



**Review**

## **Immune Privilege or Inflammation? The Paradoxical Effects of Fas Ligand**

JOE O'CONNELL\*

Department of Medicine, National University of Ireland, Cork, Ireland

**Abstract.** Fas ligand (FasL) induces apoptosis of cells, including activated lymphocytes, expressing its cognate receptor, Fas (CD95/APO-1). FasL precludes inflammatory reactions from immune privileged sites by triggering Fas-mediated apoptosis of infiltrating proinflammatory cells. Aberrant expression of FasL by cancers inhibits antitumor immune responses. The ability of FasL to impair immune responses may hold therapeutic promise as a means of protecting tissue transplants from immunological rejection. Paradoxically, FasL exhibits proinflammatory activity independent of its ability to mediate immune privilege. FasL has been shown to recruit and activate neutrophils, although the factors that determine whether FasL is pro- or anti-inflammatory are only beginning to emerge. FasL appears to contribute to cell death in Fas-sensitive endorgan cells during inflammation. Blocking of Fas-mediated endorgan apoptosis or enhancing Fas-mediated apoptosis of inflammatory cells represent potential targets for future antiinflammatory therapies.

**Key words:** Fas (CD95/APO-1); Fas ligand (FasL); apoptosis; immune privilege; transplantation; inflammation.

Fas (CD95/APO-1) is a cell surface receptor which mediates programmed cell death, or apoptosis, upon triggering by its ligand, FasL. Much of the Fas signal transduction pathway has recently been elucidated. Engagement of Fas directly activates an intracellular proteolytic cascade mediated by the caspase family of proteases. Caspase proteolysis of specific protein substrates is central to the execution of apoptosis, and specific tri-peptide inhibitors of caspases can block apoptosis. The Bcl-2 family of apoptosis regulatory proteins appears to regulate the Fas signal to some extent. This family consists of pro-(e.g. Bax, Bak, Bcl-xS) and anti-(e.g. Bcl-2, Bcl-xL) apoptotic homologues, which combine to form homo- or hetero-dimers. The ratio of

pro- to anti-apoptotic dimers acts as a molecular rheostat controlling the cell's propensity for apoptosis<sup>20</sup>.

Lymphocytes upregulate cell surface Fas and FasL upon activation, and Fas-mediated autocrine "suicide" or juxtacrine "fratricide" of lymphocytes helps to terminate immune responses. T cells delay their sensitivity to Fas-mediated apoptosis until after a period of a few days' activation. Fas-sensitization then takes place, mediated in part by a decrease in the level of the anti-apoptotic Bcl-2 homologue Bcl-xL<sup>31</sup>. In this way, potentially dangerous T cells are only permitted a limited tenure of activity followed by rapid deletion via the Fas pathway. FasL also deletes autoreactive lymphocytes during tolerance acquisition. The import-

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\* Correspondence to: Dr. Joe O'Connell, Department of Medicine, Clinical Sciences Building, University Hospital, Cork, Ireland, tel.: 00353 21 901231, fax: 00353 21 345300; e-mail: j.oconnell@ucc.ie

ance of the Fas system in normal immunoregulation is evident from the autoimmune and lymphoproliferative syndromes occurring in mice with mutations of either Fas or FasL<sup>25</sup>.

Fas is widely expressed in many cell types throughout the body, but expression of FasL occurs predominantly in activated lymphocytes and other cells within the immune system. However, cells endogenous to areas of "immune privilege" also express FasL. Endogenous expression of FasL in the eye and reproductive organs, for example, helps to eliminate infiltrating proinflammatory cells, thereby preventing hazardous inflammation in sensitive organs essential for vision and reproduction. FasL expression is aberrantly switched on in cancers, causing apoptosis of tumor-infiltrating lymphocytes. This "Fas counterattack" against antitumor immune responses helps to maintain cancers in a state of immune privilege<sup>29</sup>.

Although FasL-mediated suppression of immune responses represents a liability in cancer, the ability of FasL to suppress immune responses is being pursued as a therapeutic means of protecting tissue transplants from immunological rejection. Coupled with improved techniques for gene delivery into whole organs, introduction of the FasL gene into donor organs prior to transplant holds exciting promise in transplantation medicine. However, FasL is becoming an enigmatic molecule. In addition to its well established immune-down-regulatory roles, it has recently emerged that FasL might have proinflammatory effects in some contexts. Overexpressed FasL has been shown to recruit neutrophils by two distinct molecular mechanisms. While FasL is an inducer of apoptosis within the immune system itself, inflammatory T cells and natural killer (NK) cells also use FasL to kill Fas-sensitive target cells. This can result in unwelcome FasL-mediated apoptosis of endorgan cells during clinical inflammation. In contrast, Fas-mediated clearance of activated lymphocytes is diminished in some sites of chronic inflammation. Strategies to suppress FasL-mediated neutrophil recruitment and to manipulate apoptosis via the Fas system are emerging as potential antiinflammatory therapies.

