APOPTOSIS AND NECROSIS

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Summary

Apoptosis is a physiological process that is triggered by the activation of genetic self-destruction programs existing in the genome of all cells. In this way, the multicellular organism destroys the undesired cells from a tissue.

Apoptosis is an active form of cell death characterized by biochemical and morphological processes, especially by chromatin condensation, poly-nucleosomal DNA fragmentation and the fragmentation of the cell into apoptotic bodies. Each cell receives multiple signals, which by means of specific receptors can induce the cell to enter the cell cycle or apoptosis. The alteration of a specific receptor can lead to the appearance of a malignant clone, due to the imbalanced relation between apoptosis inducing or repressing signals and proliferation. The antiapoptotic Bcl-2 protein may inhibit apoptosis induced by the absence of growth factors, neurotrophic factors and cytokines.

In apoptosis, by the mechanisms in function, the cell actively participates in its own death. Morphologically, it is characterized by compact cytoplasm, vacuoles in the cytoplasm membrane, nuclear chromatin condensation, DNA fragmentation and the formation of apoptotic bodies. Unlike the images observed in necrosis, in the case of apoptosis chromatin does not flocculate, mitochondria are not swollen and the cell membrane is not permeable to staining agents. Apoptosis does not trigger inflammatory reactions, since lysosomal enzymes are not released.

Zeiss (24) defines apoptosis as a highly regulated process, characterized by specific morphological and biochemical properties. The apoptotic process is initiated by both physical-biological and pathological stimuli, and the full expression of apoptosis requires a signal cascade in which caspase activation plays a central part. By the elimination of the genes that control caspase-dependent apoptosis, the apoptotic phenotype is transformed into a necrotic phenotype both in vitro and in vivo. This suggests the fact that necrosis and apoptosis represent the morphological expression of a similar biochemical mechanism by both a caspase-dependent mechanism and a non-caspase-dependent mechanism with effectors such as cathepsin B and apoptosis inducing factor. The program of cell death, either by apoptosis or by necrosis, is mediated by an integrated cascade, which can be accessed from multiple sites and propagated by many ramification points. A cell can die either by apoptosis or by necrosis, depending on the physiological environment, developmental stage, type of tissue and nature of the cell death signal.

Developing tissues follow a fine line between proliferation and death; in order for a tissue to develop and grow, this should resist to apoptosis. Cell subpopulations at a given time and location must submit to apoptosis so that the tissue maintains its normal shape and function. This proves the close relationship between the mechanism of neoplastic development and proliferation and reflects the intersection between apoptotic pathogenic pathways and cell cycle mechanisms. The coexistence of apoptosis and necrosis
characterizes both developmental processes and acquired morbid processes; the morphology of apoptosis is caspase-dependent, and any deviation from this pathogenic pathway results in death through necrotic morphology.

Key words: apoptosis, necrosis

Apoptosis is the term proposed by KERR et al. (1972), being composed of apo, which means from and ptosis, which means fall. Apoptosis in Greek expresses the fall of leaves or petals. In cell biology, the term suggests the arrest of vital functions and consequently death occurring without the intervention of external factors. The cell has its own biological and chemical mechanisms that are capable of determining cell death. Apoptosis is a physiological process that is triggered by the activation of genetic self-destruction programs existing in the genome of all cells. In this way, the multicellular organism destroys the undesired cells from a tissue (10).

Apoptosis is an active form of cell death characterized by biochemical and morphological processes, especially by chromatin condensation, poly-nucleosomal DNA fragmentation and the fragmentation of the cell into apoptotic bodies. The apoptotic process plays a central role in the development and functioning of the immune system. Apoptosis can be partly genetically regulated and it can also be related to physiological and nonphysiological signals. Apoptosis can represent a defense mechanism at cellular level against cancer, by the participation of protooncogenes and tumor suppressor genes in the regulation of apoptosis (19). Each cell receives multiple signals, which by means of specific receptors can induce the cell to enter the cell cycle or apoptosis. The alteration of a specific receptor can lead to the appearance of a malignant clone, due to the imbalanced relation between apoptosis inducing or repressing signals and proliferation (6, 9, 21). The antiapoptotic Bcl-2 protein may inhibit apoptosis induced by the absence of growth factors, neurotrophic factors and cytokines (2, 15).

