Building and operating an antibody factory: Redox control during B to plasma cell terminal differentiation

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Abstract

When small B lymphocytes bind their cognate antigens in the context of suitable signals, a dramatic differentiation program is activated that leads to the formation of plasma cells. These are short-lived specialized elements, each capable of secreting several thousands antibodies per second. The massive increase in Ig synthesis and transport entails a dramatic architectural and functional metamorphosis that involves the development of the endoplasmic reticulum (ER) and secretory organelles. Massive Ig secretion poses novel metabolic requirements, particularly for what concerns aminoacid import, ATP synthesis and redox homeostasis. Ig H and L chains enter the ER in the reduced state, to be rapidly oxidised mainly via protein driven relays based on the resident enzymes PDI and Ero1. How do plasma cells cope with the ensuing metabolic and redox stresses? In this essay, we discuss the physiological implications that increased Ig production could have in the control of plasma cell generation, function and lifespan, with emphasis on the potential role of ROS generation in mitochondria and ER.

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1. Introduction

In histology textbooks, exocrine pancreas or plasma cells are often selected as prototypic examples to illustrate the morphology of cells specialized in protein secretion (professional secretory cells). The most striking common feature is a highly developed rough ER, which occupies the majority of the cytoplasm. This comes as no surprise, since the ER is the site of production of proteins destined to the extracellular world. These include soluble proteins released as ligands, membrane molecules endowed with receptor, channel or transporter activities and resident proteins of the exocytic pathway. The ionic and redox conditions found in the ER and downstream organelles resemble those encountered in the extracellular space (higher [Ca$$^{++}$$] and GSSG/GSH ratio). Therefore, the ER provides suitable conditions for secretory proteins – a term herein used to refer to proteins synthesized on ER bound ribosomes – to attain their three-dimensional structure. It also provides a test bench to ensure that ligands and receptors attain the very conformation that allows the proper signals to be transmitted and deciphered. Indeed, folding and assembly are intimately coupled to a stringent quality control schedule that restricts transport along the secretory route to native molecules [1,2].

Whilst the basic principles of folding are the same in all cell compartments, secretory proteins undergo unique covalent modifications, i.e. cleavage of the signal sequences, N-glycosylation, GPI additions and disulfide bond formation. These are catalyzed by a sophisticated network of specialized chaperones and enzymes spatially distributed along the various stations of the exocytic route, providing the most efficient assembly, quality control and transport line [1–3].

How is this assembly line built and operated? How do cells adapt their size to the synthetic demands? How do they keep track of the various activities ongoing in different compartments? These key questions are beginning to be understood and some of the underlying signalling pathways unveiled.
The differentiation of resting B lymphocytes into “antibody factories” [4–6] upon activation with antigen or mitogens provides a suitable system to analyse the molecular mechanisms that bring to the development of a professional secretory cell. Primary B cells and certain B lymphoma lines can be readily induced to undergo a real metamorphosis finalised to achieve massive antibody production. The high rate of protein synthesis and secretion achieved by plasma cells requires dealing with different kinds of stresses, as it demands metabolites and energy production, as well as the disposal of numerous catabolites, in large amounts. Physiologically, other features make B cell differentiation a unique and challenging model system. The regulation of antibody responses (proliferation of antigen specific clones, generation of memory cells, affinity maturation, etc.) is a very fascinating problem that involves the generation and interactions of a vast number of highly specialized subpopulations [4,7,8]. Suffice here to summarize a few features of the process to introduce the redox problems that terminal B cell differentiation entails. Peripheral lymphoid organs are populated by long-lived B cells, each expressing a receptor specific for a given antigen (the BCR). B cells should not secrete antibodies before they encounter their cognate antigen, as these might compete with BCRs for antigen binding. Preferential splicing toward the membrane form of heavy chains limits secretion. In the case of IgM, the isotype that dominates primary responses, post-translational events are also active. Owing to the inability of B cells to polymerize IgM, intermediates are retained and eventually degraded by thiol-dependent mechanisms [9]. Soon after the proper signal is delivered, however, some B cells must rapidly differentiate and release antibodies so as to combat the incoming enemy. Synthesis of Ig subunits increases, and redox changes ensue that allow IgM polymerization and secretion. To limit the immune response, most Antibody Secreting Cells (ASC) cells die after a few days of intense immunoglobulin production. A few of them home in the bone marrow and survive for longer periods, maintaining sufficient antibody titres to protect against re-infection [7]. Intriguing questions remain largely unanswered on the mechanisms involved in regulating plasma cell apoptosis, and the differences between long and short-lived ones.