### **FasL and Immune Privilege**

Fas-mediated apoptosis contributes to clearance of activated lymphocytes from the immune system at the end of immune responses. Tight control of immune responses limits immune-mediated collateral damage of host tissues. While some tissues can sustain the im-

mune-mediated cytotoxicity inherent in inflammatory responses, an unavoidable price for the greater benefit of pathogen elimination, certain sensitive and vital organs are incapable of tolerating inflammatory damage without jeopardizing the host's survival or perpetuation. Such organs, including the eye and reproductive organs, have inbuilt mechanisms to preclude inflammatory responses and are maintained in a state of "immune privilege", exempt from the ravages of inflammatory processes.

Among the various mechanisms of immune privilege, constitutive expression of FasL by cells endogenous to immune privileged sites helps to eliminate Fas-sensitive proinflammatory cells which infiltrate these areas. FasL expressed in the anterior chamber and in the cornea causes apoptosis of Fas-sensitive proinflammatory immunocytes which enter the eye<sup>12</sup>. This prevents inflammatory reactions from occurring within the eye, which could permanently damage tissues essential for vision. Immune privilege may be maintained in a similar way in the testis, because testicular Sertoli cells express FasL<sup>4</sup>. FasL appears to contribute to fetal immune privilege, since FasL expressed in the uterus and placenta prevents trafficking of activated leukocytes between the mother and the conceptus<sup>16</sup>. Astrocyte expression of FasL may contribute to immune privilege in the brain<sup>33</sup>. While FasL is essential to the maintenance of normal immune privilege in some organs, FasL is also subversively exploited by cancers to induce apoptosis of antitumor lymphocytes, hindering antitumor immune challenge<sup>28</sup>. FasL expression is just one of several strategies deployed to maintain immune privilege. Blood-tissue barriers, expression of down-regulatory cytokines and neuropeptides, and inhibitors of complement activation, also prevail within the eye, for example<sup>40</sup>. However, while several factors collectively maintain the eye in a state of immune privilege, FasL's contribution is critical to the process<sup>12-14, 41</sup>, and may be essential for immune privilege in other sites also.

### **FasL in Experimental Transplantation**

A number of approaches have been attempted experimentally to exploit FasL as a mediator of immunological tolerance to transplants. Success has been reported for cotransplantation of pancreatic islet grafts with testicular Sertoli cells, which constitutively express FasL<sup>19</sup>, or with myoblasts engineered to express recombinant FasL<sup>21</sup>. However, direct transfection of FasL cDNA into donor organs prior to transplant has

met with mixed outcomes. Disappointingly, some such approaches led to the unexpected finding that overexpression of recombinant FasL could actually promote rejection of the transfected graft, due to neutrophil infiltration<sup>2, 17, 36</sup>. This was most probably due to the subsequently discovered ability of soluble FasL (sFasL) to recruit neutrophils<sup>24</sup>. Soluble FasL is derived by specific proteolytic cleavage of the extracellular domain of membrane FasL by matrix metalloproteinases (MMPs), and sFasL can act as a neutrophil chemoattractant<sup>35</sup>. FasL also activates the cytotoxic activity of neutrophils, but neutrophil recruitment and activation are prevented by TGF- $\beta$ <sup>8</sup>. While FasL-transfected allografts injected subcutaneously into mice were rejected due to neutrophil infiltration, FasL-TGF- $\beta$ -double-transfected cells did not recruit neutrophils and were accepted<sup>8</sup>.

