Apoptosis, inflammatory bowel disease and carcinogenesis: Overview of international and Greek experiences

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Apoptosis is critical for organ development, tissue homeostasis, the elimination of abnormal cells and the maintenance of immune homeostasis by variable regulatory mechanisms. The death of T lymphocytes following their activation involves a series of proteases (caspases), which comprise the central executioners of apoptosis. A normal regulation of apoptosis results in disease. T-cell resistance against apoptosis contributes to inappropriate T-cell accumulation and the perpetuation of the chronic inflammatory process in inflammatory bowel disease with potential tumourigenic effect. The use of antitumour necrosis factor-alpha, anti-interleukin-6R and anti-interleukin-12 antibodies suppresses colitis activity by induction of T-cell apoptosis, thereby having important implications for the design of effective therapeutic strategies in inflammatory bowel diseases. Contrary to international data, the incidence of cancer in Greek patients with inflammatory bowel disease appears to be low. A balance between cell proliferation (Ki-67 overexpression) and apoptosis (Bax protein overexpression) may partly explain the low incidence of cancer development in Greek inflammatory bowel disease patients.

Key Words: Apoptosis; Carcinogenesis; Caspases; Inflammatory bowel disease

For each cell there is a time to live and a time to die. There are two ways in which cells die. They are either killed by harmful agents (exposure to toxic chemicals and mechanical injury) or they are induced to commit suicide. During the process of death by injury (necrosis), the cellular metabolism breaks down, the cell swells, the cellular membranes decompose and the cell contents leak out, resulting in the inflammation of surrounding tissues. On the other hand, during the process of death by suicide (programmed cell death), the cell shrinks, its mitochondria break down with the release of cytochrome c (Cyt c), it develops bubble-like blebs on its surface, the chromatin (DNA and protein) in its nucleus degrades and breaks into small, membrane-wrapped, fragments (apoptotic bodies). Consequently, the phospholipid phosphatidylserine, which is normally hidden within the plasma membrane, is exposed on the cell surface, and is then bound by receptors on phagocytic cells (ie, macrophages and dendritic cells) which then engulf the cell fragments. Lastly, the phagocytic cells secrete cytokines that inhibit inflammation. It should be emphasized that, despite the widespread occurrence of apoptosis in physiological and pathological cell death, the occurrence of cell death that fulfils neither the criteria for apoptosis nor necrosis has been well documented (paraptosis, from para meaning next to or related to, and apoptosis) (Table 1) (1). Examples that do not conform to the criteria for either of these forms of cell death include a number of neurodegenerative cell deaths, such as those in a transgenic mouse model of amyotrophic lateral sclerosis, some developmental cell deaths, such as autophagic cell death and cytoplasmic cell death, or a number of ischemia-related cell deaths featuring cell swelling, referred to as oncosis. Such an alternative nonapoptotic form of cell death is programmatic, because it requires gene expres-

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ion. Understanding the biochemical pathway(s) for nonapoptotic programmed cell death should lead to new insight into cell death events and their implications for the understanding of neurodegeneration, tumour therapeutics, development and evolutionary aspects of cell death programs (1).

There are two different reasons by which a cell commits suicide. First, programmed cell death is as important as mitosis in the development and maintenance of host homeostasis. For example, during embryogenesis, excess numbers of developing cells die and in hormone-responsive tissues, such as the uterus, cyclic depletion of specific hormones leads to the death of hormone-dependent cells (2). Similarly, the formation of the proper connections (synapses) between neurons in the brain requires that excess cells be eliminated by apoptosis (2). This concept implies that many cell populations have a propensity to undergo apoptosis (i.e., are programmed to die unless specifically protected). Namely, cells must constitutively express, or try to express, molecules that function as effectors of apoptosis, and, moreover, these effectors are actively suppressed to allow the cells to survive (2). The second reason for which a cell commits suicide is that programmed cell death is required to destroy cells that represent a threat to the integrity of the organism. Examples include cells infected with viruses (in this situation cytotoxic T lymphocytes (CTLs) execute virus-infected cells by inducing apoptosis); and cells of the immune system. A specific cell-mediated immune response is that the effector cells must be removed to prevent them from attacking body constituents. Therefore, CTLs induce apoptosis in each other and even in themselves. Defects in the apoptotic mechanisms are associated with autoimmune diseases such as rheumatoid arthritis, lupus erythematosus or inflammatory bowel disease (IBD). Moreover, prolonged immune responses of the effector cells that exhibit defective apoptosis have been associated with chronic epithelial damage that can induce tumourigenesis in many tissues. In the gastrointestinal tract, inflammation has been linked to cancers in the esophagus (reflux disease and Barrett’s esophagus), stomach, small bowel (Crohn’s disease and adenocarcinoma) and colon (IBD and adenocarcinoma) (3).

A apoptotic procedure is implicated in tumourigenesis. Thus, some cancer-causing viruses use ‘tricks’ to prevent apoptosis of the cells they have transformed. For instance, human papilloma viruses have been implicated in inducing cervical cancer, and one of them produces a protein (E6) that binds and inactivates the apoptosis promoter p53. Even tumour cells produce without the contribution of viruses may have ‘tricks’ to avoid apoptosis. For instance, some cancer cells, particularly lung and colon cancer cells, secrete elevated levels of a soluble ‘decoy’ molecule that binds to Fas ligand (FasL), plugging it so that it cannot bind Fas (CD95/APO-1). Therefore, CTLs cannot kill the cancer cells by the Fas-FasL apoptotic pathway. Lastly, other cancer cells express high levels of FasL, and can kill any Fas expressing CTL that tries to kill them.

