REVIEW

Arch. Immunol. Ther. Exp., 2008, **56**, 9–14
PL ISSN 0004-069X

DOI 10.1007/s00005-008-0002-9

Rewinding the DISC

Benjamin Chaigne-Delalande[1, 2, 3], Jean-François Moreau[1, 2, 3, 4] and Patrick Legembre[1, 2, 3]

[1] Université de Bordeaux-2, Bordeaux, France
[2] CNRS UMR 5164, Bordeaux, France
[3] IFR66, Bordeaux, France
[4] CHU de Bordeaux, Bordeaux, France

Received: 2007.10.09, **Accepted:** 2007.11.19, **Published online first:** 2008.02.05

Abstract

Fas (CD95/APO-1) belongs to the tumor necrosis factor receptor family and its signaling pathway has been extensively studied over the past 15 years. Blockade of the Fas-mediated apoptotic signal leads to abusive lymphoproliferation, auto-immunity, and an increased risk of developing lymphoma and leukemia. Fas engagement drives the formation of a complex termed DISC (death-inducing signaling complex), which contains the adaptor molecule Fas-associated protein, two members of the caspase family caspase-8 and -10, and a pseudo-caspase termed c-FLIP. According to different authors, DISC formation relies either on the redistribution of Fas into the lipid rafts or the recruitment of the actin cytoskeleton and receptor endocytosis or the production of ceramide. However, the accurate molecular ordering upstream from the formation of DISC remains very puzzling and is highly debated. Herein we review some of the factors that would potentially facilitate or limit the formation of the DISC.

Key words: apoptosis, CD95, DISC, Fas, lipid rafts.

Corresponding author: Patrick Legembre, Université de Bordeaux-2, 146 rue Léo saignat, 33076 Bordeaux, France, tel.: +33 5 57-57-11-24, fax: +33 5 57-57-14-72, e-mail: patrick.legembre@bordeaux.fr

INTRODUCTION

Fas (CD95/APO-1) is expressed at the plasma membrane as a pre-associated homotrimer [50] owing to the fact that the amino-part of its extracellular region encompasses a domain termed PLAD (pre-ligand association domain), essential for the homotypic polymerization of the receptor [53, 61]. A common feature of death receptors is the presence of a conserved stretch of 80 amino acids in their cytoplasmic tails termed the death domain (DD), which serves as a platform for death-inducing signaling complex (DISC) formation. The Fas-mediated apoptotic signal is essential for the homeostasis of the immune system and germinal mutations have been reported in patients suffering from a syndrome termed ALPS type Ia (autoimmune lymphoproliferative syndrome), a childhood disorder characterized by aberrant lymphoproliferation and autoimmunity [14, 17, 57]. These patients possess an increased risk of developing lymphomas [65]. Conversely, somatic mutations in the gene encoding Fas have been reported to be present in cells of patients suffering from various lymphomas and leukemias [22, 51]. In most cases these mutations are located inside the DD [56], altering the transmission of the apoptotic signal and thus allowing cells to escape immune surveillance.

Once Fas is engaged, its DD binds the adaptor molecule Fas-associated protein (FADD) through a homotypic interaction [4, 9]. In turn, FADD recruits and aggregates initiator caspases (caspase-8 and caspase-10). This molecular complex (Fas/FADD/caspases) is called DISC, for death-inducing signaling complex (see Fig. 1) [31]. At the DISC level, the concentration of initiator caspases favors their auto-cleavage, the activation of these proteases, and the release of activated caspases into the cytoplasm, thereby leading to the triggering of the apoptotic signal. Among the DISC components, c-FLIP$_L$ (cellular FADD-like IL-1b-converting enzyme inhibitory protein) and its smaller splice variant c-FLIP$_S$ have been reported as potent inhibitors of the caspase activation [28]. c-FLIP$_L$ shares extensive homology with the caspase-10 catalytic domain in its carboxy-terminal region, but this caspase-like protein lost a conserved cysteine residue in the catalytic site and a closely positioned histidine residue, which are essential for catalytic activity [27]. As a consequence, c-FLIP is devoid of any protease

activity and rather exerts a negative function by competing with caspase-8 and -10 for FADD-binding [3, 37].

