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Role of Fas–FasL in inflammatory diseases

Joe O'Connell

Fas ligand (FasL) induces programmed cell death, or 'apoptosis', in cells expressing its cognate receptor, Fas (CD95/APO-1). There is evidence that FasL precludes inflammatory reactions from sites of 'immune privilege' by triggering Fas-mediated apoptosis of infiltrating pro-inflammatory cells. The ability of FasL to impair immune responses is being pursued as a possible means of protecting tissue transplants from immunological rejection, and therapeutic promise has been reported in some experiments. However, FasL is becoming an enigmatic molecule, exhibiting pro-inflammatory activity independently of its ability to mediate immune downregulation. FasL can recruit and activate neutrophils and macrophages in some experimental situations. Triggering of Fas in some cell types has been shown to upregulate expression of certain pro-inflammatory cytokines and chemokines, providing an unexpected link between apoptosis and inflammation. FasL appears to contribute to the destruction of Fas-sensitive end-organ cells during inflammation. This appears to occur in two ways: (1) direct killing by cytotoxic immune effector cells expressing FasL; or (2) autocrine cell suicide of end-organ cells that upregulate their own FasL in the inflammatory context. Depending on the condition, or the site of inflammation, either or both mechanisms may occur. Prevention of Fas-mediated end-organ apoptosis and enhancement of Fas-mediated apoptosis of inflammatory cells are emerging as potential anti-inflammatory therapeutic goals.

Fas (CD95/APO-1) is a cell-surface member of the tumour necrosis factor (TNF) receptor superfamily and mediates programmed cell death, or 'apoptosis', upon engagement by its ligand, FasL (Ref. 1). Fas is widely expressed in numerous different cell types throughout the body, whereas FasL expression appears to be more restricted. Following activation, different cell types within the immune system express FasL, including T and B cells. FasL is also expressed in cells within areas of 'immune privilege', including the eye and reproductive

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organs. FasL-induced apoptosis plays both regulatory and effector functions in the immune system and appears to contribute to inflammatory processes.

Introduction to the Fas–FasL system

Apoptosis signalling via Fas

Analysis of the respective promoter sequences for the Fas and FasL genes, and studies of their regulation, have identified some of the transcription factors that control their expression. Fas expression is regulated in different cell types by transcription factors that include nuclear factor κB (NF-κB) (Ref. 2), activator protein 1 (AP-1) (Ref. 3) and p53 (Ref. 4). FasL also appears to be regulated by NF-κB and AP-1, as well as by the nuclear factor NF-AT (Ref. 5), c-Myc (Ref. 6) and the interferon regulatory factors 1 and 2 (IRF-1 and IRF-2) (Ref. 7).

Structurally, Fas has three cysteine-rich extracellular domains and an intracellular 'death domain' of approximately 80 amino acids, which is required for apoptosis signalling. FasL appears to form trimers, and trimerises Fas upon binding. Following triggering by FasL, several proteins are recruited to the death domain of Fas, to establish a death-inducing signalling complex (DISC) (Fig. 1) (Ref. 8). Triggering of Fas directly activates an intracellular proteolytic cascade mediated by the caspase family of proteases. Within the DISC, an adaptor protein, Fas-associated death domain protein (FADD), provides a bridge between stimulated Fas and pro-caspase-8. FADD-mediated recruitment of pro-caspase-8 to the activated Fas receptor results in proteolytic processing and autoactivation of pro-caspase-8. Functional caspase-8, also known as Fas-linked interleukin-1β (IL-1β)-converting enzyme-like protease (FLICE), in turn initiates activation of intracellular members of the caspase family (Ref. 9). Caspase proteolysis of specific protein targets is central to the execution of apoptosis; indeed, tri-peptide caspase inhibitors can block apoptosis (Ref. 10). Other signals also emanate from stimulated Fas, including the activation of an acidic sphingomyelinase (SMase) that leads to the production of intracellular ceramide (Ref. 11). Ceramide is a mediator of cell stress, and might play a role in Fas-mediated apoptosis.

Cells regulate their response to Fas at intracellular points in the Fas signal transduction pathway. Regulation of the Fas signal is achieved, at least in part, by members of the Bcl-2 family of apoptosis regulatory proteins (Refs 12, 13). This family consists of pro- (e.g. Bax, Bak, Bcl-x_S) and anti- (e.g. Bcl-2, Bcl-x_L) apoptotic homologues, which combine to form homo- or heterodimers. The balance between pro- and anti-apoptotic dimers acts as a molecular rheostat controlling the susceptibility of the cell to apoptosis (Ref. 14). Regulation also takes place at the level of Fas itself. Certain proteins can be recruited to the DISC, including FLICE-inhibitory protein (FLIP) (Ref. 15) and Fas-associated phosphatase-1 (FAP-1) (Ref. 16), which exert an inhibitory effect on Fas signalling. In addition, the activity of caspases can be regulated by a family of proteins called 'inhibitor of apoptosis proteins' (IAPs) (Ref. 17). Humans express at least seven different IAPs, including survivin, XIAP, cIAP1 and cIAP2. These proteins can physically interact with, and block, caspase activity. XIAP, cIAP1 and cIAP2 can specifically inhibit caspase-3, -7 and -9, and can inhibit induction of apoptosis in response to diverse stimuli, including FasL (Ref. 17).

Immunoregulatory functions of the Fas–FasL system

Although the full spectrum of physiological roles for the Fas–FasL system has yet to be determined, the receptor–ligand is best known for its roles in immunoregulation. Fas and FasL are coexpressed on the surface of activated lymphocytes, and Fas-mediated autocrine 'suicide' or juxtacrine 'fratricide' (i.e. killing of neighbouring cells upon contact via mutual triggering of Fas by FasL expressed on both cells) of lymphocytes helps to terminate immune responses (Ref. 18). Although T cells upregulate both Fas and FasL upon activation, they only become sensitive to Fas-mediated apoptosis after a period of a few days' activation. Fas sensitisation is facilitated, at least in part, by a decrease in the level of the anti-apoptotic Bcl-2 homologue Bcl-x_L (Ref. 19). In this way, potentially toxic T cells are permitted a limited tenure of activity, followed by rapid deletion via the Fas pathway, a process known as activation-induced cell death (AICD). FasL also contributes to deletion of autoreactive lymphocytes during acquisition of self-tolerance (Ref. 20). This prevents the accumulation of autoreactive immune effector cells that are directed against self-antigens.

Recently, it has emerged that the Fas system exerts other immune-downregulatory functions