In general, the process of CTL-mediated apoptosis (lysis) of target cells progresses in five key steps. Step 1: Target cell antigen recognition by CTL and conjugate formation between CTL and target cell: The CTL binds to the target cell, by using its specific antigen receptor and other accessory molecules such as CD8, CD2, and leukocyte function-associated antigen-1 (LFA-1). Therefore, the recognition of the target cell involves class I major histocompatibility complex molecules (the ligand for CD8) complexed to a foreign peptide antigen on the surface of the target (e.g., virus infected) cell, the complex serving as the ligand for the T-cell receptor (TCR), leukocyte function-associated antigen-3 (CD11a), and intercellular adhesion molecule-1 or intercellular adhesion molecule-2 (the ligands for LFA-1). Of note, transient conjugate formation may occur via CD2- or LFA-1-mediated adhesion in the absence of or before specific antigen recognition or antigen recognition may increase the ability to form conjugates by enhancing the binding function of the adhesion molecules (4).

Step 2: CTL activation by clustering of its antigen receptor, initiated by recognition of major histocompatibility molecule-peptide complexes on the target cell. The intracellular signals generated by the TCR may be increased by signals delivered through the various accessory molecules.

Step 3: Delivery of lethal hit mediated by perforin and granzyme B and/or Fas-FasL complex. During this process, CTLs develop specific membrane-bound cytolytic granules that contain numerous macromolecules, including a membrane pore-forming protein called perforin or cytolysin; enzymes frequently called granzymes that contain reactive serines in their active sites; and proteoglycans (4). In addition, CTLs share with other activated T lymphocytes (T Ls) surface expression of FasL, which can deliver apoptosis-inducing signals to target cells expressing the Fas protein. Moreover, CTLs share with other effector T Ls the ability to transcribe and secrete cytokines and other proteins upon activation, mainly tumour necrosis factor (TNF), interferon-γ (IFN-γ), lymphotixin, and, to a lesser degree, interleukin (IL)-2. Studies of CTL-mediated killing of target cells in vitro had shown that granule exocytosis-dependent (i.e., perforin/granzyme-mediated) killing and granule exo-

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**TABLE 1** Comparison of apoptosis, necrosis and paraptosis

<table>
<thead>
<tr>
<th></th>
<th>Apoptosis</th>
<th>Necrosis</th>
<th>Paraptosis</th>
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<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nuclear fragmentation</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chromatin condensation</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Apoptotic bodies</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cytoplasmic vacuolation</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Mitochondrial swelling</td>
<td>Sometimes</td>
<td>+</td>
<td>Late</td>
</tr>
<tr>
<td>Genomic effect</td>
<td>TUNEL</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Internucleosomal DNA fragmentation</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Caspase activity</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>DEVD-cleaving activity</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3 processing</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PARP cleavage</td>
<td>+ (56 kDa fragment)</td>
<td>+ (50 to 62 kDa fragments)</td>
</tr>
</tbody>
</table>

Inhibition by:

- zVAD.fmk
- BAF
- p55
- xiap
- Bcl-xL
- Actinomycin D
- Cycloheximide

**DEVD** Aspartic acid-glutamic acid-valine-aspartic acid; **PARP** poly(ADP-ribose) polymerase; **TUNEL** Terminal deoxynucleotidyltransferase-mediated uridine triphosphate end-labeling; + Present; – Absent

**Comparison of apoptosis, necrosis and paraptosis**

- Apoptotic bodies: +
- Cytoplasmic vacuolation: +
- Mitochondrial swelling: +
- TUNEL: +
- Internucleosomal DNA fragmentation: +
- Caspase activity: +
- DEVD-cleaving activity: +
- 3 processing: +
- PARP cleavage: +

**Genomic effect**

- Internucleosomal DNA fragmentation:
  - Paraptosis: +
- Caspase activity:
  - Paraptosis: +
- Mitochondrial swelling:
  - Paraptosis: +
- TUNEL:
  - Paraptosis: +

**Apoptosis Necrosis Paraptosis**

- **Morphology**
- **Necrosis**
- **Paraptosis**

- **Genomic effect**
  - **TUNEL**
  - **Internucleosomal DNA fragmentation**
  - **Caspase activity**
  - **DEVD-cleaving activity**
  - **3 processing**
  - **PARP cleavage**

**Genomic effect**

- **TUNEL**
- **Internucleosomal DNA fragmentation**
- **Caspase activity**
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cytosis-independent (ie, FasL-mediated) killing were redundant. More recent reports have suggested that perforin and granzyme are the key mediators of CTL function in immune responses to intracellular microbes. In contrast, the Fas pathway appears to be more important for immune regulation than for CTL functions (ie, for controlling excessive lymphocyte activation, particularly against self-antigens).