FAS AGGREGATION AND CONFORMATIONAL CHANGES

FasL (CD95L/CD178) is a transmembrane protein that belongs to the tumor necrosis factor (TNF) family. In contrast to the receptor Fas, which is expressed ubiquitously in different tissues, the pattern of FasL expression is cell restricted and tightly regulated. FasL is expressed *de novo* at the surface of activated T lymphocytes and natural killer cells, where it acts as a powerful weapon to eliminate transformed and infected cells [33]. Although membrane-bound FasL is well accepted as a potent apoptotic inducer [67], its soluble counterpart generated after cleavage by metalloprotease [30, 46] does not possess any cytolytic activity and even inhibits the membrane-bound FasL-mediated apoptotic signal [59, 66]. Interestingly, cross-linking of this soluble FasL using antibodies restores the proapoptotic activity, and hexameric FasL has been reported to be the minimal complex required to induce an efficient cell death signal [24]. We recently showed that at comparable levels of Fas aggregation, a home-made generated chimeric FasL is considerably more efficient in forming the DISC and in transmitting cell death than agonistic pentavalent IgM anti-Fas antibodies [3]. Besides, the agonistic antibodies and FasL do not seem to induce identical signals [25, 39, 41]. Altogether, these findings point to conformational alterations and multimerization of homotrimeric Fas as the first steps required to exclude anti-apoptotic factor(s) and to recruit molecule(s) essential to forming DISC.

THE SO-CALLED TYPE I AND TYPE II CELLS

According to the extent of the DISC formation, cells have been classified in two groups [58]. In the so-called type I cells, Fas engagement is followed by an efficient formation of the DISC and the subsequent release of a large amount of active caspases. In contrast, in type II cells, DISC formation and caspase release are impaired and in these cells the Fas-mediated apoptotic signal relies on an apoptotic amplification loop triggered by the mitochondrion [58]. Recently, Muppidi and Siegel [50] refined this model by showing that the enrichment of Fas into or outside the membrane sub-domains called lipid rafts was responsible of the type I and type II phenotypes, respectively.

The classical fluid mosaic model of the cell membrane introduced by Singer and Nicolson [63] in 1972 has been challenged in the last decade with the lipid raft theory [5, 62]. The view of the membrane bilayer has therefore changed and the extracellular leaflet is now depicted as a heterogeneous structure containing compacted proteolipidic assemblies (ordered phase) which float into the rest of the more fluid membrane (liquid phase). These sub-domains are enriched in sphingolipids and cholesterol and they are currently designated as detergent-resistant microdomains (DRMs), glycosphingolipid-enriched microdomains (GEMs), lipid rafts, membrane rafts, or simply microdomains. Cumulative data point out an essential role for the lipid rafts in different molecular processes, such as protein sorting in polarized cells [5, 62], endocytosis [55], signal transduction in B and T lymphocytes [8, 49, 69], and protein degradation [44]. Recently, we and others reported that these GEMs are essential to transmitting the Fas signal, but the molecular mechanism involved in the amplification of the Fas-mediated apoptotic signal by these membrane sub-domains remains abstruse [18, 19, 26, 40, 42, 50].

FAS REDISTRIBUTION INTO THE LIPID RAFTS

Ceramides have been described as potent inducers of cell death [52]. In the Fas signaling pathway, these lipids have been suggested as indispensable for the apoptotic signal and their production depends on acidic sphingomyelinase (aSMase) activity [10]. However, the link between the generation of ceramide and caspase activation in canonical Fas-mediated cell death is not defined. Recently, the role of ceramide in the Fas signaling pathway has been refined, and although Fas engagement generates a weak amount of active caspase-8, insufficient to induce the apoptotic signal, these proteases are essential to activating sphingomyelinase and to producing ceramide, which in turn drives the redistribution of Fas into the lipid rafts, the formation of the DISC, and the induction of the apoptotic signal [11].