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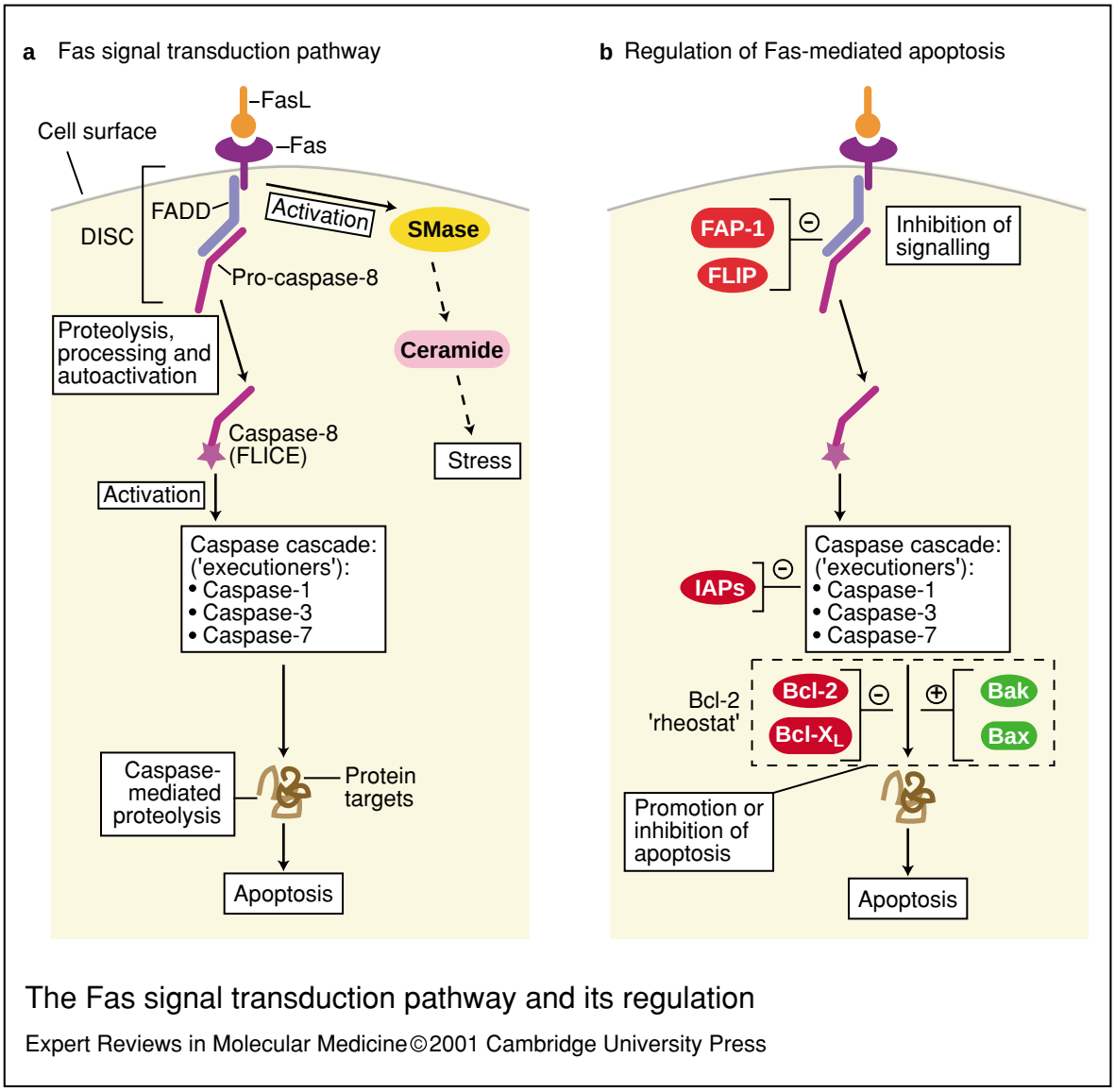

The Fas signal transduction pathway and its regulation

Figure 1. The Fas signal transduction pathway and its regulation. (a) When cell-surface Fas engages with Fas ligand (FasL), several proteins are recruited to the intracellular 'death domain' of Fas to form a death-inducing signalling complex, or DISC (Ref. 8). Of these, the Fas-associated death domain (FADD) protein acts as an adaptor, linking pro-caspase-8 to the stimulated Fas (Ref. 9). This results in the proteolytic processing and autoactivation of pro-caspase-8. The activated 'initiator' caspase-8, also known as Fas-linked interleukin-1β-converting enzyme-like protease (FLICE), then instigates activation of intracellular 'executioner' caspases. This caspase cascade, which leads to caspase-mediated proteolysis of specific protein targets, is central to the apoptotic process (Ref. 10). An acidic sphingomyelinase (SMase) is also activated in response to stimulation of Fas, resulting in the production of ceramide, a mediator of cell stress (Ref. 11). (b) The Fas signal is subject to regulation at the level of the Fas receptor and the caspase cascade. Recruitment of FLICE-inhibitory protein (FLIP) (Ref. 15) and Fas-associated phosphatase-1 (FAP-1) (Ref. 16) to the DISC inhibits Fas signalling. Caspases are inhibited by 'inhibitor of apoptosis proteins' (IAPs) (Ref. 17) The apoptosis pathway is regulated in certain cell types by the 'Bcl-2 rheostat' (Refs 12, 13), comprising Bcl-2 homologues that either inhibit (e.g. Bcl-2, Bcl-x_L) or promote (e.g. Bak, Bax) apoptosis (Ref. 14). There is also evidence that the Fas pathway can be regulated at the transcriptional level, such as by the tumour suppressor p53 (not shown). p53 positively regulates genes, including Fas and Bax, that are involved in FasL-induced apoptosis (Ref. 32) **(fig001joc)**.

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in addition to induction of lymphocyte apoptosis. Ligation of Fas results in blockade of calcium-release-activated calcium channels (CRAC) in T cells, thus inhibiting their activation (Ref. 21). Calcium-dependent T-cell activation events, such as production of IL-2, are thus inhibited by FasL. FasL has also been shown to inhibit B-cell activation and immunoglobulin (Ig) production (Ref. 22).

The importance of the Fas system in normal immunoregulation is clear from the autoimmune and lymphoproliferative syndromes occurring in mice with mutations of either Fas (*lpr*) or FasL (*gld*) (Ref. 1). The *lpr* (lymphoproliferation) mutation results in greatly diminished expression of Fas, to ~1–2% of the wild-type level. A second Fas mutation, lpr^{cg}, consists of a point mutation in the death domain, leading to expression of a Fas protein that is defective in signal transduction. The *gld* (generalised lymphoproliferative disease) mutation in the FasL gene results in expression of a non-functional FasL. Mice with either of these defects in the Fas–FasL system exhibit a similar phenotype, characterised by lymphoproliferative and autoimmune syndromes. These mice accumulate excessive numbers of T cells in the spleen and lymph nodes, and exhibit an autoimmune syndrome resembling systemic lupus erythematosis (SLE). In humans, several different Fas mutations have been found in children with autoimmune lymphoproliferative syndrome (ALPS), a rare autoimmune condition characterised by massive lymphadenopathy (Refs 23, 24). This finding has emphasised the crucial role of Fas in immune homeostasis and peripheral tolerance.

FasL and immune privilege

Another immunoregulatory role for the Fas system emerged with the finding that FasL was constitutively expressed in certain cells within sites of 'immune privilege'. These sensitive areas, including the eye and reproductive organs, cannot tolerate the tissue damage inherent in inflammatory responses and have evolved strategies to suppress local inflammation, one of which appears to be the expression of FasL (Fig. 2). In the eye, for example, endogenous expression of FasL in the retina and cornea helps to eliminate infiltrating pro-inflammatory cells, thereby preventing hazardous inflammation that could permanently damage tissues essential for vision (Refs 25, 26). There is evidence that expression of FasL contributes to the transplantability of immune-privileged tissues (Ref. 27) and, in particular, to the remarkable success of human corneal transplants (Refs 28, 29).

In maintaining immune privilege, FasL does not act in isolation. Other mechanisms, including blood–tissue barriers, expression of downregulatory cytokines and neuropeptides, and inhibitors of complement activation, also prevail within the eye and other areas of immune privilege (Ref. 30). However, while several mechanisms collectively maintain the eye in a state of immune privilege, FasL has been shown to be critical to the process (Refs 25, 26, 28, 31), and might also be essential for immune privilege in other sites. Ocular immune privilege has been found to be deficient in *gld* mice that have a mutated FasL. Aberrant expression of FasL by tumours appears to impair anti-tumour immune responses, helping to maintain cancers in a state of immune privilege (Ref. 32).

FasL in experimental transplantation

The ability of FasL to suppress immune responses has been pursued as a potential strategy to protect experimental tissue transplants from immunological rejection. Success has been reported for cotransplantation of pancreatic islet cells with testicular Sertoli cells, which constitutively express FasL (Ref. 33), or with myoblasts engineered to express recombinant FasL (Ref. 34). Coupled with new techniques for gene transfer into whole tissues, introduction of the gene encoding FasL into donor organs prior to transplant holds exciting promise in transplantation medicine. Recently, novel transfection protocols for gene delivery into whole organs have led to successful alloengraftment of FasL-transfected pancreatic islets (Ref. 35), liver (Ref. 36) and kidney (Ref. 37) in animal transplantations. However, direct transfection of FasL cDNA into donor organ cells has also met with unwelcome outcomes. Disappointingly, in many such approaches, it was found that inducing expression of recombinant FasL actually accelerated rejection of the transfected graft, mediated largely by a neutrophilic infiltrate (Refs 38, 39, 40). In stark contrast to its immune-downregulatory roles, these experiments revealed – quite unexpectedly – that FasL might have pro-inflammatory effects in some contexts.

