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Review

Insights into the molecular regulation of FasL (CD178) biology

Marcus Lettau, Maren Paulsen, Hendrik Schmidt, Ottmar Janssen*

Christian-Albrechts-University, Institute of Immunology, D-24105 Kiel, Germany

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ABSTRACT

Fas ligand (FasL, CD95L, APO-1L, CD178, TNFSF6, APT1LG1) is the key death factor of receptor-triggered programmed cell death in immune cells. FasL/Fas-dependent apoptosis plays a pivotal role in activation-induced cell death, termination of immune responses, elimination of autoreactive cells, cytotoxic effector function of T and NK cells, and the establishment of immune privilege. Deregulation or functional impairment of FasL threatens the maintenance of immune homeostasis and defense and results in severe autoimmunity. In addition, FasL has been implicated as an accessory or costimulatory receptor in T cell activation. The molecular mechanisms underlying this reverse signaling capacity are, however, poorly understood and still controversially discussed. Many aspects of FasL biology have been ascribed to selective protein–protein interactions mediated by a unique polyproline region located in the membrane-proximal intracellular part of FasL. Over the past decade, we and others identified a large number of putative FasL-interacting molecules that bind to this polyproline stretch via Src homology 3 or WW domains. Individual interactions were analyzed in more detail and turned out to be crucial for the lysosomal storage, the transport and the surface appearance of the death factor and potentially also for reverse signaling. This review summarizes the work in the framework of the Collaborative Research Consortium 415 (CRC 415) and provides facts and hypotheses about FasL-interacting proteins and their potential role in FasL biology.

Introduction

Fas ligand (FasL, APO-1L, CD95L, CD178, TNFSF6) is a member of the tumor necrosis factor (TNF) family of type II transmembrane proteins. Although FasL has been initially described as a T cell-associated effector protein, over the past years, it turned out that the molecule is also detectable in/on various other cell populations and tissues (Janssen et al., 2003). FasL was discovered as the prototypic death factor that induces cell death by apoptosis upon binding to its default receptor Fas (APO-1, CD95, TNFRSF6) (Suda et al., 1993). It was noted that cytotoxic T lymphocytes (CTLs) and Natural killer (NK) cells employ the FasL/Fas system to complement the perforin/granzyme-mediated pathway for eliminating virus-infected or tumorigenic cells (Brunner et al., 2003; Lowin et al., 1994).

Apart from its role in CTL- and NK cell-mediated target killing, FasL is crucial for maintaining immune homeostasis and preventing autoimmunity (Krammer, 2000). Whereas activation of naïve T cells results in clonal expansion and differentiation, the repeated stimulation of previously activated cells with antigen triggers an activation-induced cell death (AICD). Although AICD has been mostly studied in *in vitro* systems for cellular activation, it is regarded as a means to eliminate (auto-) reactive T cells and to terminate immune responses. AICD can be prevented by blocking the FasL/Fas interaction and, at the T cell level, is associated with an activation-induced surface appearance of FasL, which in turn triggers death via Fas in an autocrine or paracrine fashion (Krammer, 2000).

Based on naturally occurring mutant mice defective for either Fas (*lpr*/*lpr*) or FasL (*gld*/*gld*), it became apparent that functionally relevant mutations in FasL and Fas result in severe autoimmunity due to the accumulation of potentially autoreactive T cells in the absence of regulatory cell death induction (Takahashi et al., 1994; Watanabe-Fukunaga et al., 1992). The same was observed in humans suffering from Fas- or FasL-associated autoimmune lymphoproliferative syndromes (ALPS). Since defect mutants of FasL are very rare in humans, it was suggested that FasL deficiency is not compatible with life (Del-Rey et al., 2006; Rieux-Laucat et al., 2003). Thus, unlike *gld* mice that carry a mutation in the extracellular receptor-binding region, FasL knockout mice display a much more severe autoimmune phenotype and die early after birth (Karray et al., 2004).

* Corresponding author at: Christian-Albrechts-University, Institute of Immunology, UK S-H Campus Kiel, Arnold-Heller-Str. 3, Bldg. 17, D-24105 Kiel, Germany. Tel.: +49 431 5973377; fax: +49 431 5973335.
E-mail addresses: lettau@immunologie.uni-kiel.de (M. Lettau), paulsen@immunologie.uni-kiel.de (M. Paulsen), schmidt@immunologie.uni-kiel.de (H. Schmidt), ojanssen@email.uni-kiel.de (O. Janssen).

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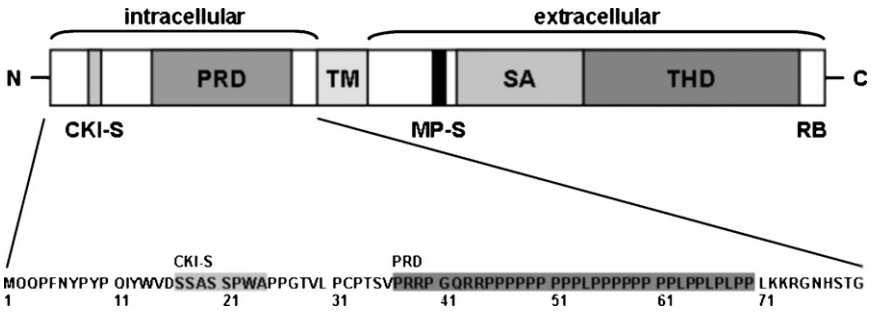

Fig. 1. Schematic representation of FasL structure. FasL is a type II transmembrane protein. Its unique cytosolic tail contains a binary casein kinase substrate motif (CKI-S) and a proline-rich domain (PRD). A stretch comprising aa 137–183 within the extracellular part is suggested to be essential for trimerization and self-assembly (SA). The receptor-binding site (RB) is at the very C-terminus of the molecule adjacent to the TNF homology domain (THD), that contains several potential N-glycosylation sites. Cleavage sites for metalloproteases (MP-S) have been mapped to Ser126/Leu127 and are thus located outside the SA region, indicating that sFasL may form functional trimers.

