Apoptosis

A01

NUCLEAR PATTERNS OF DNA FRAGMENTATION DURING APOPTOSIS IN THE INVOLUTING MAMMARY GLAND

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A majority of secretory epithelial cells of the mouse mammary gland is eliminated by programmed cell death (apoptosis) during post-lactational involution. Apoptotic cells are characterized by an oligonucleosomal fragmentation of chromosomal DNA which can be visualized by DNA laddering on an agarose gel or by *in situ* terminal transferase assays. We have investigated the distribution of intranuclear DNA strand breaks during involution by analyzing 25 µm thick sections in a Bio-Rad confocal microscope followed by reconstructing the distribution of fragmentation by image processing (by Imaris on a Silicon graphics workstation). We typically found distinct classes of nuclear staining patterns: in addition to homogeneously stained normal and pycnotic nuclei a class of nuclei with a predominant staining of regions close to the inner nuclear membrane was observed. These classes of nuclei may represent different stages or different pathways of cell death. Data will be presented on the distribution of DNA fragmentation with respect to the topography of the nucleus during mouse mammary involution. The results strongly suggest a non-random degradation of nuclear DNA during cell death and rise important questions about the kinetics and coordination of DNA fragmentation in this system.

A02

BCL-2 PROTECTS FROM CELL DEATH INDUCED BY A TOXIC OVERLOAD OF ABERRANT PROTEIN

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Programmed cell death involves the activation of IL-1β converting enzyme (ICE)-related proteases. Although substrates of these enzymes are known, it is unclear whether their products are specific mediators of cell death or proteolytic fragments contributing to the accumulation of a critical mass of toxic "junk" protein. To obtain evidence for the latter possibility, we artifically created high amounts of aberrant protein by blocking the ubiquitin/proteasome pathway in two ways: eliminating the ubiquitin-activating enzyme E1 in a conditional manner and/or blocking the proteasome with selective inhibitors. In both cases, cells died by apoptosis effectively blockable by Bcl-2 overexpression. Further analysis showed that Bcl-2 neither acts as E1 enzyme, nor prevents the generation of aberrant protein. By contrast, it may stimulate a novel pathway to neutralize the "junk" protein created under apoptotic situations.

A03

CHIMERA BETWEEN BCL-2 AND BAX: A TOOL TO DISSECT DOMAINS FOR SURVIVAL AND DEATH

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Bcl-2 and Bax belong to a family of homologous proteins which control programmed cell death. Whereas overexpression of Bcl-2 confers death protection, overexpression of Bax mitigates the survival effect of Bcl-2 and can even kill cells on its own. The opposite activities of Bcl-2 and Bax have been partially explained by their heterodimerization capacities. Heterodimerization is mediated by two conserved regions present in all Bcl-2 homologs, called BH1 and BH2. A third region, called BH0 is present only in the class of Bcl-2 homologs which act as survival factors. Here we present data on survival and intracellular localization of mutant proteins in which the BH0 and BH2 regions have been swapped between Bcl-2 and Bax. Our results indicate that both regions are required for the survival function of Bcl-2 and cannot be replaced by homologous regions from Bax. In contrast, Bax does not require its BH2 for death activity. Possible models for Bcl-2/Bax interactions are discussed.

A04

A LYSOSOMAL COMPONENT REQUIRED FOR CELL DEATH INDUCED BY TUMOR NECROSIS FACTOR α

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Programmed cell death induced by tumor necrosis factor α (TNFα) has been shown to involve a series of molecular events including activation of serine and cysteine proteases, the generation of reactive oxygen species and the production of ceramide. How these events are set in motion is largely unknown. Here we show that the integrity of lysosomes is crucial for the death-promoting effect of TNFα. Cellular treatment of two TNF-sensitive cells with lysosomotropic agents delays TNFα-induced cell death even in the presence of actinomycin D. Neither inhibitors against defined serine- or cysteine proteases, nor antioxidants exert such a death-protective effect as lysosomotropic agents. Importantly, cell death induced by other physiological or non-physiological agents is not impeded by the destruction of lysosomal activity. These results point towards a lysosomal component being a specific mediator of TNFα-induced cell death.

A05

ANALYSIS OF PROTEINS THAT BIND TO BCL-2 DURING APOPTOTIC STRESSES

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The oncogene product Bcl-2 effectively spares mammalian cells from programmed cell death induced by numerous stimuli. The molecular basis for this death-protective activity is still a matter of debate. In order to find partners of Bcl-2 mediating its action, we devised an immunoprecipitation approach to identify proteins which specifically associate with Bcl-2 under physiological and non-physiological apoptotic stresses. Here we present a well-known antagonist of Bcl-2, called Bax whose interaction with Bcl-2 remains intact during apoptotic stresses. In addition, a novel protein is shown which appears to co-precipitate with Bcl-2 only under stress conditions. The identification of the latter protein may pave the way to uncover the impatiently awaited molecular function of Bcl-2.

A06

DYNAMIC AND ULTRASTRUCTURAL FEATURES OF APOPTOSIS IN HUMAN BREAST CANCER CELL LINES.

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Apoptosis, or programmed cell death, was studied in oestrogen-sensitive MCF-7 and in non-sensitive-oestrogen SKBR-3 human mammary cell lines.Sequential serum (FCS) and glutamine deprivation, oestrogen-withdrawal by treatement with pure anti-oestrogen ICI 182,780 were used to induced apoptosis in both cell lines. The dynamic features of apoptosis were followed by propidium iodide (PI) uptake (permeability test) and DNA strand breaks labeled by tailing with Terminal deoxynucleotidyl Transferase (TdT), measured by means of flow cytometry. Ultrastuctural properties of both nuclear chromatin and cell surface were assessed by transmission electron microscopy.Our results reveal that by changes of culture conditions, oestrogen-sensitive MCF-7 cells have both lower growth capability and increased apoptotic rate than non-oestrogen-sensitive SKBR-3 cells.