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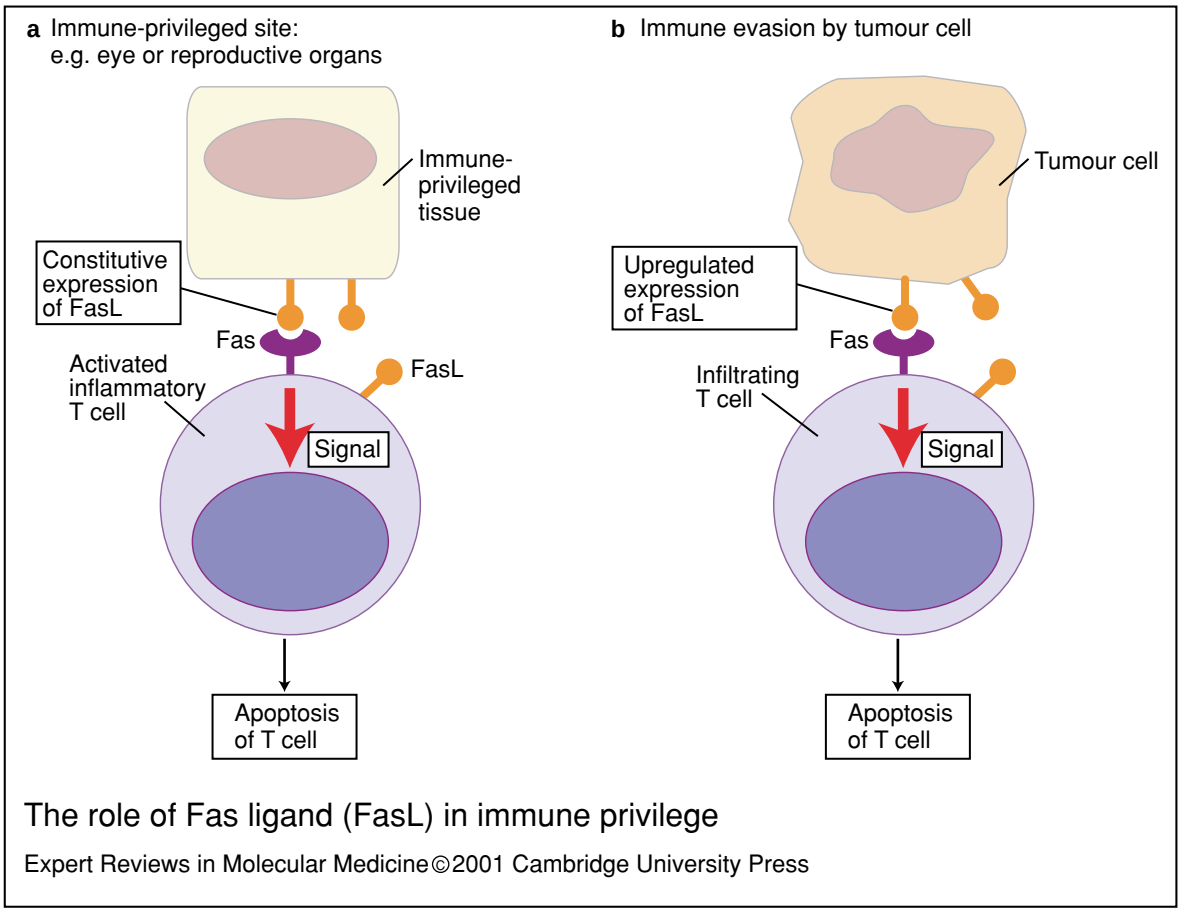

Figure 2. The role of Fas ligand (FasL) in immune privilege. (a) Inflammatory reactions are necessarily suppressed in certain organs where inflammatory damage could be detrimental to the host. Cells in these sites of 'immune privilege', such as the eye (Ref. 25) and reproductive organs (Ref. 27), express FasL constitutively, inducing Fas-mediated apoptosis of activated inflammatory cells that enter these sites. (b) Upregulated expression of FasL by tumour cells might contribute to immune evasion by 'counterattacking' anti-tumour immune effector cells. In some human cancers – including colon, oesophageal and ovarian cancers – FasL has been associated with apoptosis and loss of tumour-infiltrating lymphocytes (TILs) (Ref. 32) **(fig002joc)**.

Pro-inflammatory activities of FasL

Neutrophil-stimulatory effects of FasL: recruitment and activation

Soluble FasL: a neutrophil chemoattractant?

Transfection of recombinant FasL into experimental murine allograft cells prior to transplantation triggers the recruitment of neutrophils in many instances (Refs 38, 39, 40). It was suggested that this is due to an ability of soluble FasL (sFasL) to act as a neutrophil chemoattractant, since soluble forms of FasL seem to stimulate neutrophil migration in in vitro assays (Refs 41, 42). sFasL is derived by specific proteolytic cleavage of the extracellular domain of membraneous FasL by matrix metalloproteinases (MMPs). However, as will shortly be discussed, more-recent evidence has suggested that sFasL is not responsible for neutrophil recruitment in vivo, and may even oppose this FasL-mediated inflammatory effect. The discrepancy in experimental observations may be due to the fact that some earlier experiments used a recombinant form of sFasL that contained the entire extracellular domain of FasL, rather than the somewhat shorter, authentic, proteolytically cleaved version.

Further work is needed to confirm whether sFasL plays pro- or anti-inflammatory roles in human inflammatory diseases. The contribution of sFasL to inflammatory disorders is suggested by the finding of elevated sFasL in the serum of patients with conditions such as myocarditis

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(Ref. 43), alcoholic liver disease (Ref. 44) and rheumatoid arthritis (Ref. 45). In rheumatoid arthritis, the level of sFasL in the synovial fluid of the inflamed joints correlated with disease severity (Ref. 46), which would tend to suggest a pro-inflammatory role for sFasL. However, an alternative pro-inflammatory role for sFasL might be the inhibition of lymphocyte apoptosis, as will be discussed below.

FasL-mediated neutrophil recruitment: an indirect mechanism

While the suggestion that sFasL can directly chemoattract neutrophils remains somewhat controversial, it is highly probable that FasL can mediate neutrophil recruitment by an indirect mechanism. FasL induces secretion of IL-1β, a neutrophil chemoattractant, from neutrophils themselves (Ref. 42), and probably from other cell types. This occurs because triggering of Fas activates caspases, including caspase-1 (originally known as IL-1β-converting enzyme, or ICE), which proteolytically process pro-IL-1β, resulting in the secretion of active IL-1β. Thus, experimental allografts that overexpress FasL can trigger secretion of IL-1β, either from the limited number of 'vanguard' neutrophils that initially infiltrate the graft, or indeed from other host cells. This local, FasL-mediated IL-1β production may then result in chemoattraction of an amplified wave of neutrophil infiltration (Fig. 3). Support for this mechanism comes from the finding that FasL-mediated neutrophil recruitment does not occur in IL-1-knockout mice (Ref. 42).

