

Apoptosis Versus Polemosis. Different Mechanisms Leading to Non-necrotic Cell Death

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Abstract. Apoptosis is a physiological auto-suicidal, genetically-induced cell deletion process of senescent effete normal cells. Apoptosis guarantees genetic fidelity, minimizes phenotypic variation and eliminates genotypic alteration. The auto-destruction-induced by a cascade of caspases- results in the breakdown of normal cells and the formation of apoptotic bodies. Those bodies are rapidly phagocytized by macrophages and internalized by cells of the same type. On the other hand, polemosis (from Greek polemos: war) is a more dynamic mechanism of cell destruction between two different cell systems. Polemosis is focal, haphazardly distributed at the time of observation, probably erratic and influenced by the chemotactical attraction of committed lymphocytes by neoplastic cells. Polemosis is implemented by the interaction of the Fas-Fas ligand compulsory cellular system of self-defence. The end result of that struggle is manifested by the destruction of committed lymphocytes and the appearance of polemotic bodies (nuclear fragmentation with or without cytoplasmic remnants). The question arises as to whether polemotic bodies are also engulfed by macrophages, as is the case with apoptotic granules. In this work, the possible association between CD68-positive macrophages and intraepithelial polemotic bodies was investigated in sections from 50 colorectal adenomas. Polemotic bodies were found in groups of dysplastic cells in 84% ($n=42$) of the 50 adenomas, but none of them showed an accumulation of CD68 macrophages around the polemotic bodies. As polemotic bodies (autologous T-cells DNA) are not engulfed by macrophages it is hypothesized that the DNA of these bodies might be incorporated into the nuclei of dysplastic cells. This would satisfy the avidity of the DNA of rapidly proliferating dysplastic cells, a process that takes place, unremittingly, at any given time.

More than 30 years ago the Australian pathologist John Kerr and co-workers (1) described a particular form of physiological cell death which they coined "apoptosis". That term had been suggested by James Cormack, Professor of classical Greek at the University of Aberdeen, Scotland, and meant "falling off" or "dropping off"; it was used by Homer to describe the dropping off of leaves from trees during autumn (2). The microscopic residues of that auto-suicidal process were called *apoptotic granules*.

During the past 30 years much research has centred on the study of the phenomenon of apoptosis, resulting in 10,400,000 entries in Google (2005-09-13). That vast number of publications undoubtedly mirrors the enormous interest in apoptosis as a physiological mechanism involving the final outcome of normal cells. That physiological pathway contrasts with the one taking place in cell necrosis, a process conveyed by one or more deleterious causes such as anoxia, trauma, burning, or bacteria, to name a few.

Thirty years previously, in 1954, Leuchtemberger *et al.* (3) reported *basophilic* inclusions in the dysplastic cells of rectal adenomas. Subsequently, Helwig (4) detected similar bodies in the rectal mucosa in patients with ulcerative colitis. As Feulgen, a DNA stain, evidenced these intracellular inclusions, the authors assumed that the inclusions were virus particles (3,4). In the literature those inclusions are referred to as Leuchtemberger bodies (LBs).

Years later (5), we studied, in detail, the occurrence of intraepithelial LBs in sections of colorectal adenomas. We found moderate to high numbers of LBs in up to 82% of the adenomas. In addition, we found intraepithelial lymphocytes (IELs) in 70% of 102 hyperplastic polyps and tubular adenomas, in ≥80% of 75 villous and serrated adenomas and in 50% of 28 incipient adenocarcinomas. LBs were found at the basal aspect, but also within the adenomatous cells (5-8); LBs were not present in the adjacent *lamina propria mucosa*. Those findings suggested that LBs were remnants from the cells that had trespassed the dysplastic epithelium through their basement membrane (5,6). The cells accountable for the LBs were T-lymphocytes (7-10). Studies of cell proliferation (Ki67) and with transmission

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electron microscope (5) provided no evidence that the adenomatous cells were undergoing cell destruction, despite a high number of intraepithelial LBs. Other studies (11,12) showed that Fas molecules are down-regulated in adenomatous cells but not in IELs, indicating that the Fas-ligand molecules of IELs cannot exert their deleterious effect upon the adenomatous cells, not binding to the Fas of adenomatous cells. On the other hand, the intact Fas-ligand molecules of adenomatous cells are able to bind to the Fas molecules of T-lymphocytes, leading to the structural disintegration of T-cells. The interaction between adenomatous cells and T-lymphocytes was later confirmed in experimental animals (13). Rats developing slow-growing colonic adenomas induced by glutamic acid pyrolysate (GLU 1) often showed IELs and/or a high number of LBs, whereas rats with dimethylhydrazin-induced fast-growing colonic adenomas seldom had IELs or LBs (13).

Hence, two dissimilar mechanisms of non-necrotic cell death appear to exist. One is apoptosis, a physiological process of cell auto-suicide (*i.e.* auto-destruction) scheduled in normal tissues for the homeostatic mechanism of cell renewal. That process is activated by a family of cysteine proteases, called caspases. The other process, that hereafter will be referred to as *polemosis* (from Greek *polemos*: war), is a more dynamic mechanism of cell destruction between two different cell systems, one being committed lymphocytes and the other neoplastic cells. The term *polemotic bodies* is herein coined to highlight the microscopic fragments resulting from the breakdown of intruding T-cells.

