

# Apoptosis during lymphoid development

Sue J Sohn, Arvind Rajpal and Astar Winoto\*

Recent investigations have provided important insights into how signaling through the antigen receptors determines whether a cell survives or dies. In T cells, Grb2 and MAP kinases play essential roles in differentiating between apoptotic and survival signals. The PTEN phosphatase and Bim, a pro-apoptotic Bcl-2 family member, regulate apoptosis in both T and B cells. In B cells, antigen receptor-mediated death can be rescued by co-stimulation, in which the roles of protein kinase C and BAFF, a TNF family member, have been recently elucidated. In a recently identified mechanism of regulating inflammation, receptors such as c-mer and glycoproteins such as MFG-E8 were found to participate in the clearance of apoptotic cells.

## Addresses

Department of Molecular and Cell Biology, Division of Immunology and Cancer Research Laboratory 469, Life Science Addition, University of California, Berkeley, CA 94720-3200, USA

\*e-mail: winoto@uclink4.berkeley.edu

Current Opinion in Immunology 2003, 15:209–216

This review comes from a themed issue on  
Lymphocyte development  
Edited by Ellen Robey and Mark Schlessel

0952-7915/03/\$ – see front matter  
© 2003 Elsevier Science Ltd. All rights reserved.

DOI 10.1016/S0952-7915(03)00004-9

## Abbreviations

<b>Apaf-1</b>	apoptotic protease-activating factor 1
<b>BCR</b>	B-cell receptor
<b>DP</b>	CD4 <sup>+</sup> CD8 <sup>+</sup> double-positive
<b>ERK</b>	extracellular signal-related kinase
<b>GR</b>	glucocorticoid receptor
<b>JNK</b>	c-Jun N-terminal kinase
<b>MAP</b>	mitogen-activated protein
<b>MEK/MKK</b>	MAP kinase kinase
<b>MEKK/MKKK</b>	MEK/MKK kinase
<b>MFG-E8</b>	milk fat globule EGF factor 8
<b>PI3K</b>	phosphatidylinositol-3 kinase
<b>PKC</b>	protein kinase C
<b>PS</b>	phosphatidylserine
<b>PTEN</b>	phosphatase and tensin homolog
<b>PTK</b>	protein tyrosine kinase
<b>TCR</b>	T-cell receptor
<b>TNF</b>	tumor necrosis factor

## Introduction

Apoptosis plays a central role in the generation of the lymphoid system. Developing T and B cells are destined to die, unless a functional antigen receptor is produced through gene rearrangements to trigger a rescue signal. The lymphocytes with functional receptors are screened on the basis of individual receptor specificity, and cells

bearing 'self-reactive' receptors are eliminated by apoptosis to protect the organism from inappropriate assault. Mature lymphocytes are further subjected to apoptotic death after a normal immune response to regulate activated lymphocytes, in response to self-antigen, or via homeostatic mechanisms that balance the relative representation of various components of the immune system. In this review, we focus primarily on recent advances made in our understanding of the apoptotic regulation of developing lymphocytes. For a review of homeostatic regulation of lymphocytes through the processes of activation-induced cell death, we refer the readers to a recent review [1]. We will discuss molecular mechanisms of the T-cell default death pathway (death by neglect), T-cell antigen receptor-dependent death (negative selection), and B-cell antigen receptor-mediated death. We also include in our discussion the regulation involved in the removal of apoptotic cells.

## Apoptosis of developing T cells

Immature T cells that fail to generate functional antigen receptors or fail to receive any T-cell receptor (TCR) signals die by default, a process commonly referred to as 'death by neglect'. The precise mechanism of death by neglect remains unresolved; however, recent studies have centered around defining the mechanisms that sensitize the CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) thymocytes to apoptosis. Data from several earlier studies suggested that sensitivity to glucocorticoids plays a role. The ultimate fate of an immature T cell thus poised for death may be reversed by a signal through the surface TCR, if the interaction between the TCR and its ligand is 'weak'. A 'strong' signal immediately triggers apoptosis (negative selection). The molecular basis of how the survival- and death-inducing signals are differentiated by the TCR remains an important fundamental question. In addition, the regulation of effector mechanisms of apoptosis in developing T cells has been of great interest. We omit a discussion of the role of the TNF receptor family in thymocyte death in this review because studies conducted in mice expressing a dominant-negative mutant FADD, which inhibits signals from all death domain-containing receptors, show that these receptors play a relatively minor role in the regulation of apoptosis in developing T cells [2].

## Death by neglect

DP thymocytes are exquisitely sensitive to apoptotic signals. When cultured *in vitro* most of them die within 72 hours. The addition of outside stimuli, such as glucocorticoids, irradiation or anti-CD3/CD28 antibodies, accelerates their demise. What determines the sensitivity of these DP cells is still an open and interesting question.

DP T cells express a reduced level of Bcl-2, which could partly explain their apoptotic tendency. Other factors, such as endogenous glucocorticoids, have also been suggested to play an essential role in death by neglect, as adrenalectomized mice display increased thymic cellularity and DP cells display keen sensitivity to corticosteroids in the absence of TCR stimulation. Signals from the TCR can antagonize the apoptotic effect of glucocorticoids, leading to the hypothesis that the combined signals from steroid hormones and TCR determine the fate of individual thymocytes [3]. However, mice reconstituted with glucocorticoid receptor-negative ( $GR^{-/-}$ ) fetal liver cells display normal thymic composition and cellularity as well as death by neglect [4<sup>•</sup>], suggesting either that glucocorticoids might not play a requisite role in this process or that alternative corticosteroid receptors exist to mediate thymocyte cell death.