TGF-β inhibits neutrophil-stimulatory effects of FasL

Upon cell–cell contact between a target cell and a neutrophil, FasL on the surface of the target cell appears to activate the cytotoxic activity of the neutrophil directly. Neutrophil recruitment and activation are inhibitable by transforming growth factor β (TGF-β) (Ref. 47). While allografts of FasL-transfected cells injected subcutaneously into mice were rejected in association with a neutrophilic infiltration, FasL–TGF-β double-transfected cells were accepted (Ref. 47). Interestingly, solutions of aqueous humour extracted from within murine eyes also prevented the neutrophil-activating effect of FasL in vitro, and this inhibitory effect was abolished by the addition of an anti-TGF-β neutralising antibody. Thus, TGF-β present within the eye may prevent inflammatory effects of ocular-expressed FasL, favouring instead its role in mediating immune privilege (Ref. 47). These results suggest that the local cytokine microenvironment might play an important role in determining the effect – pro- versus anti-inflammatory – of FasL. For instance, the relative absence of downregulatory cytokines such as TGF-β at sites of active inflammation might promote the neutrophil-recruiting activity of FasL.

Full-length FasL: the pro-inflammatory form of FasL in vivo

In contrast to the view that sFasL represents the pro-inflammatory form of FasL, a recent experiment provided evidence that in fact full-length, membrane-bound FasL was the predominant mediator of inflammatory effects (Ref. 48). A comprehensive study was performed using cells expressing recombinant constructs of various forms of FasL, including soluble-only FasL, full-length 'native' FasL, and non-cleavable, membrane-only FasL. Cells expressing the various constructs were injected into the peritoneal cavity of syngeneic mice and the ensuing inflammatory response was analysed. It was found that full-length, apoptosis-active FasL was the predominant pro-inflammatory form of FasL in vivo (Ref. 48). Furthermore, the ability of the various forms of FasL to elicit inflammation correlated with their potential to induce apoptosis, and the MMP-cleaved, natural sFasL form opposed FasL-mediated inflammation in vivo. It was also previously shown that sFasL has markedly reduced apoptosis-inducing activity compared with membrane-bound FasL (Ref. 49). The results of these experiments would therefore suggest that the rate of MMP-mediated shedding of FasL regulates both its inflammatory (Ref. 48) and its apoptotic (Ref. 49) effects.

Unexpected links between apoptosis and inflammation

One of the main findings of the study of cells expressing recombinant constructs of FasL (Ref. 48) was that Fas signalling of host cells was a critical factor in triggering inflammatory responses to FasL-overexpressing cells. FasL-overexpressing allografts grown in the peritoneal cavity did not induce inflammation in host mice (lpr^{cg}) that express a cell-surface Fas with a mutated, non-signalling death domain (Ref. 48). However, the inflammatory response, including

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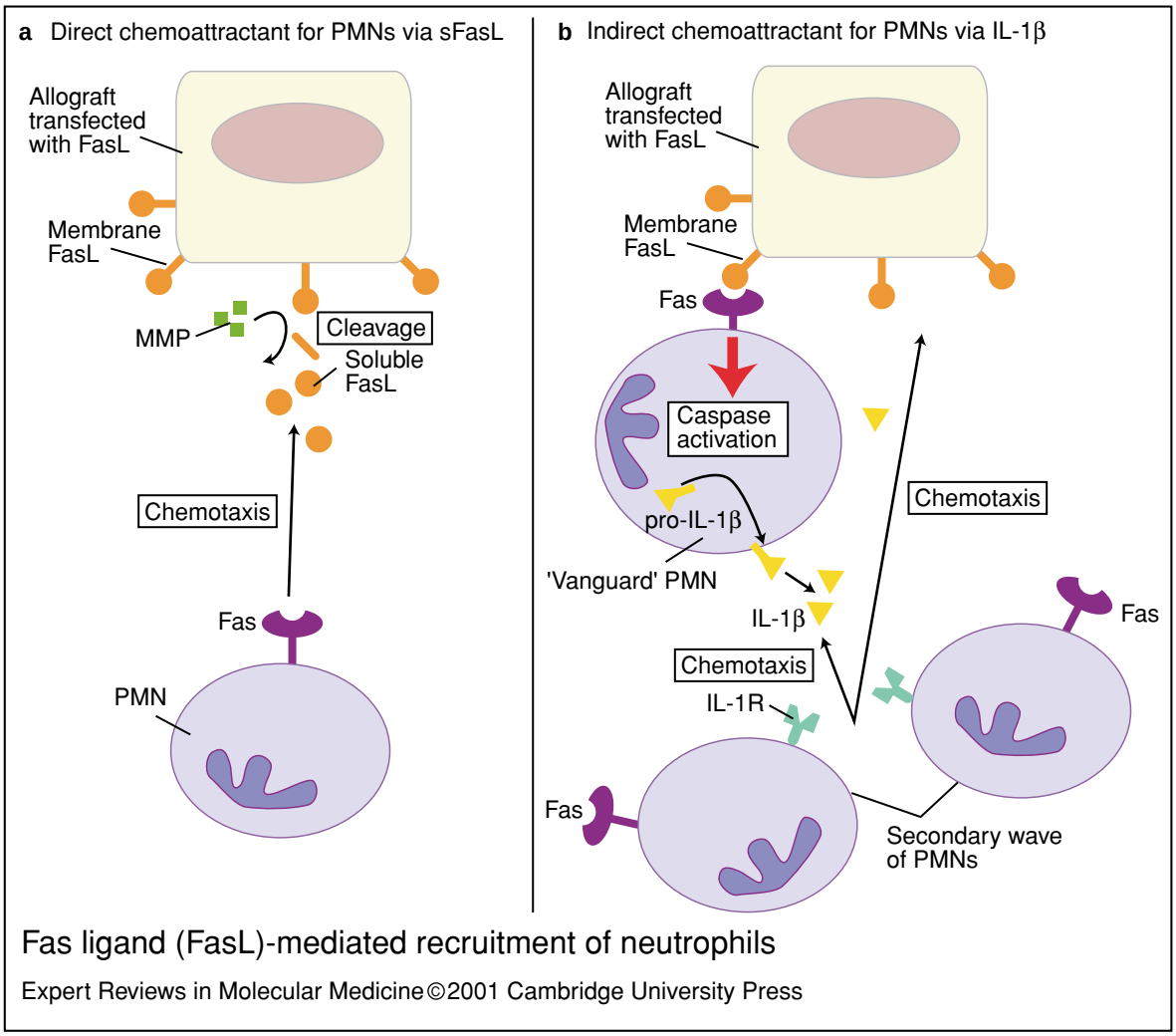

Figure 3. Fas ligand (FasL)-mediated recruitment of neutrophils. Two potential mechanisms appear to account for the recruitment of polymorphonuclear leukocytes (PMNs), or neutrophils, to some allografts overexpressing recombinant FasL. (a) Direct chemoattraction via soluble FasL (sFasL). sFasL is derived by matrix metalloproteinase (MMP)-mediated cleavage of the extracellular domain of membraneous FasL, and has been shown to act as a direct neutrophil chemoattractant in vitro (Ref 41). (b) Indirect chemoattraction via FasL-initiated production of interleukin-1β (IL-1β). When FasL on allograft cells engages Fas on neutrophils – and indeed other cell types such as macrophages – this activates caspases that process pro-IL-1β, leading to secretion of active IL-1β. The secreted IL-1β then chemoattracts an amplified wave of neutrophils to the site where FasL stimulation of the 'vanguard' neutrophils occurred. IL-1β binds to its receptor, IL-1R, on neutrophils (Ref. 42). In addition to chemotaxis, IL-1β activates neutrophils, and enhances neutrophil adhesion, cytotoxicity and phagocytosis **(fig003joc)**.

neutrophil recruitment, was restored when Fas-responsive cells isolated from the peritoneum of syngeneic wild-type mice were injected into the peritoneal microenvironment of the allograft in the *lpr*^{cg} hosts. This indicated that inflammation was secondary to FasL-mediated stimulation of host cells, and that the process depended on FasL-mediated production of neutrophil chemoattractants by Fas-sensitive cells in the allograft microenvironment, rather than on any direct effect of FasL on the neutrophils themselves.