The redistribution of Fas into the DRMs when cells are treated with various anti-tumoral agents was also recently reported [19–21, 34]. Gajate and Mollinedo showed that the ether lipid 1-*O*-octadecyl-2-*O*-methyl-*-rac*-glycero-3-phosphocholine (Et-18-OCH_3; edelfosine) induces the redistribution of Fas into the DRMs, which represents a self-sufficient step mediating a Fas-dependent apoptotic signal independently of FasL expression or Fas/FasL interaction [18]. Although edelfosine treatment does not seem to require sphingomyelinase activity, cisplatin treatment drives the migration of Fas into lipid rafts via the induction of aSMase [34]. The novel anti-tumoral drug Aplidin involves the actin cytoskeleton [20] to promote the lateral mobility of Fas and its stabilization on supramolecular platforms, thus allowing the formation of the DISC and the induction of apoptosis. Taken together, these findings suggest that the redistribution of Fas into the lipid rafts is sufficient to mediate the activation of the apoptotic signal independently of the presence of FasL or to decrease the apoptotic threshold required to induce the death signal by altering the balance between the negative and positive molecules involved in the DISC formation.

In the earlier steps of the Fas molecular ordering, high-molecular-mass forms of Fas are easily observable by Western blotting using denaturing and reducing conditions. We and others did not observe any correlation between the formation of these aggregates and the intensity of the death signal [36, 39]. However, using sucrose density gradient centrifugation to separate proteins and native protein complexes according to their Stoke's radius, various forms of Fas microaggregates have recently been isolated upon Fas engagement and one type of these complexes has been shown to contain DISC components [16]. Feig et al. [16] suggested that the occurrence of Fas microaggregates and DISC formation depend on the palmitoylation of a cysteine located inside the Fas intracellular tail (C199). In contrast, Chakrabandhu et al. [7] pointed out that Fas palmitoylation was involved in the redistribution of the receptor into the lipid rafts, a mandatory step in triggering cell death. Experiments have to be performed to clarify the exact function of Fas palmitoylation, but the post-translational modification of the cysteine at position 199 remains the only genetic clue supporting the redistribution of Fas into the DRMs and its essential role in the apoptotic signal. Characterization of the molecules involved in Fas palmitoylation will be of great interest to fully understand the initial events of the Fas engagement cascade.

ACTIN CYTOSKELETON AND FAS-MEDIATED APOPTOSIS

The capping of Fas which takes place following the binding of Fas ligands is supposed to rely on actin polymers [1], and sphingomyelinase activation via the ceramide production has been described to modulate the actin cytoskeletal network [23]. Based on elegant studies carried out on T-lymphocyte activation and the "immune synapse" formation, it has been shown that one way in which the cytoskeleton is essential in this process is by maintaining membrane rigidity and by regulating supra-molecular complex formation [15]. Remarkably, activation of Fas leads to the formation of a cell polarized cap reminiscent of the "immune synapse" [68]. Since cell polarization is an actin cytoskeleton-driven process and disruption of actin polymers disturbs the transmission of the apoptotic signal [1, 7, 20, 35, 54], we can hypothesize that the actin cytoskeleton plays an essential role in the Fas signal. However, contradictory studies have reported that the actin cytoskeleton network is not implicated in the Fas-mediated apoptotic signal in type II cells [1] and that preventing actin filament elongation with cytochalasin D amplifies or does not at all disturb the Fas signal in epithelial cells (e.g. ovarian cancer cell lines or in airway epithelial cells) or in the Jurkat cell line (type II cell), respectively [6, 47]. Fas engagement transmits non-apoptotic signals [2, 37, 38], and the actin cytoskeleton has also been involved in the transduction of this signal [64]. These results are debated, since recent data put forward that, whereas the actin cytoskeleton is essential to the DISC formation and the induction of apoptosis, it does not participate in the triggering of non-apoptotic signals [35]. Altogether, these findings are ambiguous and highlight the fact that more experiments are needed to define the exact role of the actin cytoskeleton and the connecting factors required to associate the transmembrane receptor Fas to it. Among ezrin, radixin, and moesin, the so-called ERM proteins which act as linkers between the plasma membrane and the actin cytoskeleton, ezrin has been involved in the Fas-mediated apoptotic signal [45, 54] and represents one molecular link between the apoptotic signal and the actin cytoskeleton network.