Inside the cells, Bcl-2 family members clearly play an essential role in the process of thymocyte death by neglect. Overexpression of either Bcl-2 or Bcl-xL protects DP thymocytes from dying and leads to an increased thymic cellularity. Conversely, deletion of Bim (a Bcl-2 homology region 3 [BH3]-only pro-apoptotic Bcl-2 family member) leads to delayed thymocyte apoptosis [5]. Abrogation of both Bax and Bak, two of the pro-apoptotic Bcl-2 family proteins downstream of Bim, also results in the prolonged survival of thymocytes in culture [6<sup>••</sup>].  $Bax^{-/-}bak^{-/-}$  DP thymocytes are additionally resistant to cytokine withdrawal and glucocorticoid-induced apoptosis, suggesting that Bcl-2 family members play a crucial role in many aspects of cell death, including death by neglect [6<sup>••</sup>]. In contrast to the requirement of Bcl-2 family members, the mitochondrial release of cytochrome-c to activate apoptotic protease-activating factor 1 (Apaf-1) and caspase-9, long thought to be the central event in initiating apoptosis, was recently shown not to be essential in thymocyte death. Thymocytes isolated from mice reconstituted with  $apaf-1^{-/-}$  or  $caspase-9^{-/-}$  fetal liver cells are as sensitive as their wild-type counterparts to death by neglect and other apoptotic stimuli [7<sup>••</sup>]. These data lead to an interesting notion that the cytochrome-c/Apaf-1/caspase-9 'apoptosome' amplifies rather than initiates apoptotic signals, and that Bcl-2 regulates an alternative caspase pathway independent of the apoptosome complex (see Figure 1).

### Death by negative selection

Engagement of the TCR results in the rapid induction of intracellular events, including the activation of Src- and Syk-family protein tyrosine kinases (PTKs), protein kinase C (PKC) and mitogen-activated protein (MAP) kinases. Induction of a 'strong' signal, generated by self-antigen, commits the cell to apoptosis. This indicates that the life-versus-death choice can be made at the level of the TCR, on the basis of TCR–ligand interactions. It is thought that antigen receptor-proximal events in an

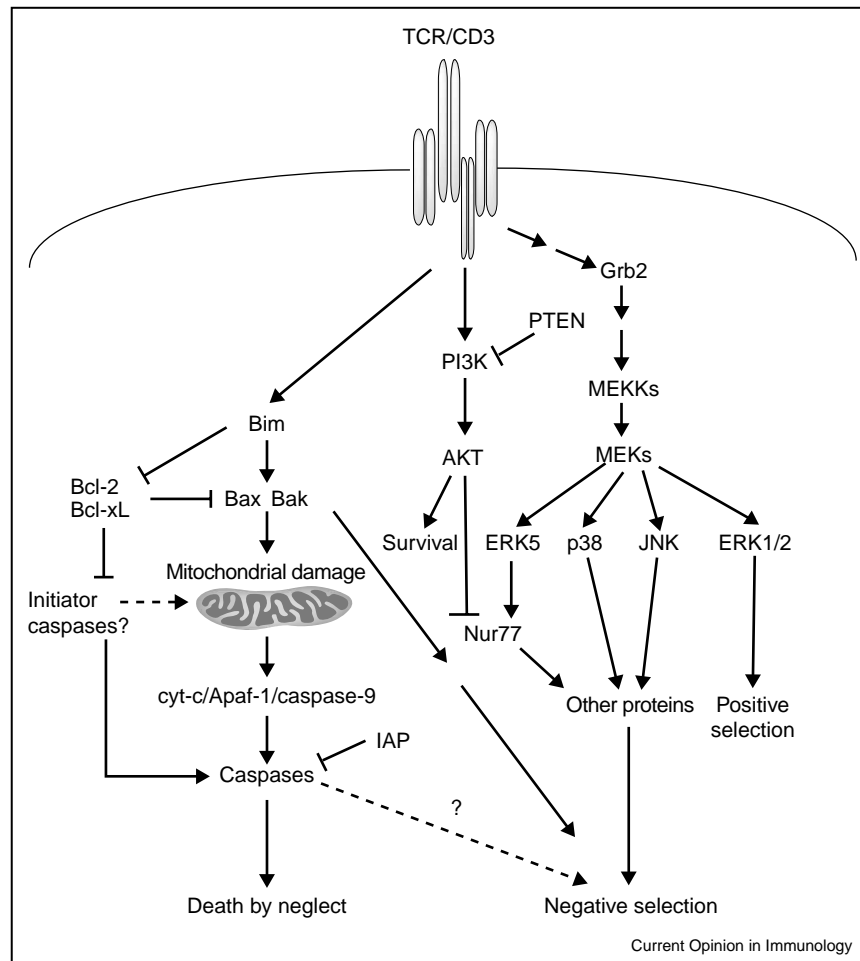
apoptotic signal (negative selection) are similar to those in a survival signal (positive selection), whereas downstream events diverge (for reviews, see [8–10]). Consistent with this view, activation of PTKs is required for both signals, but the MAP kinase pathways differentially regulate the apoptotic and survival signals in DP thymocytes.

Activation of the MAP kinase (MAPK) pathways involves sequential phosphorylation and activation of a three-kinase cascade: MEKK-MEK-MAPK. In T cells, the MAP kinases ERK1/2, Jnk1/2, p38 [11] and ERK5 (A Winoto, unpublished data) are activated in response to TCR crosslinking. Targeted disruption of the *erk1* gene, or expression of dominant-negative Ras (activator of ERK1/2), specifically blocks positive selection [12,13]. Inhibition of p38 with a pharmacologic inhibitor, disruption of the *jnk* genes [14,15], or expression of dominant-negative Jnk, renders thymocytes resistant to TCR-induced apoptosis and results in a block in negative selection. Because of embryonic lethality of *p38 $\alpha$*  gene disruption, the requirement for p38 $\alpha$  in T-cell death has not been directly assessed [16,17]. Taken together, these results implicate the MAP kinase pathways in being able to differentiate apoptotic and survival signals generated by the interactions of TCR with a negatively or a positively selecting ligand, respectively (see Figure 1).

Nonetheless, the activation of specific MAP kinases does not correlate completely with the ultimate decision to live or die. For example, ERK1/2 are activated in response to negatively and positively selecting ligands [18]. Disruption of the genes encoding the upstream activators MAP kinase kinase (MKK, also called MEK) and MKK kinase (MKKK, also called MEKK) has also led to somewhat confusing results. Deletion of *MKK6*, a p38 MKK, leads to decreased apoptosis in thymocytes [19]; however, the absence of MKK4 (an MKK that activates Jnk) potentiates T cell apoptosis in the thymus [20] and MKK7 (another Jnk MKK)-deficiency does not block activation or apoptosis. Interestingly, deficiency of MEKK2 (an MKKK that activates both Jnk and p38) results in increased thymocyte death in response to treatment with anti-CD3 antibody [21], inconsistent with the prediction that immature T cells with reduced Jnk and p38 activation would be resistant to apoptosis. These apparently conflicting data probably reflect the existence of a compensatory mechanism that ultimately results in the adjustment of activation thresholds for downstream pathways.