It is emerging that not only are apoptosis and inflammation *not* mutually exclusive – as had originally been thought – but they may actually be intimately linked. This is particularly evident from the finding that some caspases (including caspase-1) have evolved dual roles, both in the execution of apoptosis and in the secretion of

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pro-inflammatory cytokines (including IL-1β and IL-18) (Refs 50, 51). It might be that this double function facilitates cytokine-mediated recruitment of inflammatory phagocytes to engulf and clear apoptotic bodies in areas of excessive apoptosis. In addition to stimulating caspase-mediated processing and release of pro-inflammatory cytokines, FasL also upregulates the expression of various chemokines and cytokines in different cell types. Indeed, IL-8 expression is induced in response to FasL in several cell types (Ref. 52). Furthermore, rat vascular smooth muscle cells engineered to overexpress FADD – which activates the Fas apoptosis pathway downstream from Fas itself – were found to upregulate several cytokines / chemokines, including IL-8, IL-1α, macrophage chemotactic protein 1 (MCP-1), growth-regulated oncogene 1 (GRO-1) and IL-6 (Ref. 53). Moreover, this triggered macrophage recruitment when these cells underwent apoptosis in vivo (Ref. 53), providing further evidence that inflammation is triggered by activation of the Fas apoptosis pathway, rather than by any ability of the FasL protein itself to chemoattract inflammatory cells. The study showed that locally excessive Fas-mediated apoptosis might not be 'silent' with respect to the immune system, but might instead be 'flagged' for recruitment of inflammatory cells.

Apoptosis of end-organ cells during inflammation: the role of Fas

FasL-mediated cell death: immune-mediated killing and autocrine suicide

Despite the involvement of common inflammatory cells and mediators, different inflammatory diseases affect different tissues or organs in the body. For example, rheumatoid arthritis is predominantly a disease arising from inflammation of the joints, whereas hepatitis results from inflammation of the liver. The term 'end-organ' refers to the target organ where the inflammatory response is directed. Despite the different anatomical locations, there are some common features among the inflammatory processes that can occur in different affected end-organs. Death of end-organ cells is inherent in the pathogenesis of many inflammatory conditions, and is considered to be due largely to rampant immune-mediated cytotoxicity. As discussed earlier, Fas is widely expressed in many tissues, and FasL is expressed as a cytotoxic mediator by immune effector cells. Fas-mediated killing of end-organ cells by FasL-expressing cytotoxic cells has been implicated during inflammation in various sites (Fig. 4a). For instance, Fas-expressing end-organ tissues are infiltrated by FasL-expressing inflammatory cells in Sjorgren's syndrome (Ref. 54), ulcerative colitis (Ref. 55), and viral hepatitis induced by either hepatitis virus B (Ref. 56) or C (Ref. 57).

In some inflammatory conditions, the end-organ cells themselves appear to upregulate their own FasL. Hence, it would appear that, in addition to direct immune-mediated killing, Fas-mediated autocrine suicide or juxtacrine fratricide might contribute to the inflammatory pathology (Fig. 4b). Such conditions include alcohol-induced liver inflammation (Ref. 56) and *Helicobacter pylori*-induced gastritis (Ref. 58). In Hashimoto's thyroiditis (HT), thyrocytes upregulate expression of Fas and, because these cells have been reported to express FasL constitutively, this results in cell suicide and clinical hypothyroidism (Ref. 59). However, this proposed autocrine mechanism of thyrocyte death in HT remains controversial; some investigators have shown thyrocyte expression of FasL mRNA and protein, and bioactivity of FasL (Ref. 59), while others have been unable to detect FasL expression in thyrocytes (Ref. 60). Doubts have been raised about the specificity of some commercially available anti-FasL antibodies. However, other groups have rigorously demonstrated the specificity of the antibodies concerned (Ref. 61). The controversy has emphasised the need for careful specificity and sensitivity of controls when attempting to detect FasL. Concordant detection of FasL mRNA and protein, or detection using more than one reagent, are necessary to demonstrate FasL expression convincingly.

Inflammatory cytokines exacerbate cell death

End-organ cells are probably particularly susceptible to apoptosis in the inflammatory microenvironment. The Fas sensitivity of many cell types is increased by the pro-inflammatory cytokine interferon γ (IFN-γ). Although this is partly due to the ability of IFN-γ to upregulate expression of Fas (Ref. 62), IFN-γ also has other effects that increase the susceptibility of the cell to apoptosis. Strong expression of cell-surface Fas is a prerequisite for induction of Fas-mediated apoptosis, but expression of Fas alone is not sufficient. As discussed above, the Fas signal

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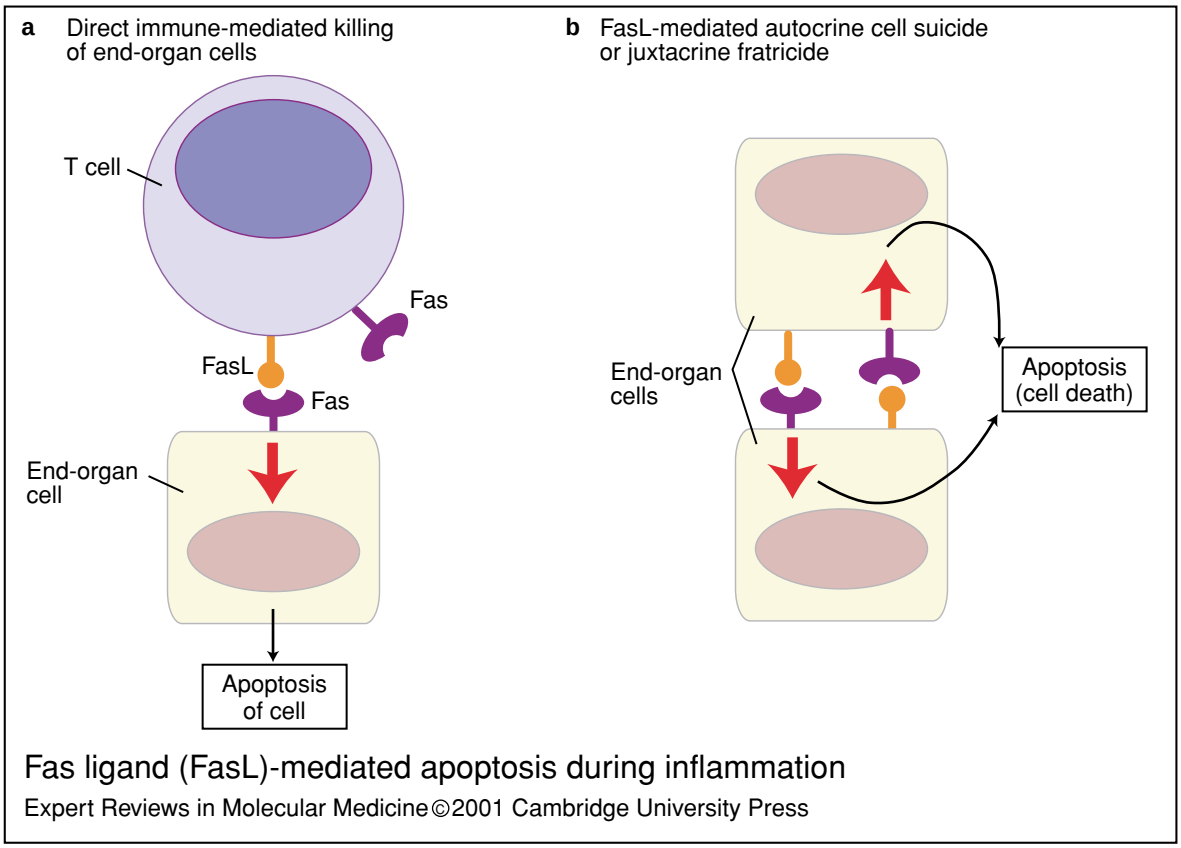