ENDOCYTOSIS IN THE FAS SIGNAL

The role of endocytosis in the signal transduction of members of the TNF receptor has recently been revisited [48]. The TNF/TNFR1 death-inducing complex is drastically inhibited by the adenoviral protein E3-14.7K, which blocks TNF-induced TNFR1 internalization, whereas the transmission of the non-apoptotic signal NF-κB is conserved [60]. Furthermore, in type I cells, the DISC is found efficiently constituted at the level of the endosomal compartment, indicating that Fas endocytosis is essential to inducing cell death [35]. The redistribution of Fas into the lipid rafts has been reported as essential to binding ezrin, which in turn favors the endocytosis of the bound complex [7]. Nevertheless, it is noteworthy that another member of the TNF receptor family, the TNF-related apoptosis-inducing ligand receptor, does not require internalization to induce cell death [32].

It therefore appears that the study of endocytosis in the Fas signaling pathway may be a very promising new research area, since besides adding a spatial dimension in the tight regulatory mechanisms of the Fas signaling pathway, it would bring together the actin cytoskeleton, the DRMs, and the adaptor molecule ezrin. Moreover, it would be tempting to explain the higher threshold required to induce apoptosis compared with the non-apoptotic signal [38], as blocking the process of endocytosis solely inhibits the induction of the apoptotic signal. Altogether, these findings support the conclusion that several molecular processes are taking place upstream of DISC formation and the induction of active caspases, while its exact molecular ordering and the complete list of actors remain a subject of intense research.

REMARKS

Herein we reported on different molecular processes that have been described in the literature as participating in the formation of the DISC and the induction

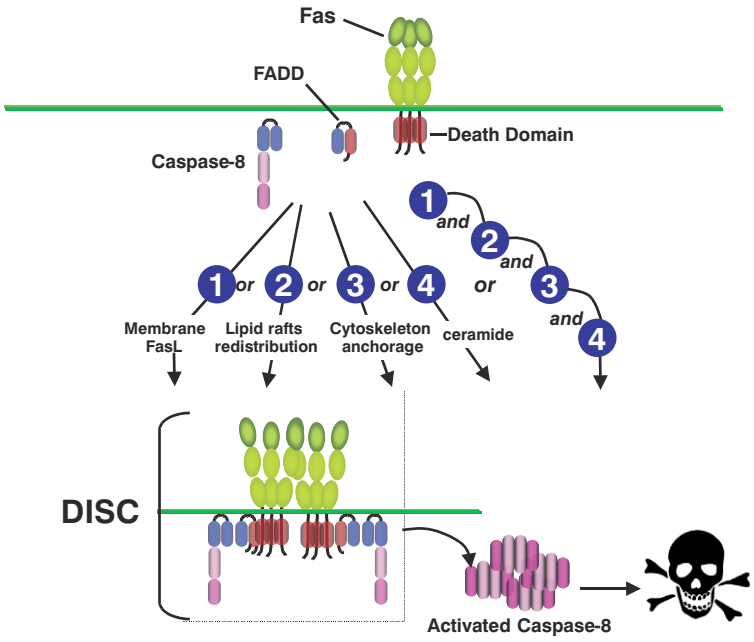

Fig. 1. Investigating the molecular ordering of the initial step(s) potentially involved in the formation of DISC and the induction of the apoptotic signal.

of the apoptotic signal. The question now is to decipher whether each process acts independently and is specific to a certain cellular context or whether they are all interconnected (see Fig. 1). In the latter situation it would be interesting to identify the molecular ordering upstream DISC formation.

Recently the apoptotic function of Fas has been challenged *in vivo* and *in vitro* since more and more data are accumulating extending the role of this receptor to areas other than apoptosis, such as in neutrophil attraction [29], liver regeneration [13], neurone outgrowth [12], and favoring tumorigenesis and metastasis [2, 43]. Understanding the initial steps of Fas signal progression would be essential to explaining the dichotomy of the Fas-mediated signal.

Acknowledgment: This work is supported by grants from the Association pour la Recherche sur le Cancer (grant 3798), from la Fondation de France (Leucémie), from la Ligue Contre le Cancer (comité de la Dordogne), and from l'Agence de Biomédecine. Patrick Legembre is an employee by the French Institute of Health and Medical Research (INSERM). Benjamin Chaigne-Delalande is supported by a grant from the Fondation de France (Leucémie).