More recently, the disruption of a single allele of the *grb2* gene was shown to block thymic negative selection [22<sup>••</sup>]. Grb2 is a src homology 2 (SH2)/SH3 domain-containing adaptor molecule that interacts with Sos (a guanine nucleotide exchange factor) and is thought to activate the MAP kinase pathways. This defect correlates with the

Figure 1



A schematic diagram of signaling pathways involved in TCR-mediated apoptosis in death by neglect and negative selection. DP thymocytes undergo death by neglect via a pro-apoptotic Bcl-2 protein-regulated pathway (Bim, Bax and Bak). Activation of Bax and Bak by Bim leads to cytochrome-c (cyt-c) release from mitochondria, which in turn activates Apaf-1 and caspase-9. Caspase-9 then activates downstream caspases, leading to apoptosis. Death by neglect can be inhibited by the expression of anti-apoptotic proteins, such as Bcl-2 and Bcl-xL. It is not clear if an additional death by neglect pathway to cyt-c/Apaf-1/caspase-9 exists but, in thymocytes, the cyt-c/Apaf-1/caspase-9 pathway is dispensable for apoptosis. Engagement of TCR/CD3 by a negatively selecting antigen leads to activation of the pro-apoptotic Bim → Bax/Bak pathway and the four MAP kinase pathways (ERK1/2, p38, Jnk and possibly ERK5) leading to apoptosis. Engagement of TCR/CD3 by a positively selecting antigen activates PI3K and the ERK1/2 MAP kinases, leading to survival. Activation of MAP kinases appears to be regulated by the quantity of Grb2 adaptor protein. PTEN, a negative regulator of the PI3K pathway, is implicated in apoptosis induced by negative selection. Broken lines depict putative pathways that remain to be fully elucidated. IAP, inhibitor of apoptosis.

inhibition of Jnk and p38 activation, but not ERK1/2 activation. Furthermore, in support of the model that a 'strong' signal generates an apoptotic signal, Jnk and p38 are shown to exhibit higher activation thresholds, requiring higher concentrations of phorbol myristate acetate (PMA) to reach the equivalent level of activation as ERK1/2 [22\*\*]. Thus, diminution of Grb2 protein levels attenuates MAP kinase signals, affecting Jnk and p38 first because of their intrinsically higher activation thresholds. These results further suggest that the different signal 'strengths' are translated into the extent of Grb2- and Sos-dependent activation of downstream MAP kinases. The events that follow Jnk or p38 activation in thymocytes

undergoing negative selection remain unclear. However, a p38 inhibitor has been shown to block TCR-mediated translocation of Bax to mitochondria, suggesting that the activation of MAP kinases may ultimately trigger the mitochondrial pathway of apoptosis [23].

A recently described MAP kinase, ERK5, which activates the transcription factor myocyte enhancer factor (MEF)-2 [24,25], may also regulate thymocyte apoptosis by its ability to induce Nur77, an orphan steroid receptor implicated in T-cell apoptosis (see Figure 1; [25]). Interestingly, the catalytic domain of ERK5 is most closely related to ERK1/2, and pharmacological inhibitors of

the ERK1/2 pathway also block ERK5 [26]. ERK5 can be activated by MEK5 and the upstream MEKK2 and MEKK3 [27,28]. ERK5 is distinct from other MAP kinases as it contains a unique large carboxy-terminal region. This region contains a binding site for the MEF2 transcription factors and a powerful transcription activation domain [25]. Truncation of this activation domain leads to a dead ERK5 protein that can inhibit Nur77 expression. Conversely, a catalytically inactive ERK5 fails to induce transcription. This unusual mechanism allows a rapid response to outside stimuli to induce a high level transcription of a normally weakly transcribed gene. The role of ERK5 in thymocyte selection has yet to be explored, as ERK5-deficient mice die at day 11 of gestation due to angiogenesis defects [29,30].

Nur77, and its family member Nor-1, are orphan steroid nuclear proteins that are rapidly upregulated in response to TCR stimulation in thymocytes (for a recent review, see [31]; [32,33]). Disruption of either *nur77* or *nor-1* alone does not lead to any obvious thymic defects, but a dominant-negative protein can inhibit negative selection. These data suggest overlapping roles for Nur77 and Nor-1 in negative selection [34,35]. Expression of either Nur77 or Nor-1 in thymocytes leads to massive cell death that is dependent on the transcriptional activity of Nur77 [36]. Although the downstream transcriptional targets of Nur77 in thymocytes are unknown, there is some evidence to suggest that Nur77-dependent death in a prostate cancer cell line (LNCaP) may involve direct recruitment of the molecule to the mitochondria [37]. However, only 15–20% of the LNCaP cells transfected with a green fluorescent protein (GFP)–Nur77 fusion protein display mitochondrial translocation of the molecule when treated with phorbol ester, calcium ionophore and synthetic retinoid analogs. The mitochondrial targeting of Nur77 in LNCaP cells leads to the release of cytochrome-c, which is followed by apoptosis and can be antagonized by the expression of Bcl-2. However, in transgenic animals, Bcl-2 coexpression cannot rescue the Nur77 apoptotic activity (A Winoto, unpublished data). Furthermore, AKT (also called protein kinase B)-dependent phosphorylation of Nur77 decreases its transcriptional activity with a corresponding reduction in apoptotic activity in RAT1 fibroblasts [38\*,39\*]. These data suggest that, although mitochondrial translocation could be a putative mechanism of action for Nur77-mediated apoptosis in some cell lines, transcriptional activity of Nur77 is required for thymic negative selection.