Figure 4. Fas ligand (FasL)-mediated apoptosis during inflammation. Excessive cell death of end-organ cells is a feature of many inflammatory diseases. The term 'end-organ' refers to the target organ where the inflammatory response is directed. (a) In several inflammatory conditions, FasL might be involved in direct immune-mediated killing of end-organ cells. FasL-expressing inflammatory cells (such as T cells) have been found in association with Fas-expressing end-organ cells in conditions such as viral hepatitis (Refs 56, 57), ulcerative colitis (Ref. 55) and Sjorgren's syndrome (Ref. 54). (b) FasL-mediated autocrine cell suicide, or juxtacrine fratricide, appears to occur in some inflammatory conditions, such as alcohol-induced liver inflammation (Ref. 56) and *Helicobacter pylori*-induced gastritis (Ref. 58). In these instances, Fas-expressing end-organ cells appear to upregulate expression of their own FasL, triggering autocrine cell death **(fig004joc)**.

is also regulated at intracellular levels, so that Fas-expressing cells are not always Fas sensitive. Some Fas-resistant cell lines that already express abundant cell-surface Fas are sensitised to Fas-mediated apoptosis by IFN-γ (Refs 63, 64). This indicates that IFN-γ can sensitise cells at intracellular levels in the Fas pathway. IFN-γ upregulates caspase-1 (Refs 64, 65) and possibly other caspases (Ref. 65), and there is some evidence that IFN-γ may also increase apoptotic sensitivity at the level of the Bcl-2 rheostat, by upregulation of the pro-apoptotic protein Bak (Ref. 65). Hence, by producing pro-apoptotic cytokines such as IFN-γ, inflammatory T cells can sensitise end-organ cells to receive a FasL-mediated apoptotic 'hit' (Fig. 5).

The proposed model for autocrine FasL-mediated suicide of thyrocytes in HT provides an intriguing scenario for how the pro-inflammatory microenvironment may sensitise end-organ cells to Fas-mediated suicide. The stimulus for thyrocyte upregulation of Fas is evidently IL-1β produced by inflammatory cells (Ref. 59). Thus, even though thyroid-infiltrating inflammatory cells express little FasL (Ref. 66), they condition the microenvironment such that thyrocytes upregulate Fas, resulting in suicide via their own FasL.

As mentioned earlier, there is evidence that Fas and FasL can be regulated by the transcription factor NF-κB; this could conceivably account for upregulation of both proteins during

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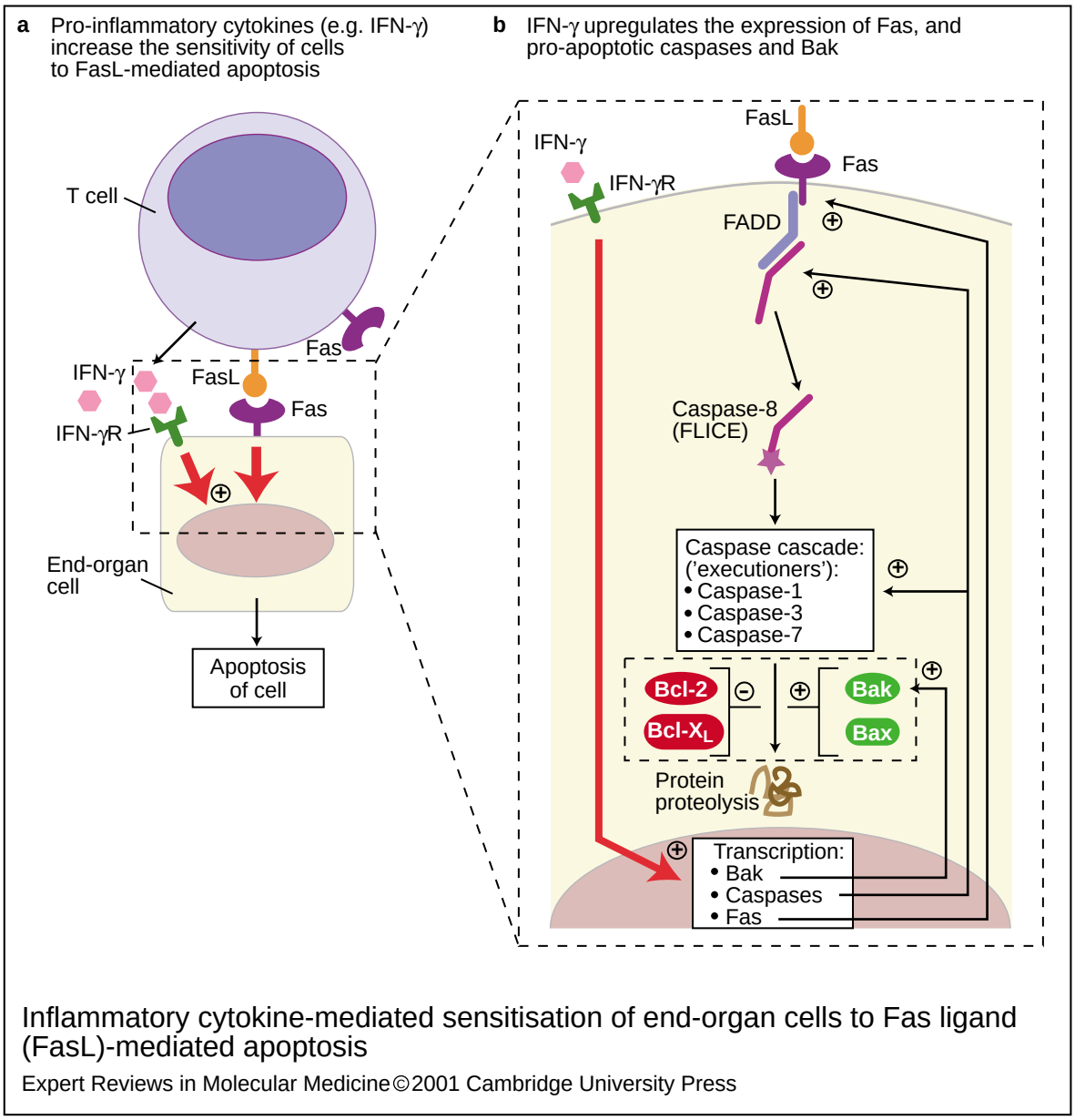

Inflammatory cytokine-mediated sensitisation of end-organ cells to Fas ligand (FasL)-mediated apoptosis

Figure 5. Inflammatory cytokine-mediated sensitisation of end-organ cells to Fas ligand (FasL)-mediated apoptosis. (a) The pro-inflammatory cytokine microenvironment appears to exacerbate cell death during inflammation. Pro-inflammatory cytokines, particularly interferon γ (IFN-γ; shown here), have been shown to increase the sensitivity of many cell types to FasL-mediated apoptosis (Refs 63, 64). (b) Indeed, following binding to IFN-γ receptor (IFN-γR), IFN-γ upregulates expression of Fas itself in many cell types (Ref. 62). Apoptosis sensitisation in response to IFN-γ is also effected at intracellular points, through upregulation of caspases (Refs 64, 65). Finally, there is evidence that IFN-γ may 'pitch' the 'Bcl-2 rheostat' in favour of apoptosis, by upregulation of the pro-apoptotic Bak (Ref. 65) **(fig005joc)**.

inflammation, since the inflammatory cytokines IL-1β and TNF-α are potent activators of NF-κB. Another potentially important link between inflammation and Fas-mediated apoptosis is the double-stranded RNA-activated protein kinase, PKR (Ref. 67). PKR is induced in response to infection with RNA viruses, and also by the pro-inflammatory cytokine TNF-α and the IFNs. In response to these cytokines, PKR phosphorylates inhibitory-κB (I-κB), thereby inducing NF-κB

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activity. PKR causes upregulation of several pro-apoptotic genes, including Fas, Bax and p53 (Ref. 68). While these effects might potentiate apoptosis in response to TNF-α, and possibly Fas, PKR has also been shown to trigger apoptosis by promoting FADD-mediated activation of caspase-8 independently of Fas (Ref. 69).