A07

REQUIREMENT OF LYN AND SYK TYROSINE KINASES FOR THE PREVENTION OF APOPTOSIS BY CYTOKINES IN HUMAN EOSINOPHILS

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In allergic diseases, the cytokines IL-5 and GM-CSF are upregulated and have been proposed to cause blood and tissue eosinophilia by inhibition of eosinophil apoptosis. We demonstrate herein, in freshly isolated human eosinophils, that the IL-3/IL-5/GM-CSF receptor β subunit interacts with cytoplasmic tyrosine kinases to induce phosphorylation of several cellular substrates, including the β subunit itself. The Lyn and Syk intracellular tyrosine kinases constitutively associate at a low level with the IL-3/IL-5/GM-CSF receptor β subunit in human eosinophils. Stimulation with GM-CSF or IL-5 results in a rapid and transient increase in the amount of Lyn and Syk associated with the IL-3/IL-5/GM-CSF receptor β subunit. Lyn is required for optimal tyrosine phosphorylation and activation of Syk. In contrast, Syk is not required for optimal tyrosine phosphorylation and activation of Lyn. These data suggest that Lyn is proximal to Syk in a tyrosine kinase cascade which transduces IL-3, IL-5, or GM-CSF signals. Compatible with this model, both Lyn and Syk are essential for the activation of the anti-apoptotic pathway(s) induced through the IL-3/IL-5/GM-CSF receptor β subunit in human eosinophils.

A08

FUNCTIONAL FAS RECEPTORS ARE EXPRESSED BY HUMAN EOSINOPHILS

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The interaction between activated T cells and eosinophils has been proposed to play an important role in the pathogenesis of allergic diseases. T-cell derived cytokines such as IL-5 and GM-CSF inhibit eosinophil apoptosis and may therefore contribute to the development of tissue and blood eosinophilia in these disorders. Withdrawal of these cytokines leads to eosinophil apoptosis in vitro. However, whether mechanisms exist to actively induce apoptosis in eosinophils is at present unknown. We demonstrate herein, that freshly isolated human eosinophils express mRNA and protein for the Fas receptor. Using anti-Fas mAb, we show that Fas activation accelerates apoptotic eosinophil death in vitro. Eosinophil apoptosis induced by ligation of the Fas receptor is dependent on the activation of tyrosine kinases which induce rapid protein-tyrosine phosphorylation. In contrast, activation of the Fas receptor is not associated with increases in intracellular Ca^{2+} levels indicating that phospholipase C is not a target for activated tyrosine kinases in this signaling pathway. Moreover, we observed that eosinophils derived from some hypereosinophilic donors do not express functional Fas receptors although Fas protein is normally expressed in these cells. This implies that the susceptibility of the Fas receptor is a matter of regulation in eosinophils as it has been previously observed in other systems. Cytokines seem to be not involved in the regulation of Fas susceptibility since Fas induced death occured even in the presence of GM-CSF or IL-5. These data suggest that Fas ligand - Fas interactions are involved in the regulation of eosinophil apoptosis and that defects in this system could contribute to the accumulation of these cells in allergic and asthmatic diseases.

A09

DIRECT DEMONSTRATION OF DELAYED EOSINOPHIL APOPTOSIS AS A MECHANISM TO CAUSE TISSUE EOSINOPHILIA

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A multicellular organisms must balance the rates of cell generation and cell death to maintain cellular homeostasis. Therefore, abnormalities in the regulation of this balance may contribute to a number of different pathogenic processes. Nasal polyps, which often occur in association with allergic rhinitis and asthma, are characterized by a marked infiltration of eosinophils. Using a method for detecting eosinophils with DNA strand breaks, we present direct evidence for an inhibition of eosinophil apoptosis in this model of tissue eosinophilia. The delay of the death process in these cells is likely to be responsible for eosinophil accumulation in nasal polyps. By using Southern blot analysis linked to the reverse transcription polymerase chain reaction, we detected a mRNA signal specific for the eosinophil survival factor IL-5 in all nasal polyps. Treatment of the eosinophil infiltrated tissue with specific anti - IL-5 monoclonal antibody decreased tissue eosinophilia. Therefore, IL-5 may represent an important cytokine responsible for the prevention of eosinophil apoptosis in nasal polyps. Moreover, a previously suggested IL-4 dependent specific recruitment of eosinophils into the inflammed tissue could be excluded by our studies. Together, these findings suggest a novel mechanism by which eosinophils specifically accumulate in human tissues.

A10

A RAPID SCREEN FOR VARIOUS FORMS OF CYTOTOXICITY USING FLOW CYTOMETRY

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A method of rapid screening for cytotoxicity is presented. The technique simultaneously studies parameters indicative of the vital and fatal states of lymphoid cells. Cell death includes both apoptotic and necrotic processes. TK6 human lymphoblastoid cells were treated with various cytotoxic agents. Subsequently cell-death rates and viability were evaluated by flow cytometry. Morphological changes were analysed by transmission electron microscopy. Various cytotoxic agents such as X-rays, proton irradiation, photodynamic-therapy and taxol treatment effectively induced different forms of cytotoxicity which could be distinguished. We conclude, that the method presented provides a rapid indicator of cytotoxicity and permits characterization of different modes of cell death such as necrosis and apoptosis.