In line with their physiological role of cells of the B lineage, the early phases of B cell development are regulated by signalling pathways based on the capability of assembling functional BCRs [10,11]. Along the same lines, it is tempting to speculate that the terminal plasma cell differentiation also obeys the same rules, and devoted sensors exist that keep track of the type and quantity of antibody produced. How can a cell remember the number of molecules it had secreted? We have recently shown that as Ig production increases upon B cell differentiation, the proteasomal capacity decreases. As a result, polyubiquitinated proteins increase, and certain proteins normally degraded by proteasomes (i.e. IκBα, Bax and Bim) are stabilised. The process is accompanied by increased sensitivity to proteasome inhibitors and spontaneous apoptosis [12]. If an increased proteasomal load vs capacity ratio predisposes ASC to apoptosis, the nature of the final blow remains to be determined [13]. Among other factors, reactive oxygen species (ROS) production could be an important source of signals (and stress) for plasma cells. Recent evidences prove that the process of oxidative folding can generate ROS [14–17]. These molecules are generally associated with cell damaging events, senescence and death. A new exciting field is emerging though, that recognizes ROS as second messenger molecules, able to produce rapid and reversible post-translational modifications on proteins similar to phosphorylation. Indeed more and more findings indicate that ROS, in particular H2O2, are essential to the proper development and proliferation of cells [18–22]. Intriguingly, a role for H2O2 has been proposed for the BCR [23]. Since Ig are rich in disulphide bonds, their increasing production in differentiating B cells could generate ROS that might act as signalling devices.

In this essay, we will review the available evidence on the regulation of redox homeostasis during B to plasma cell differentiation, and discuss possible models that may couple Ig production to lifespan control.

2. Oxidative protein folding

Disulphide bonds are essential for the folding and assembly of many proteins synthesized in the ER. They increase stability, an important feature for Ig to recognize and neutralize foreign elements in the body fluids [24]. Disulfide bond formation is based on redox reactions in which two cysteines are oxidised by removal of two electrons forming a covalent bond [25]. Although direct oxidation could also take place, in living cells oxidative folding utilizes primarily disulphide interchange reactions in which an oxidised donor molecule (D) transfers the bond to a reduced acceptor (A) via formation of a transient mixed disulphide intermediate:

\[
\text{D-SS} + \text{HS-A-SH} \rightarrow \text{HS-D-SS-A-SH} \rightarrow \text{HS-D-SH} + \text{A-SS}.
\]

Cargo proteins enter reduced in the ER and are rapidly oxidised by protein disulphide isomerase (PDI). PDI is then re-oxidised by Ero1 flavoproteins (Fig. 1). Electrons are finally transferred to molecular oxygen and possibly to other electron acceptors, particularly in anaerobic conditions [14,16,26–30].

In yeast, Ero1p is an essential gene, whose expression is induced in conditions of ER stress (see C. Kaiser and D. Fass articles in this issue). Also PDI is essential in yeast, despite the presence of numerous other oxidoreductases in the ER. The forced expression of some of these can partially complement the absence of PDI.

This pathway is fundamentally conserved in higher eukaryotes, although some important differences exist. First, whilst a single Ero1 gene exists in Saccharomyces cerevisiae, two isoforms, Ero1α and Ero1β, are found in mammals. Both are able to complement yeast ero1 mutants, underscoring the conservation of the oxidative folding pathways. Ero1α is induced by hypoxia [31] and PPARγ and inhibited by SIRT1 ([32] and references therein). In contrast, Ero1β increases during the UPR [33], and is constitutively expressed at high levels in certain secretory tissues [34], confirming that ER stress responses are important for the acquisition of the secretory phenotype [5,35,36]. The reasons
underlying the functional differentiation between Ero1α and Ero1β in mammals are not yet clear.