Given the crucial role of phosphatidylinositol-3 kinase (PI3K)–AKT in lymphocyte development and homeostasis, phosphatase and tensin homolog (PTEN), a negative regulator of the PI3K pathway and a known tumor suppressor, is an essential candidate for study. T-cell specific deficiency of PTEN leads to increased thymic cellularity due to a defect in negative selection. In male HY TCR transgenic mice, in which most DP thymocytes

normally undergo apoptosis, deficiency of PTEN increases the absolute number and the proportion of DP cells. In PTEN-deficient mice, CD4<sup>+</sup> cells accumulate in the periphery and the mice die prematurely at 17 weeks of age due to the formation of lymphomas. T cells from these mice display autoreactivity, a reduced apoptotic response to superantigen and serum, and cytokine withdrawal [40\*\*]. How the loss of PTEN protects thymocytes from negative selection is not clear, but the most likely scenario involves an increased activity of AKT, which leads to overproduction of anti-apoptotic molecules or enhanced repression of pro-apoptotic proteins.

In addition to their role in death by neglect, the Bcl-2 family members are also involved in negative selection. Bim deficiency renders thymocytes resistant to apoptosis induced by anti-CD3 antibodies or antigen in OT-II TCR transgenic mice and male HY TCR transgenic mice. These mice display a twofold to threefold increase in mature B and T cells in the periphery and, with age, they are susceptible to lymphadenopathy and autoimmune diseases mimicking Bcl-2 transgenic mice [41\*\*]. Similarly, mice with deletions of Bax and Bak, two pro-apoptotic Bcl-2 family members that are required for the Bim activity, also exhibit defects in negative selection [6\*\*]. The mechanism of Bim-mediated apoptosis requires translocation of the molecule from the dynein motor complex to the mitochondria where it interacts with Bcl-2 and Bcl-xL [42]. Overexpression of Bcl-2 or Bcl-xL protein, however, does not protect thymocytes from negative selection, suggesting that many pathways lead to negative selection, only one of which involves the Bcl-2 family members. How TCR signaling via the MAP kinases and PTEN in turn regulates Bim translocation remains to be elucidated.

It is widely established that the activation of caspases is an essential part of the general mechanism of apoptosis. However, the role of caspases in negative selection is still not clear. Although it had previously been reported that peptide-mediated thymocyte deletion can be blocked by the pan-caspase inhibitor zVAD-FMK [43], more recent reports show that zVAD-FMK is ineffective in halting negative selection in TCR transgenic mice [44,45]. The analysis of transgenic mice expressing p35, a general caspase inhibitor that binds to caspases-1, -3, -4, -6, -7 and -8, has led to mixed results regarding negative selection [45,46]. Abrogation of Apaf-1, which is part of the cytochrome-c/Apaf-1/caspase-9 complex, does not perturb negative selection [44]. However, overexpression of a baculoviral protein inhibitor of apoptosis (IAP), a protein that binds to and inhibits caspase activities, leads to partial inhibition of negative selection [47]. Taken together, these data argue that caspases might play a part in negative selection but, in the absence of caspase activation, caspase-independent pathways are sufficient to induce negative selection. These pathways may

include the translocation of apoptosis-inducing factor (AIF) or DNase endoG from mitochondria to the nucleus [48,49], or other yet-to-be identified pathways.

### Regulation of B-cell apoptosis

In contrast to T cells, the apoptotic response of B cells triggered by B-cell receptor (BCR) engagement in many cases simulates death by neglect. The crosslinking of BCRs induces immature and mature B cells to undergo apoptosis, unless co-stimulation by IL-4, CD40L or B-cell activating factor (BAFF) amongst others, provides a counteractive survival signal. Thus, apoptosis of a B cell reflects the lack of appropriate co-stimulation. Biochemically, inhibition of apoptosis has been linked to activation of NF- $\kappa$ B and the PI3K pathway, and the induction of anti-apoptotic Bcl-2 family members.

BAFF (also called BLys, TALL-1, THANK or zTNF4) is a fairly recent addition to the growing list of B-cell co-stimulatory molecules. It is a TNF family cytokine that interacts with three different receptors (BCMA, TACI and BAFF-R) to regulate the NF- $\kappa$ B pathway. *In vitro*, BAFF increases the survival of immature and mature B cells [50]. Analyses of mice lacking BAFF, TACI or BAFF-R have indicated that BAFF, for the most part, inhibits the apoptosis of B cells, so that its absence reduces the number of circulating B cells [51,52\*,53\*].

The events immediately following BCR engagement resemble TCR-mediated signaling, including the activation of Src- and Syk-family PTKs, PKC and MAP kinase pathways (reviewed in [54]). Recent data suggest that the activation of PKC plays an important role in regulating BCR signals. Mice lacking PKC $\beta$  exhibit immunodeficiency with overall increases in the phosphorylation of PTK and Ca<sup>2+</sup> signaling [55], suggesting that hyper-responsiveness may also induce apoptosis in B cells. Deficiency of PKC $\zeta$  in mice was recently reported to accelerate B-cell apoptosis *in vitro*, with defects in the induction of NF- $\kappa$ B-dependent genes and an impaired humoral response [56]. Interestingly, deficiency of PKC $\delta$  in mice results in an accumulation of hyperproliferative B cells and production of autoreactive antibodies without a block in the apoptotic response [57\*,58\*]. Taken together, these data suggest that PKC-dependent regulation of pro- and anti-apoptotic signals in B cells (modifiable by co-stimulation) is complex, perhaps involving direct and feedback mechanisms to modulate the 'strengths' of BCR signals. Moreover, employing multiple species of PKC may permit the divergence of BCR signals to a multitude of downstream pathways.

The importance of PI3K in B-cell apoptosis was revealed by disruption of the gene encoding the PI3K catalytic subunit p110 $\delta$ . Mice lacking p110 $\delta$  exhibit a block in differentiation of B1 and marginal zone B cells, together with a concomitant increase in apoptosis, decreased AKT

activity and reduced Bcl-xL levels [59\*]. In addition, an increased number of B cells accumulate in mice lacking PTEN, a negative regulator of PI3K.

It remains unclear if MAP kinases are required for B-cell apoptosis, but most data suggest that MAP kinases play a minor role. B cells develop normally and are not reduced in number in Jnk1- [15], Jnk2- [14], or Mekk2- [21] deficient mice. Deficiency of MKK4 in B cells does not lead to defects in IgM-mediated apoptosis *in vitro*, although a fraction of mice develop lymphadenopathy with T- and B-cell expansion and activation [60], suggesting that the MKK4-Jnk pathway may play some role in the apoptosis of peripheral lymphocytes. Recently, the survival of pre-B cells transformed by the BCR-ABL translocation product has been shown to depend on the Jnk1 protein [61], indicating that the activation of Jnk1 may play an anti-apoptotic role in pre-B cells.