The level of expression of recombinant FasL in transfected allografts appears to be of immense importance in determining the response – pro- versus anti-inflammatory – to FasL. Overexpression may be more likely to trigger a neutrophilic inflammatory response, whereas moderate levels of expression may be sufficient to mediate immune privilege. Recently, novel transfection protocols for gene delivery into whole organs have resulted in successful allograftment of FasL-transfected pancreatic islets<sup>9</sup>, liver<sup>22</sup> and kidney<sup>44</sup> in animal transplantations. Delivery of a plasmid encoding the FasL cDNA (pFasL) to transplanted livers *in vivo* was achieved by liposome vesicles conjugated with an inactivated hemagglutinating virus<sup>22</sup>. The pFasL-liposome vector complex was injected into the liver recipients via the hepatic portal vein. The level of pFasL was adjusted so that FasL was expressed in about 10% of the transplanted liver cells. While this moderate expression of FasL was found to prolong survival of the transplants, higher FasL levels led to graft destruction, and lower levels had no positive effect.

### Proinflammatory Effects of FasL

In contrast to its well established immune down-regulatory roles, FasL can exhibit proinflammatory effects in some contexts. As already mentioned, FasL can cause neutrophil recruitment and activation – proinflammatory effects which may be suppressed by TGF- $\beta$  in the microenvironment of immune privileged sites such as the eye<sup>8</sup>. FasL has been shown to stimulate the release of proinflammatory cytokines from some cell types: interleukin (IL) -1 from neutrophils<sup>24</sup>, and IL-8 from a colonic epithelial cell line<sup>1</sup>. While these proinflammatory effects of FasL have been demonstrated in

experimental contexts, it remains to be ascertained to what extent they contribute to clinical inflammation in humans. During clinical inflammation at several locations in the body, Fas-mediated apoptosis of endorgan cells appears to occur. Although such apoptosis may be immune-mediated via FasL-expressing inflammatory cells, autocrine Fas-mediated suicide may also occur in some inflammatory conditions. Apoptosis of endorgan cells may be exacerbated by a local proinflammatory cytokine microenvironment, which increases sensitivity to Fas-mediated apoptosis. While Fas-mediated apoptosis helps to delete unwanted activated T cells during normal immune homeostasis, there is evidence that this process may be impaired in certain inflammatory contexts. Provision of exogenous FasL to experimental rheumatoid joints has been shown to alleviate inflammation, suggesting that enhancement of Fas-mediated apoptosis among inflammatory cells may hold therapeutic potential.

### FasL in Neutrophil Recruitment and Activation

As already mentioned, recombinant FasL overexpressed in transfected allograft cells can trigger the recruitment of neutrophils, and sFasL is a neutrophil chemoattractant<sup>35</sup>. Chemotactic signaling via Fas is apparently distinct from Fas-mediated apoptosis signaling, since sFasL can chemoattract neutrophils from *lpr*<sup>cg</sup> mice that express a Fas with a mutated death domain. An involvement of sFasL in inflammatory diseases is suggested by the finding of elevated serum levels of sFasL in patients with myocarditis<sup>46</sup>, alcoholic liver disease<sup>45</sup>, and rheumatoid arthritis<sup>26</sup>. In rheumatoid arthritis, the level of sFasL present in the synovial fluid of the inflamed joints correlated with disease severity<sup>15</sup>. This suggests that the neutrophilic infiltrate frequently observed in rheumatoid joints may be partly due to sFasL-mediated chemotaxis. Hence, in addition to causing tissue destruction, the increased MMP activity associated with rheumatoid arthritis might also contribute to inflammation by increasing production of sFasL.

While sFasL directly chemoattracts neutrophils, a second mechanism of FasL-mediated neutrophil recruitment has also been identified. FasL induces secretion of IL-1 $\beta$ , another neutrophil chemoattractant, from neutrophils themselves<sup>24</sup>. This occurs via Fas-mediated activation of caspases, including caspases in addition to caspase-1 (IL-1 $\beta$ -converting enzyme, or ICE), which proteolytically process pro-IL-1 $\beta$ , leading to secretion of active IL-1 $\beta$ . Secretion of IL-1 $\beta$  by a limited number of “vanguard” neutrophils which initially infiltrate

FasL-overexpressing experimental allografts appears to chemoattract an amplified second wave of neutrophil infiltration. As already mentioned, FasL also activates the cytotoxic activity of neutrophils, but FasL-mediated neutrophil activation is inhibited in the presence of TGF- $\beta$ <sup>8</sup>. The local cytokine microenvironment, therefore, appears to play a role in determining whether or not FasL will exert a proinflammatory effect. Insufficient downregulatory cytokines, such as TGF- $\beta$ , at sites of active inflammation might, therefore, promote the neutrophil-recruiting activity rather than the immune downregulatory role of FasL.