The lethal hit is initiated by exocytosis of the granule contents onto the membrane of the target cell. As a consequence of granule content exocytosis, perforin, present as a monomer in the granule, comes in contact with extracellular levels of calcium (typically 1 to 2 mM) and undergoes polymerization. Polymerization of perforin leads to the formation of a large water-filled channel that preferentially occurs in a lipid bilayer, such as the plasma membrane of the target cell. If an adequate number of polymerized channels are assembled, the target cell will be unable to exclude ions and water, resulting in osmotic swelling and lysis. A second, equally important, function of perforin is to provide a means for introducing granzymes into the target cell. Granzyme B is a serine protease that preferentially cleaves protein substrates at aspartic acid residues. Target proteins that are recognized by granzyme B include IL-1 converting enzymes (ICE) or related cysteine proteases (caspases) that are themselves aspartic acid-directed. ICE and ICE-like proteases become catalytically activated upon cleavage at susceptible aspartic acid residues, so that by cleaving ICE, granzyme B initiates an ICE protease cascade that is comparable to the activation of the caspase cascade induced by ligation of cell surface Fas. The net result is that the insertion of granzyme B into the cytoplasm of a target cell, like the ligation of Fas, initiates apoptotic cell death. Thus, by activating an apoptotic death pathway, a perforin/granzyme B-induced cascade of caspases can lead to the destruction of viral DNA genomes as well as the target cell genome, thereby eliminating potentially infectious DNA.

**Step 4:** Detachment of CTL

**Step 5:** Death of the target cell by osmotic lysis and apoptosis (4).

With regard to lymphocytes, programmed cell death plays a key role in controlling the size of the lymphocyte pool at several stages of lymphocyte maturation and activation. Immature lymphocytes that do not express functional antigen receptors undergo programmed death. After their maturation, if lymphocytes never encounter antigens, they die by apoptosis. Even if lymphocytes are activated by antigen, fractions of the progeny that do not receive sufficient growth factors or sustained stimulation also die. During lymphocyte maturation and activation, fluctuations in the levels of expression of Bcl-2 or Bcl-xL appear to correlate inversely with susceptibility to apoptosis (2). Overexpression of Bcl-2 or Bcl-xL leads to enhanced survival of immature lymphocytes and prolonged antibody responses. Conversely, knockdown of Bcl-2 or Bcl-xL results in reduced survival of mature or immature lymphocytes. It has also been suggested that the long life span of memory lymphocytes may be due to constitutive expression of Bcl-2 and/or Bcl-xL (2). Besides, lymphocytes undergo a second form of apoptotic death, that occurs not because of lack of stimulation or deficient of growth factors but as a consequence of receptor-mediated activation. This is termed activation-induced cell death (AICD) and is considered to be induced and regulated by mechanisms that are different from those that control Bcl-2-regulated programmed cell death. In particular, AICD can be induced through activation of surface death receptors (such as Fas and TNF-receptor [TNFR]) (5,6). AICD is essential for the death of lymphocytes that recognize self-antigens and also plays a role in the induction of tolerance to some foreign antigens (2).

At the intestinal mucosal level, the apoptosis of lamina propria (LP) TLs is regulated via active and passive mechanisms. Immune responses in the mucosa are commonly characterized by major expansions of antigen-specific TLs that have potent effector function (7). Although this may be essential for host defense, it may also result in effector cell populations with considerable autoreactivity that can cause mucosal inflammation. To deal with this latter possibility, the mucosal immune system has evolved several strategies for the control of mucosal immune responses. Among these is the regulation of apoptosis that either occurs by an active mechanism following TCR stimulation (AICD) or by a passive mechanism following lymphokine (for instance, IL-2) withdrawal (7). The active mechanism involves death receptors such as Fas and TNFR and/or their ligands (FasL and TNF), while the passive mechanisms do not (7).

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**Death of intestinal TLs mediated by Fas-FasL**