For the correct use of terminology, it should be emphasized that a differentiation is proposed between apoptosis, which strictly refers to morphological cell changes, and programmed death, which occurs in the course of biochemical (physiological) processes specific for cell death.

The term apoptosis is currently used either as a synonym for programmed cell death, or to indicate morphological cell changes that are associated with programmed cell death. According to some authors (10) the correct expression would be “programmed cell death by apoptosis”; at present, the concepts of apoptosis and programmed cell death are used with the same meaning, being considered synonymous. The authors consider that apoptosis can be defined as a strictly physiologically regulated process, which is partly characterized by nuclear condensation and cell volume decrease, with the maintenance of an intact plasma membrane, and which reaches its highest point with the irreversible destruction of nuclear chromatin and genomic DNA digestion.

In apoptosis, by the mechanisms in function, the cell actively participates in its own death. Morphologically, it is characterized by compact cytoplasm, vacuoles
in the cytoplasmic membrane, nuclear chromatin condensation, DNA fragmentation and the formation of apoptotic bodies. Unlike the images observed in necrosis, in the case of apoptosis chromatin does not flocculate, mitochondria are not swollen and the cell membrane is not permeable to staining agents. Apoptosis does not trigger inflammatory reactions, since lysosomal enzymes are not released (8).

For the understanding of the phenomenon of cell death through apoptosis or necrosis, the clarifications made by Zeiss (24) are edifying and essential. The author defines apoptosis as a highly regulated process, characterized by specific morphological and biochemical properties. The apoptotic process is initiated by both physical-biological and pathological stimuli, and the full expression of apoptosis requires a signal cascade in which caspase activation plays a central part. By the elimination of the genes that control caspase-dependent apoptosis, the apoptotic phenotype is transformed into a necrotic phenotype both in vitro and in vivo. This suggests the fact that necrosis and apoptosis represent the morphological expression of a similar biochemical mechanism by both a caspase-dependent mechanism and a non-caspase-dependent mechanism with effectors such as cathepsin B and apoptosis inducing factor. The program of cell death, either by apoptosis or by necrosis, is mediated by an integrated cascade, which can be accessed from multiple sites and propagated by many ramification points. A cell can die either by apoptosis or by necrosis, depending on the physiological environment, developmental stage, type of tissue and nature of the cell death signal.

Developing tissues follow a fine line between proliferation and death; in order for a tissue to develop and grow, this should resist to apoptosis. Cell subpopulations at a given time and location must submit to apoptosis so that the tissue maintains its normal shape and function. This proves the close relationship between the mechanism of neoplastic development and proliferation and reflects the intersection between apoptotic pathogenic pathways and cell cycle mechanisms. The coexistence of apoptosis and necrosis characterizes both developmental processes and acquired morbid processes; the morphology of apoptosis is caspase-dependent, and any deviation from this pathogenic pathway results in death through necrotic morphology (24).

Table 1

<table>
<thead>
<tr>
<th>Morphological comparison between apoptosis and necrosis</th>
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<tbody>
<tr>
<td><strong>According to Wyllie and Duval, 1998</strong></td>
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<tr>
<td><strong>APOPTOSIS</strong></td>
</tr>
<tr>
<td>Histology: Isolated cells affected in healthy tissues</td>
</tr>
<tr>
<td>Cytology: Pyknotic nuclei, condensed cytoplasm, round cell fragments</td>
</tr>
<tr>
<td>Exclusion tests: Staining agents do not</td>
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<tr>
<td><strong>NECROSIS</strong></td>
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<tr>
<td>Histology: Cells that die together, with structural disintegration</td>
</tr>
<tr>
<td>Cytology: Cellular edema, Intact but poorly stained nuclei</td>
</tr>
<tr>
<td>Exclusion tests: Staining agents penetrate the</td>
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In the mechanisms of apoptosis, endonuclease activity plays a major role, being responsible for chromatin condensation, nuclear segregation and dispersion. As a result of chromatin cleavage and chromatin condensation, a dramatic reduction in nuclear volume occurs. Small masses of nuclear chromatin are formed, *apoptotic bodies*, which contain morphologically intact cell organelles. Apoptotic bodies are phagocytosed by viable neighboring cells or macrophages. DNA cleavage in low molecular weight material is extremely important and represents a protective function, limiting the probability of transfer of potentially active genes from dead cells to the nucleus of viable neighboring cells (1, 2, 3).

