreased pH, hypoxia and an increase level of ROS, which collectively lead to excessive tissue destruction. At this later stage, this excessive tissue destruction could promote the accessibility to cryptic neoantigens favoring the progression and exacerbation of autoimmunity (126).

**ROS IN THE PROCESS OF CYTOTOXIC LYMPHOCYTE KILLING**

Cytotoxic lymphocytes are particularly efficient at eliminating target cancer cells and virally infected cells. They mainly used the cytotoxic granule pathway relying on the degranulation of the pore forming protein perforin and a family of five serine proteases call granzymes in human (127–129). Although the granzymes trigger very distinct cell death pathways, we found that granzyme A and B (GA and GB) share the ability to induce ROS-dependent cell death. It was demonstrated that GA induces ROS-dependent death that is independent of the mitochondrial outer membrane permeabilization (MOMP) and insensitive to BCL2 but has all the morphological features of apoptosis (127, 130–133). We also showed that ROS are necessary for the rapid cell death induction by GB. We found that K562 cells treated with a sublytic concentration of perforin (P) and GB undergo a rapid increase in ROS production and cell death that is inhibited in the presence of the well-characterized antioxidants N-acetyl cysteine (NAC), superoxide scavenger MnTBAP, or the mitochondrial targeted superoxide scavenger MitoQ (134). Moreover, GB and P-induced ROS and cell death are completely absent in pseudo rho cells deficient for mitochondrial DNA (mtDNA) and therefore lacking a functional respiratory chain (134). Both GA and GB induce ROS release from isolated intact mitochondria in the absence of cytoplasmic fraction S100 (130). Using organelle proteomics and bioinformatics, we found that GA and GB cleave NDUF53, NDUFV1, NDUS1 and NDUS2 iron-sulfur (Fe-S) cluster-containing subunits of the respiratory chain complex I (86, 130, 131).
CANCER, OXIDATIVE STRESS, AND CYTOTOXIC LYMPHOCYTES