Activated T-cells are more susceptible to apoptosis than resting T-cells. Among T-cells in the LP normally exhibit a higher state of activation, and express memory cell markers such as CD45RO, the activation-induced cell death might be very important to downregulate effector cell function and cytokine production in the gut, thereby maintaining the immune homeostasis in the specialized microenvironment of the mucosa (7). Memory T-cells are known to express Fas constitutively at high levels, and normal LP T-cells (LPTs) exhibit an enhanced susceptibility to Fas-mediated cell death (7). Death-receptor-induced apoptosis comprises signaling processes via Fas, TNFRI, TNF-related apoptosis-inducing ligand-R1, and DR3 or DR6. Upon triggering of Fas by FasL, the adaptor molecule Fas-associated death-domain-containing protein (FADD) and pro-caspase 8 are recruited to the Fas receptor, thereby forming a death-induced signaling complex (DISC) (7). In particular, trimerization, or more likely oligomerization of Fas results to the recruitment of the Fas-adaptor protein, FADD, through their mutual death domains (DDs) (8). The other end of FADD contains two death effector domains (DEDs) through which it can recruit caspase 8 or its enzymatically inactive homolog and Fas inhibitor, FADD-like IL-1p-converting enzyme (FLICE) inhibitory proteins (8). Of note, Ced-3 gene encoding a protein related to mammalian, IL-1p-converting enzyme (ICE; caspase 1), which exerts proteolytic activation of the caspase cascade, thereby emerging as the most central step of apoptosis (9). To date, 14 mammalian caspases have been identified and involved in different aspects of cell death, although the exact function of each individual caspase is still mostly unidentified (9). Caspases are created as proenzymes containing an aminoterminal prodomain and the p20 and p10 domains that are cleaved to form the active enzyme as a tetramer of the two p20-p10 heterodimers; each tetramer contains two active sites (8). Caspases cleave precursors to create mature cytokines (caspase 1 [ICE, IL-1β converting enzyme] and caspase 11), thereby activating upstream processes.
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Figure 1) A poptosis of mucosal T-cell by the Fas-Fas ligand (Fadl) system and suppression of Fas-mediated apoptosis by interleukin (IL)-12. Normal lamina propria T (LPT) cells demonstrate an enhanced susceptibility to Fas-mediated cell death. Upon activation of Fas, the proteins Fas-associated death domain (FADD), cytotoxicity-dependent A po-1-associated protein 3 (CAP3) and pro-caspase 8 are recruited to the death domain (DD) of the receptor, forming the death induced signaling complex (DISC). DISC signaling events result in the activation of caspase 8 which, in turn, either directly activates caspase 9 or induces cytochrome {C (Cyt c) release from mitochondria. Cyt c in the cytoplasm binds to apoptotic protease activation factor (Apaf)-1, forming a complex that binds caspase 9 and causes its activation. Subsequently, activated caspase 9 activates other proteases, including caspase 3, and therefore causes cleavage of various substrates that subsequently lead to the death of the cell. However, in chronic intestinal inflammation, increased production of IL-12 prevents Fas-mediated T-cell apoptosis, resulting to T-cell accumulation in the intestine. This mechanism of disease perpetuation can be successfully blocked by antibodies to IL-12 (7,13). STAT4 Signal transducer and activator of transcription 4 (initiator) and downstream (effector) caspases. Initiator caspases, such as caspases 8 and 9, exert regulatory roles. Upon binding to signal-transducing molecules, they subsequently activate downstream effector caspases, such as caspases 3, 6, or 7, which finally cleave different cellular substrates, thereby inducing the apoptotic cell death (9-11). Moreover, there is recent evidence indicating a participation of caspases and other apoptotic regulatory molecules beyond that of cell death, including the control of T-cell proliferation and cell cycle progression (9).

DISC signalling events result from activation of caspase 8 (recruitment of pro-caspase 8 leads to its autoproteolytic activation and the release of active caspase 8 [FLICE] into the cytosol). Of note, cytotoxicity-dependent A po-1-associated protein 3 is identified as the FLICE homologue (BH1-BH4), as well as a carboxy-terminal hydrophobic transmembrane tail domain, which localizes the proteins to the outer mitochondrial membrane and endoplasmic reticulum (ER). Group B members exhibit proapoptotic activity. This group includes Bax and Bak and resembles Group A except for the lack of the amino-terminal BH4 domain. Group C contains a large group of proapoptotic molecules (eg, Bid and Bik) that are much less similar than Groups A and B and contain only the BH3 domain (8).

The proto-oncogene Bcl-2 is expressed in the mitochondrial membrane as well as in the contiguous membrane of the ER and nuclear envelope, mediating resistance against a wide range of apoptotic stimuli (11,14,15). The ER plays an important role in intracellular calcium homeostasis, and alterations in this homeostasis are implicated in the control of apoptosis. Bcl-2 decreases the free calcium concentration within the ER and suppresses apoptotic cell death (16,17). Moreover, experimental evidence suggests that Bcl-2 targeted to the ER can inhibit Myc-, but not etoposide-induced apoptosis in the rat-1 fibroblast cell line (14). On the other hand, the Bcl-2 family...
members Bax and Bak involve direct effects on the ER calcium pool with consequent sensitization of mitochondria to calcium-mediated fluxes and Cyt c release. These effects modulate the kinetics of Cyt c release and promote apoptosis (18).

Because Bcl-2 and Bcl-xL only inhibit apoptotic activities of mitochondria, the first (activated by low amounts of caspase 8) but not the second (activated by large amounts of caspase 8) pathway of active apoptosis is inhibited by these antiapoptotic proteins. On the other hand, passive forms of apoptosis involve the activation of Bid proteins that act exclusively via mitochondrial mechanisms; therefore, passive apoptosis, as caused by lymphokine withdrawal, is strongly inhibited by Bcl-2 and Bcl-xL (7).

With regard to chronic intestinal inflammation, recent data indicate that increased production of cytokines (IL-12) prevents Fas-mediated T-cell apoptosis, resulting in T-cell accumulation in the gut. This mechanism of disease perpetuation can be successfully blocked by antibodies to IL-12 (7).

