Apoptosis in disease: about shortage and excess

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Abstract

The death of cells by apoptosis is a fundamental event in development and the maintenance of cell homoeostasis. The other side of the coin, however, is that excessive cell death by apoptosis or the lack of apoptosis is often the driving force of many diseases. Whereas reduced apoptosis sensitivity is a basic characteristic of many tumour cells, accelerated tissue cell death and loss of tissue functions is the underlying cause of many auto-immune and inflammatory diseases.

Introduction

In multicellular organisms the total number of cells has to be tightly regulated, both quantitatively and qualitatively. Various examples demonstrate that either uncontrolled cell growth or cell depletion may result in significant loss of vital functions, development of various diseases and possibly even the death of the affected individual. Whereas necrotic changes in tissues have been and still are considered an important aspect of various pathologies in humans, there is accumulating evidence that impaired cell death induced by apoptosis is crucially involved in many diseases. In this review, we will discuss the role of excessive and reduced apoptosis in the induction of disease in general, and will elaborate further this issue around T-cell-mediated immunopathologies.

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Apoptosis and disease: too little, too much...

Apoptosis inhibition is involved in tumour formation

Disease is a pathological disturbance of the normal physiological status, with severe consequences for the integrity of the affected organ or individual where the physiological processes are temporarily, or permanently, out of their dynamic balance. Thus, whenever apoptotic cell death contributes to the development of disease, this may be due to either too much, or too little, apoptosis. There are numerous examples for either situation in the various pathologies. If reduced apoptosis is the underlying cause of a given disease, we have to assume that the cell population inappropriately surviving may significantly harm the surrounding tissue and directly affect its function (Figure 1). A prime example for such a pathological situation is the formation of tumours. Tumour cells are genetically altered by mutations, which give rise to a cell population that is (relatively) insensitive to growth control and apoptosis induction. Although tumour cells are usually not directly cytotoxic

Figure 1. Reduced or excessive apoptosis can cause disease

If apoptosis induction can be compensated, normal tissue functions are preserved and disease does not develop. Reduced apoptosis sensitivity of a target-cell population, combined with uncontrolled growth, may cause damage to the neighbouring tissue. Excessive apoptosis may lead to loss of a target-cell population and its effector functions.
for normal parenchymal cells, they do compete for blood supply, growth factors and space, thus gradually affecting the viability and function of the neighbouring normal tissue. For example, a brain tumour may displace normal brain tissue, leading to severe loss of its function, but surgical removal of the tumour may often allow resumption of normal brain functions.

Why do tumour cells grow in an apparently uncontrolled manner? Obviously, the reduced control of cell-cycle progression and inappropriate growth factor expression contribute to this cell behaviour. Mutations in cell-cycle control or growth-factor-receptor genes leading to uncontrolled growth are often found in various tumours. However, there is increasing evidence that enhanced proliferation is compensated in normal cells by enhanced cell death, and that successful tumour formation frequently requires mutations that enhance the resistance to apoptosis induction. Evidence for this hypothesis can be tested in most tumour models, and is elegantly illustrated in a recent experimental study by Evan and co-workers [1]. In many tumours, the transcription factor and oncogene c-Myc is over-expressed and supports cell-cycle progression. Similarly, transgenic over-expression of c-Myc in pancreatic β-cells of the murine pancreas causes an initial burst of β-cell proliferation, which is, however, followed by extensive induction of apoptosis. Thus enhancement of cell growth is not sufficient for tumour formation, but may instead result in subsequent cell death. In contrast, if the anti-apoptotic molecule Bcl-x<sub>L</sub> is co-expressed with c-Myc in β-cells, massive expansion of the transformed cells is observed with the formation of angiogenic, invasive tumours (insulinomas) [1]. Thus resistance to apoptosis induction is a key event in certain tumour development. Apoptosis-related genes are frequently mutated in tumour cells. Often, anti-apoptotic gene products are over-expressed, whereas pro-apoptotic genes are inactivated by mutations or deletions. It is thus not surprising that the first anti-apoptotic gene identified, Bcl-2, was isolated from a B-cell lymphoma [2]. Similarly, the tumour-suppressor-gene product p53 can induce apoptosis and p53 mutations are among the most common genetic alterations in malignant tumours [3].