Therapeutic inhibition and induction of apoptosis

Strategies to prevent Fas-mediated apoptosis of end-organ cells during inflammation offer exciting therapeutic potential, particularly in diseases such as viral hepatitis, ulcerative colitis and HT. The peptide inhibitors of caspases might hold therapeutic promise in this regard. On the basis of the amino acid sequences of their target sites, chemically modified peptide inhibitors are available with specificity for the different caspases (Ref. 70). The chemical group attached to the peptide, commonly fluoromethyl ketone (fmk), modifies the cysteine side-chain in the active site of the caspase, preventing its activity. Broad-spectrum caspase inhibitors, such as the tri-peptide z-VAD-fmk, appear to inhibit all caspases and protect cells from various inducers of apoptosis, including FasL (Ref. 71). Indeed, caspase inhibitors have been used to prevent Fas-mediated apoptosis in vivo in mouse liver – a tissue that appears to be particularly sensitive to Fas-mediated apoptosis. z-VAD-fmk has been shown to prevent inflammatory-mediated cell death of hippocampal neurones in experimental pneumococcal meningitis in rabbits, reducing brain damage (Ref. 72).

A potential obstacle to therapeutic prevention of end-organ apoptosis is the fact that anti-apoptosis agents might also prevent the normal apoptotic clearance of lymphocytes and other inflammatory cells. Inhibition of lymphocyte apoptosis could conceivably prolong inflammatory responses, off-setting the benefits of end-organ protection. However, it may be possible in the future to find agents that have selective effects on apoptosis pathways in specific cell types.

Attempts have also been made to induce Fas-mediated apoptosis of tumours. However, in studies of experimental murine tumours, it was found that circulating anti-Fas agonists were lethal, due largely to Fas-mediated apoptosis of hepatocytes (Ref. 73). An additional systemic injection of a caspase inhibitor protected murine hepatocytes from apoptosis in response to circulating anti-Fas agonists, preventing Fas-mediated hepatotoxicity and delaying the lethal side-effects of such agonists (Ref. 74). This suggests the possibility that caspase inhibitors might protect hepatocytes from apoptosis during viral or other instances of liver inflammation.

Fas-mediated apoptosis of inflammatory lymphocytes: therapeutic possibilities

Possible reasons for lack of Fas sensitivity

As already mentioned, Fas-mediated apoptosis of activated lymphocytes helps to terminate immune responses (Fig. 6a). Insufficient clearance of lymphocytes by apoptosis might be one reason why inflammatory responses become chronic. For example, a lack of T-cell apoptosis has been observed within rheumatoid joints (Ref. 75). Upon isolation, rheumatoid T cells have been found to express high levels of the anti-apoptotic Bcl-2 homologue Bcl-x_L (Refs 75, 76). Sensitisation of activated T cells to FasL-mediated clearance is normally associated with downregulation of Bcl-x_L. Failure to downregulate Bcl-x_L might therefore account for the relative resistance to apoptosis of T cells in situ in rheumatoid joints (Fig. 6b).

When cultured ex vivo, synovial T cells downregulate Bcl-x_L and undergo apoptosis. This indicates that factors in the microenvironment of the rheumatoid joint inhibit T-cell apoptosis in vivo and that, once removed from these protective factors, T cells undergo spontaneous apoptosis. There is some evidence to suggest that survival signals from fibroblasts might modulate the Fas sensitivity of T cells within rheumatoid joints. When synovial T cells were co-cultured with fibroblasts in vitro, the T cells maintained high Bcl-x_L expression and their apoptosis was inhibited (Ref. 75). The apoptosis-protective signal appeared to involve specific integrin–ligand interactions between the fibroblasts and the T cells.

Another potential factor that could conceivably impair T-cell apoptosis in the rheumatoid joint is sFasL. Evidently, sFasL, which has very little apoptosis-inducing activity, has been shown to competitively inhibit Fas-mediated apoptosis triggered by membrane-bound FasL (Refs 77, 78). The sFasL present in rheumatoid joints (Refs 45, 46) might impair intercellular Fas signalling between autoreactive lymphocytes, preventing their clearance by apoptosis (Fig. 6b).

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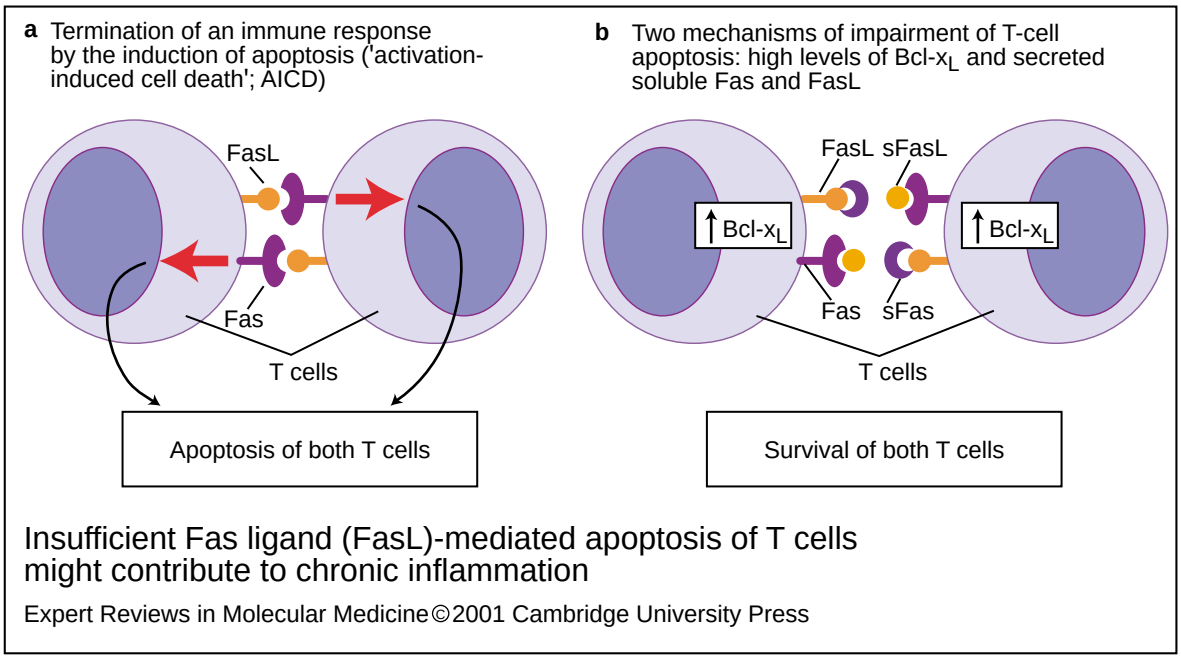

Figure 6. Insufficient Fas ligand (FasL)-mediated apoptosis of T cells might contribute to chronic inflammation. (a) Termination of an immune response by the induction of apoptosis in T cells, a process known as 'activation-induced cell death' (AICD) (Ref. 18). (b) Two mechanisms of impairment of T-cell apoptosis, such as might ocur in inflammatory conditions (e.g. rheumatoid arthritis) (Ref. 75). Freshly isolated T cells from rheumatoid joints have been shown to express high levels of the anti-apoptotic Bcl-2 homologue, Bcl-x_L, which may impair normal FasL-mediated T-cell turnover (Refs 75, 76). Secreted, soluble forms of both FasL (sFasL) and Fas (sFas) have been reported to occur at elevated levels in rheumatoid synovium (Refs 45, 46, 79). These soluble variants might act as receptor antagonists, precluding the interaction of functional cell-surface Fas and FasL, and consequently inhibiting apoptosis (Refs 78, 81) **(fig006joc)**.

Elevated levels of a soluble form of Fas (sFas) have also been demonstrated in rheumatoid joints (Ref. 79). sFas is translated from a splice variant of Fas mRNA that lacks the transmembrane domain (Refs 80, 81). Thus, sFas secreted from cells might act as a receptor antagonist, blocking ligation of membrane-bound Fas by FasL (Fig. 6b).