Most authors concur that the *apoptotic* granules are rapidly phagocytized by macrophages and internalized by cells of the same type (14-21). Enzymes emanating from lysosomes finally digest the engulfed bodies. In contrast, there is no information regarding the fate of *polemotic bodies*. The question arises: are polemotic bodies also engulfed by macrophages, as is the case with *apoptotic* granules? Alternatively, are these bodies incorporated into other cells or "vanish" without the participation of macrophages?

In this work these questions were explored. For this purpose, the distribution of immunohistochemically-labelled macrophages was assessed in a cohort of colorectal adenomas. Particular attention was paid to areas with dysplastic cells having *polemotic bodies* and to areas with dysplastic cells lacking *polemotic bodies*.

Materials and Methods

The material comprised 50 consecutive endoscopically-removed colorectal adenomas without invasive growth.

The sections were stained with H&E, Feulgen and CD68 (Dako Cytomation, Glostrup, Denmark). CD68 is a LPG glycosylated lysosomal membrane protein that is expressed in the cytoplasmic granules in macrophages and other mononuclear cells with phagocytic properties (22).

The disintegration of nuclear material into residual fragments is the end-product of the process of *polemosis*. As a result of that nuclear fragmentation, *polemotic bodies* appear as basophilic granules in H&E stain. Sometimes, a rim of eosinophilic cytoplasm is seen around those basophilic granules.

According to the degree of cellular dysplasia, the adenomas reviewed were classified into those with low-grade (LGD) and with high-grade (HGD) dysplasia.

The presence of *polemotic bodies* in individual adenomas was assessed in H&E- and in Feulgen-stained sections. The frequency of glands having *polemotic bodies* was graded as none (0), occasional (+), moderate (++) or high (+++).

The number of macrophages in the *lamina propria* (as detected by CD68) facing adenomatous cells showing *polemotic bodies* was graded as negative (0), infrequent (+), moderate (++) or high (+++). The results were compared to the number of macrophages facing adenomatous cells lacking *polemotic bodies*.

Results

Polemotic bodies were found at the basal aspect but also within dysplastic cells of colorectal adenomas (Figure 1). This phenomenon occurred in one or more groups of dysplastic glands in 42 (84%) of the 50 adenomas.

Of the dysplastic glands having occasional (+), moderate (++) or high (+++) numbers of polemotic bodies, none showed CD68 macrophages gathering about those bodies (Figure 1). Of the glands without *polemotic bodies* the majority showed no accumulation of CD68 macrophages around dysplastic glands. In some other dysplastic glands without *polemotic bodies*, however, CD68 macrophages were seen to accumulate around the glands.

Of the 35 adenomas with LGD, 29 (82.8%) had dysplastic glands showing *polemotic bodies*. Of the 15 adenomas with HGD, 13 (86.7%) had dysplastic glands showing *polemotic bodies*. The difference was non-significant ($p=0.6$).

Discussion

Apoptosis is an auto-suicidal, unremitting, predictable physiological programmed cell death. It is a direct consequence of the "in and out" principle scheduled by the homeostatic process of cell renewal that governs all normal tissues. *Apoptosis*, a genetically-induced cell deletion, guarantees genetic fidelity, minimizes phenotypic variation and eliminates genotypic alteration. Triggered by a cascade of caspases (14-21), the microscopic end-point of *apoptosis* is cell disintegration with formation of *apoptotic* granules. In contrast, *polemosis* is a more dynamic fight-back mechanism between two different cell systems, namely committed lymphocytes and rapidly proliferating neoplastic cells (7-10, 23). *Polemosis* is focal, haphazardly distributed at the time of observation, probably erratic and possibly influenced by the chemotactical attraction of committed lymphocytes by adenoma cells. *Polemosis* is implemented by the interaction of the Fas-Fas ligand compulsory cellular system of self-