Uncontrolled proliferation and neoplastic transformation come with enormous demands for energy and macromolecule building blocks. These demands impose a severe metabolic stress, requiring a striking reprogramming of the cancer cell metabolism (146, 147). The resulting altered metabolism combined with the hypoxic nature of the TME is accompanied by marked production of ROS (148–151). This overproduction of ROS activates the cellular antioxidant response based on enzymatic and non-enzymatic antioxidant molecules, which is under the transcriptional control of the transcription factor nuclear factor (erythroid-derived 2)-like 2 (NRF2). Three isoforms of superoxide dismutase (SOD), cytosolic CuZn-SOD (SOD1), mitochondrial Mn-SOD (SOD2), and extracellular EC-SOD (SOD3), are involved in the rapid dismutation of $O_2^-$ into H$_2$O$_2$ (15, 16). The homotetrameric catalase converts H$_2$O$_2$ into water using NADPH as a cofactor (15, 17). The glutathione peroxidases (GPx) use glutathione (GSH) and reduce H$_2$O$_2$ and lipid hydroperoxides (15, 18). H$_2$O$_2$ removal also involves thioredoxin (TRX), thioredoxin reductase (TRR), thioredoxin peroxidase (PRX) and glutaredoxins (15). The most abundant non-enzymatic antioxidant molecule in the cell is GSH, which participates in the reduction of H$_2$O$_2$ into H$_2$O and O$_2$, and is thereby oxidized to form GSSG. GSSG is then recycled into GSH by glutathione reductase still using as electron donor NAD(P)H. GSH also maintains aqueous and lipophilic levels of the antioxidant ascorbic acid (vitamin C) and α-tocopherol (vitamin E), respectively. Nevertheless, when this antioxidant system is overwhelmed, the pro-oxidant/anti-oxidant equilibrium is lost, and a state of oxidative stress is reached where the cells accumulate oxidative damage in all type of macromolecules, including DNA, RNA, lipids and proteins, which could lead to cell death (152–154). Oxidative DNA modifications generate 8-hydroxy-2′-deoxyguanosine, which contributes to the accumulation of mutations that enhance aging and carcinogenesis (155). Consequently, transformed cells adapt and reach new redox balance, and paradoxically, ROS instead of killing, stimulate tumor development and progression by promoting cell proliferation through their mitogenic action as activator of extracellular-regulated kinase 1/2 (ERK1/2). This induces ligand-independent receptor tyrosine kinase (RTK) activation, activating Src kinase, NF-κB and phosphatidylinositol-3 kinase (PI3K)/Akt, to enable evasion of apoptosis and anoikis as well as to induce metalloproteinase (MMP) release in the extracellular matrix to favor invasion and promote angiogenesis (156–161). ROS also contribute to epithelial to mesenchymal transition (EMT), an important process in the metastatic dissemination of cancer cells (2). Importantly, in the TME, cancer cells reprogram other cells, such as cancer-associated fibroblasts (CAFs), endothelial cells and cancer-associated macrophages (CAMs), in a ROS-dependent manner to favor tumor progression. CAFs contribute to tumor growth by promoting the tumor angiogenesis by secreting VEGF and angiopeptin, by generating anti-apoptotic factors and by the secretion of chemokines (CCL2 and CCL5) and MMPs to promote the dissemination while blocking the immune response through the secretion of immunosuppressive cytokines IL-6, IL-10 and TGF-β (162–164). This marked production of ROS also alters the phenotype of innate immune cells infiltrating the tumor parenchyma, contributing to the noxious nature of the TME (165–168). Interestingly, it is worth noting that a direct link exists between the environmental ROS of the TME and inflammation (169). Intracellular ROS may regulate EMT in a NF-κB– and hypoxia-inducible factor 1 (HIF-1α)–dependent manner in a process requiring the activity of cyclooxygenase-2 (COX-2), the first enzyme in the synthesis of prostaglandins, prostacyclin and thromboxanes including prostaglandin E2 (PGE2). This suggests that this oxidized microenvironment favors a state of chronic inflammation in the TME. As stated earlier cytotoxic lymphocytes (NK cells and cytotoxic T lymphocytes) play an essential role in the immune response against cancer (97, 170–177). It is therefore not surprising that harnessing the power of these innate and adaptive cytotoxic immune cells during immune check point blockade (ICB) or CAR-T/NK cell immunotherapies has produced very...
encouraging results (178–180). As we have discussed earlier, NK cells and CD8+ effector memory T cells are particularly sensitive to even low doses of H$_2$O$_2$ while the T regulatory cells are protected from H$_2$O$_2$-dependent inhibition of their suppressive function and H$_2$O$_2$-induced death (87, 88, 90). Accordingly, tumor-infiltrating lymphocytes must adapt to this oxidized microenvironment among other things by modulating the ratio ORAI1:ORAI3 expression (106, 112). Despite these adaptation mechanisms, as we have seen earlier, exposure to exogenous ROS can severely dampen lymphocytes’ effector function, making the TME particularly hostile to infiltrating lymphocytes (181). Interestingly and counterintuitively, the inflamed nature of the TME further contributes to making the TME hostile for lymphocytes and NK cells. Indeed, it was recently reported that tumor-derived PGE2 achieves immune evasion by inhibiting NK cell-mediated remodeling of the TME and unleashing of cytotoxic T cells (173). Interestingly, F2-isoprostanes (F2-IsoPs) and isolevuglandins (IsoLGs), which are oxidized derivatives of PGE2, are extremely relevant disease biomarkers, as they are directly involved in the pathological processes (induction of inflammatory pathways, modulation of immune response, and induction of cell death) (182–184). Since inflammation is closely linked to ROS production, whether the oxidized form of PGE2 contributes to this immune evasion needs to be investigated. Collectively, the evidence supports that the oxidized nature of the TME is likely to affect the efficiency of infiltrating anti-tumor lymphocytes and the development of strategies to enable lymphocytes to withstand the oxidized nature of the TME could improve immunotherapies (Figure 3).

CONCLUDING REMARKS

Based on the available evidence, both NOX- and mitochondrial-derived ROS play critical roles in lymphocyte activation, development, effector function, cytotoxicity, viability but also dysfunction. To this regard ROS directly contribute to both physiological and pathological adaptive immune responses.
ROS from both sources contribute to the activation process of lymphocytes, however, based on strong genetic evidence relying on hypomorphic allele of the Ncf1 gene encoding for p47^phox, it is tempting to suggest that NOX-derived ROS would have a preponderant role as modulators to fine-tune the inflammatory response depending on the timing, the site and the level of their production. But more investigations are still required to seal this case. Moreover, since ROS also participate in innate cell function by potentiating the killing ability of phagocytes, an essential step in the antigen processing and presentation function of these phagocytes, ROS also indirectly contribute to adaptive immunity though the interplay between innate and adaptive immunity. Further characterization of the complex functions of ROS in lymphocyte biology will bring new insight for understanding the pathological conditions in which lymphocyte function is either detrimental or beneficial.

AUTHOR CONTRIBUTIONS

DM, EB, and MW wrote and made the illustrations. All authors contributed to the article and approved the submitted version.

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