### Fas-Mediated Endorgan Apoptosis during Inflammation

Increased apoptosis of endorgan cells has been reported at several sites of inflammation. Fas is widely expressed in many tissues, and since FasL is expressed as a cytotoxic mediator by activated immune effector cells, Fas-mediated apoptosis has been implicated in inflammatory tissue damage. Infiltration of Fas-expressing endorgan tissues by FasL-expressing inflammatory cells has been reported in Sjogren's syndrome<sup>43</sup>, hepatitis B-<sup>10</sup> and hepatitis C-<sup>23</sup> associated liver inflammation, and in ulcerative colitis<sup>39</sup>. Furthermore, endorgan cells in some inflammatory conditions appear to upregulate their own FasL, suggesting that Fas-mediated autocrine suicide or juxtacrine fratricide may contribute to cell death. Such conditions include alcohol-induced liver inflammation<sup>10</sup> and *Helicobacter pylori*-induced gastritis<sup>5, 32</sup>. In Hashimoto's thyroiditis, thyrocytes, which constitutively express FasL, upregulate expression of Fas, leading to cell suicide and clinical hypothyroidism<sup>11</sup>.

A proinflammatory cytokine microenvironment appears to exacerbate tissues' susceptibility to Fas-mediated apoptosis. Specifically, the Fas-sensitivity of many cell types is increased by the proinflammatory cytokine interferon (IFN)- $\gamma$ . Sensitization is due to upregulation of Fas in some cell types, although IFN- $\gamma$  also sensitizes Fas-resistant cells which already express abundant cell-surface Fas<sup>18, 27</sup>. Caspase-1 is upregulated by IFN- $\gamma$ <sup>18, 30</sup>, and there is evidence that IFN- $\gamma$  may upregulate other caspases also<sup>30</sup>. IFN- $\gamma$  may also increase apoptotic sensitivity at the level of the Bcl-2 rheostat, since there is evidence for upregulation of the proapoptotic Bcl-2 homologue Bak in response to IFN- $\gamma$ <sup>30</sup>. By producing apoptosis-enhancing cytokines such as IFN- $\gamma$ , inflammatory T cells can therefore set endorgan cells up to receive a FasL-mediated apoptotic "hit". Hashimoto's

thyroiditis provides an intriguing example of how the proinflammatory microenvironment can sensitize endorgan cells to Fas-mediated suicide. Thyrocyte upregulation of Fas in Hashimoto's thyroiditis appears to be stimulated by IL-1 $\beta$  produced by inflammatory cells<sup>11</sup>. Hence, even though thyroid-infiltrating inflammatory cells express little FasL<sup>38</sup>, they condition the microenvironment such that thyrocytes upregulate Fas, resulting in suicide via their own FasL.

The contribution of endorgan apoptosis to inflammatory tissue damage is highlighted by the recent finding that the pan-caspase inhibitor z-VAD-fmk was shown to prevent inflammatory-mediated cell death of hippocampal neurons in experimental pneumococcal meningitis in rabbits. This suggests exciting therapeutic potential for caspase inhibitors in preventing brain damage during pneumococcal meningitis<sup>6</sup>. However, therapeutic approaches to limit endorgan apoptosis must be carefully designed so that apoptosis of lymphocytes is not also significantly impeded, which would prolong inflammatory activity.

### Fas-Mediated Apoptosis of Inflammatory Lymphocytes

There is a paucity of T cell apoptosis within rheumatoid joints<sup>34</sup>, suggesting that insufficient apoptosis of lymphocytes might be one reason why inflammatory responses become chronic. Activated T cells are normally sensitized to FasL, at least in part, by downregulation of the antiapoptotic Bcl-2 homologue, Bcl-xL. Rheumatoid T cells were found to express high levels of Bcl-xL when isolated<sup>34, 37</sup>, which might account for their apparent lack of apoptosis *in situ*. Coculture of synovial T cells with fibroblasts *in vitro* maintained high Bcl-xL expression, and inhibited apoptosis in the T cells<sup>34</sup>. Fibroblast protection of T cells from apoptosis involved integrin-ligand interactions. This finding suggests that the Fas-sensitivity of T cells might be modulated within rheumatoid joints via survival signals from fibroblasts. In addition, sFasL, which has very little apoptosis-inducing activity, can actually inhibit proper induction of apoptosis by membranous FasL. Hence, sFasL present in rheumatoid joints might inhibit intercellular FasL-Fas signaling between autoreactive lymphocytes, preventing their apoptotic clearance.