Another remarkable difference between yeast and mammalian Ero1 molecules concerns the mechanisms of sub-cellular localisation. A C-terminal tail is present in yEro1p that mediates membrane association and is essential for function. Neither Ero1α nor Ero1β possesses this tail. Both are secreted by mammalian cells when over-expressed, implying saturable retention mechanisms. It was demonstrated that the localisation of Ero1α and Ero1β depends on dynamic interactions with PDI or ERp44 [37], two soluble proteins that despite the presence of ER localisation motifs (KDEL and RDEL, respectively) are differentially distributed in the early secretory pathway. ERp44 accumulates in ERGIC and cis-Golgi, distally with respect to PDI [38,39]. Of the three trx-like domains of ERp44, only the N-terminal one possesses a catalytic CRFS motif. It is via this cysteine that ERp44 forms mixed disulfides with Ero1α, Ero1β, unpolymerized IgM subunits, adiponectin and other substrates of thiol-dependent protein quality control [9, 32,39]. The differential distribution of PDI and ERp44 is important in the biogenesis of IgM polymers, dictating their step-wise assembly and quality control and coupling efficiency and fidelity in the antibody factory [38; see also below]. The dynamic retention of Ero1α may allow the oxidoreductin to distribute in distinct subcompartments, and provide the oxidative power needed in the sequential steps in IgM biogenesis [38]. Moreover, it offers an elegant mechanism to regulate the secretion of certain proteins; indeed, the over-expression of Ero1α allows secretion of unassembled IgM [9] and adiponectin [32,39] by competitive binding with ERp44, as well as by favouring oxidation of the substrate proteins.

2.1. Regulating oxidative power in the ER

As recalled above, Ero1β is expressed in conditions of ER stress, or in certain tissues specialised in protein secretion. A mechanism can be hence envisaged that adjusts oxidative power in the ER. When Ero1α becomes insufficient, reduced proteins would accumulate in the ER. The consequent UPR increases Ero1β, thus generating the required oxidative power. However, cells must also prevent over-oxidation. In the ER, disulfide bonds must be constantly isomerised for many proteins to attain their final three-dimensional structure. Furthermore, proteins that fail to fold or assemble are not allowed to proceed along the secretory pathway and inter-chain disulfide bonds are reduced prior to their retro-translocation to the cytosol for degradation [40–42]. Many PDI homologs reside in the ER of mammalian cells (e.g. ERp57, ERp72, P5, PDIR, PDlp and ERp27) (see L. Ellgaard in this issue, [43–45] and [33,46]). Since Ero1 molecules selectively interact with a subset of them [15,47], some members could exert reductase activities. Recent results indicate that ERdj5, an ER resident protein containing a DnaJ domain and four thioredoxin-like domains, favours ER associated degradation of certain substrate proteins reducing their inter-chain disulfide bonds (Nagata et al., personal communication). An exciting question that emerges from these findings is what maintains ERdj5 in the reduced state.

Despite the specificity of protein relays which allow opposite redox reactions to coexist in the ER (disulfide bond formation in nascent proteins, reduction in terminally misfolded ones), Ero1 activity must be nonetheless tightly controlled. Its main substrate, PDI must remain in part reduced to act as an isomerase and as a redox-sensitive chaperone, catalyzing ERAD substrate unfolding before dislocation [48–50]. Moreover, elegant in vitro assays demonstrated that Ero1p generates H2O2 in equimolar amounts to the number of disulfide bonds formed [16]. H2O2 can be converted to other ROS which can be highly detrimental for the cell [22]. Suggestions of a significant contribution of Ero1 in cellular ROS production in metazoans come from the observation that down-regulation of Ero1 strongly reduces ROS levels in ER stressed Caenorhabditis elegans [51]. Since human Ero1α and β can complement yeast defective cells, it would be extremely important to determine whether oxidative folding generates peroxide also in mammalian cells, and if so in what molar ratio.