Earlier studies indicated that the mitochondrial pathway of apoptosis plays a significant role in B-cell apoptosis. In further support of this notion, and in parallel to their effects on T cells, Bim-deficient mice also exhibit defects in B-cell apoptosis, leading to an accumulation of B cells [5]. However, biochemical events leading from the antigen receptor to the mitochondria remain unresolved.

### Clearance of apoptotic cells

Despite the high numbers of apoptotic cells that are generated in the course of lymphocyte maturation and immune responses, apoptotic cells do not normally accumulate because of their rapid removal by scavenger cells. This is important for the prevention of secondary necrosis and the release of pro-inflammatory cytokines, which can injure surrounding tissues (reviewed in [62]). Recent advances in understanding the regulation of clearance of apoptotic cells have been facilitated by identification of receptors that specifically recognize apoptotic cells. Among these are the scavenger receptors, complement receptors, CD14, CD36, and phosphatidylserine (PS)-specific receptors. Defects in several of these receptors have been directly linked to increased accumulation of apoptotic cells, heightened inflammatory responses and autoimmune phenotypes in mice. A PS-specific receptor, c-mer, is a membrane tyrosine kinase expressed on macrophages and it binds to PS exposed on the surfaces of apoptotic cells via GAS6 [63]. Elimination of the intracellular domain of c-mer greatly inhibits the uptake of apoptotic cells by macrophages, leading to an accumulation of apoptotic bodies in lymphoid organs [64]. Moreover, these mice develop lupus-like autoimmune disease over time, including increased production of anti-chromatin antibodies and renal glomerulonephritis [65\*]. These phenotypes recapitulate the characteristics of C1q-deficient mice [66], which are also implicated in the clearance of apoptotic cells [67]. These data support the hypothesis that, in certain autoimmune diseases, the

impaired removal of dying cells may be directly responsible for the inappropriate priming of lymphocytes and a dysregulated inflammatory response. More recently, MFG-E8, a secreted glycoprotein expressed abundantly in mammary glands, was shown to mediate the binding of aminophospholipids (expressed on apoptotic cells) to  $\alpha_v\beta_3$  integrin (expressed on scavenger cells) [68\*\*]. It was proposed that MFG-E8 facilitates the rapid clearance of apoptotic epithelial cells during involution of mammary tissue. Thus, the employment of secreted factors that act as adaptors between apoptotic and scavenger cells may constitute an additional regulatory process in the clearance of apoptotic cells.

## Conclusions

Many unresolved questions remain in our understanding of lymphoid development. For example, the precise nature of antigen receptor-mediated survival versus death signaling and the effector phase of apoptosis in central tolerance are still unclear. Recent advances have revealed the roles of the Bcl-2 family proteins Bim, Bax and Bak in T- and B-cell apoptosis. However, the mitochondrial 'apoptosome' was shown to be dispensable for thymocyte apoptosis, implying a potentially novel mechanism of regulation by the Bcl-2 family proteins. Recent studies also suggest a new level of apoptotic regulation imposed by PTEN, which antagonizes the PI3K/AKT pathway, although activation of PTEN by antigen receptor engagement has yet to be demonstrated. The requirement for caspases in T cell negative selection remains controversial, but the apparent inconsistencies obtained in different studies point to an existence of caspase-independent pathways. Despite many similarities, T and B cells employ different mechanisms to distinguish apoptotic and survival signals. In T cells, a quantitative interpretation of the effects of *grb2*-heterozygosity adds to the model of signal strength in cell fate decisions. In B cells, the inclusion of co-stimulatory signals leads to activation of the anti-apoptotic pathways, reflecting that a more qualitative distinction is involved in cell fate decisions. Further studies of the signaling pathways in T and B cells should lead to a significant understanding of how the immune system develops and how the decision to die or live is made.

## Acknowledgements

The first two authors contributed equally to the writing of this review. We thank Zheng Xing and Yuri Cho for the critical reading of this manuscript and apologize to those whose references we cannot cite due to space constraint. The work performed in this laboratory is supported by grants from the National Institutes of Health CA66236, CA75162 and CA92000.