**Reduced T-cell apoptosis as an underlying defect in auto-immunity**

Resistance to apoptosis is not only a central element in tumour formation, but is also involved in the pathogenesis a variety of other diseases. The ability of T-cells to die by apoptotic cell death is as important for induction of a proper immune response as is the induction of T-cell activation and proliferation. Recent years of research have substantiated the role of members of the TNF (tumour necrosis factor) family in the maintenance of immune homoeostasis and in the induction of T-cell apoptosis. In particular, Fas ligand (also called CD95 or APO-1 ligand) plays a predominant role in the regulation of T- and B-cell homoeostasis. Failure of mature T- and B-cells to undergo apoptosis is closely associated with development of auto-immunity [4]. Interestingly, mutations in the Fas receptor or Fas ligand are frequently found in a group of
patients, suffering from the so-called ALPS (auto-immune lymphoproliferative syndrome). This disease is characterized by uncontrolled T- and B-cell proliferation, splenomegaly and formation of auto-antibodies, and strongly resembles the phenotype of the Fas-receptor-knockout mouse (reviewed in [5]). In many other auto-immune diseases, including rheumatoid arthritis [6], reduced T-cell apoptosis has also been suggested as one of the underlying defects leading to induction of disease.

**Excessive target-cell apoptosis in disease**

In contrast, in organ-specific auto-immune diseases, uncontrolled excessive target-cell apoptosis may be the disease-initiating event. As shown in Figure 1, excessive induction of apoptosis in a distinct target-cell population may gradually lead to their disappearance and loss of their effector functions. Type I diabetes (also termed juvenile diabetes or insulin-dependent diabetes mellitus) is a frequent auto-immune disease, in which self-reactive T-cells cause the destruction of the β-cells in the pancreas. As a result, the insulin-producing cells gradually disappear and affected patients have to compensate for reduced endogenous production by insulin injections. Why do autoreactive T-cells suddenly attack β-cells of the pancreas and cause their destruction? A likely possibility is that the central deletion process in the thymus, responsible for the elimination of the vast majority of autoreactive immature T-cells, is not as stringent as necessary. Thus, in most individuals, the presence of T-cells specific for β-cell antigens can be demonstrated in the peripheral blood. Overt type I diabetes, however, only develops in less than 1% of the Caucasian population, implicating that normally other mechanisms prevent the onset of the disease. Hengartner and colleagues [7] have demonstrated that viral antigens can be over-expressed in the pancreas of fully immunocompetent mice, without developing any pathological alterations. In contrast, upon challenge with the infectious virus, the immune system is stimulated and the resulting immune response is capable of eliminating the viral load, but the virus-specific T-cells now also attack the virus-protein-expressing β-cells, resulting in overt type I diabetes [7] (Figure 2). This experimental model system illustrates that self-tolerance can be broken by the induction of a potent pro-inflammatory immune response. Similar observations are made in other auto-immune diseases. For example, rheumatoid arthritis or EAE (experimental allergic encephalomyelitis), a disease similar to multiple sclerosis in humans, can be induced experimentally in rodents by injecting self-antigen in an inflammatory context, i.e. by co-injecting complete Freund’s adjuvant. This induces an inflammatory response and causes the breakdown of self tolerance. Therefore, strong inflammatory responses and viral infections may often be initial triggers leading to auto-immune disorders.