Apoptosis is involved in physiological processes and especially pathological ones, the latter aspect being extremely important for oncogenesis, evolution and even treatment. The understanding of the mechanisms of apoptosis and the clarification of the biochemical pathways in the activation of the apoptosis inducing genetic program will change the actual concept of treating diseases, in general, and cancer, in particular (1, 4).

Some bacteria are pathogenic through the activation of the genetic program that induces the “suicide” of the affected cell. Some substances elaborated by microbes can enter the genetic programs of cells, causing

<table>
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<th>by staining agents</th>
<th>penetrate the cell</th>
<th>The cell membrane is not permeable to staining agents</th>
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<tbody>
<tr>
<td>Cytoplasmic ultrastructure</td>
<td>Intact, compact organelles</td>
<td>Significantly increased, swollen mitochondria and matrix densification</td>
</tr>
<tr>
<td></td>
<td>Ergastoplasmic dilation</td>
<td>Dilated organelle contour</td>
</tr>
<tr>
<td></td>
<td>Intact cytoplasmic membrane</td>
<td>Rupture of plasma and internal membranes</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Capsular and toroidal chromatin condensations</td>
<td>The primary chromatin pattern is maintained with a normal distribution</td>
</tr>
<tr>
<td>Circumstances</td>
<td>Frequently in &quot;programmed death&quot;</td>
<td>Never physiological Completion</td>
</tr>
<tr>
<td></td>
<td>Atrophy</td>
<td>Hypoxia</td>
</tr>
<tr>
<td></td>
<td>Immunomediated cell death</td>
<td>Toxins (high doses)</td>
</tr>
<tr>
<td>Tissue effects</td>
<td>Non-inflammatory Phagocytosis induced by adjacent cells</td>
<td>Acute inflammatory</td>
</tr>
<tr>
<td></td>
<td>Rapid involution without general tissue collapse</td>
<td>Subsequent scarification</td>
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apoptosis. This could explain the affinity of some pathogenic bacteria for certain cells or tissues (25).

### Alteration of apoptotic characteristics

(According to SOLARY et al., 1993)

<table>
<thead>
<tr>
<th>Biochemical changes associated with apoptosis</th>
<th>Consequences</th>
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<tbody>
<tr>
<td><strong>Activation of an endonuclease:</strong>&lt;br&gt;Non-identified enzyme(s): internucleosomal DNA cleavage</td>
<td>“Ladder” aspect of DNA migrating in agar gel</td>
</tr>
<tr>
<td><strong>Cytoskeletal changes:</strong>&lt;br&gt;Transglutaminase and protease activation that results in the formation of an insoluble protein network</td>
<td>It prevents lysosomal and membrane rupture and the appearance of an inflammatory reaction (“cage effect”)</td>
</tr>
<tr>
<td><strong>Plasma membrane changes:</strong>&lt;br&gt;Increased isoprenoid synthesis and loss of membrane phospholipid dissymmetry&lt;br&gt;Alteration of membrane surface receptors, especially their sugar composition&lt;br&gt;Probable activation of ATP dependent pumps, allowing the flow of intracellular water against a concentration gradient</td>
<td>It helps in the differentiation of apoptotic cells from phagocytic cells. It explains the reduction in the size of apoptotic cells, sometimes being useful for the isolation of these cells on the Percoll gradient</td>
</tr>
<tr>
<td><strong>Inconsistent changes:</strong>&lt;br&gt;Increased intracellular calcium levels</td>
<td>In the transduction of a membrane signal or the transcription of a specific gene (e.g. calmodulin)</td>
</tr>
<tr>
<td>Increased synthesis of 3-galactoside, a link protein&lt;br&gt;Increase of TRPM-2 gene transcription&lt;br&gt;Activation of collagenases and metalloproteases&lt;br&gt;TGF 31 synthesis</td>
<td>It inhibits cell proliferation&lt;br&gt;It increases intracellular calcium levels&lt;br&gt;It allows the cell to separate from the neighboring cells&lt;br&gt;Regulation of the apoptosis/proliferation balance (cells, epithelia)</td>
</tr>
</tbody>
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Role of oncogenes in the regulation of programmed cell death
(According to Lane, 1992)