## References and recommended readings

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Hildeman DA, Zhu Y, Mitchell TC, Kappler J, Marrack P: **Molecular mechanisms of activated T cell death *in vivo***. *Curr Opin Immunol* 2002, **14**:354-359.
  2. Newton K, Harris AW, Bath ML, Smith KGC, Strasser A: **A dominant interfering mutant of FADD/MORT1 enhance deletion of autoreactive thymocytes and inhibits proliferation of mature T lymphocytes**. *EMBO J* 1998, **17**:706-718.
  3. Ashwell JD, Lu FW, Vacchio MS: **Glucocorticoids in T cell development and function**. *Annu Rev Immunol* 2000, **18**:309-345.
  4. Brewer JA, Kanagawa O, Sleckman BP, Muglia LJ: **Thymocyte apoptosis induced by T cell activation is mediated by glucocorticoids *in vivo***. *J Immunol* 2002, **169**:1837-1843.  
GR-deficient thymocytes, derived from *GR<sup>-/-</sup>* fetal liver, develop normally and exhibit normal sensitivity to anti-CD3 antibody stimulation *in vitro*, whereas sensitivity to corticosterone is reduced. Interestingly, apoptosis mediated by anti-CD3 *in vivo* is abolished in these mice, demonstrating for the first time that anti-CD3 antibody mediates apoptosis through induction of glucocorticoid.
  5. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, Adams JM, Strasser A: **Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity**. *Science* 1999, **286**:1735-1738.
  6. Rathmell JC, Lindsten T, Zong WX, Cinalli RM, Thompson CB: **Deficiency in Bak and Bax perturbs thymic selection and lymphoid homeostasis**. *Nat Immun* 2002, **3**:932-939.  
The authors show that *bak<sup>-/-</sup>bax<sup>-/-</sup>* double-knockout thymocytes are resistant to death by neglect and apoptosis triggered by dexamethasone, superantigen or anti-CD3 antibodies. Peripheral T cells are also defective in apoptosis, as *bak<sup>-/-</sup>bax<sup>-/-</sup>* mice exhibit splenomegaly.
  7. Marsden VS, O'Connor L, O'Reilly LA, Silke J, Metcalf D, Ekert PG, Huang DC, Cecconi F, Kuida K, Tomaselli KJ *et al.*: **Apoptosis initiated by Bcl-2-regulated caspase activation independently of the cytochrome c/Apaf-1/caspase-9 apoptosome**. *Nature* 2002, **419**:634-637.  
This paper shows that loss of either Apaf-1 or caspase-9 does not affect cell death in all the apoptotic conditions examined. This is in contrast to the widely believed notion that cytochrome-c/caspase-9/apaf-1 is the initiator of cell death. These data lead the authors to propose that there are Apaf-1/Caspase-9 independent pathways that can be antagonized by Bcl-2 and the apoptosome mainly acts as an amplification system in apoptosis.
  8. Alberola-Ila J, Takaki S, Kerner JD, Perlmutter RM: **Differential signaling by lymphocyte antigen receptors**. *Annu Rev Immunol* 1997, **15**:125-154.
  9. Hogquist KA: **Signal strength in thymic selection and lineage commitment**. *Curr Opin Immunol* 2001, **13**:225-231.
  10. Berg LJ, Kang J: **Molecular determinants of TCR expression and selection**. *Curr Opin Immunol* 2001, **13**:232-241.
  11. Dong C, Davis RJ, Flavell RA: **MAP kinases in the immune response**. *Annu Rev Immunol* 2002, **20**:55-72.
  12. Pages G, Guerin S, Grall D, Bonino F, Smith A, Anjuere F, Auberger P, Pouyssegur J: **Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice**. *Science* 1999, **286**:1374-1377.
  13. Swan KA, Alberola-Ila J, Gross JA, Appleby MW, Forbush KA, Thomas JF, Perlmutter RM: **Involvement of p21ras distinguishes positive and negative selection in thymocytes**. *EMBO J* 1995, **14**:276-285.
  14. Sabapathy K, Hu Y, Kallunki T, Schreiber M, David JP, Jochum W, Wagner EF, Karin M: **JNK2 is required for efficient T-cell activation and apoptosis but not for normal lymphocyte development**. *Curr Biol* 1999, **9**:116-125.
  15. Sabapathy K, Kallunki T, David JP, Graef I, Karin M, Wagner EF: **c-Jun NH2-terminal kinase (JNK)1 and JNK2 have similar and stage-dependent roles in regulating T cell apoptosis and proliferation**. *J Exp Med* 2001, **193**:317-328.
  16. Adams RH, Porras A, Alonso G, Jones M, Vintersten K, Panelli S, Valladares A, Perez L, Klein R, Nebreda AR: **Essential role of p38alpha MAP kinase in placental but not embryonic cardiovascular development**. *Mol Cell* 2000, **6**:109-116.
  17. Mudgett JS, Ding J, Guh-Siesel L, Chartrain NA, Yang L, Gopal S, Shen MM: **Essential role for p38alpha mitogen-activated protein kinase in placental angiogenesis**. *Proc Natl Acad Sci USA* 2000, **97**:10454-10459.