Although still a matter of debate with regard to its physiological relevance, FasL has been linked to the establishment of immune privilege and to protection from inflammation (Griffith et al., 1995). These functions are again associated with a selective surface expression of FasL for instance on cells of the anterior chamber of the eye or in neurons and astrocytes of the central nervous system, respectively (Linkermann et al., 2003). Similarly, the constitutive surface expression of FasL on certain tumor cells or its secretion on tumor-derived exosomes may generate a tumor-associated immune privilege allowing malignantly transformed cells to evade immune surveillance and potentially even kill tumor infiltrating lymphocytes (O'Connell et al., 1999). More recently, however, the biological significance of FasL-mediated immune privilege and "tumor counterattack" has been challenged since *in vivo*, tumor-associated FasL expression might rather yield pro-inflammatory effects (Igney and Krammer, 2005).

Like several other members of the TNF superfamily, FasL has also been discussed as an accessory or costimulatory molecule for T cell activation. Comparing *gld*, *lpr* and wildtype mice, it was reported that FasL ligation inhibits the proliferation of murine $CD4^+$ T cells (Desbarats et al., 1998). On the other hand, it was shown that FasL ligation by functional Fas augmented the proliferation of murine $CD8^+$ (but not $CD4^+$) T cells both *in vitro* and *in vivo* (Suzuki et al., 2000; Suzuki and Fink, 1998). A reverse signaling capacity of FasL was also implicated in positive selection in the thymus when FasL-expressing thymocytes interact with Fas-positive stroma cells or thymic antigen-presenting cells (APC) (Boursalian and Fink, 2003).

Considering the diverse functions of FasL within and outside the immune system and based on our previous studies on AICD and on protein–protein-interactions, we initiated the CRC 415 project to define molecular interactions that control FasL surface appearance and function. Over the past decade, we and others worked out that the unique intracellular tail of FasL with its extended polyproline stretch forms the docking site for regulatory proteins that dictate the fate of the death factor. Moreover, we identified the sheddase for FasL and analyzed the capacity of the FasL/Fas system to modulate TCR-associated cellular activation.

Modular composition of FasL

Being a member of the TNF superfamily (TNFSF6), FasL is a type II transmembrane protein that displays a significant homology to related proteins within the extracellular "TNF homology domain" (THD) (Fig. 1). The specific receptor binding site is located at the very C-terminus and mediates selective binding to the cystein-rich domains of the Fas receptor. Within its ectodomain, FasL contains three putative sites for N-linked glycosylation (N184, N250, and N260) which might be posttranslationally modified but do not seem to alter self-aggregation or receptor binding significantly. Mutations of these residues, however, correlate with reduced expression of FasL. Notably, N-linked glycosylation often protects lysosome-associated membrane proteins from degradation and therefore might also play a role in FasL maturation, storage, stability, surface appearance and secretion (Lettau et al., 2008; Voss et al., 2008).

It is now common sense that FasL exhibits its biological activity as a membrane-associated homo-trimeric or even hexameric complex (Holler et al., 2003). Oligomerization depends on a self assembly (SA) region spanning aa 137–183 of the FasL ectodomain. Importantly, the extracellular parts of several TNF family members contain cleavage sites for matrix metalloproteases (MMPs) to deliberate a soluble cytokine. In the case of FasL, putative cleavage sites are located N-terminal of the self assembly region, thereby allowing the formation of different soluble complexes (Lettau et al., 2008; Voss et al., 2008). The consequences of FasL shedding are quite substantial since only membrane-bound FasL (mFasL) triggers death induction whereas soluble FasL (sFasL) counteracts it (O'Reilly et al., 2009). In addition, mFasL and sFasL might differentially modulate the activation of resting T cells (Paulsen et al., 2010) and also the intracellular remnants of FasL might be further processed and function as signaling elements (see below).

The transmembrane area of FasL (aa 82–102) is followed by an intracellular region (aa 1–81 in humans), where FasL significantly differs from all other members of the TNF family (Janssen et al., 2003). Remarkably, the intracellular tail of FasL is significantly longer than those of the functionally most related TNF family members TNF (35 aa), Lymphotoxin β (LTβ, 18 aa), "TNF-related apoptosis-inducing ligand" (TRAIL, 17 aa). Within this tail, only FasL contains a unique and highly conserved proline-rich domain (PRD) that enables interactions with proteins containing Src homology 3 (SH3) or WW interaction domains. Similar to respective motifs in other TNF family members, FasL harbors a conserved casein kinase (CK) substrate region (aa 17–21 in humans). In the case of TNF, serine phosphorylation of this motif was implicated in reverse signaling and the regulation of expression (Watts et al., 1999). Likewise, a TCR- or FasFc-induced CK-mediated serine phosphorylation within the murine FasL cytosolic tail was associated with FasL reverse signaling in $CD8^+$ T cells (Sun et al., 2006; Sun and Fink, 2007). Last but not least, the very N-terminal part of FasL contains several potential tyrosine phosphorylation sites (aa 7, 9, 13 in humans). Phosphorylation of these residues and ubiquitinylation at lysine residues flanking the PRD seem to affect FasL sorting to secretory lysosomes (Zuccato et al., 2007).

FasL-interacting proteins

Apparently, the expression and surface appearance of a potentially dangerous death factor have to be controlled by a reliable molecular machinery. Given the potential of FasL to interact with