Viral and chemical oncogenesis offers attractive research directions regarding the balance/imbalance between apoptosis and proliferation. Spontaneous or induced tumor cell apoptosis regulates tumor growth. Tumor growth represents an imbalance between proliferation and apoptosis, starting with the first stages of carcinogenesis. Experiments have demonstrated that in a preneoplastic hepatic focus, initiated by a chemical carcinoma, the number of apoptotic cells is much higher than that of the unaffected adjacent tissues. Contrary to the generally accepted idea, the tumor initiation stage is not irreversible, because 80-90% of the initiated cells are eliminated by apoptosis. The promotion stage is also subject to apoptosis (7, 21).

Cytotoxic cells induce in target cells morphological changes that trigger apoptosis. Apoptotic cells are in higher numbers in tumors that are strongly infiltrated with cytotoxic T lymphocytes or NK cells.

New hypotheses and researches have appeared considering oncogenesis as a possible deregulation of apoptosis. Thus, it has been assumed that an oncogene, such as bcl-2, may not stimulate cell proliferation, but inhibit cell apoptosis. It has been shown that under the action of growth stimuli, the c-myc oncogene is involved in cell proliferation but, in the absence of mitogenic signals, this oncogene can induce apoptosis. Finally, p53, whose disappearance or inactivation is frequently observed in malignant tumors, can orient the cell towards proliferation or apoptosis, depending on internal or external signals and the physiological cell status (12, 13, 20).

In veterinary oncology, there are few studies on the apoptotic index in various types of neoplasms. In mammary carcinoma in dogs, the apoptotic index is very low, between 0 and 1% (11).

The inhibition of the apoptosis of cells from germinative centers plays an important role in the development of malignant lymphomas. The mitotic index in the
germinative centers from malignant non-Hodgkin lymphoma is significantly lower compared to unchanged lymph nodes. So, apoptotic inhibition in malignant lymphomas results in the increase of the number of cells per volume unit, not by an increased mitotic index, but by a low apoptotic index (14, 18, 23).

In pluricellular organisms, survival and functioning according to physiological parameters is mediated by a balance between cell proliferation and destruction, an optimal number of various cellular categories being ensured. The elimination of undesired (old, abnormal, tumor) cells from the organism occurs by a genetically controlled process, which has structures for the transmission of messages to effector elements (caspases), a process termed programmed cell death or apoptosis. This process is initiated by a multitude of external and internal cellular factors that, in a certain context, trigger apoptosis. The cells that are about to enter apoptosis show a series of changes, some of which are evident (nuclear material alterations), others inapparent or undetectable by actual investigation means (10). The authors reproduce the data published by THOMPSON (1995) regarding diseases in which apoptosis is inhibited and diseases in which it are stimulated.

The diminution in the capacity of cells to enter the apoptotic process when they receive internal or external signals to enter this process, and to be eliminated, has two major causes: either the cell has no functional apoptotic equipment, which is destroyed, or the cellular pathways for apoptosis are blocked.

**Diseases associated with apoptotic inhibition (22):**

1. **Cancer:**
   - follicular lymphomas;
   - cancers with $p53$ mutations;
   - hormone dependent tumors: mammary neoplasms, prostatic neoplasms and ovarian neoplasms.

2. **Autoimmune diseases:**
   - systemic lupus erythematosus;
   - immune glomerulonephritis.

3. **Viral infections:**
   - herpetic viruses;
   - poxviruses;
   - adenoviruses.