![Fig. 1. Oxidative folding and the generation of ROS. In eukaryotic cells, oxidative folding of cargo proteins is based on serial disulfide interchange reactions involving PDI and Ero1 molecules. In vitro, yeast Ero1 is oxidised by molecular oxygen generating H2O2 in stoichiometric amounts to the disulfide bonds formed. Most mammalian cells express constitutively Ero1α. If cargo is produced in excess, Ero1β is induced via UPR pathways activated by inefficient oxidative folding.](image-url)
Recent findings revealed an elegant, in-built mechanism for regulating Ero1p activity [29,45]. The oxidation of two non-catalytic cysteine pairs, leads to inactivation of the enzyme. This rapid feedback mechanism could be particularly important in physiological situations when the requirements for oxidative folding vary transiently and frequently, as in certain secretory cells. The translocation of reduced substrates into the ER lumen shifts the redox balance toward reducing conditions, and rapidly activates pre-existing Ero1 molecules, thus fulfilling the increased oxidative demand. If this pool proves insufficient, the transcription of Ero1 (Ero1α in mammalian cells) is induced. The two redox isoforms observed in human Ero1α (Ox1 and Ox2) might correspond to distinct functional states [27,52].

Another redox-sensitive feedback mechanism could operate at the transcriptional level. Ero1α is regulated by the hypoxia-inducible factor 1 (HIF1) [31]. The O2 sensing subunit of HIF1, HIF1α, has been found at the ER where it is kept silent by O2-dependent generation of •OH. The hydroxyl radical is produced by an ER localised Fenton reaction \( \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{H}_2\text{O} \) [53]. It is tempting to speculate that Ero1 is the source of \( \text{H}_2\text{O}_2 \) production at the ER.

Mammalian Ero1α is generally expressed at rather low levels in cultured cell lines and the assumption is that its catalytic activity is enough to drive multiple PDI oxidation cycles and hence ensure the folding of many substrate molecules. Low expression would help in avoiding the risk of oxidative stress. However, data concerning Ero1β suggest that this model is maybe too simplistic. Ero1β is expressed at high levels in specific cell types of human stomach and pancreas. It can form homo- and hetero-dimers with Ero1α. In vivo, the ratio between Ero1β monomers and dimers differs in stomach and pancreas [34]. Even though the biological meaning of Ero1β homo- and hetero-dimers is not clear, their tissue specific regulation hints to a complex mode of control of Ero1 activity.

Besides the existence of Ero1-centered regulatory mechanisms, ER redox homeostasis heavily relies on a variety of cellular buffering systems, including glutathione and NADP+/NADPH (recently reviewed in [54]). The cellular concentration of GSH ranges from 1 to 10 mM [55] but the ratio between oxidised (GSSG) and reduced (GSH) species is very different in the cytosol (1:30–100) and in the ER (1:1–3) [56,57]. This has been long considered the driving force for disulphide bond formation, but the discovery of the Ero1-PDI relays has changed this point of view. It seems now clear that GSH is crucial in balancing Ero1 function, avoiding ER hyper-oxidation and formation of non-native disulphide bonds [58–60].

Like the GSH/GSSG couple, NADP+/NADH and NADP+/NADPH have a pivotal role in protecting the cell against ROS damage, being involved in the main anti-oxidant defence systems comprising glutathione peroxidases (Gpx), the thioredoxin–thioredoxin reductase system (Trx), peroxiredoxins (Prx), glutaredoxins, superoxide dismutases (SOD), catalases and exogenous micronutrients and vitamins [21,54]. Open questions concern the mechanisms that drive the import of GSH, NADH and NADPH into the ER lumen, and their regulation during differentiation or stress.

3. Redox-dependency of BCR activation

Many lines of evidence confirm that ROS play a direct role in the development, survival and terminal differentiation of mature B lymphocytes. These cells express surface BCR, a complex formed by membrane Ig (in charge of recognizing antigen) and the kinases Igα and Igβ, involved in signal transduction (Fig. 2). BCR expression is indispensable for B cell survival even in the absence of antigen, implying that basal signals are generated and deciphered [61,62]. Signalling from the BCR was demonstrated to involve ROS. Suitable doses of \( \text{H}_2\text{O}_2 \) can mimic antigen binding and trigger the same phosphorylation cascade as BCR cross-linking [23]. ROS generation by the DUOX1 NADPH oxidase is critical for BCR signalling upon antigen binding. Oxidants and Ca++ act in a cooperative manner to regulate the strength and duration of BCR signalling [63], crucial parameters for determining the subtype of B cell generated from transitional, immature B cells [64]. It is still unclear what the source of the basal BCR signalling is and how the strength of the signals could be achieved and interpreted. Binding of ligands (e.g. LPS) to Toll like receptors (TLR) activates NAPDH oxidases [65,66] suggesting a main role for superoxides as second messengers in the activation of the innate and humoral immune responses. Upon stimulation with LPS or INFγ, macrophages and dendritic cells produce B-cell-activating factor (BAFF), essential for B cell generation and maintenance. LPS-induced BAFF expression is potentiated by ROS and inhibited by PrxII [67].

