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News and Commentary

The *Bcl2a1* gene cluster finally knocked out: first clues to understanding the enigmatic role of the Bcl-2 protein A1

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A tightly controlled balance between cell survival and cell death assures correct development and normal physiology of multicellular organisms. Members of the Bcl-2 family that regulate apoptosis often control this decision between cell death and survival. The generation of gene-deficient mice has greatly aided our understanding of the function of most individual Bcl-2 family members. However, a function for Bcl2A1 (A1) has been elusive until the Herold group recently generated A1-deficient mice, filling a long-awaited gap. Their initial characterization is now reported in two papers in this issue of *Cell Death & Differentiation*.[1,2]

Bcl-2 proteins come in three flavors that have antiapoptotic or proapoptotic function (Figure 1). The proapoptotic group is further divided into BH3-only proteins ('activators' and 'sensitizers') as well as non-BH3-only 'executioners'. Enhanced expression and/or post-transcriptional modification empowers 'activators' (Bim, Puma, tBid and Bad) to induce a conformational change in 'executioners' (Bax and Bak) to polymerize on the surface of mitochondria, thereby creating holes in the outer membrane and allowing cytochrome *c* (cyto *c*) to escape from the intermembrane space. In the cytoplasm, cyto *c* initiates the formation of high-molecular-weight scaffolds to activate dormant caspases, which catalyze proteolytic intracellular disintegration. Destruction of the cell culminates in the formation of apoptotic bodies that are engulfed by macrophages. Antiapoptotic Bcl-2 proteins like Bcl-2, Mcl-1, Bcl-X_L and A1, also known as 'guardians', interfere with the induction of apoptosis by binding and thereby neutralizing the proapoptotic members (Figure 1). However, being more than just a balance between pro- and antiapoptotic Bcl-2 family members, it is becoming quite clear that specific interactions between particular anti- and proapoptotic Bcl-2 family members regulate apoptosis.[3] Finally, sensitizers like Noxa tune the system by sequestering the guardians, thereby enhancing the threshold for apoptosis induction. Thus, regulated induction of apoptosis – dictated by specific interactions between individual Bcl-2 family members – controls cell fate.

To clarify the function of antiapoptotic Bcl-2 proteins, total and conditional gene knockout mice have been generated. Conditional knockout mice for Bcl-2 proteins have varying phenotypes (reviewed in Delbridge *et al.*[4]). Chimeric mice lacking Bcl-X_L in the hematopoetic system indicate a critical role for the survival of developing lymphocytes. Similarly, Mcl-1-deficient mature lymphocytes have profoundly reduced survival. Finally, lack of Bcl-2 expression in the lymphocyte lineage leads to thymic and splenic atrophy due to increased cell death of immature and mature lymphocytes. To date, A1 remained the only pro-survival Bcl-2 family member whose function has not yet been determined using a mouse strain lacking expression of the protein in all or selected tissues. The reason for that is that the gene for A1 (*Bcl2a1*) had been quadruplicated in the mouse genome. Three genes (*Bcl2a1a*, *-b and -d*) code for almost identical functional isoforms of A1 suggesting a high degree of redundancy among these three proteins.[5] The fourth gene *Bcl2a1c* is a pseudogene due to a point mutation resulting in a premature stop codon.

In mice, A1 expression is mostly limited to hematopoietic cells. As a strongly NF-κB-induced gene, it is upregulated in immune cells on contact with antigen, that is, via the antigen receptors in T cells (TCR) or B cells (BCR) and Toll-like receptors in all immune cells.[6] In addition, signals coming from the pre-TCR or pre-BCR in developing thymocytes and pre-B cells, respectively, also upregulate A1 expression. Thus, deletion of A1 might impinge on development and/or activation of immune cells. Surprisingly, initial studies examining mice with ablated *Bcl2a1a*, revealed a relatively minimal impact of A1a on hematopoietic/immune cell survival.[7,8] The only significant phenotype was that *Bcl2a1a*$^{-/-}$ mice had moderately reduced survival of activated neutrophils and mast cells in response to particular stimuli, although basal neutrophil homeostasis was not perturbed. An obvious

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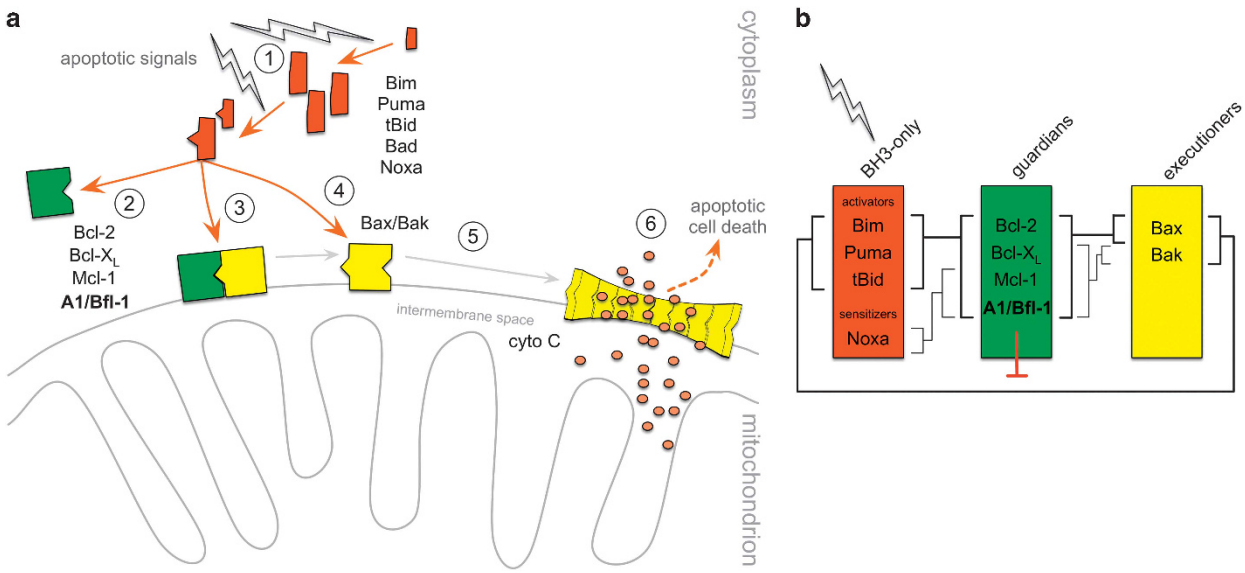