Cell-mediated cytotoxicity has most probably not evolved to destroy tissue cells expressing self-antigen, but to defend the host against pathogens hiding within the cells of the body, such as viruses and intracellular bacteria.
However, the same mechanisms responsible for the elimination of infected target cells also mediate the death of innocent tissue cells. T-cells kill their targets predominately via death receptor ligation and the perforin/granzyme B pathway (reviewed in [8]). Activated T-cells and natural killer cells express death ligands, like Fas ligand, TNFα and TRAIL (TNF-related apoptosis-inducing ligand). Upon receptor ligation on the corresponding target cell, the DISC (death-inducing signalling complex) forms and caspases (cysteine proteases with a preference for aspartic acid) become activated. Caspases are the major effector proteases in apoptosis induction, responsible for the demolition of the cell. Details of the signalling events that are initiated upon death receptor ligation have been extensively reviewed elsewhere [9] and will not be further discussed here. Although the initial events between death receptor-induced apoptosis and perforin/granzyme-B-induced target-cell killing are different, they both eventually activate the same suicide programme in the target cell. After the release of the cytotoxic granules from the activated T-cell, granzyme B binds to the target cells through the mannose-6-phosphate receptor and translocates with the help of perforin to the cytoplasm. Granzyme B, itself a protease, can cleave and activate caspases and initiate the apoptosis programme. Thus both cytotoxic effector mechanisms engage the target cell’s own suicide programme (Figure 3) [8].

The question remains why different cytotoxic effector mechanisms are used to eventually engage the same signalling events, namely the apoptosis machinery. Target cells not only express different patterns and levels of death receptors, a prerequisite for the induction of apoptosis via this pathway, but also different patterns and levels of apoptosis inhibitors, capable of inhibiting either the death-receptor- or cytotoxic-granule-mediated pathway. For example, the experimental inhibition of the Fas pathway has a marked beneficial
effect on the course of EAE, suggesting that Fas plays a crucial role in
demyelination and neuron destruction [10]. Similarly, Fas-induced apoptosis
appears to be the predominant mechanism of hepatocyte killing during virus-
induced hepatitis. Hepatocytes are known to express relatively high levels of
Fas, which is even further up-regulated in an inflammatory environment. In
addition, upon injection of an agonistic anti-Fas antibody, mice rapidly suc-
cumb due to massive liver cell apoptosis [11]. Thus hepatocyte killing via the
Fas pathway appears to dominate. In contrast, in the elimination of other
virus-infected cells or transplant rejection, the perforin/granzyme B pathway
appears to be more important.

Excessive tissue cell apoptosis is not only observed during auto-immune
diseases or, as will be discussed below, during excessive inflammatory responses,
but is also a characteristic of other diseases. The loss of CD4+ T-cells is a hall-
mark of HIV infection in humans. As a result, the host becomes immunodefi-
cient due to the lack of T-cell help in the induction of protective immune
responses against opportunistic infections. Recently, it was recognized that this
loss of CD4+ T-cells is also mediated by apoptosis. CD4+ T-cells are either
directly killed by the virus upon infection, or become innocent bystander targets of activated cytotoxic T-cells (reviewed in [12]). Thus, similar to the disorders described above, apoptosis induction in a distinct target-cell population (CD4+ T-cells) may result in severe consequences for the HIV-infected individual.

The examples discussed above indicate that either reduced or excessive cellular apoptosis can result in a severe impairment of specific tissue functions, often leading to disease (Figure 4). Whereas some tissues may be extremely sensitive to cell loss, others may well have a certain buffering capacity, i.e. can tolerate the loss of a significant number of cells due to subsequent compensation or regeneration. Disease may thus represent a situation where cell loss cannot be compensated anymore.

The intestinal mucosa: a site of death and life

The intestinal mucosa is constantly confronted with two apparently conflicting duties, i.e. to allow uptake of nutrients and to prevent invasion of the intestinal mucosa by the luminal microflora. This is certainly not a trivial task given the huge number (>10^{14}) of bacteria present in the intestine, which even exceeds the total number of cells in the rest of the human body, and the total surface of the intestinal mucosa (approx. 300 m²) that needs to be protected from invasive microbes. Furthermore, the luminal content may contain potentially toxic or mutagenic metabolites derived from ingested food and possibly also bacterially derived metabolites, which may threaten the integrity of the intestinal mucosa, especially the epithelium (reviewed in [13]). This makes it clear that local immune responses against invading pathogens have to be tightly regulated and need to be carried out with the appropriate immune mechanisms that limit the spread of infiltrating microbes, however, without causing excessive bystander damage to non-infected cells in the intestinal epithelium and lamina propria.