In the appearance and development of the neoplastic process, the role of apoptosis consists of an insufficient development of programmed cell death, accompanied or not by excessive proliferation. Apoptosis counteracts a tumor proliferation process, but at the same time the majority of substances used in anticancer therapy induce apoptosis by triggering cell lesions that cannot be repaired by cellular mechanisms (10).

In neurodegenerative and heart diseases, apoptosis is increased, these diseases being according to THOMSON (22):

1. AIDS;
2. neurodegenerative diseases: Alzheimer’s disease; Parkinson’s disease; lateral amyotrophic sclerosis and cerebellar degeneration;
3. myelodysplastic syndrome: anaplastic anemia;
4. ischemic lesions: myocardial infarction and changes related to reperfusion;
5. toxic hepatic diseases: alcohol.

Apoptosis occurs as a physiological mechanism for the protection of the organism that allows the elimination of undesired cells, in particular those containing pathological mutations. Recent studies have shown that a deficiency in the apoptotic process can play a fundamental role in the genesis or development of cancer. Numerous cellular genes involved in the regulation of apoptosis have already been identified, such as protooncogenes (c-myc, bcl-2) and tumor suppressor genes (p53). It seems that apoptosis is a complex network of pathways that interact, causing the intervention of proapoptotic and antiapoptotic antagonist factors (19).

In the neoplastic tissue, even in the case of a high growth rate, many cells die at a rate similar to that of cell formation and multiplication. In the proliferation or the death of neoplastic tissue cells, a major role is played by vascularization. Endothelial cells from tumor capillaries are directly involved in the proliferation process. The study of vascular endothelium has revealed the fact that the proportion of cells in the S phase is approximately 10 times higher than in endothelial cells from normal blood vessels. Blood vessels within tumors are formed by endothelium arranged on the basal membrane, having a continuous structure or partially lacking vascular walls. The tumor vascular network is poorly oriented, which allows the blood flow to change its direction or have stasis periods. The result of this irregular flow is that tumors larger than several millimeters show deep hypoxic areas, with low pH, due to the accumulation of acid metabolites of anaerobic glycolysis. The cell groups from the proximity of blood vessels, which are in increased supply, continue proliferation, and old cells will be progressively driven towards hypoxic areas, where they will gradually die. Necrotic areas will be surrounded by tumor cell cords that benefit from oxygen supply and substances necessary for proliferation.

Tumor cells usually produce angiogenic factors (TAF), which stimulate endothelial growth. However, by the alert rate of development of the neoplastic tissue, their vascularization capacity is exceeded. Hypoxic tumor necrosis is a sign that rapid tumor cell proliferation continues. In numerous neoplastic types (gliomas, sarcomas, soft tissues, etc.) the presence of necrosis is correlated with an unfavorable prognosis. The death of endothelial cells through apoptosis can be induced by the fluctuation of angiogenic factors; tumor necrosis factors, tumor macrophage and lymphocyte products may have a similar effect.

Apoptotic cells within tumors may appear among neoplastic cells during proliferation, but they appear in great numbers around and at the periphery of necrotic foci. The incidence of apoptotic cells varies widely from one tumor type to another or even from one patient to another, always depending on the phase and
developmental stage of the tumor. In some cases, the presence of apoptotic cells represents a diagnostic and/or prognostic factor.

The factors responsible for tumor cell apoptosis are not well understood; internal factors, the action of cytotoxic cells, NK cells and macrophages are suspected. The intimate phenomena by which cytostatics act are not completely elucidated. The fact that tumor apoptosis can be intrinsically programmed also has interesting implications for therapy and has led to attempts to discover the regulation mechanisms of apoptosis in tumor cells. Thus, human c-myc and H-ras oncogenes were inserted in a rodent fibroblast cell line. The cells obtained in this way were tumorigenic, but their capacity of local invasion and metastasising varied. All c-myc tumors had high mitotic and apoptotic rates, with a slow general expansion rate. In contrast, ras tumors had low apoptotic rates and were more aggressive. In ras tumors, necrosis was much more extensive, as a result of the non-proliferation of vascular endothelium, which favors hypoxia and necrosis.