18. Werlen G, Hausmann B, Palmer E: **A motif in the alphabeta T-cell receptor controls positive selection by modulating ERK activity.** *Nature* 2000, **406**:422-426.
19. Tanaka N, Kamanaka M, Enslin H, Dong C, Wysk M, Davis RJ, Flavell RA: **Differential involvement of p38 mitogen-activated protein kinase kinases MKK3 and MKK6 in T-cell apoptosis.** *EMBO Rep* 2002, **3**:785-791.
20. Nishina H, Radvanyi L, Raju K, Sasaki T, Kozieradzki I, Penninger JM: **Impaired TCR-mediated apoptosis and Bcl-XL expression in T cells lacking the stress kinase activator SEK1/MKK4.** *J Immunol* 1998, **161**:3416-3420.
21. Guo Z, Clydesdale G, Cheng J, Kim K, Gan L, McConkey DJ, Ullrich SE, Zhuang Y, Su B: **Disruption of MekK2 in mice reveals an unexpected role for MEKK2 in modulating T-cell receptor signal transduction.** *Mol Cell Biol* 2002, **22**:5761-5768.
22. Gong Q, Cheng AM, Akk AM, Alberola-Ila J, Gong G, Pawson T, Chan AC: **Disruption of T cell signaling networks and development by Grb2 haploid insufficiency.** *Nat Immun* 2001, **2**:29-36.
- The authors show that mice with a disruption of a single allele of *grb2* exhibit attenuated activation of Jnk and p38, but normal ERK1/2 activation, in response to treatment with anti-CD3/CD28 antibodies. In transgenic TCR models, *grb2* heterozygosity correlates with defects specifically in negative selection, demonstrating that quantitative differences in Grb2 activity correlate with the activation of distinct MAP kinase pathways and the decision of apoptosis and survival.
23. Yoshino T, Kishi H, Nagata T, Tsukada K, Saito S, Muraguchi A: **Differential involvement of p38 MAP kinase pathway and Bax translocation in the mitochondria-mediated cell death in TCR- and dexamethasone-stimulated thymocytes.** *Eur J Immunol* 2001, **31**:2702-2708.
24. Yang CC, Ornatsky OI, McDermott JC, Cruz TF, Prody CA: **Interaction of myocyte enhancer factor 2 (MEF2) with a mitogen-activated protein kinase, ERK5/BMK1.** *Nucleic Acids Res* 1998, **26**:4771-4777.
25. Kasler HG, Victoria J, Duramad O, Winoto A: **ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain.** *Mol Cell Biol* 2000, **20**:8382-8389.
26. Mody N, Leitch J, Armstrong C, Dixon J, Cohen P: **Effects of MAP kinase cascade inhibitors on the MKK5/ERK5 pathway.** *FEBS Lett* 2001, **502**:21-24.
27. Sun W, Kesavan K, Schaefer BC, Garrington TP, Ware M, Johnson NL, Gelfand EW, Johnson GL: **MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway.** *J Biol Chem* 2001, **276**:5093-5100.
28. Chao TH, Hayashi M, Tapping RI, Kato Y, Lee JD: **MEKK3 directly regulates MEK5 activity as part of the big mitogen-activated protein kinase 1 (BMK1) signaling pathway.** *J Biol Chem* 1999, **274**:36035-36038.
29. Regan CP, Li W, Boucher DM, Spatz S, Su MS, Kuida K: **Erk5 null mice display multiple extraembryonic vascular and embryonic cardiovascular defects.** *Proc Natl Acad Sci USA* 2002, **99**:9248-9253.
30. Sohn SJ, Sarvis BK, Cado D, Winoto A: **ERK5 MAP kinase regulates embryonic angiogenesis and acts as a hypoxia-sensitive repressor of VEGF expression.** *J Biol Chem* 2002, **277**:43344-43351.
31. Winoto A, Littman DR: **Nuclear hormone receptors in T lymphocytes.** *Cell* 2002, **109**(Suppl):S57-S66.
32. Liu ZG, Smith SW, McLaughlin KA, Schwartz LM, Osborne BA: **Apoptotic signals delivered through the T-cell receptor of a T-cell hybrid require the immediate-early gene *nur77*.** *Nature* 1994, **367**:281-284.
33. Woronicz JD, Calnan B, Ngo V, Winoto A: **Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas.** *Nature* 1994, **367**:277-281.
34. Lee SL, Wesselschmidt RL, Linette GP, Kanagawa O, Russell JH, Milbrandt J: **Unimpaired thymic and peripheral T cell death in mice lacking the nuclear receptor NGFI-B (Nur77).** *Science* 1995, **269**:532-535.
35. Ponnio T, Burton Q, Pereira FA, Wu DK, Conneely OM: **The nuclear receptor Nor-1 is essential for proliferation of the semicircular canals of the mouse inner ear.** *Mol Cell Biol* 2002, **22**:935-945.
36. Kuang AA, Cado D, Winoto A: **Nur77 transcription activity correlates with its apoptotic function *in vivo*.** *Eur J Immunol* 1999, **29**:3722-3728.
37. Li H, Kolluri SK, Gu J, Dawson MI, Cao X, Hobbs PD, Lin B, Chen G, Lu J, Lin F *et al.*: **Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3.** *Science* 2000, **289**:1159-1164.
38. Pekarsky Y, Hallas C, Palamarchuk A, Koval A, Bullrich F, Hirata Y, Bichi R, Letofsky J, Croce CM: **Akt phosphorylates and regulates the orphan nuclear receptor Nur77.** *Proc Natl Acad Sci USA* 2001, **98**:3690-3694.
- See annotation to [39\*].
39. Masuyama N, Oishi K, Mori Y, Ueno T, Takahama Y, Gotoh Y: **Akt inhibits the orphan nuclear receptor Nur77 and T-cell apoptosis.** *J Biol Chem* 2001, **276**:32799-32805.
- These two papers [38\*,39\*] demonstrate how AKT phosphorylation can affect transcriptional and apoptotic activities of Nur77.
40. Suzuki A, Yamaguchi MT, Ohteki T, Sasaki T, Kaisho T, Kimura Y, Yoshida R, Wakeham A, Higuchi T, Fukumoto M *et al.*: **T cell-specific loss of Pten leads to defects in central and peripheral tolerance.** *Immunity* 2001, **14**:523-534.
- Deficiency of PTEN, a negative regulator of the PI3K pathway, leads to defective negative selection, autoimmunity and increased mortality due to the accumulation of CD4<sup>+</sup> T cell lymphomas. Of great interest is how the loss of PTEN from T cells leads to defects in central and peripheral tolerance.
41. Bouillet P, Purton JF, Godfrey DI, Zhang LC, Coultas L, Puthalakath H, Pellegrini M, Cory S, Adams JM, Strasser A: **BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes.** *Nature* 2002, **415**:922-926.
- Bim-deficient thymocytes display increased resistance to apoptosis induced by *in vitro* treatment with anti-CD3 antibodies as well as *in vivo* stimulation with superantigen and cognate peptide antigen.
42. Puthalakath H, Huang DC, O'Reilly LA, King SM, Strasser A: **The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex.** *Mol Cell* 1999, **3**:287-296.
43. Clayton LK, Ghendler Y, Mizoguchi E, Patch RJ, Ocain TD, Orth K, Bhan AK, Dixit VM, Reinherz EL: **T-cell receptor ligation by peptide/MHC induces activation of a caspase in immature thymocytes: the molecular basis of negative selection.** *EMBO J* 1997, **16**:2282-2293.
44. Hara H, Takeda A, Takeuchi M, Wakeham AC, Itie A, Sasaki M, Mak TW, Yoshimura A, Nomoto K, Yoshida H: **The apoptotic protease-activating factor 1-mediated pathway of apoptosis is dispensable for negative selection of thymocytes.** *J Immunol* 2002, **168**:2288-2295.
45. Doerfler P, Forbush KA, Perlmutter RM: **Caspase enzyme activity is not essential for apoptosis during thymocyte development.** *J Immunol* 2000, **164**:4071-4079.
46. Izquierdo M, Grandien A, Criado LM, Robles S, Leonardo E, Albar JP, de Buitrago GG, Martinez AC: **Blocked negative selection of developing T cells in mice expressing the baculovirus p35 caspase inhibitor.** *EMBO J* 1999, **18**:156-166.
47. Robles MS, Leonardo E, Criado LM, Izquierdo M, Martinez AC: **Inhibitor of apoptosis protein from *Orgyia pseudotsugata* nuclear polyhedrosis virus provides a costimulatory signal required for optimal proliferation of developing thymocytes.** *J Immunol* 2002, **168**:1770-1779.
48. Joza N, Susin SA, Dugas E, Stanford WL, Cho SK, Li CY, Sasaki T, Elia AJ, Cheng HY, Ravagnan L *et al.*: **Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death.** *Nature* 2001, **410**:549-554.
49. Li LY, Luo X, Wang X: **Endonuclease G is an apoptotic DNase when released from mitochondria.** *Nature* 2001, **412**:95-99.

