Chapter 4
Molecules Involved in Recognition and Clearance of Apoptotic/Necrotic Cells and Cell Debris

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Abstract: Disposal of apoptotic or necrotic cells or cell remnants is of paramount importance for the survival of multicellular organisms. Plasma membrane rupture of accumulating dying cells would lead to a deluge of cellular components into the extracellular spaces and could lead to impairment of tissue organisation, occlusion of vessels and the induction of inflammatory and autoimmune reactions. Therefore, efficient mechanisms exist, that secure safe disposal of dying cells and their contents. Neighbouring cells and professional phagocytes like macrophages and immature dendritic cells are the main effector cells of these processes. The mechanism of engulfment of dying cells and the signalling pathways leading to phagocytosis are described. In addition, extracellular mechanisms seem to exist that are activated for the disposal of necrotic cells and their remnants.

Keywords: Apoptosis • Necrosis • Phagocytosis • Phagocyte receptors

4.1 Programmed Versus Accidental Cell Death, in Simple Apoptosis Versus Necrosis

Cell death is a characteristic feature in the lifespan of eukaryotic cells and essential for the survival of multicellular organisms (for reviews see Majno and Joris 1995; Zakeri et al. 1995; Fiers et al. 1999; Van Cruchten and Van Den Broeck 2002; Fink and Cookson 2005). Coordinated cell demise is necessary for sculpturing tissues during embryogenesis (organogenesis), for preserving a constant organ cell number.
by balancing continuous renewal with elimination of aged or non-functional cells (tissue homeostasis), and in the immune system during the processes that lead to optimal antigen recognition or for maintaining self-tolerance by eliminating lymphocytes with autoimmune activity. During these physiological situations cell death occurs by genetically determined pathways and has therefore been termed programmed cell death (PCD). However, there are different modes of PCD like apoptosis and autophagy, type I and type II cell death, respectively (Krysko et al., Chap. 1; Diez-Fraile et al., Chap. 2, this Vol.). The former being the most frequently occurring form of PCD. The morphological features of apoptosis were described in detail by Kerr and colleagues (Kerr et al. 1972). Characteristically apoptotic cells detach from neighbouring cells and the extracellular matrix, exhibit cytoplasmic shrinkage, pyknosis, i.e. nuclear shrinkage and chromatin condensation at the inner nuclear lamina, nuclear fragmentation (karyorrhexis), and subsequently they disintegrate into membrane-surrounded cell fragments (apoptotic bodies; Fig. 4.1a–d and Fig. 4.2c). The morphological features of apoptosis are caused by the selective cleavage of essential cellular proteins of the nuclear scaffold, focal adhesions, cytoskeleton and the DNA-repair machinery after activation of a group of aspartate-specific cysteine proteases called caspases. Caspase activation results from the transmission of death signals by cell death receptors (extrinsic pathway) and/or release of pro-apoptotic factors from mitochondria (intrinsic pathway). However, also under pathological conditions (infections, irradiation, intoxication, and mutagenic events) PCD is initiated with the aim to protect the organism against neoplasia, invading micro-organisms and to overcome inflammatory reactions.

In contrast to PCD, accidental cell death (ACD), also better known as necrosis or type III cell death, is usually regarded as the consequence of non-physiological insults. It can occur when an organism is exposed to extreme physical forces and/or to aggressive chemicals and in particular under pathological conditions such as anoxia caused by ischemia, mechanical trauma, heat, chill, irradiation, and/or drug intoxication. In a number of instances necrosis is initiated by suppression of

Fig. 4.1 Apoptosis and necrosis in MCF-7 cells. a–d: Human mamma adenocarcinoma MCF-7 cells treated with 2 μM staurosporin exhibit typical morphological signs of apoptosis. Early after induction of apoptosis (2 hours) the cells shrink (b) and expose PS on the plasma membrane (b'', positive plasma membrane staining with annexin V-FLUOS). After 4 hours, the cells display chromatin condensation (c’), however, the plasma membrane remains intact (c‘’, negative nuclear staining with propidium iodide, PI). Secondary (apoptotic) necrosis with osmotic swelling (d) due to plasma membrane damage occurs after 24 hours (d‘’, positive nuclear staining with PI). e–h: MCF-7 cells treated with 1 mM H2O2 and 1 mm sodium azide exhibit typical signs of primary necrosis, like pyknosis and osmotic cell swelling (oncosis). Early after induction of necrosis the cells display membrane rupture and PS exposure in parallel (e’’ and f’’, positive nuclear and plasma membrane staining with PI and annexin V-FLUOS, respectively). In the presence of murine wild-type serum, chromatin breakdown occurs with ongoing time (16 h) and DNA-fragments diffuse from the nucleus into the cytoplasm (g’ and g’’). Chromatin breakdown depends on DNASE1 as revealed by its lack in the presence of serum derived from Dnase1 KO mice (h’ and h’’). Parts of this figure were already published in (Napirei et al. 2004). a–h: phase contrast microscopy. a’–h’: DNA-staining by the plasma membrane-permeable dye Hoechst 33258. a’’ and c’’–h’’: DNA-staining with the plasma membrane-impermeable dye PI. b’’ and f’’: staining of PS by annexin V-FLUOS. Bars: 20 μm.