In therapy, it has been demonstrated that some cytotoxic drugs act on cancerous cells, inducing apoptosis. Experimental studies aim to discover cytostatics with a maximum efficiency on tumor cells, by either preventing apoptotic block or decreasing the apoptosis induction threshold, while increasing the difference between the therapeutic dose and the toxic threshold in the action of these drugs.

By synthesizing the results of the researches in this field, KAHN (1994) noted the existence of cell apoptosis in the absence of growth factors, neurotrophic factors or cytokinins. Apoptosis can be avoided by an increased synthesis of Bcl-2, which suggests that this antia apoptotic protein can alter at least certain effects of the above mentioned factors. Between Bcl-2 (apoptotic inhibitors) and Fas (apoptotic inducers) proteins there are no known direct interactions with the control systems of the cell cycle, and proliferation and apoptosis may both be supposed to act on tumor growth, but their mechanisms are different. Each cell receives multiple signals (hormones, cytokines, etc.) that, by means of specific receptors, can induce the cell to enter the normal cycle or apoptosis. The alteration of a specific receptor may result in the appearance of a malignant clone, due to the imbalanced relation between the inducing or repressing signals of apoptosis and proliferation. The stimulation of proliferation and apoptosis occurs by the activation of common genes, such as c-fas and c-myc genes (21).

Viral oncogenesis offers interesting research alternatives regarding the interaction between apoptosis and proliferation, providing at the same time, like in the case of c-myc and bcl-2 genes, an exciting hypothesis on the mechanisms of cooperation of oncogenes.

The spontaneous or induced apoptosis of tumor cells regulates tumor growth. Tissue homeostasis requires a constant balance between cell death and proliferation. Apoptosis mediates the selective elimination of undesired cells (damaged, old, preneoplastic cells). Tumor growth represents an imbalance between proliferation and apoptosis, starting with the first stages of carcinogenesis.
SOLARY et al. (1993) have experimentally demonstrated that in a preneoplastic hepatic focus initiated by a chemical carcinoma, the number of apoptotic cells is much higher compared to the surrounding tissue. So, contrary to the generally accepted idea, the tumor initiation phase is not irreversible, because 80-90% of the initiated cells are eliminated by apoptosis. The promotion stage is also subject to apoptosis. In a tumor, cells disappear periodically by differentiation, desquamation, migration or death.

Cytotoxic cells induce in target cells morphological changes that evoke apoptosis. The number of apoptotic cells has been found to be much higher in tumors that are markedly infiltrated with cytotoxic T lymphocytes or NK cells.

The consequence of oncogenesis being regarded as a possible deregulation of apoptosis has been an enlargement of perspective in the formulation of new work hypotheses in this field in which scientific research has an unlimited scope. Thus, it has been supposed that an oncogene such as bcl-2 can play a role not in the stimulation of cell proliferation, but in the inhibition of cell apoptosis. Recently, it has been demonstrated that under the action of various growth factors, the c-myc oncogene is involved in cell proliferation but, in the absence of mitogenic signals, this oncogene is capable of inducing apoptosis. Finally, p53, whose disappearance or inactivation is frequently found in malignant tumors, can orient the cell towards proliferation or apoptosis, depending on various internal or external signals and the time of the cell cycle.

The mechanisms responsible for apoptotic cell death in untreated malignant non-Hodgkin lymphoma, as well as in other tissue neoplasms, are not yet completely understood.

The results of the study performed by LEONCINI et al. (1993) suggest that an increase in the apoptotic index and the lack of the cell protein Bcl-2 could be an unfavorable prognostic factor, regardless of the histologically established malignancy grade, for malignant non-Hodgkin lymphoma. However, it can be estimated that a relative increase in cell apoptosis and a better understanding of its mechanisms could have major implications in the establishment of new principles in the treatment of malignant diseases. A deeper knowledge of apoptosis and its induction by various means could assist in the more effective destruction of neoplastic cells.

