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# The CD95 signaling pathway

## To not die and fly

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**O**ur recent findings indicate that cells exposed to transmembrane (m-CD95L) or metalloprotease-cleaved CD95L (cl-CD95L) undergo a localized Ca<sup>2+</sup> entry that not only inhibits the initial steps of the CD95-mediated apoptotic signal but also promotes cell motility. Based on recent findings published on the non-apoptotic signals induced by CD95, we discuss how m-CD95L and cl-CD95L diverging by their stoichiometry could both contribute to the immune response by first recruiting activated T lymphocytes in the inflamed area and later by eliminating infected and transformed cells.

### Emergent Functions for the Death Receptor CD95

CD95 (also known as Fas) belongs to the TNF (Tumour Necrosis Factor) -receptor superfamily. Fifteen years ago, it has been shown that when exposed to an agonistic anti-CD95 mAb (APO1–3), the aggregated receptor recruits the adaptor protein FADD (Fas-Associated protein with Death Domain), which then binds caspase-8/-10 and ultimately elicits the apoptotic signal and the death of the cell. This complex was designated the DISC for Death Inducing Signaling Complex<sup>1</sup> and since numerous factors have been found to modulate the implementation of this complex and thus, the transmission of death receptor-mediated apoptotic signal. The cognate CD95 ligand, CD95L (also known as FasL or CD178) is a transmembrane “cytokine” belonging to the TNF family.

CD95L exhibits a restricted expression pattern, being expressed primarily at the surface of activated T lymphocytes and NK cells, where it contributes to the elimination of infected and transformed cells. However, CD95L is also found under inflammatory conditions, at the surface of epithelial cells, macrophages or dendritic cells where its biological function remains elusive. This type II transmembrane protein can be cleaved by metalloproteases such as MMP3,<sup>2</sup> MMP7,<sup>3</sup> MMP9<sup>4</sup> or ADAM-10 (A Disintegrin And Metalloproteinase 10)<sup>5,6</sup> and released as a soluble ligand into the connective tissue and the bloodstream. Cleaved CD95L (cl-CD95L) was described initially as an inert ligand competing with its membrane-bound and pro-apoptotic counterpart (m-CD95L) for binding to CD95.<sup>7,8</sup> More recent studies confirmed that the homotrimeric cl-CD95L fails to trigger cell death but more importantly, they also bring to light that this soluble ligand possesses indeed a biological function by eliciting non-apoptotic signals leading to cell migration<sup>9–13</sup> and/or proliferation.<sup>14</sup> In this regard, we and others demonstrated that the metalloprotease-processed CD95L actively participates in aggravating inflammation and autoimmunity both in mouse model<sup>12</sup> and humans affected by systemic lupus erythematosus (SLE).<sup>13</sup> Overall, these findings ascribe non-apoptotic roles to CD95 through the implementation of different signals (i.e., JNK, PI3K and NF-κB). The role(s) of each CD95-mediated non-apoptotic signal remains however to be finely characterized in pathophysiological contexts.

**Keywords:** lymphocyte, calcium, Orai1, Fas, FasL, TNF, apoptosis, migration, PI3K

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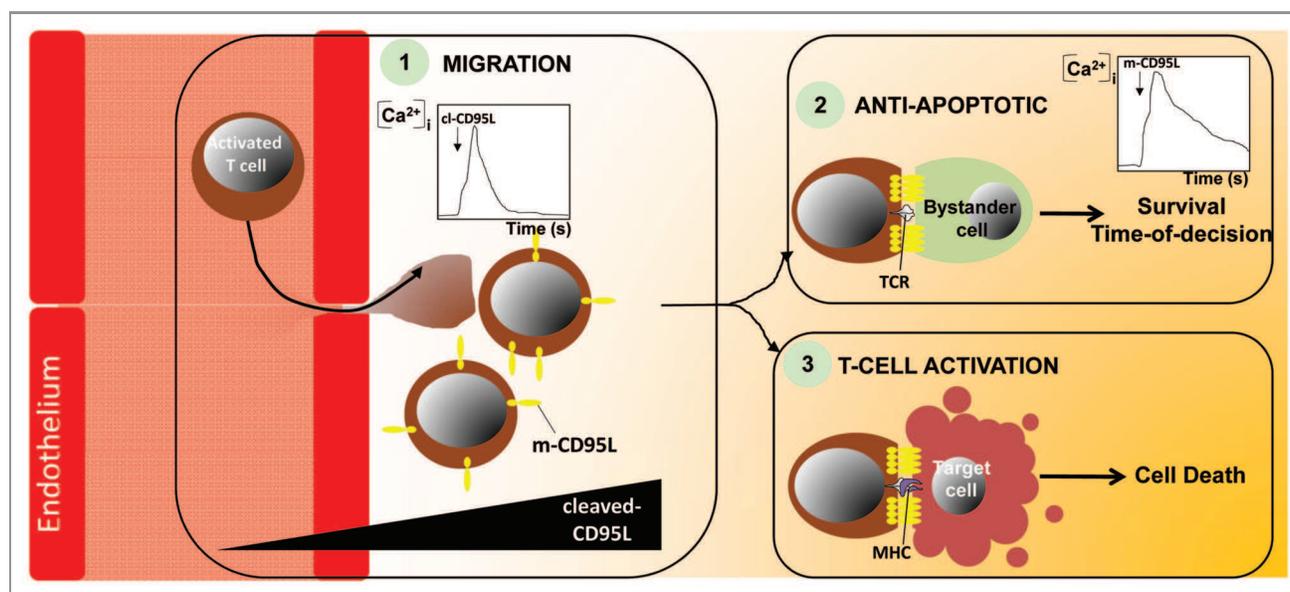
## A Novel Actor in the Initial Events of the CD95 Pathway