In Hashimoto's thyroiditis, infiltrating T cells are apparently sensitive to Fas-mediated apoptosis, yet these T cells lack significant expression of FasL<sup>38</sup>. While expression of Fas was found to be upregulated in both infiltrating lymphocytes and hyperactivated sy-

novial cells in arthritic joints, FasL expression was largely absent<sup>7, 48</sup>. These findings suggest that in some conditions, Fas-expressing inflammatory cells do not express sufficient FasL to induce self-regulatory apoptosis, and that these inflammatory cells might be eliminated therapeutically by supplying FasL to the site of inflammation. Adenoviral-mediated gene transfer of FasL ameliorated collagen-induced arthritis in mouse ankle joints<sup>47</sup>. In addition to causing increased local apoptosis of lymphocytes and hyperactivated synovial cells, provision of FasL to the inflamed joint also induced systemic tolerance of the causative autoantigen (collagen in this model). This is consistent with the finding that FasL-mediated suppression of an antigen-specific immune response within the eye could also induce systemic tolerance to that antigen<sup>14</sup>. In experimental autoimmune thyroiditis, gene therapy with an expression vector encoding FasL, which was injected directly into the inflamed thyroid, resulted in apoptotic clearance of infiltrating autoreactive T cells<sup>3</sup>. While FasL has been demonstrated to have therapeutic efficacy in these instances, there is always a risk that FasL might exacerbate Fas-mediated apoptosis of endorgan cells. If FasL is to have therapeutic potential, a delicate balance must be achieved between Fas-mediated clearance of inflammatory cells and limitation of Fas-mediated endorgan apoptosis.

Hematopoietic cell protein tyrosine phosphatase (HCP) is required for Fas-mediated apoptosis signaling in lymphoid cells<sup>42</sup>. Indeed, protein phosphorylation is generally protective against apoptosis, whereas protein dephosphorylation is associated with pro-apoptotic signaling. Members of a family of protein kinase C (PKC)-inhibitory compounds, the bisindolymaleimides I–XI, can selectively increase the sensitivity to Fas-mediated apoptosis of activated, but not resting, lymphocytes<sup>49</sup>. Bisindolymaleimide VIII was shown to prevent the onset of experimental autoimmunity in mice, suggesting therapeutic potential for this compound in selectively eliminating autoreactive lymphocytes. Whether bisindolymaleimide VIII could have therapeutic efficacy as an antiinflammatory agent would depend on whether or not it would also increase the Fas-sensitivity of endorgan cells. Since the Fas-resistance of T cells in rheumatoid joints appears to be due to prolonged expression of Bcl-xL, antisense oligonucleotides to Bcl-xL directed to the site of inflammation might promote Fas-mediated clearance of rheumatoid T cells. The efficacy of such an approach would again depend on whether antisense to Bcl-xL would preferentially sensitize inflammatory cells rather than endorgan cells to apoptosis.

## Conclusions

As a potent inducer of local immune privilege, FasL was initially perceived as a “holy grail” in transplantation medicine. However, initial excitement surrounding the therapeutic possibility that FasL could mediate immune privilege in transplants was tempered by the unexpected finding that overexpressed FasL could exert proinflammatory effects, particularly neutrophil recruitment. Recently, novel techniques for gene delivery into whole organs, and adjustment of expression to obtain moderate levels of FasL, have led to success in achieving tolerance to experimental transplants. Inclusion of TGF- $\beta$  might complement such a strategy, by inhibiting proinflammatory effects of FasL. In addition to its proinflammatory activity, FasL also appears to induce apoptosis of endorgan cells during inflammation. Sources of FasL in the inflammatory context include activated immune effector cells, or upregulated FasL expression in endorgan cells themselves. Caspase inhibitors might offer therapeutic potential in preventing inflammatory-mediated endorgan apoptosis. On the other hand, insufficient apoptosis of inflammatory cells seems to contribute to chronic inflammation in rheumatoid arthritis. Lack of FasL means that rheumatoid T cells elude normal Fas-mediated self-regulation, and gene therapy with FasL ameliorated inflammation in a mouse model of arthritis. In addition to provision of FasL, future antiinflammatory therapies might also include agents, such as bisindolymaleimides or antisense to Bcl-xL, to enhance apoptosis of inflammatory cells. However, such strategies must be tailored to achieve apoptosis of inflammatory cells without exacerbating endorgan apoptosis. Therapeutic manipulation of Fas-mediated apoptosis clearly holds exciting clinical potential in suppressing unwelcome inflammatory activity both in transplantation and inflammatory diseases.

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