In summary, the death-receptor pathway as typified by Fas (CD95/APO-1) includes the following. Oligomerization of Fas by FasL (on the same cell or a neighbouring one) causes recruitment of FADD to the cytoplasmic tail of Fas by their mutual DDs. The opposite end of FADD contains DEDs that allow recruitment of either procaspase 8 or FLICE inhibitory proteins. The latter contains a mutation in the enzymatic domain rendering it enzymatically inactive. Caspase 8 can cleave the BH3-only protein, Bid, and the resulting truncated Bid can inactivate Bcl-2 in the mitochondrial membrane (as can Bax following DNA damage). This allows the escape of Cyt c, which clusters with A paf-1 and caspase 9 in the presence of dATP to activate the caspase 9. Second mitochondria-derived activator of caspase/direct IAP-binding protein with low pi is also released from the mitochondria and inactivates IA Ps. Active caspase 9 can cleave and activate procaspase 3 to its active form, resulting in activation of other caspases, breakdown of several cytoskeletal proteins and degradation of the caspase-activated DNAse inhibitor, thereby releasing caspase-activated DNAse that leads to DNA degradation, cell death and phagocytosis of cell debris (Figure 1) (8).

A poptosis of LP CD4+ T-cells in the normal and inflamed intestine

Naive T-cells are mostly Fas-negative and thus resistant to Fas-mediated apoptosis, unlike memory T-cells that are largely Fas-positive. As the majority of T-cells in the LP express memory cell markers, and, moreover, memory T-cells express high levels of Fas, in the unstimulated state (before TCR stimulation), LPT cells display increased susceptibility to Fas-mediated apoptosis compared with unstimulated peripheral-blood T-cells; this is due to as yet unknown downstream changes in the Fas signaling pathway. In addition, LPT cells show augmented spontaneous apoptosis compared with peripheral blood cells; this is possibly due to a passive apoptotic mechanism associated with IL-2 withdrawal, as apoptosis is decreased by addition of IL-2 and is induced by addition of anti-IL-2 antibodies. Similarly, after stimulation of LPT cells with anti-CD2 and anti-CD28 antibodies, apoptosis is further increased compared with stimulated peripheral blood T-cells (19). This augmented apoptosis is the consequence of activation-induced apoptosis involving death receptors, because it is observed when the cells are activated by the CD2 rather than the CD3 activation pathway, thereby CD2 pathway is a more effective means of activating T-cells (20). Besides, this apoptosis is extinguished by antibodies that block Fas, while addition of IL-2 or anti-IL-2 antibodies has a minor effect (7). In contrast to LPT cells from control patients, those from patients with ulcerative colitis and Crohn's display defective Fas-induced apoptosis upon stimulation by CD2. Even though LPT cells in IBD express the similar amount of Fas on their surface, they are less sensitive to Fas-mediated apoptosis when compared with control cells. Nevertheless, resistance of LPT cells against apoptosis in Crohn's disease is not limited by the Fas-Fasl system, as recent data have shown that T-cells in Crohn's disease grow more rapidly in response to IL-2 than do control cells and are more resistant to IL-2-deprivation-induced apoptosis and nitric oxide-mediated apoptosis (21). This wide resistance to apoptosis is further supported by the fact that inflamed intestinal mucosal T-cells express augmented levels of Bcl-2 and therefore may be resistant to a range of apoptotic mechanisms that involve mitochondrial activity (7).

In view of above mentioned data, the consideration that emerges is that, in the normal (uninflamed) gut mucosa, T-cells exhibit increased spontaneous or activation-induced apoptosis mediated by the Fas pathway that places a limit on the expansion of T-cells following direct and bystander stimulation by specific antigen. On the contrary, in inflamed gut tissue, T-cells are resistant to apoptosis, thereby exhibiting prolonged survival and augmented cytokine production that might, in turn, significantly aggravate the inflammation. Indeed, this defective apoptosis results in inappropriate T-cell accumulation that fosters mucosal inflammation in IBD (22). Resistance of T-cells to apoptotic death can extend their life-span, and retained activated T-cells in IBD mucosa results in an exceedingly prolonged immune response leading to perpetuation of chronic inflammation. These deleterious events appear to occur particularly in Crohn's disease, as indicated by studies showing that LPT cells have a reduced expression of the proapoptotic Bax protein, while an imbalance between Bax and the antiapoptotic Bcl-2 protein is present in the inflamed mucosa, thereby favoring resistance to apoptosis and contributing to the chronicity of inflammation (9,21-24). Finally, chronic inflammatory epithelial injury can cause tumourigenesis in the gastrointestinal tract, including cancers in the small bowel (Crohn's disease and adenocarcinoma) and colon (IBD and adenocarcinoma) (25). Such resistance to apoptosis also appears to be important in various autoimmune diseases such as the autoimmune lymphoproliferative syndrome. Although a severe defect in Fas-mediated apoptosis in autoimmune lymphoproliferative syndrome can cause nongastrointestinal conditions of autoimmunity, the resistance to apoptosis also appears to be important in various autoimmune diseases such as the autoimmune lymphoproliferative syndrome. Although a severe defect in Fas-mediated apoptosis in autoimmune lymphoproliferative syndrome can cause chronic intestinal inflammatory conditions of autoimmunity, the resistance to apoptosis also appears to be important in various autoimmune diseases such as the autoimmune lymphoproliferative syndrome. Although a severe defect in Fas-mediated apoptosis in autoimmune lymphoproliferative syndrome can cause chronic intestinal inflammatory conditions of autoimmunity, the resistance to apoptosis also appears to be important in various autoimmune diseases such as the autoimmune lymphoproliferative syndrome.