4. Preparing for antibody secretion

Increased demand for protein folding power in the ER triggers the Unfolded Protein Response (UPR), a multidimensional signalling pathway that regulates the expression of ER resident proteins and lipids [36,68,69], translation, the cell cycle and apoptosis [70–72]. Three resident membrane proteins, IRE1, ATF6 and PERK, sense ER stress. Not only is the response activated in stress or disease: to achieve a high rate of protein synthesis and transport, secretory cells utilize arms of the UPR. Interestingly, they do so in a tissue specific way, suggesting subtle regulatory pathways [5]. IRE1 and the downstream transcription factor XBP1 are essential for plasma cell differentiation [35,73], whilst PERK and CHOP ([74] and S. Masciarelli, L. Hendershot and R. Sitia, unpublished results) are not. The role and regulation of the UPR in B cell differentiation is matter of extensive discussion [5,6,74]. The easiest model would predict that exuberant Ig production triggers the UPR, thus favouring the expansion of the ER and other secretory organelles [75–79]. However, studies on in vitro differentiating B lymphoma cells showed that B cells increase the transcription of UPR genes well before starting massive Ig production [80,81], which implies additional cross-talk(s) between differentiation signals and the UPR. A connection seems to be provided by Blimp1, a transcription factor whose expression is sufficient to induce plasma cell differentiation. Interestingly, the finding that XBP-1 is a target of Blimp1 [36], provides an explanation as to how B cells can expand the secretory pathway by selectively activating arm(s) of the UPR.
5. Coupling efficiency and fidelity in the antibody factory

Once the secretory route and metabolic supplies have been sufficiently expanded, IgM production increases exponentially. The translational apparatus focuses on Ig-μ and L chains, somehow resembling the situation encountered in virus infected cells, and for a few days a differentiating B cell masters the production and release of thousands of IgM per second. As

Fig. 2. B cell differentiation. Mature B lymphocytes are long-lived resting cells with scarce cytosol that do not secrete antibodies. They express on their surface antigen specific B cell receptors (BCR) composed by the membrane-specific isoform of Ig (μmL2). Membrane and secreted Ig-μ (μm and μs) differ in their C-terminal segments. μm chains possess a hydrophobic tail essential for membrane insertion and assembly with BCR signalling components (Igα–Igβ). The μs tailpiece mediates the assembly, retention and degradation of unpolymerized secretory IgM subunits [149–152]. Antigen binding initiates a signalling cascade which is amplified and sustained by H2O2 generation. After an initial phase of clonal expansion, differentiation entails an increase in the cell volume largely sustained by the development of the ER and other secretory organelles, together with empowered metabolic and synthetic capacities [80]. The ATF6 and Ire1 UPR arms are activated and likely contribute in driving ER expansion. The Blimp-1-dependent transcription of XBP-1 may provide a further link between the UPR and the increase in secretory capacity of the cell. After a couple of days, ASC increase IgM synthesis, and polymerization and secretion ensue. ERp44 and ERGIC53 may promote polymerization [38]. After a few days of intensive Ig secretion most ASC undergo apoptosis. Different types of stress (ER stress, proteotoxicity, redox imbalance) likely concur in causing cell death.
mentioned above, this poses many homeostatic problems, including aminoacid import, energy production and redox balance. To satisfy the increased demand for oxidative folding, more Ero1α and β are produced. Also few proteins involved in redox control, peroxiredoxin 4 (Prx4) and glutathione S-transferase theta 2 increase in this phase. Interestingly, whilst Ero1α is activated soon, with kinetics similar to other ER residents (depicted in light blue in Fig. 3), Ero1 β increases in the last days of differentiation, concomitantly with the onset of IgM polymerization and massive release ([80,81] M. Otsu, S. Masciarelli and R. Sitia, unpublished data). A similar temporal expression pattern also characterizes ERp44 and ERGIC-53, two proteins shown to facilitate IgM polymerization [38], as well as Sel1L, a protein that in association with the E3 ligase Hrd1, mediates the degradation of unassembled μ chains [82] (Cattaneo et al., 2008). These observations possibly reflect the attempts to optimize Ig assembly, quality control and release. It will be of interest to identify additional proteins with similar kinetics, as these could play an important role in the antibody factory.