Figure 1 The mitochondrial death pathway is controlled by Bcl-2 proteins. (**a**) Apoptotic signals induce transcriptional and/or post-transcriptional activation of proapoptotic BH3-only proteins (1). These proteins neutralize the antiapoptotic Bcl-2 proteins ('guardians') leading to release 'executioners', that is, Bax and Bak (2/3). Certain BH3-only ('activators') instruct free Bax and Bak to polymerize to form pores in the outer mitochondrial membrane (4/5). Release of cytochrome (cyto) *c* leads to the formation of high molecular complexes in which dormant caspases are activated. (**b**) Selectivity of interaction in the Bcl-2 family. Brackets and lines indicate interactions between proteins. The 'guardians' interfere with the interaction of BH3-only and 'executioners' (—|). For the sake of clarity, not all members of the Bcl-2 family are shown. For detailed discussion, see Czabotar *et al.*[3]

explanation for such a mild phenotype was the potential redundancy of the nearly identical proteins A1b and A1d. One step closer to a full A1 knockout state was taken with a mouse model expressing constitutive or inducible shRNAs, targeting all three active isoforms of A1.[9] Interestingly, in this model, the number of mast cells in connective tissues was reduced and mice were protected from anaphylaxis.[10] However, this and another RNAi transgenic mouse line showed somehow conflicting data on B- and T cell development and activated B cells.[9] This might be explained by incomplete knockdown efficiency by or off-target effects of RNAi. Thus, it appears that A1 might be critical for the survival of developing B and T cells, as well as certain innate cells (neutrophils and mast cells) in response to activation or inflammatory stimuli. However, due to the potential problems with RNAi discussed above, a specific role for A1 has been elusive.

Obviously, this has been a challenge especially to the Herold group that has a long-standing interest in the role and regulation of A1. Consequently, this group has undertaken the heroic task of generating an A1-conditional mouse model. In this model, A1a and A1d are constitutively knocked out, while A1b is flanked with LoxP sites and is deletable by Cre recombinase. The Herold group – in close cooperation with the Villunger group – analyzed the complete A1-deficient ($A1^{-/-}$) mouse and found, surprisingly, that the mice are viable and fertile.[1] Notably, in contrast to the siRNA mouse models, they showed that the complete loss of A1 resulted in small decreases in $\gamma\delta$T cells and regulatory $CD4^+$ $Foxp3^+$ T cells, and more substantial decreases in memory $CD4^+$ T cells and conventional dendritic cells under steady state conditions. Given the role for A1 previously reported for activated T cell survival[11] and the fact that endogenous memory $CD4^+$ T cells were decreased in $A1^{-/-}$ mice, the authors also examined T cell homeostasis in response to viral infection.[2] To avoid potential lethal infection if T cell responses depended upon A1, the authors used a 50/50 mixed bone marrow chimera approach with WT and $A1^{-/-}$ cells. At basal homeostasis, WT $CD4^+$ and $CD8^+$ T cells experienced no competitive advantage. Similarly, after influenza infection, the authors quantified viral-specific $CD8^+$ T cells using NP tetramers, and found no difference in the expansion or contraction of the response between WT and $A1^{-/-}$ cells. In another series of experiments, the authors examined the role of A1 in chronic lymphocytic choriomeningitis virus (LCMV) infection. In this model, chronic infection drives early Bim-mediated attrition of viral-specific $CD8^+$ T cells.[12,13] Again, using a mixed bone marrow chimera approach, the authors found no difference in the numbers of LCMV-specific $CD8^+$ T cells between WT and $A1^{-/-}$ cells. Similar results were obtained for $CD4^+$ T cells. However, viral-specific $CD4^+$ T cells were not examined specifically in either model. Thus, at least for viral-specific $CD8^+$ T cells, it is clear that Bcl2A1 is not obligatory for survival, although more work is required to determine the role for A1 in antigen-specific effector/memory $CD4^+$ T cell survival. Having this combined *Bcl2a1a/b/d* knockout mouse available, those studies are finally manageable.

In conclusion, the generation of this mouse line is an important step forward to further clarify the role and interplay of the Bcl-2 family members. Furthermore, this mouse line will greatly aid in unravelling the involvement of A1 in immune responses during infection and autoimmune disease as well as in cancer.

Conflict of Interest

The authors declare no conflict of interest.

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