Considering the above-mentioned data, it is reasonable to assume that eliminating excessive T-cells could restore the intestine to its normal state or, at least, a condition of controlled inflammation (Figures 2,3). Strong evidence for this effect is provided by recent studies in animal models of IBD showing that various antibodies to proinflammatory cytokines and their receptors, such as TNF, IL-12, and IL-6 receptor (IL-6R), appear to suppress chronic intestinal inflammation by the induction of T-cell apoptosis (7,22). A similar effect has been noticed by deletion of CD44v7+ cells (26). Indeed, with two such approaches, the administration of antibodies against IL-
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**Figure 2**) Different outcomes resulting from the balance between T-cell proliferation and apoptosis (cell death) in the normal gut mucosa, or imbalance during chronic gut inflammation. In health, mucosal T-cell proliferation induced by dietary and enteric flora antigens is counterbalanced by a normal quantity of apoptosis resulting in the low degree of physiological inflammation found in the normal intestine. In Crohn’s disease there is augmented T-cell proliferation induced by still undetermined stimuli derived from bacterial antigens, food and possibly unrecognized pathogens. Simultaneously, T-cells die less because of their resistance to apoptosis. This defective apoptosis leads to inappropriate T-cell accumulation that fosters inflammation. If the degree of T-cell apoptosis is augmented in Crohn’s disease, an effect apparently mediated, for instance, by anti-tumour necrosis factor-alpha (TNF-α) therapy (infliximab) (22), a new balance is established between the increased rate of proliferation due to inflammation and the increased rate of apoptosis. A condition of controlled inflammation is then established resulting in clinical improvement, and later on decreased cellularity of the gut mucosa (22).

IL-12 and apoptosis

In animal (mice) models of trinitrobenzene sulfonic acid-induced colitis, a T helper 1 (Th1)-mediated inflammation replete with cells producing large amounts of IFN-γ, IL-12 and TNF-α, administration of a neutralizing anti-IL-12 antibody is followed after two days by the appearance of apoptotic (terminal deoxynucleotidyltransferase-mediated uridine triphosphate end-labeling positive) CD4+ cells at the site of inflammation in the large intestine. This T cell inducing apoptosis is followed by rapid resolution of the inflammatory process, and might explain the remarkable suppression of intestinal inflammation in various Th1 models of colitis after treatment with anti-IL-12 antibodies. The anti-IL-12-induced apoptosis is a Fas-mediated phenomenon in that the cells being lost are preferentially Fas-bearing cells. Moreover, the therapeutic effect of the antibody is greatly diminished in MRL/lpr mice and cannot mediate apoptosis by the Fas pathway, as well as in normal (SJL/J) mice administered Fas-Fc, an agent that blocks Fas-signalling via Fas, but does not itself signal via Fas. These data strongly suggest that activated Th1 cells producing inflammatory cytokines require the sustained presence of IL-12 if they are to avoid a Fas-mediated apoptotic death. The mechanism by which IL-12 counteracts the Fas pathway in such cells is not yet completely understood, although it is recognized that it does not involve the induction of the anti-apoptotic proteins, Bcl-2 or Bcl-xL, and therefore may act on mitochondria-independent DISC signalling events (Figure 1) (7).
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(STAT)-3 dependent activation of a cascade of antiapoptotic proteins such as Bcl-2 and Bcl-XL, and then to apoptosis resistance (27). By blocking the IL-6 trans-signalling pathway, restoration of T-cell susceptibility to apoptosis leads to suppression of Th1-mediated colitis in several animal models of chronic intestinal inflammation, including colitis associated with IL-10 deficiency, trinitrobenzene sulfonic acid-induced colitis, and colitis occurring in severe combined immunodeficiency mice upon transfer of naive (CD45Rbhi, CD45Rblow) T-cells (7,27). Support for such IL-6 trans-signalling comes from the information that a gp130-Fc fusion protein that interacts with sIL-6R but not with membrane-bound IL-6R could also ameliorate various forms of colitis, presumably by blocking the interaction of the complex with membrane-bound IL-6R and thus the anti-apoptotic IL-6 signal. Furthermore, it has been shown in patients with Crohn's disease (a Th1-mediated inflammation in humans) that, while T-cells from the LP lack IL-6R, they appear to have transducted an IL-6-specific signal in vivo in which they contain activated STAT3. Additionally, IL-6-sIL-6R complexes have been observed in the serum of patients with Crohn's disease, and treatment of Crohn's disease LPT cells with anti-IL-6R results in both the disappearance of cells containing activated STAT3 and augmented cell apoptosis (Figure 4) (7). In contrast to the antiapoptotic effect mediated by IL-12, the antiapoptotic effect mediated by IL-6 trans-signalling, may involve Bcl-2 and Bcl-xL. Indeed, these proteins are increased in LP cells from patients with Crohn's disease leading to an increase of the Bcl-2/Bax ratio and are known to be induced by STAT3 (7). In view of these new data involving IL-6R signalling, the reduced apoptosis in the inflammatory milieu of the inflamed gastrointestinal tract can be attributed to the fact that in this milieu the cells are subject to IL-6R trans-signalling and the induction of antiapoptotic proteins (7).