REFERENCES

1. Algeciras-Schimnich A., Shen L., Barnhart B. C., Murmann A. E., Burkhardt J. K. and Peter M. E. (2002): Molecular ordering of the initial signaling events of CD95. Mol. Cell Biol., **22**, 207–220.
2. Barnhart B. C., Legembre P., Pietras E., Bubici C., Franzoso G. and Peter M. E. (2004): CD95 ligand induces motility and invasiveness of apoptosis-resistant tumor cells. EMBO J., **23**, 3175–3185.
3. Beneteau M., Daburon S., Moreau J. F., Taupin J. L. and Legembre P. (2007): Dominant-negative Fas mutation is reversed by down-expression of c-FLIP. Cancer Res., **67**, 108–115.
4. Boldin M. P., Goncharov T. M., Goltsev Y. V. and Wallach D. (1996): Involvement of MACH, a novel MORT1/ /FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell, **85**, 803–815.
5. Brown D. A. and Rose J. K. (1992): Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell, **68**, 533–544.
6. Celeste Morley S., Sun G. P. and Bierer B. E. (2003): Inhibition of actin polymerization enhances commitment to and execution of apoptosis induced by withdrawal of trophic support. J. Cell Biochem., **88**, 1066–1076.
7. Chakrabandhu K., Herincs Z., Huault S., Dost B., Peng L., Conchonaud F., Marguet D., He H. T. and Hueber A. O. (2007): Palmitoylation is required for efficient Fas cell death signaling. EMBO J., **26**, 209–220.
8. Cheng P. C., Dykstra M. L., Mitchell R. N. and Pierce S. K. (1999): A role for lipid rafts in B cell antigen receptor signaling and antigen targeting. J. Exp. Med., **190**, 1549–1560.
9. Chinnaiyan A. M., Tepper C. G., Seldin M. F., O'Rourke K., Kischkel F. C., Hellbardt S., Krammer P. H., Peter M. E. and Dixit V. M. (1996): FADD/MORT1 is a common mediator of CD95 (Fas/APO-1) and tumor necrosis factor receptor-induced apoptosis. J. Biol. Chem., **271**, 4961–4965.
10. Cifone M. G., De Maria R., Roncaioli P., Rippo M. R., Azuma M., Lanier L. L., Santoni A. and Testi R. (1994): Apoptotic signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. J. Exp. Med., **180**, 1547-1552.
11. Cremesti A., Paris F., Grassme H., Holler N., Tschopp J., Fuks Z., Gulbins E. and Kolesnick R. (2001): Ceramide enables fas to cap and kill. J. Biol. Chem., **276**, 23954–23961.
12. Desbarats J., Birge R. B., Mimouni-Rongy M., Weinstein D. E., Palerme J. S. and Newell M. K. (2003): Fas engage-