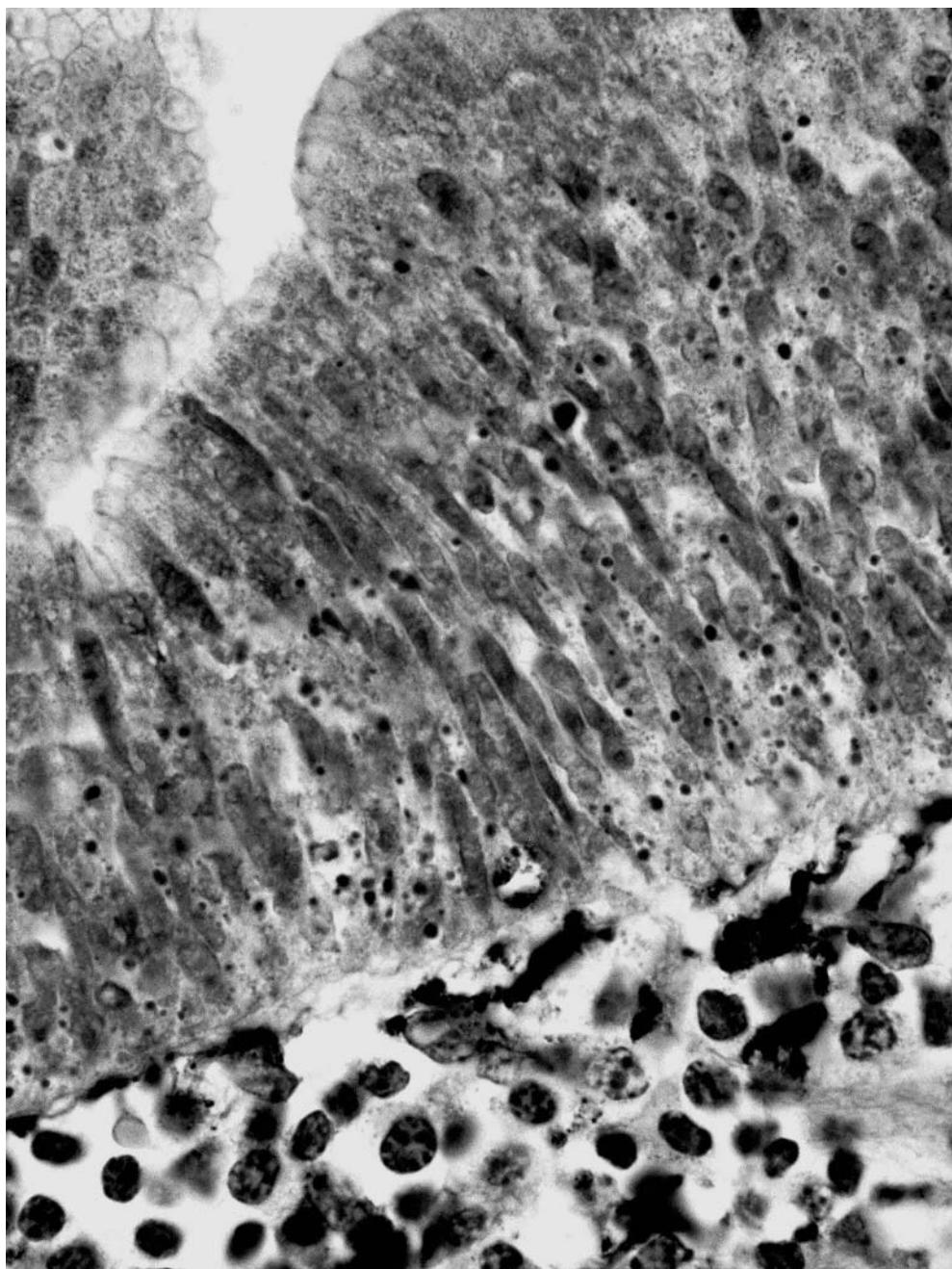


Figure 1. High-power view of a colonic adenoma with high-grade dysplasia showing numerous intraepithelial polemotic bodies. Note lack of accumulation of macrophages around that gland (CD68 immunostain, $\times 100$ oil immersion).

defence (10-12). The end result of that struggle is manifested by the destruction of committed lymphocytes and the appearance of *polemotic bodies* (nuclear fragmentation with or without cytoplasmic remnants). It is unclear, however, whether *polemosis* encompasses only a group of dysplastic glands at all times (detected at the time of observation) or whether another group of glands will alternatively be

affected by that process but at different time-intervals. Rationally, the epithelial "assault" by T-cells should take place in waves, obviously focussed to different groups of adenomatous glands, but at different time-intervals. If that is the case, much research is needed to understand why only few groups of dysplastic glands (at the time of observation) attract lymphocytes to the fields of holocaust.

It should be understood that the end-product of cell fragmentation caused either by *apoptosis* or by *polemosis* is indistinguishable at histological examination. Perhaps that is the reason why the concept of *polemosis* as an alternative pathway to that of *apoptosis* has not been suggested previously in the literature. The understanding that *apoptosis* is a physiological mechanism of homeostasis aimed to remove senescent effete normal cells and that *polemosis* is the annihilation of trespassing immuno-competent cells deceived by more shrewd and rapidly proliferating neoplastic cells, may cast more light onto the phenomenon of non-necrotic cell death.

In *apoptosis* the granules are removed by mononuclear phagocytes and by adjacent cells (14-21). Once phagocytosed, lysosomal enzymes derived from ingesting cells degrade the *apoptotic bodies*. In *polemosis*, however, the *bodies* are apparently not removed by macrophages. These results thus confirm previous studies using Feulgen-stained sections and transmission electron microscopy (5).

As *polemic bodies* (autologous T-cells DNA) are not engulfed by macrophages it is hypothesized that the DNA of these *bodies* might be incorporated into the nuclei of dysplastic cells. This would satisfy the avidity of the DNA of rapidly proliferating dysplastic cells, a process that takes place, unremittingly, at any given time (23).

Although only colorectal adenomas were studied here, it is conceivable that the concept of *polemosis* applies to other neoplastic lesions in other organs.

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