50. Do RK, Hatada E, Lee H, Tourigny MR, Hilbert D, Chen-Kiang S: **Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response.** *J Exp Med* 2000, **192**:953-964.
51. Yan M, Marsters SA, Grewal IS, Wang H, Ashkenazi A, Dixit VM: **Identification of a receptor for BLYS demonstrates a crucial role in humoral immunity.** *Nat Immun* 2000, **1**:37-41.
52. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-  
 • Morskaya S, Dobles M, Frew E, Scott ML: **An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway.** *Science* 2001, **293**:2111-2114.  
 BAFF-deficient mice display a dramatic loss of follicular and marginal zone B cells and lack a serum antibody response. Of note is the finding that a deficiency of BCMA, one of the BAFF receptors, leads to no significant alterations in B cell survival response or development, indicating a redundancy in BAFF and the BAFF receptor system.
53. Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T,  
 • Tumas D, Grewal IS, Dixit VM: **Activation and accumulation of B cells in TACI-deficient mice.** *Nat Immun* 2001, **2**:638-643.  
 Transmembrane activator and calcium-modulating and cyclophilin ligand interactor (TACI)-deficient mice possess increased numbers of B cells, which hyperproliferate and produce elevated levels of immunoglobulin *in vitro*. This suggests a role for TACI in mediating apoptotic signals in B cells, a notion that appears to contradict the presumed role of BAFF in promoting B cell survival. *In vivo*, TACI<sup>-/-</sup> B cells mount an enhanced response to T cell-dependent antigens while mounting a decreased response to T cell-independent antigens, indicating that the outcome of BAFF-mediated signals may be context-dependent.
54. DeFranco AL: **The complexity of signaling pathways activated by the BCR.** *Curr Opin Immunol* 1997, **9**:296-308.
55. Leitges M, Schmedt C, Guinamard R, Davoust J, Schaal S, Stabel S, Tarakhovskiy A: **Immunodeficiency in protein kinase cbeta-deficient mice.** *Science* 1996, **273**:788-791.
56. Martin P, Duran A, Minguet S, Gaspar ML, Diaz-Meco MT, Rennert P, Leitges M, Moscat J: **Role of zeta PKC in B-cell signaling and function.** *EMBO J* 2002, **21**:4049-4057.
57. Mecklenbrauker I, Saijo K, Zheng NY, Leitges M, Tarakhovskiy A:  
 • **Protein kinase Cδ controls self-antigen-induced B-cell tolerance.** *Nature* 2002, **416**:860-865.  
 Mice deficient for PKCδ develop splenomegaly and lymphadenopathy due to an increased number of B cells in peripheral lymphoid organs. In addition, B cell antigen-specific tolerance is defective in these mice.
58. Miyamoto A, Nakayama K, Imaki H, Hirose S, Jiang Y, Abe M,  
 • Tsukiyama T, Nagahama H, Ohno S, Hatakeyama S *et al.*: **Increased proliferation of B cells and auto-immunity in mice lacking protein kinase Cδ.** *Nature* 2002, **416**:865-869.  
 This paper also demonstrates the hyperproliferative response and accumulation of B cells in mice deficient for PKCδ.
59. Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A,  
 • Humphries LA, Rawlings D, Reynolds H, Vigorito E *et al.*: **A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase in B cell development and activation.** *J Exp Med* 2002, **196**:753-763.  
 p110δ<sup>-/-</sup> mice possess reduced numbers of B cells. These cells are hyporesponsive *in vitro* when stimulated with anti-IgM antibodies and are unresponsive to co-stimulatory signals triggered through CD40 or IL-4. In addition, p110δ<sup>-/-</sup> B cells fail to activate the AKT (PKB) and NF-κB pathways in response to anti-IgM crosslinking.
60. Swat W, Fujikawa K, Ganiatsas S, Yang D, Xavier RJ, Harris NL, Davidson L, Ferrini R, Davis RJ, Labow MA *et al.*: **SEK1/MKK4 is required for maintenance of a normal peripheral lymphoid compartment but not for lymphocyte development.** *Immunity* 1998, **8**:625-634.
61. Hess P, Pihan G, Sawyers CL, Flavell RA, Davis RJ: **Survival signaling mediated by c-Jun NH<sub>2</sub>-terminal kinase in transformed B lymphoblasts.** *Nat Genet* 2002, **32**:201-205.
62. Savill J, Fadok V: **Corpse clearance defines the meaning of cell death.** *Nature* 2000, **407**:784-788.
63. Funakoshi H, Yonemasu T, Nakano T, Matumoto K, Nakamura T: **Identification of Gas6, a putative ligand for Sky and Axl receptor tyrosine kinases, as a novel neurotrophic factor for hippocampal neurons.** *J Neurosci Res* 2002, **68**:150-160.
64. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK: **Phagocytosis and clearance of apoptotic cells is mediated by MER.** *Nature* 2001, **411**:207-211.
65. Cohen PL, Caricchio R, Abraham V, Camenisch TD, Jennette JC,  
 • Roubey RA, Earp HS, Matsushima G, Reap EA: **Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase.** *J Exp Med* 2002, **196**:135-140.  
 Mice expressing an inactive form of c-mer (mer<sup>kd</sup>) develop a lupus-like autoimmune phenotype with age, confirming the significance of phagocytosis and clearance of apoptotic cells in preventing an inflammatory response and potential cross-reactivity to self-antigen.
66. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, Loos M, Pandolfi PP, Walport MJ: **Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies.** *Nat Genet* 1998, **19**:56-59.
67. Taylor PR, Carugati A, Fadok VA, Cook HT, Andrews M, Carroll MC, Savill JS, Henson PM, Botto M, Walport MJ: **A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells *in vivo*.** *J Exp Med* 2000, **192**:359-366.
68. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata  
 •• S: **Identification of a factor that links apoptotic cells to phagocytes.** *Nature* 2002, **417**:182-187.  
 This paper describes the identification of MFG-E8, a glycoprotein secreted by activated macrophages. MFG-E8 acts as a linker molecule that simultaneously interacts with aminophospholipids displayed on the surface of apoptotic cells and α<sub>v</sub>β<sub>3</sub> integrin on phagocytes, thus aiding in the phagocytosis and clearance of apoptotic cells.