Recombinant FasL and therapeutic apoptosis of inflammatory cells

Gene therapy using FasL

Thyroid-infiltrating T cells in HT exhibit pronounced sensitivity to Fas-mediated apoptosis when isolated, although these T cells express little FasL themselves (Ref. 66). Fas expression has been shown to be upregulated in both infiltrating lymphocytes and hyperactivated synovial cells in arthritic joints, yet FasL expression is largely lacking (Refs 82, 83). These findings raise the possibility that Fas-expressing inflammatory cells could be eliminated therapeutically by supplying FasL to the site of inflammation.

Adenovirus-mediated gene transfer of FasL has been shown to ameliorate collagen-induced arthritis in mouse ankle joints (Ref. 84). Forced expression of FasL in the inflamed joint led to an increase in local apoptosis of lymphocytes and hyperactivated synovial cells. Interestingly, provision of FasL to the inflamed joint also resulted in the induction of systemic tolerance of the causative autoantigen (collagen in this model). This is consistent with findings regarding FasL expressed in the eye, in which FasL-mediated suppression of an antigen-specific immune response within the eye led to systemic tolerance to the antigen (Ref. 31). Furthermore, in an experimental model of autoimmune thyroiditis, it was shown that direct injection of expression vectors encoding FasL into the inflamed thyroid led to apoptotic clearance of infiltrating autoreactive T cells (Ref. 85).

Although introduction of exogenous FasL elicited therapeutic efficacy in all of these instances, FasL would probably exacerbate the

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pathology in some sites of inflammation by promoting end-organ destruction. For FasL to have therapeutic potential, a balance must be achieved between FasL-mediated clearance of inflammatory cells and restriction of FasL-mediated end-organ destruction.

FasL-transfected 'killer' dendritic cells

FasL has recently been employed in a novel paradigm for inducing antigen-specific immunological tolerance. The protocol involves engineering expression of recombinant FasL in antigen-primed dendritic cells (Ref. 86). As professional antigen-presenting cells (APCs), dendritic cells normally provide activation signals to T cells in an antigen-specific manner. However, when transfected with FasL, dendritic cells instead deliver a FasL-mediated death signal to antigen-specific T cells. These FasL-transfected 'killer' dendritic cells hold potential for inducing tolerance to tissue transplants, and may also represent a potential strategy for induction of apoptosis in autoantigen-specific inflammatory T cells. While killer dendritic cells might have the potential to inhibit inflammatory responses selectively without causing generalised immunosuppression, one limitation is the requirement to prime the cells with appropriate antigens. Ideally, this would necessitate prior knowledge of the specific autoantigens driving the inflammatory process, which may be difficult to identify and could vary from patient to patient.

Sensitisation of inflammatory cells to FasL: protein kinase inhibitors

The sensitivity of cells to apoptosis appears to be influenced to some extent by the phosphorylation status of intracellular proteins. Apoptosis is associated with protein dephosphorylation, whereas protein phosphorylation seems to be generally protective against apoptosis. This is also true for the Fas system: protein kinase inhibitors increase the Fas sensitivity of colon epithelial cell lines (Ref. 87), and haematopoietic cell protein tyrosine phosphatase (HCP) is essential for apoptosis signalling via Fas in lymphoid cells (Ref. 88).

Interference with protein phosphorylation is emerging as a potential therapeutic strategy to promote apoptosis of inflammatory cells. In particular, a family of protein kinase C (PKC)-inhibitory compounds, the bisindoylmaleimides I-XI, were found to increase selectively the sensitivity to Fas-mediated apoptosis of activated, but not resting, lymphocytes (Ref. 89). The compounds varied in their ability to sensitise lymphocytes to Fas-mediated apoptosis, and the most potent of these compounds (bisindoylmaleimide VIII) was shown to prevent the onset of experimental autoimmunity in mice. Whether these compounds alter the Fas sensitivity of other cell types to the same extent has not yet been established. If these or similar agents could preferentially enhance Fas-mediated apoptosis in activated lymphocytes without significantly sensitising end-organ cells, such agents could have therapeutic promise to facilitate FasL-mediated clearance of inflammatory cells from sites of inflammation.

Conclusions: research in progress and outstanding research questions

The overexpression of recombinant FasL in experimental allografts has led to the unexpected finding that FasL, an established mediator of immune downregulation and immune privilege, could exert pro-inflammatory effects. In particular, the apparent ability of FasL to trigger the recruitment of neutrophils tempered initial excitement surrounding the therapeutic possibility that FasL could mediate immune privilege in transplants. However, this pro-inflammatory activity of FasL has only been demonstrated in somewhat artificial experimental contexts, involving engineered overexpression of FasL in allografts. In addition, a pro-inflammatory response to overexpressed FasL did not occur in all cases. Furthermore, several groups have reported that recombinant FasL prolonged the survival of various experimental allografts. It therefore appears that the opposing effects of FasL – immune-downregulatory versus pro-inflammatory – might be governed by other factors, such as the local cytokine microenvironment. TGF-β, for example, was found to inhibit neutrophil-stimulatory effects of FasL. It remains to be determined what other factors influence the role of FasL.

As discussed earlier, new evidence suggests that pro-inflammatory responses to FasL-expressing allografts are probably a consequence of the release of pro-inflammatory cytokines from host cells undergoing FasL-mediated apoptosis in the allograft microenvironment. Triggering of the Fas pathway induces caspase-mediated processing and secretion of IL-1β and

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IL-18, as well as upregulation of IL-8 and other inflammatory cytokines. It is not yet known to what extent these pro-inflammatory effects of FasL contribute to human inflammatory diseases.

FasL appears to induce apoptosis of end-organ cells during certain inflammatory conditions. Both FasL expressed on the surface of immune effector cells and FasL upregulated in cells of the end-organ itself may contribute to tissue damage in the inflammatory context. Agents such as caspase inhibitors offer potential in preventing inflammatory mediated end-organ apoptosis. However, in rheumatoid arthritis, lack of FasL means that activated inflammatory T cells elude normal Fas-mediated apoptotic control. Strategies must therefore be devised that inhibit end-organ apoptosis without significantly preventing apoptosis of inflammatory cells. Indeed, future anti-inflammatory therapies might include agents, such as recombinant FasL or bisindoylmaleimides, that enhance apoptosis of inflammatory cells. In addition, FasL-transfected 'killer' dendritic cells are potentially capable of inducing apoptosis in autoantigen-specific inflammatory T cells.

The complexity of the involvement of FasL in inflammation is compounded by the possibility that it might exert pro- or anti-inflammatory roles at different stages of the inflammatory process. In experimental autoimmune encephalomyelitis, for example, evidence was recently provided that FasL contributed to the onset of inflammation, but was also essential for the natural resolution of inflammation, presumably by clearing inflammatory cells (Refs 90, 91). While manipulation of apoptosis via the Fas–FasL pathway offers exciting therapeutic potential in ameliorating inflammatory diseases, increased knowledge of the precise roles of FasL in inflammation is required and, in particular, how the opposing roles of FasL are regulated.

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The Inflammation Research Association website provides information about conferences and meetings organised by the Association on various aspects of inflammation. It also contains links to other relevant organisations and societies, conferences and journals:

http://www.inflammationresearch.org

The International Association of Inflammation Societies website provides details of the biennial World Congress of Inflammation:

http://www.inflammation-IAIS.org

The American Association of Immunologists website provides details of conferences, sources of research funding and awards, training courses and educational material, available research positions, and the *Journal of Immunology* online:

http://12.17.12.70/aai/default.asp

The Apoptosis Online website provides 'a cohesive collection of information regarding apoptosis research' and serves as a communication channel for researchers involved in apoptosis studies, in particular hosting an interactive discussion board:

http://www.apopnet.com/

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