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The Paracaspase MALT1 Controls Caspase-8 Activation during Lymphocyte Proliferation

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SUMMARY

Caspase-8, an initiator caspase involved in lymphocyte apoptosis, is paradoxically required for lymphocyte proliferation. It is not understood how caspase-8 is controlled during antigenic signaling to allow for activation while averting the triggering of apoptosis. Here, we show that caspase-8 undergoes limited activation upon antigenic stimulation, and this activation is dependent on the paracaspase MALT1. The paracaspase domain of MALT1, in a protease-independent manner, induces caspase-8 activation through direct association. MALT1 diminishes the activation of apoptotic effector caspases, but it does not alter the activity of caspase-8 toward c-FLIP$_L$, which is required for antigenic signaling. Mutants of MALT1 that fail to activate caspase-8 and permit c-FLIP$_L$ cleavage cannot facilitate NF-κB activation or IL-2 induction. Our results reveal a mechanism that utilizes a protease potentially deadly to the cell for proliferative signaling and demonstrate a functional connection between the caspase and paracaspase families to enable non-apoptotic processes.

INTRODUCTION

Maintenance of immune homeostasis is critical for the elimination of foreign antigens while preventing autoimmunity and hyperproliferative diseases. This balance is characterized by a rapid clonal expansion of antigen-reactive lymphocytes followed by targeted apoptosis of activated cells. Caspase-8 plays an integral role in lymphocyte apoptosis through engagement by death receptors including CD95 (Fas/Apo-1), tumor necrosis factor receptor 1 (TNFR1), and TRAIL receptors (Krammer et al., 2007). Upon death receptor stimulation, the precursor of caspase-8 (procaspase-8) is recruited to the oligomeric membrane-associated death-inducing signaling complex (DISC). There, procaspase-8 acquires protease activity upon dimerization (Boatright et al., 2003; Chang et al., 2003) and subsequently undergoes two autocleavage events via an interdimer processing mechanism to yield the active mature form (Chang et al., 2003). Activation of caspase-8 in the DISC is regulated by the proteolytically inactive homolog c-FLIP$_L$, which is also a caspase-8 substrate (Chang et al., 2002; Micheau et al., 2002). Mature caspase-8 is released from the DISC and transcleaves effector caspases such as caspase-3 and caspase-7. The effector caspases then undergo a second autocleavage event, generating mature forms (Liu et al., 2005), which, in turn, cleave a large number of proteins to dismantle the cell (Figure 1A).

Paradoxical to its established role in lymphocyte apoptosis, caspase-8 is also essential for lymphocyte activation. Human and mouse lymphocytes defective in caspase-8 show profound defects in proliferation in response to antigen receptor engagement (Chun et al., 2002; Salmena et al., 2003), and this function of caspase-8 requires proteolytic activity (Su et al., 2005). During antigenic signaling, procaspase-8 associates with a complex formed by Bcl10 and MALT1 (Su et al., 2005), which links the receptor proximal signaling events to activation of the transcription factor NF-κB and induction of interleukin-2 (IL-2) (Thome, 2004). Chromosomal translocations resulting in upregulation and/or gain-of-function mutations of Bcl10 and MALT1 are associated with uncontrolled lymphocyte proliferation and lymphomas (Isaacson and Du, 2004). Bcl10 is an adaptor protein that recruits MALT1 to the receptor-associated lipid rafts, whereas MALT1 is a member of the paracaspase family, classified by a paracaspase domain that is most similar, yet still distantly related, to the protease domain of caspases (Uren et al., 2000). Recent studies demonstrated that the paracaspase domain of MALT1 possesses protease activity, cleaving Bcl10 and the NF-κB inhibitor A20 (Coornaert et al., 2008; Rebeaud et al., 2008). However, the protease activity of MALT1 plays a fine-tuning rather than an essential role in antigenic signaling. Paracaspases, like caspases, are found in metazoans ranging from *Caenorhabditis elegans* to human (Uren et al., 2000), yet the functional relationship between these two related proteases remains unclear.

The dual role of caspase-8 in apoptosis and cell proliferation raises a central question as to how caspase-8 becomes activated in antigenic signaling to enable proliferative signaling while averting the triggering of apoptosis. In this study, we uncover a mechanism of caspase activation involving heterodimerization between caspase-8 and the paracaspase MALT1. The MALT1 paracaspase domain, independently of protease activity, promotes procaspase-8 to undergo limited autoproteolytic processing upon heterodimerization. This generates an active form of caspase-8 that exhibits diminished activity toward caspase-3. Yet, MALT1 still enables caspase-8 to cleave c-FLIP$_L$, which,