![Figure 4. Disease as an unbalance between pro- and anti-apoptotic signals](image)

Increased resistance to apoptosis induction in immune cells and decreased apoptosis resistance in tissue cells can cause disease.
The importance of a tightly regulated local immune system in the maintenance of tissue homoeostasis has been demonstrated clearly in mouse strains that are deficient for functional genes normally involved in mounting (and regulating) immune responses, e.g. IL-2 (interleukin 2), IL-10 and transforming growth factor β (reviewed in [14]). Intriguingly, in the presence of conventional bacterial flora, these mouse strains spontaneously develop a chronic inflammatory disorder of the intestine, primarily of the colon where the largest bacterial load in the intestinal lumen is found. Whereas the absence of the immunosuppressive cytokines IL-10 or transforming growth factor β may lead to an impaired development of regulatory T-cells (T-cells capable of controlling immune responses, e.g. via the production of immunosuppressive cytokines), and hence to an excessive expansion of inflammatory T-cells in the colonic mucosa, it is believed that impaired apoptosis induction in T-cells in the absence of IL-2 production is the underlying cause of colitis (inflammatory disease in the colon) induction in IL-2-deficient mice. IL-2 is well known as a T-cell growth factor. Surprisingly, T-cells in the IL-2-deficient mouse show no defects in proliferation, but reduced sensitivity to apoptosis. Chronically stimulated T-cells become increasingly sensitive to Fas receptor ligation. IL-2 not only enhances the expression of Fas ligand on activated T-cells and thus contributes to their elimination, but also down-regulates the specific inhibitor of the Fas signalling pathway cFLIP (cellular Flice-like inhibitory protein) [15]. cFLIP is a caspase-8 homologue that lacks its catalytic domain. It acts as a dominant-negative inhibitor and blocks the Fas signalling pathway at the receptor-complex level. Failure to produce sufficiently high levels of IL-2 and down-regulate cFLIP may thus lead to enhanced apoptosis resistance in effector T-cells, and eventually to uncontrolled immune reactions and severe tissue damage. Interestingly, impaired apoptosis induction in colitis-inducing T-cells is not only observed in experimental animal models, but also in human patients. Crohn’s disease and ulcerative colitis are two main forms of inflammatory bowel disease in humans. Intestinal T-cells isolated from Crohn’s disease and ulcerative colitis patients show reduced sensitivity to Fas in vivo when compared with cells isolated from controls. Similarly, reduced in situ T-cell apoptosis is observed in the colonic mucosa of Crohn’s disease patients (reviewed in [16]). These observations from human patients and experimental model systems strongly support the idea that T-cell apoptosis in the intestinal mucosa is an important immunomodulatory mechanism and has to be tightly regulated to avoid uncontrolled, destructive immune responses.

Altered apoptosis sensitivity may lead to inappropriate survival of intestinal cytotoxic T-cells and apoptosis induction in innocent bystander targets, e.g. epithelial cells. Epithelial cells are Fas-sensitive and are readily killed by Fas ligand-expressing T-cells. Apart from Fas ligand, another death-inducing ligand appears to play an important role in the pathogenesis of inflammatory bowel disease. TNFα is produced by activated T-cells and macrophages and can induce apoptosis upon binding to TNF receptors I and II and engaging the caspase cas-
cade. Experimental work in animal models clearly demonstrated a crucial role for TNFα in the induction of inflammatory disorders of the intestine, such as colitis and graft-versus-host disease. The mechanism of TNFα-mediated disease induction has not yet been fully defined. However, the local release of TNFα leads to rapid apoptosis induction in epithelial cells, immediately followed by shedding of the dying cells into the intestinal lumen [17]. This strongly suggests that TNFα-induced epithelial cell apoptosis also affects the permeability of the intestinal epithelium. The uncontrolled entry of immunostimulatory bacterial products will activate macrophages and T-cells further, leading to a vicious circle of cell activation and tissue destruction. The central role for TNFα in the pathogenesis of inflammatory bowel disease in human patients, in particular in patients with Crohn’s disease, has been demonstrated by the observed dramatic response rate in patients with active disease following administration of a humanized anti-TNFα antibody (Infliximab) [18]. Anti-TNFα probably produces its effect through the neutralization of TNFα; however, it is also possible that the TNFα-binding antibody mediates the specific depletion of cell-surface TNFα-expressing immune effector cells, e.g. via complement activation or natural-killer-cell activation through Fc receptors. Evidence for an apoptosis-inducing effect of Infliximab in transmembrane TNFα-expressing monocytes in vitro has been reported recently [19].