Specific features of the apoptotic process in ulcerative colitis and Crohn's disease patients have been described. Delayed neutrophil apoptosis is a feature of persistent acute inflammation and neutrophil-mediated damage has been shown to be associated with the development of IBD. Neutrophils isolated from patients with IBD have shown reduced spontaneous apoptosis compared with those of cancer patients. Mesenteric venous serum of IBD patients contributed to this delay, which contained higher levels of IL-8. Furthermore, procaspase 3 expression was also decreased in IBD neutrophils, contributing to reduced spontaneous and Fas antibody-induced apoptosis. Therefore, neutrophil apoptosis may be altered in Crohn's disease and ulcerative colitis through the release of antiapoptotic cytokines and altered caspase expression (28-30). Thus, alterations in cell death mechanisms may lead to persistence of the inflammatory response associated with IBD (30).

It has been shown that FasL mRNA is strongly expressed in active ulcerative colitis lesions, but not in those with active proctitis-type ulcerative colitis and/or active Crohn's disease (31). The FasL mRNA positive cells infiltrating ulcerative colitis lesions are largely CD3 T lymphocytes. Therefore, the CD3 T lymphocytes with surface FasL may be involved, at least in part, in the pathogenesis of this disease. The high expression of FasL in ulcerative colitis may indicate IL-8 production and secretion from colon epithelia, promoting the migration and activation of lymphocytes and neutrophils. As a consequence, the increase in FasL on CD3 T lymphocytes is thought to play a role in the chronic inflammation, directly through the induction of apoptosis in Fas-expressing colon epithelia, and indirectly through IL-8-mediated migration and

**Figure 4** Prevention of T-cell apoptosis in chronic gut inflammation via interleukin (IL)-6 trans-signalling and tumour necrosis factor (TNF) signalling events. Chronic gut inflammation is associated with increased production of both IL-6 and TNF. IL-6 can bind to IL-6R lamina propria T-cells (LPT) upon binding to its soluble receptor (sIL-6R) and the gp 130 molecule. Signalling by gp 130 leads to activation of signal transducer and activator of transcription 3 (STAT3) and STAT3 target molecules such as Pim-1/2, c-myc and lastly Bcl-2 and Bcl-xL. This pathway induces increased cell survival and T-cell resistance against apoptosis in inflammatory bowel disease (IBD) patients. On the other hand, blockade of IL-6 trans-signalling causes remission of gut inflammation by suppression of T-cell resistance against apoptosis. Equally to IL-6, TNF can cause increased cell survival by signalling via the tumour necrosis factor receptor (TNFR) II/II and activation of nuclear factor (NF)-κB, even though activation of TNFR-I can also induce apoptosis by activation of caspase 6. Nevertheless, recent data indicate that antibodies to TNF (which are clinically effective in inducing rapid suppression of gut inflammation in IBD patients) (42) seem to mediate their effects at least in part by rapid induction of T-cell apoptosis in the inflamed intestine, signifying an important function for TNF in regulating gut cell survival in IBD patients (7). A pro-1 A apoptotic protease activation factor; c–AIP Cellular inhibitor-of-apoptosis proteins; Cyt c Cytochrome c; DD Death domain; FADD Fas-associated protein with death domain; RIP Receptor-interacting protein; SODD Silencer of death domains; TRADD TNFR-I-associated death domain protein; TRAF TNF receptor-associated factor; VCP Valosine-containing protein

### IL-6 and apoptosis

Some data indicate a direct pathogenic role for the complex of IL-6 and its soluble receptor (sIL-6R) in IBD (Crohn's disease), thereby suggesting a therapeutic potential of disrupting this form of cytokine signalling. It has been demonstrated that LP macrophages release sIL-6R, which may complex with IL-6 and stimulate gp 130 on the surface of intestinal LPT cells, leading to a signal transducer and activator of transcription (STAT)-3 dependent activation of a cascade of antiapoptotic genes such as Bcl-2 and Bcl-xL, and then to apoptosis resistance...
extensive colitis, defined as contiguous disease from the rectum to the cecum in patients with ulcerative colitis begins with a disease duration of seven to ten years, affecting approximately 30% at 25 years. Those at greatest risk are patients with ulcerative colitis who have not responded to treatment, have intestinal strictures, or have recurrent bleeding. Most studies have agreed that the risk of cancer in patients with IBD increases with duration of disease and with severity of inflammation, with an increased incidence of neoplastic transformation (35). Most studies have agreed that the risk of cancer in patients with ulcerative colitis is increased in the long-term, with a higher incidence of dysplasia and carcinoma development (33). Inactive ulcerative colitis, defined by the absence of symptoms and endoscopic evidence of inflammation, is associated with a lower risk of cancer compared to active ulcerative colitis (34). One exception is the proctitis type, which has unique features, such as a shorter disease duration and lower risk of dysplasia and carcinoma development (36). While the risk of cancer is lower in inactive ulcerative colitis, the risk of dysplasia and carcinoma development is still higher than in the normal colon and inactive ulcerative colitis (37). Therefore, the accelerated epithelial cell turnover caused by chronic inflammation and epithelial damage may predispose the mucosa to DNA damage, resulting in an increased risk of mutation in line with dysplasia and carcinoma development in IBD patients (37).