ment induces neurite growth through ERK activation and p35 upregulation. Nat. Cell Biol., **5**, 118–125.
13. Desbarats J. and Newell M. K. (2000): Fas engagement accelerates liver regeneration after partial hepatectomy. Nat. Med., **6**, 920–923.
14. Drappa J., Vaishnaw A. K., Sullivan K. E., Chu J. L. and Elkon K. B. (1996): Fas gene mutations in the Canale--Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. N. Engl. J. Med., **335**, 1643–1649.
15. Faure S., Salazar-Fontana L. I., Semichon M., Tybulewicz V. L., Bismuth G., Trautmann A., Germain R. N. and Delon J. (2004): ERM proteins regulate cytoskeleton relaxation promoting T cell-APC conjugation. Nat. Immunol., **5**, 272–279.
16. Feig C., Tchikov V., Schutze S. and Peter M. E. (2007): Palmitoylation of CD95 facilitates formation of SDS-stable receptor aggregates that initiate apoptosis signaling. EMBO J., **26**, 221–231.
17. Fisher G. H., Rosenberg F. J., Straus S. E., Dale J. K., Middleton L. A., Lin A. Y., Strober W., Lenardo M. J. and Puck J. M. (1995): Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell, **81**, 935–946.
18. Gajate C., Del Canto-Janez E., Acuna A. U., Amat-Guerri F., Geijo E., Santos-Beneit A. M., Veldman R. J. and Mollinedo F. (2004): Intracellular triggering of Fas aggregation and recruitment of apoptotic molecules into Fas-enriched rafts in selective tumor cell apoptosis. J. Exp. Med., **200**, 353–365.
19. Gajate C. and Mollinedo F. (2001): The antitumor ether lipid ET-18-OCH(3) induces apoptosis through translocation and capping of Fas/CD95 into membrane rafts in human leukemic cells. Blood, **98**, 3860–3863.
20. Gajate C. and Mollinedo F. (2005): Cytoskeleton-mediated death receptor and ligand concentration in lipid rafts forms apoptosis-promoting clusters in cancer chemotherapy. J. Biol. Chem., **280**, 11641–11647.
21. Gajate C. and Mollinedo F. (2007): Edelfosine and perifosine induce selective apoptosis in multiple myeloma by recruitment of death receptors and downstream signaling molecules into lipid rafts. Blood, **109**, 711–719.
22. Gronbaek K., Straten P. T., Ralfkiaer E., Ahrenkiel V., Andersen M. K., Hansen N. E., Zeuthen J., Hou-Jensen K. and Guldberg P. (1998): Somatic Fas mutations in non--Hodgkin's lymphoma: association with extranodal disease and autoimmunity. Blood, **92**, 3018–3024.
23. Hanna A. N., Berthiaume L. G., Kikuchi Y., Begg D., Bourgoin S. and Brindley D. N. (2001): Tumor necrosis factor-alpha induces stress fiber formation through ceramide production: role of sphingosine kinase. Mol. Biol. Cell, **12**, 3618–3630.
24. Holler N., Tardivel A., Kovacsovics-Bankowski M., Hertig S., Gaide O., Martinon F., Tinel A., Deperthes D., Calderara S., Schulthess T., Engel J., Schneider P. and Tschopp J. (2003): Two adjacent trimeric Fas ligands are required for Fas signaling and formation of a death-inducing signaling complex. Mol. Cell Biol., **23**, 1428–1440.
25. Huang D. C., Hahne M., Schroeter M., Frei K., Fontana A., Villunger A., Newton K., Tschopp J. and Strasser A. (1999): Activation of Fas by FasL induces apoptosis by a mechanism that cannot be blocked by Bcl-2 or Bcl-x(L). Proc. Natl. Acad. Sci. USA, **96**, 14871–14876.