6. Ending the antibody response

After a few days of intense Ig secretion most ASCs die [7]. Their short lifespan is probably important to limit the immune response. If the antigen is not yet defeated, new specific B lymphocytes, meanwhile expanded in numbers and equipped with better receptors by affinity maturation and isotype switching in germinal centers, will be activated. What causes apoptosis of ASCs? Ig production itself could cause death via different mechanisms. One could be a prolonged UPR sustained by continuous Ig production. The UPR can lead to apoptosis through multiple pathways [83–86]: activation of ER stress specific caspases (caspase 4 in human, 12 in mouse), mitochondrial activation by ER Ca++ release, activation of JNK via IRE1, and suppression of anti-apoptotic bcl-2 and sensitization to ROS via the transcription factor CHOP (downstream of the PERK branch of the UPR) [87–96]. However, recent data exclude a role for PERK and the downstream factor CHOP in plasma cell apoptosis ([74] and S. Masciarelli, L. Hendershot and R. Sitia, unpublished results). A recent study supports a direct role of Bax, caspase 4 and the ER in plasma cell apoptosis [97], though autocrine or paracrine loops involving the death ligand TRAIL may also be important [98].

In all likelihood, different factors concur in rendering ASCs susceptible to apoptosis. One predisposing factor could be the chaperone overload [99,100] that might arise at the high rate of protein synthesis achieved by plasma cells. In addition, we recently showed that in the late phases of differentiation, when antibody production becomes maximal, proteasomal activity decreases. This correlates with parallel increased sensitivity to proteasome inhibitors, and stabilisation of endogenous proteasomal substrates, including IκBα, and the proapoptotic factors Bim and Bax [12]. Aminoacid supply and energy production could also become limiting as Ig synthesis becomes preponderant, further lowering the threshold for apoptotic commitment [13].

Perturbation of the redox homeostasis could be yet another factor leading to plasma cell death. On the basis of the present knowledge about oxidative folding, the massive production and assembly of Ig could generate abundant ROS in differentiating plasma cells. The production of 10^7 IgM per second, each containing 10^2 disulfides, implies that 10^5 bonds are formed solely to satisfy the requirements of antibody production, explaining the increase in Ero1α and β. If O2 is the ultimate electron acceptor also in living mammalian cells, up to 10^5 H2O2 molecules per second could be generated as a byproduct of oxidative folding in IgM secreting cells.

Besides oxidative folding, additional processes could contribute to ROS production. It has been estimated that nearly 2% of the total oxygen consumed by mitochondria leaks from mitochondrial respiratory chain in a partially reduced form [101]. It is reasonable to imagine that the higher metabolic requirements in plasma cells would cause a corresponding increase in mitochondrial ROS generation. Such mechanism has been proposed to explain the increase in ROS upon T lymphocytes activation ([102] and references therein).

A regulated source of ROS in differentiating B cells could be NADPH oxidases. These are complex enzymes known to generate superoxide in phagocytes during inflammatory processes [103]. Different isoforms of NADPH oxidase (NOX) and dual oxidase (DUOX) have been described in non-phagocytic cells to generate ROS in a regulated manner, with important consequences upon signalling [104,105]. B lymphocytes express NOX2 and DUOX1 [23,63,105]. Intriguingly, we found increased expression of NOX2a (p67phox) transcripts in murine ASC with respect to unstimulated B lymphocytes (S. Masciarelli, L. Hendershot and R. Sitia, unpublished observations). These preliminary findings deserve further analyses, especially since previous reports failed to detect NOX2a in plasma cells [106].