In 1989, LIU et al., and in 1990, WILLIAMS et al. suggested that the apoptotic inhibition of cells from germinative centers could play an important role in the development of malignant lymphomas. Starting from this hypothesis, HOLLOWOOD and MACARTNEY (1991) have studied the apoptotic index and mitotic index in the germinative centers from malignant non-Hodgkin lymphoma. Results have demonstrated an extremely high apoptotic index in the germinative follicles of the control group compared to the neoplastic group. This favors the hypothesis that, by the inhibition of apoptosis in malignant lymphomas, apoptosis is directly involved in the pathogenesis of these tumors.

Results suggest that an increase in the apoptotic index and the absence of the Bcl-2 cell protein could be more reliable predictive elements in establishing a
prognosis for malignant lymphomas compared to histological prognosis. The precise evaluation of the relation between apoptotic diminution and cell proliferation from malignant follicles requires further investigations, as well as the development of adequate supporting techniques.

Regarding the perspectives and implications of the apoptotic process in cancer therapy, we note the observation of some researchers, according to which the sensitivity of cancer cells to chemotherapy, ionizing radiation, etc., seems to be related to the induction of an apoptotic program.

Based on the results obtained in vitro, FUKUDA, KOJIRO and CHIU (1993) suggest that tumor apoptosis can represent a residual autoregulation attempt, aimed at the expanding tumor population and/or could be the result of mild cell injuries, such as hypoxia, nutritional deficiencies or other unknown noxious factors. The authors have demonstrated that apoptosis can be induced in vitro, in hepatoma cells, by the successive action of low intensity injuries or stimuli. The clarification of the biochemical pathway in the activation of apoptosis should lead to the finding of new possibilities in cancer treatment.

Spontaneous apoptosis in tumors can be amplified by therapy, e.g. by hormonal deprivation of hormone dependent tumors. It should be considered that the induction of apoptosis depends on the cellular terrain, the inducing signal and its intensity (5, 21). Apoptosis can be activated by an increase in speed as well as by the activation of cell cycle regulating genes. The increase of the apoptotic speed starts with the activation of non-specific oncogenes (c-fas; c-jun; c-myc), in response to extracellular signals, which modulate their activity (inhibitory signals or their absence). The cell is at the same time unable to complete its cell cycle, probably due to the absence of the mitogenic factor, and dies through apoptosis.

Some chemical substances used in the treatment of tumors result in the arrest of the cell cycle in phase $G_1$ or especially in phase $G_2$. This arrest of the cell cycle evolution is interpreted as a possibility for the treated cell to gain time for lesion repair, in order to resume its cell cycle. If during malignant transformation the control points, such as $p53$, have disappeared, the cell does not interrupt its cycle for lesion repair and rapidly accumulates lethal lesions. If the processes involved in DNA repair are deficient, cells cannot eliminate DNA lesions, such as those produced by ultraviolet rays, when the cell accumulates genetic abnormalities. Over the past years, the first human genes involved in DNA repair have been successfully isolated.

The apoptosis of a great number of cells, shortly after anticancer treatment, suggests that apoptosis induction does not depend on the cell cycle (21). All happens as if the cell was programmed for the activation of the final phase of apoptosis, probably by the activation of an endonuclease. The alteration of the genetic programme of the cell undergoing differentiation or the change of signals sent to the cell can modify cellular sensitivity to apoptosis induction. The capacity of tumor cells to enter apoptosis becomes the key of the response to the tumor disease treatment.
SOLARY et al. (1994) estimate that apoptosis modulation can have various implications, such as:

- potential antitumor activity of cytotoxic agents;
- reduction of undesired (toxic) effects, inhibiting apoptosis in non-tumor cells;
- orientation of research towards the obtaining of new products, which selectively induce apoptosis in malignant cells.

It may be concluded that a knowledge of the mechanisms and development of the apoptotic process phenomena and the clarification of the biochemical pathway in the activation of genetically programmed death will change the current concept regarding the treatment of diseases, in general, and of neoplasms, in particular. Researches in this direction bring hope that more efficient means to fight tumor disease and, why not, to prevent the multiplication of neoplastic cells, may be found. The possibility to influence the apoptotic process, sometimes by accelerating it, at other times by slowing it down, gives hope for the efficient treatment of some diseases, but also for the prolongation of life.

References