The combination of the above-mentioned data may lead to the consideration that the increased cell proliferation, as indicated by the elevated expression of oncogene Ki-67, and the defective apoptosis represent two risk factors for cancer development in IBD. It is also known that the progressive accumulation of genetic changes in both oncogenes and tumor-suppressor genes parallels the clinical and histopathological progression from normal colon to adenomas to dysplasia and frank colon cancer (38). Contrary to the data of other countries, the incidence of cancer in Greek patients with IBD is lower (39,40), although as yet there is no possible explanation for this observation. However, in a recent pilot study involving 22 Greek patients (16 with Crohn's disease and 6 with ulcerative colitis), an increased mucosal expression of Bax protein was noticed in 50% to 62% of the patients. Moreover, an elevated expression of oncogene Ki-67 was also observed in three quarters of the patients (41). These preliminary results suggest a possible existence of a balance between cell proliferation (indicated by Ki-67 overexpression) and apoptosis (indicated by Bax protein overexpression), thereby reflecting no defective apoptotic process that may explain, at least in part, the low incidence of cancer development in Greek IBD patients.

CLOSING COMMENTS
A poptosis of LPTs seems to be necessary to maintain immune homeostasis in the specialized microenvironment of the gut mucosa by downregulating the gut immune response and elimi-
An ingat reactive clones. Whereas normal LPTs manifest an increased susceptibility to Fas-mediated apoptosis, IBD is associated with the resistance of LPTs to multiple apoptotic pathways. Such defective apoptosis may interfere with clonal deletion and maintenance of tolerance that may be a key factor for inappropriate T-cell accumulation contributing to chronic mucosal inflammation in IBD. Moreover, abnormal regulation of apoptosis results in disease, including cancer (24). Novel therapeutic strategies such as anti-IL-6R and anti-IL-12 antibodies appear to mediate their rapid beneficial effects on colitis activity at least in part by the suppression of T-cell resistance against apoptosis and the consecutive induction of mucosal T-cell death resulting in the limited expansion of activated T-cells and deletion of autoreactive T-cell clones. In addition, anti-TNF-α antibodies that are currently used with considerable success in the treatment of Crohn’s disease have also been suggested to act by inducing rapid and specific increase in apoptosis of T-cells in the gut mucosa, possibly altering (increasing) the Bax/Bcl-2 ratio (42). It should be noted that the infiximab (anti-TNF-α) antibodies therapy is based on the consideration that Crohn’s disease is mainly a Th1-related inflammatory condition, in which cytokine profiles of the Th1 variety (including TNF-α) have been particularly implicated in the pathogenesis of the tissue damage. On the other hand, ulcerative colitis is probably a Th2-related inflammation. Although therapy with anti-IL-6R or anti-IL-12 antibodies also induces death of LP Th1 T-cells, it is not clear whether such treatment would also induce cell death of T regulatory 1 or Th3 cells: an event that would question the long-term beneficial effects of this therapeutic strategy. However, several findings indicate that Th3 and T regulatory 1 cells are not subject to anti-IL-12-mediated apoptosis. Indeed, these cells are not Th1 cells and thus lack a fully competent IL-12 receptor. Moreover, there is good evidence that transforming growth factor-beta extinguishes IL-12Rβ2 chain expression so that Th3 cells would primarily downregulate IL-12R expression, and lastly, administration of anti-IL-12 to mice has been shown to increase transforming growth factor-beta and IL-10 secretion (7).

The experimental observations on anti-IL-6R or anti-IL-12 therapeutic approaches are relevant to human IBD, and to other human Th1- or Th2-mediated inflammatory conditions. Therefore, in Th1-mediated inflammation such as Crohn’s disease, the supply of an IL-12 inhibitor such as anti-IL-12 will not only lead to resolution of disease by neutralization of the cytokines that initiate the Th1 pathway of inflammation, but also by the death of induced CD4+ Th1 cells. A similar effect can be obtained by the provision of an IL-6 inhibitor such as anti-IL-6R because such treatment also results in both neutralization of cytokines and cell death. In this case, however, the therapy is probably not limited to Th1-mediated inflammation because it applies to end-stage CD4+ effector T-cells arising from any T-cell differentiation pathway. Thus, anti-IL-6R antibodies may be used in ulcerative colitis, more like a Th2-mediated than a Th1-mediated inflammation, in that neither IL-12 nor IFN-γ secretion is increased by isolated LP macrophages or T cells, respectively, and Th2 T-cell-mediated animal models of inflammation resemble ulcerative colitis pathologically (7).

The potential relevance of modulating T-cell resistance against apoptosis as a therapeutic strategy in IBD is underlined by the recent observation that drugs that facilitate Fas-mediated apoptosis can be used for suppression of T-cell-mediated autoimmune disease. However, the T-cell death brought about by anti-IL-12 and anti-IL-6R antibodies in intestinal inflammation is likely to occur via largely independent mechanisms. This introduces the possibility of inducing death of inflammatory CD4+ T-cells in independent and possibly synergistic ways. Therefore, the future of therapeutic strategy of IBD might lie in combined anticytokine therapy rather than therapy with a single agent.

Such therapeutic approaches, by inducing T-cell apoptosis, may alter the potential for tumourigenesis observed in IBD. Moreover, future investigation of genetic alterations of oncogenes involved in apoptosis may identify specific population groups associated with a high or low (observed in a small number of Greek patients) risk of cancer development in IBD.

REFERENCES