Calcium ions ( $\text{Ca}^{2+}$ ) participate in cell signaling as a second messenger that relies on magnitude (cytosolic concentration), temporal parameters (i.e., duration and frequency) and spatial localization to trigger a variety of cellular responses. Following membrane receptor stimulation,  $\text{Ca}^{2+}$  responses mainly occur through a biphasic signal caused by activation of IP3 receptors and the release of  $\text{Ca}^{2+}$  from the endoplasmic reticulum (ER) followed by a  $\text{Ca}^{2+}$  entry across the plasma membrane.<sup>15</sup> This store-operated  $\text{Ca}^{2+}$  entry (SOCE), mediated in T-lymphocytes by  $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  (CRAC) channels, plays a pivotal role in both the replenishment of the ER store and in cell signaling.<sup>16</sup> Recently, STIM1 was identified as the ER-stored  $\text{Ca}^{2+}$  sensor that links ER depletion to activation of the plasma membrane CRAC channel formed by Orai1 subunits, allowing  $\text{Ca}^{2+}$  to selectively enter the cell.<sup>17</sup> Following contact of a T cell with an antigen-presenting dendritic cell, STIM1 and Orai1 colocalize with T cell receptors (TCRs) in the immunological synapse and contribute to a localized  $\text{Ca}^{2+}$  influx.<sup>18</sup>

Tissues in which infected or transformed cells are disseminated require the recruitment of immune cells to specifically eliminate these threats. Based on our findings, we surmise that the first line of activated T lymphocytes infiltrating the transformed or infected area expresses high amount of membrane-bound CD95L to trigger cell death in affected cells but also to provide a pool of ligand that will be processed by metalloproteases therefore engendering a cl-CD95L gradient. This gradient would in turn recruit a second wave of activated T cells, which ultimately amplifies if necessary the immune response and leads to the total eradication of the target cells. Of note, we established that the amount of cl-CD95L is dramatically increased in sera of SLE patients and contributes to the endothelial transmigration of activated T cells that accumulate in the damaged organs. We also observed that cl-CD95L evokes a transient and localized SOCE (Fig. 1), which is instrumental in enhancing PI3K activation, actin remodeling and thus migration of activated T cells.<sup>13</sup> Seeking for their cellular targets, these migrating T cells may encounter CD95-expressing bystander cells in the inflamed tissue raising the question of how is prevented in these

healthy cells an accidental and irreversible activation of the apoptotic signal that will lead to their deleterious elimination. Our recent findings uncovered that engagement of CD95 by the membrane-bound form of CD95L (experimentally replaced by a home-made IgCD95L that mimics the membrane-bound multi-aggregated physiologic ligand) evokes a sustained and localized  $\text{Ca}^{2+}$  entry (Fig. 1), which freezes the initial steps of the CD95 apoptotic signal and doing so delays its delivery.<sup>19</sup> Although intensity and temporal parameters of the CD95-mediated  $\text{Ca}^{2+}$  signal diverge between cells exposed to cl-CD95L and m-CD95L, both ligands implement SOCE through the co-localization of CRAC channel Orai1 with CD95. In addition, we observed that whereas in presence of cl-CD95L, cells undergo the formation of a caspase and FADD-independent Motility-inducing signaling complex (MISC), m-CD95L stimulates the transient and  $\text{Ca}^{2+}$ -dependent recruitment of PKC-beta2 within DISC that participates in delaying the multi-protein complex formation and the transduction of the apoptotic signal.

Accordingly, these findings support the hypothesis that a non-specific T lymphocyte/bystander cell contact would



**Figure 1.** Role of the Orai1-driven  $\text{Ca}^{2+}$  entry in T lymphocytes challenged with the different forms of CD95L. When exposed to the two forms of CD95L, cleaved and membrane-bound, activated T lymphocytes, target cells (infected or transformed cells) or bystander cells undergo an Orai1-driven  $\text{Ca}^{2+}$  entry that modulates differently the CD95-mediated signaling pathway. The  $\text{Ca}^{2+}$  traces obtained with cells exposed to cleaved-CD95L (1) or membrane-bound CD95L (2) are depicted. MHC: Major Histocompatibility Complex.

transiently engage the CD95 receptor and achieve a Ca<sup>2+</sup>-dependent Time-Of-Decision (TOD) preventing the transmission of the CD95-mediated apoptotic signal in the target cell. In contrast, the selective recognition of the MHC/peptide by cytotoxic T lymphocytes may provide a sustained interaction that overrides the Ca<sup>2+</sup>-driven TOD allowing the selective elimination of infected or malignant cells. Numerous questions still remain to be addressed. First, whether cl-CD95L

induces cell motility of activated T cells in general or only on specific T-cell subpopulations involved in the etiology of the autoimmune disorders is still unknown. Second, how two ligands only distinguishable by their divergent stoichiometries, are able to evoke such different intracellular Ca<sup>2+</sup> patterns and biological outcomes using the same receptor is very puzzling, and finally, the molecular ordering leading to the activation of the Ca<sup>2+</sup> signal remain to be identified.

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