Although formal evidence of ROS generation during plasma cell differentiation remains to be gathered, many are the indications that ASCs undergo oxidative stress. The up-regulation of different enzymes involved in thiol redox balance upon stimulation of 1.29μ+ with LPS [80,81] has been already
recalled (Fig. 3). Prx1 expression was detected by immunohistochemistry in human plasma cells but not in B lymphocytes infiltrating inflammatory tissue of the oral cavity, independently of the inflammation-inducing agent [107]. An ER resident oxidoreductase (plasma cell-Trx related protein, PC-TRP) is specifically expressed in murine primary plasma cells [108]. Trx1 expression is higher in human cell lines with plasma cell-like features than earlier stages of differentiation, even though immortalized lines could be selected for stronger anti-oxidant responses [109].

Further indication of ongoing oxidative stress in ASC comes from the observation that differentiating B lymphoma cells trigger a Nr2-dependent anti-oxidant response (S. Cozza and R. Sitia, unpublished data). Nrf-2 is a redox-sensitive transcription factor that mediates the induction of glutathione S-transferase, glutamyl cysteine ligase (GCLM/GCLC, the limiting enzymes for GSH synthesis), heme oxygenase-1 (HO-1), phase II detoxifying enzymes and other anti-oxidant factors [110–116].

The above observations indicate that oxidative stress [117,118] could contribute to limit plasma cell lifespan, in a fashion possibly dependent on the rate and intensity of antibody secretion. Proteasomal insufficiency, a chronic UPR and ROS production could hence conjure in inducing death when enough antibodies have been produced. It is worth recalling that the UPR and ROS signalling share a common apoptotic pathway through the apoptosis signal-regulated kinase 1 (ASK1) [119–121]. Activated Ire1 recruits the TNF receptor associated factor 2 (TRAF2), which in turn binds to ASK1 leading to apoptosis via JNK activation. ASK1 deficient cells are unable to activate JNK and are much more resistant to ER stress-dependent death [122]. ASK1 is negatively regulated by reduced Trx1 [123,124].

Ig synthesis could hence activate the ASK1/JNK apoptotic cascade, via the different mechanisms elicited by UPR/Ire1 pathway and ROS [124].

Furthermore, the proapoptotic activity of JNK is executed via the bcl-2 family members, known to be involved in plasma cell death. JNK can inhibit bcl-2 [125] and mcl-1 [126], and activate Bax [127,128], Bim and Bmf [129,130]. Recent studies point to a direct proapoptotic role for Bax in human plasma cells: Bax is recruited at the ER and contributes to caspase 4 cleavage before the release of mitochondrial apoptotic factors [97]. Bax is stabilised and accumulates in differentiating B lymphoma cells whilst the level of bcl-2 expression remains constant [12]. An additional link among ER stress, ROS generation and Bax proapoptotic activity comes from studies regarding pharmacologically induced UPR and apoptosis. The ER resident protein Bax inhibitor 1 (BI-1) regulates ER stress associated ROS generation via up-regulation of HO-1 through Nrf-2 and protects cells from UPR induced apoptosis [131].

Increased ROS could favour plasma cell apoptosis also perturbing Ca++ homeostasis. The activity of both sarcoplasmic/endoplasmic reticulum calcium ATPases (SERCA2b in particular) and IP3 Receptors type 1 are regulated in a redox dependent manner [132,133]. In turn, ER Ca++ release potentiates mitochondria-dependant apoptosis (reviewed in [25]).

Both ER stress [134,135] and ROS [136] reviewed in [137]) were recently shown to induce autophagy. Since the latter degradative pathway may integrate proteasome function [138–141], its stimulation by ROS and ER stress could in part compensate proteasome impairment in ASC [12]. However, autophagy is known to mediate caspase-independent apoptosis in response to DNA damage [142] or cytotoxic stressors, including ROS [143]. The role played by autophagy in the control of plasma cell lifespan clearly deserves further investigation.

7. Concluding remarks

Terminal B lymphocyte differentiation provides a unique system to investigate the molecular mechanisms that dynamically regulate protein synthesis and secretion and lifespan control. Several lines of evidence suggest that the production of ROS could be exploited as a signal reporting on the protidogenic efficiency within the secretory apparatus. ROS play a key role in activation-induced apoptosis of T lymphocytes [102,144–146]. The development of suitable imaging technologies (147,148) and reference therein) should allow to determine the generality of this mechanism, and its role in limiting plasma cell lifespan.

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