26. Hueber A. O., Bernard A. M., Herincs Z., Couzinet A. and He H. T. (2002): An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes. EMBO Rep., **3**, 190–196.
27. Inohara N., Koseki T., Hu Y., Chen S. and Nunez G. (1997): CLARP, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis. Proc. Natl. Acad. Sci. USA, **94**, 10717–10722.
28. Irmler M., Thome M., Hahne M., Schneider P., Hofmann K., Steiner V., Bodmer J. L., Schroter M., Burns K., Mattmann C., Rimoldi D., French L. E. and Tschopp J. (1997): Inhibition of death receptor signals by cellular FLIP. Nature, **388**, 190–195.
29. Kang S. M., Schneider D. B., Lin Z., Hanahan D., Dichek D. A., Stock P. G. and Baekkeskov S. (1997): Fas ligand expression in islets of Langerhans does not confer immune privilege and instead targets them for rapid destruction. Nat. Med., **3**, 738–743.
30. Kayagaki N., Kawasaki A., Ebata T., Ohmoto H., Ikeda S., Inoue S., Yoshino K., Okumura K. and Yagita H. (1995): Metalloproteinase-mediated release of human Fas ligand. J. Exp. Med., **182**, 1777–1783.
31. Kischkel F. C., Hellbardt S., Behrmann I., Germer M., Pawlita M., Krammer P. H. and Peter M. E. (1995): Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J., **14**, 5579–5588.
32. Kohlhaas S. L., Craxton A., Sun X. M., Pinkoski M. J. and Cohen G. M. (2007): Receptor-mediated endocytosis is not required for tumor necrosis factor-related apoptosis--inducing ligand (TRAIL)-induced apoptosis. J. Biol. Chem., **282**, 12831–12841.
33. Krueger A., Fas S. C., Baumann S. and Krammer P. H. (2003): The role of CD95 in the regulation of peripheral T-cell apoptosis. Immunol. Rev., **193**, 58–69.
34. Lacour S., Hammann A., Grazide S., Lagadic-Gossmann D., Athias A., Sergent O., Laurent G., Gambert P., Solary E. and Dimanche-Boitrel M. T. (2004): Cisplatin-induced CD95 redistribution into membrane lipid rafts of HT29 human colon cancer cells. Cancer Res., **64**, 3593–3598.
35. Lee K. H., Feig C., Tchikov V., Schickel R., Hallas C., Schutze S., Peter M. E. and Chan A. C. (2006): The role of receptor internalization in CD95 signaling. EMBO J., **25**, 1009–1023.
36. Lee Y. and Shacter E. (2001): Fas aggregation does not correlate with Fas-mediated apoptosis. J. Immunol., **167**, 82–89.
37. Legembre P., Barnhart B. C. and Peter M. E. (2004): The relevance of NF-kappaB for CD95 signaling in tumor cells. Cell Cycle, **3**, 1235–1239.
38. Legembre P., Barnhart B. C., Zheng L., Vijayan S., Straus S. E., Puck J., Dale J. K., Lenardo M. and Peter M. E. (2004): Induction of apoptosis and activation of NF--kappaB by CD95 require different signalling thresholds. EMBO Rep., **5**, 1084–1089.
39. Legembre P., Beneteau M., Daburon S., Moreau J. F. and Taupin J. L. (2003): Cutting edge: SDS-stable Fas microaggregates: an early event of Fas activation occurring with agonistic anti-Fas antibody but not with Fas ligand. J. Immunol., **171**, 5659–5662.
40. Legembre P., Daburon S., Moreau P., Ichas F., de Giorgi F., Moreau J. F. and Taupin J. L. (2005): Amplification of Fas-mediated apoptosis in type II cells via microdomain recruitment. Mol. Cell Biol., **25**, 6811–6820.
41. Legembre P., Daburon S., Moreau P., Moreau J. F. and Taupin J. L. (2006): Cutting edge: modulation of fas-medi-