Apart from direct apoptosis induction in epithelial cells, TNFα may also affect the development of inflammatory bowel disease through other mechanisms. As mentioned above, epithelial cells express Fas and can be killed by Fas-ligand-expressing T-cells. Inflammatory cytokines, such as TNFα and interferon γ, not only enhance the expression of the Fas receptor, but also sensitize the cells for Fas-induced apoptosis, presumably by down-regulating anti-apoptotic molecules. Such sensitized epithelial cells may then become easy prey of activated cytotoxic T-cells, infiltrating the lamina propria and the epithelial cell layer during inflammatory bowel disease.

In summary, there is increasing evidence that apoptosis induction plays a crucial role in the development of inflammatory disease of the intestinal mucosa, such as Crohn’s disease and ulcerative colitis. The inflammatory environment may lead to reduced homoeostatic T-cell apoptosis as well as enhanced target cell killing and tissue destruction (Figure 5).

**Therapeutic approaches**

Obviously, progress in the understanding of the role of apoptosis in the development of various diseases must also be translated into therapeutic approaches. Multiple approaches aim at preventing apoptosis of vital target cells during diseases characterized by rapid and excessive target cell death. For example, small-molecule caspase inhibitors may have promising protective effects on neuronal cell loss during stroke [20].
In diseases characterized by excessive tissue cell apoptosis due to uncontrolled activation of T-cells, however, another approach may prove to be successful. The examples described above have shown that T-cell apoptosis may represent an interesting target. Compounds or neutralizing antibodies that enhance the apoptosis sensitivity of T-cells due to down-regulation of anti-apoptotic molecules, such as cFLIP or Bcl-xL, have already been used successfully to inhibit disease induction in experimental animal models. For example, the protein kinase C inhibitor bisindolylmaleimide VIII can specifically down-regulate cFLIP expression, and *in vivo* administration has a strong ameliorating effect during experimental rheumatoid arthritis and EAE [4,21]. These experiments show that disease treatment by sensitizing T-cells to apoptosis induction is an interesting and applicable concept. However, a profound understanding of effector-cell apoptosis regulation may be required to specifically interfere with the pathogenesis of the various diseases.

Figure 5. Uncontrolled T-cell activation in the intestinal mucosa causes excessive tissue cell apoptosis

(A) Tissue section through normal intestinal mucosa (mouse colon) showing the crypt section. (B) Detection of apoptotic nuclei (black arrowhead) in the epithelial layer during experimental colitis in the mouse. Note the epithelial cell layer infiltrating lymphocyte (grey arrowhead). (C) Compensation of excessive epithelial cell apoptosis (black arrowheads) by increased proliferation (cells in mitosis, white arrowhead). Experimental colitis in the mouse: (D) apoptotic epithelial cells are shed into the gut lumen during experimental colitis in the mouse.
Conclusion
A more profound understanding of the molecules and pathways leading to apoptotic cell death, and their regulation during the pathogenesis of different diseases, may allow the identification of crucial target molecules and promote the development of therapeutic protocols and compounds to specifically target a disease-inducing cell population or prevent target cell death.

Summary

- In multicellular organisms, the balance between cell growth and cell loss has to be tightly regulated.
- Reduced or excessive cell death by apoptosis can lead to an imbalance of this cellular homeostasis, and may lead to a loss of tissue function and eventually to disease.
- Uncontrolled T-cell activation can lead to excessive tissue cell apoptosis and loss of vital functions.
- Failure to control T-cell effector functions by apoptosis is an underlying cause of many diseases.

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References


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