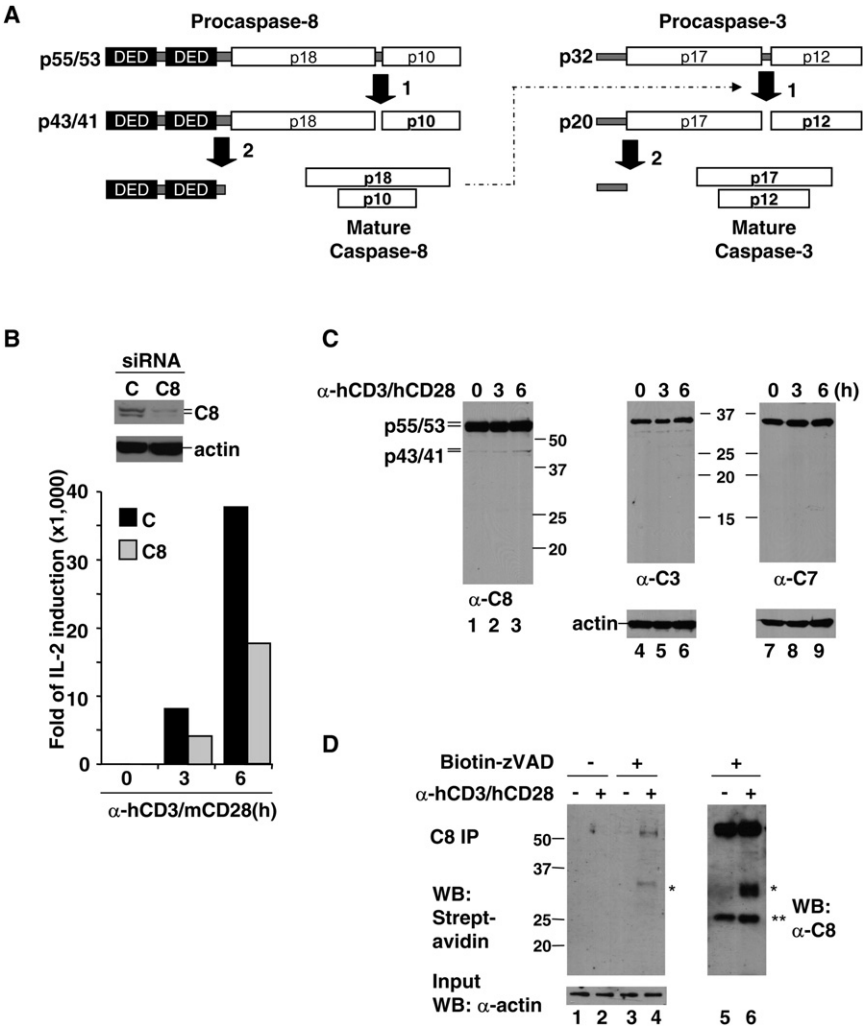

Figure 1. Proliferative Function of Caspase-8 Is Related to Its Activation, but Not Full Processing

(A) Schematic of caspase-8 and caspase-3 processing.

(B) Human primary CD4+ T cells transfected with control (C) or caspase-8 (C8) siRNA plus m/hCD28 were stimulated with α-hCD3/mCD28 antibodies. Caspase-8 expression was analyzed by immunoblotting (top) and IL-2 induction by quantitative RT-PCR (bottom).

(C) CD4+ T cells were stimulated with α-hCD3/hCD28 for the indicated durations, and processing of caspase-8, caspase-3 (C3), and caspase-7 (C7) was analyzed by immunoblotting.

(D) CD4+ cells treated with α-hCD3/hCD28 were incubated with biotin-zVAD-fmk or DMSO. Lysates were immunoprecipitated with α-caspase-8 and were analyzed with streptavidin-HRP (left) or α-caspase-8 (right). *Uncharacterized bands. **IgG light-chain bands.

akin to caspase-8, is involved in lymphocyte proliferation (Chau et al., 2005; Zhang and He, 2005). Thus, MALT1 controls caspase-8 activation to favor proliferative over apoptotic signaling.

RESULTS

Procaspase-8 Is Activated, but Not Significantly Processed, at Early Stages of T Cell Activation

To assess the involvement of caspase-8 processing in antigen receptor signaling, we knocked down the expression of caspase-8 in purified human primary CD4+ T cells by using siRNA (Figure 1B, top panel). To limit the antigenic response to siRNA-treated cells, we also used a chimeric CD28—consisting of the mouse CD28 extracellular domain and the human CD28 cytoplasmic tail (m/hCD28)—that transmits costimulatory signals in transfected human T cells in response to an agonistic anti-mouse CD28 antibody (Parry et al., 2003). Treatment with anti-human CD3 plus anti-mouse CD28 antibodies strongly induced the expression of IL-2 in control siRNA-treated cells as early as 3–6 hr after treatment. However, IL-2 induction was significantly impaired when caspase-8 was knocked down (Figure 1B, bottom panel), consistent with previous results and indicating a critical function for caspase-8 at the early stages of T cell activation. Yet, during this timeframe, only a small amount of the caspase-8 p43/41 processing intermediate was generated, and the fully processed, apoptotic, mature p18 form was not detectable (Figure 1C).

This limited processing nevertheless suggested that procaspase-8 acquires enzymatic activity upon antigenic stimulation. To confirm, we applied a biotinylated labeling agent that covalently binds the active site of caspases, biotin-zVAD-fmk, to stimulated primary T cells. Procaspase-8 became activated within 1 hr upon anti-CD3/CD28 treatment (Figure 1D). Yet, despite its activation, caspase-8 did not engage the apoptotic pathway, as shown by the lack of appreciable processing and activation of its apoptotic substrates, effector caspase-3 and caspase-7 (Figure 1C). Therefore, during T cell receptor (TCR) signaling, caspase-8 is activated to a limited extent and does not initiate the apoptotic caspase cascade.

MALT1 Promotes Procaspase-8 Activation through the Paracaspase Domain

In human primary T cells, caspase-8 associates with MALT1 both prior to and after antigenic stimulation (Figure 2A). To examine the functional relationship between caspase-8 and MALT1-induced NF-κB activation, we used caspase-8 wild-type or -deficient Jurkat cells. In wild-type Jurkat cells, coexpression of both MALT1 and its activator Bcl10 strongly activated NF-κB (Figure 2B). However, this activation was impaired in caspase-8-deficient cells, indicating that caspase-8 functions downstream of