ated apoptosis by lipid rafts in T lymphocytes. J. Immunol., **176**, 716–720.
42. Legembre P., Moreau P., Daburon S., Moreau J. F. and Taupin J. L. (2002): Potentiation of Fas-mediated apoptosis by an engineered glycosylphosphatidylinositol-linked Fas. Cell Death Differ., **9**, 329–339.
43. Legembre P., Schickel R., Barnhart B. C. and Peter M. E. (2004): Identification of SNF1/AMP kinase-related kinase as an NF-kappaB-regulated anti-apoptotic kinase involved in CD95-induced motility and invasiveness. J. Biol. Chem., **279**, 46742–46747.
44. Legler D. F., Micheau O., Doucey M. A., Tschopp J. and Bron C. (2003): Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalpha-mediated NF-kappaB activation. Immunity, **18**, 655–664.
45. Lozupone F., Lugini L., Matarrese P., Luciani F., Federici C., Iessi E., Margutti P., Stassi G., Malorni W. and Fais S. (2004): Identification and relevance of the CD95-binding domain in the N-terminal region of ezrin. J. Biol. Chem., **279**, 9199–9207.
46. Mariani S. M., Matiba B., Baumler C. and Krammer P. H. (1995): Regulation of cell surface APO-1/Fas (CD95) ligand expression by metalloproteases. Eur. J. Immunol., **25**, 2303–2307.
47. Meng Y., Kang S. and Fishman D. A. (2005): Lysophosphatidic acid inhibits anti-Fas-mediated apoptosis enhanced by actin depolymerization in epithelial ovarian cancer. FEBS Lett., **579**, 1311–1319.
48. Miaczynska M., Pelkmans L. and Zerial M. (2004): Not just a sink: endosomes in control of signal transduction. Curr. Opin. Cell Biol., **16**, 400–406.
49. Montixi C., Langlet C., Bernard A. M., Thimonier J., Dubois C., Wurbel M. A., Chauvin J. P., Pierres M. and He H. T. (1998): Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. EMBO J., **17**, 5334–5348.
50. Muppidi J. R. and Siegel R. M. (2004): Ligand-independent redistribution of Fas (CD95) into lipid rafts mediates clonotypic T cell death. Nat. Immunol., **5**, 182–189.
51. Muschen M., Rajewsky K., Kronke M. and Kuppers R. (2002): The origin of CD95-gene mutations in B-cell lymphoma. Trends Immunol., **23**, 75–80.
52. Obeid L. M., Linardic C. M., Karolak L. A. and Hannun Y. A. (1993): Programmed cell death induced by ceramide. Science, **259**, 1769–1771.
53. Papoff G., Hausler P., Eramo A., Pagano M. G., Di Leve G., Signore A. and Ruberti G. (1999): Identification and characterization of a ligand-independent oligomerization domain in the extracellular region of the CD95 death receptor. J. Biol. Chem., **274**, 38241–38250.
54. Parlato S., Giammarioli A. M., Logozzi M., Lozupone F., Matarrese P., Luciani F., Falchi M., Malorni W. and Fais S. (2000): CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. EMBO J., **19**, 5123–5134.
55. Parton R. G. and Richards A. A. (2003): Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. Traffic, **4**, 724–738.
56. Peter M. E., Legembre P. and Barnhart B. C. (2005): Does CD95 have tumor promoting activities? Biochim. Biophys. Acta, **1755**, 25–36.
57. Rieux-Laucat F., Le Deist F., Hivroz C., Roberts I. A., Debatin K. M., Fischer A. and de Villartay J. P. (1995): Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science, **268**, 1347–1349.
58. Scaffidi C., Fulda S., Srinivasan A., Friesen C., Li F., Tomaselli K. J., Debatin K. M., Krammer P. H. and Peter M. E. (1998): Two CD95 (APO-1/Fas) signaling pathways. EMBO J., **17**, 1675–1687.
59. Schneider P., Holler N., Bodmer J. L., Hahne M., Frei K., Fontana A. and Tschopp J. (1998): Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. J. Exp. Med., **187**, 1205–1213.
60. Schneider-Brachert W., Tchikov V., Merkel O., Jakob M., Hallas C., Kruse M. L., Groitl P., Lehn A., Hildt E., Held-Feindt J., Dobner T., Kabelitz D., Kronke M. and Schutze S. (2006): Inhibition of TNF receptor 1 internalization by adenovirus 14.7K as a novel immune escape mechanism. J. Clin. Invest., **116**, 2901–2913.
61. Siegel R. M., Frederiksen J. K., Zacharias D. A., Chan F. K., Johnson M., Lynch D., Tsien R. Y. and Lenardo M. J. (2000): Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. Science, **288**, 2354–2357.
62. Simons K. and Ikonen E. (1997): Functional rafts in cell membranes. Nature, **387**, 569-572.
63. Singer S. J. and Nicolson G. L. (1972): The fluid mosaic model of the structure of cell membranes. Science, **175**, 720–731.
64. Soderstrom T. S., Nyberg S. D. and Eriksson J. E. (2005): CD95 capping is ROCK-dependent and dispensable for apoptosis. J. Cell Sci., **118**, 2211–2223.
65. Straus S. E., Jaffe E. S., Puck J. M., Dale J. K., Elkon K. B., Rosen-Wolff A., Peters A. M., Sneller M. C., Hallahan C. W., Wang J., Fischer R. E., Jackson C. M., Lin A. Y., Baumler C., Siegert E., Marx A., Vaishnaw A. K., Grodzicky T., Fleisher T. A. and Lenardo M. J. (2001): The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. Blood, **98**, 194–200.
66. Suda T., Hashimoto H., Tanaka M., Ochi T. and Nagata S. (1997): Membrane Fas ligand kills human peripheral blood T lymphocytes, and soluble Fas ligand blocks the killing. J. Exp. Med., **186**, 2045–2050.
67. Suda T., Takahashi T., Golstein P. and Nagata S. (1993): Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell, **75**, 1169–1178.
68. Viola A., Schroeder S., Sakakibara Y. and Lanzavecchia A. (1999): T lymphocyte costimulation mediated by reorganization of membrane microdomains. Science, **283**, 680–682.
69. Xavier R., Brennan T., Li Q., McCormack C. and Seed B. (1998): Membrane compartmentation is required for efficient T cell activation. Immunity, **8**, 723–732.