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Granzyme B generates unique fragments from autoantigens

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Keywords

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Context

Systemic autoantigens have diverse functions and subcellular distributions. However, they cluster in surface structures during apoptosis. Cysteine proteases, called caspases, are involved in the initiation (eg caspase-8) and the effector (eg caspases-3, -6, and -7) phases of the apoptotic cascade. Caspases cleave many autoantigens, generating identical fragments in all forms of apoptotic death described. It is therefore unlikely that these fragments would initiate autoimmunity. Granzyme B is a serine protease located in the cytoplasmic granules of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. While both CTL and NK cells can trigger Fas/FasL-mediated apoptosis (where L is ligand), they can also effect cell death by granule-exocytosis-induced cytotoxicity. This may be accompanied by distinct patterns of cleavage of autoantigens, a process which could release cryptic epitopes and initiate systemic autoimmunity. To investigate whether granzyme B cleaves systemic autoantigens, to generate fragments which are distinct from those liberated by caspases during apoptosis.

Significant findings

Studies *in vitro* demonstrated that 12 autoantigens were cleaved by both caspase-3 and granzyme B but at distinct sites. Nine autoantigens were cleaved exclusively by granzyme B. Eight autoantigens tested were cleaved by neither protease. Finally, 16 molecules proved resistant to cleavage by granzyme B. Similar proteolytic activity of caspase-3 and granzyme B was evident in intact cells during granule-induced cell death. In cases where the cleavage of protein by caspase-3 was more than ~200-fold more efficient than cleavage by granzyme B, the fragments unique to granzyme B activity were only visualised if caspase activity was inhibited with iodoacetamide. Granzyme B was shown to cleave autoantigens at highly conserved tetrapeptide sites, with aspartic acid at P1. Subtle differences between these sites and the caspase-8 cleavage sites were defined. CTLs and NK cells can trigger cell death by either granule exocytosis (granzyme B) or by a Fas/FasL-mediated pathway, involving the initiator protease caspase-8. However, none of the autoantigenic substrates cleaved by granzyme B in this study were cleaved by caspase-8.

Comments

This study makes the exciting observation that CTL- or NK-mediated cytotoxicity could lower the threshold for the induction of systemic autoimmune disease. It is striking that the majority of well-defined autoantigens liberate unique proteolytic fragments following degradation with granzyme B. However, the definition of a non-autoantigen is problematic and is necessarily based on the absence of proof that a molecule is autoantigenic, rather than proof that the molecule is autoantigenic. Furthermore, the distinct cleavage patterns of granzyme B operate in all healthy subjects without induction of autoaggressive autoimmunity. Despite these reservations, this is a novel and important study, focusing attention on the potential for proteases as culprits and potential therapeutic targets in autoimmune diseases.

Methods

In total, 29 well-defined systemic autoantigens and 17 other proteins, believed to be non-autoantigenic, were studied. Cleavage of endogenous cell proteins *in vitro* by either granzyme B or caspases (-3 or -8) was assessed by incubating HeLa cell lysates with either caspase-3/dithiothreitol, caspase-8/dithiothreitol or granzyme B/iodoacetamide. In some experiments, purified granule contents from a cytotoxic T cell line (YT) were used instead of purified granzyme B. Pretreatment of lysates with iodoacetamide abolished endogenous caspase activity (by covalently modifying their active sites) and enabled the isolated effect of added protease to be observed. Following incubation, the samples were subjected to SDS-PAGE and individual proteins and their fragments were identified by immunoblotting with specific antisera. Additionally, an *in vitro* transcription/translation system was used to examine the cleavage of [^{35}S]methionine-labelled autoantigen substrates. After SDS-PAGE, proteins and their fragments were visualized by fluorography. Cleavage of endogenous substrates *in vivo*was also studied by incubating a Fas-negative erythroleukemia cell line (K562) with either YT granule contents or lymphokine-activated killer (LAK) cells. The incubation was terminated by adding SDS gel buffer directly to the cell suspension, and the proteolytic fragments were analysed. The granzyme B cleavage sites within proteins were identified by a combination of observing fragment sizes and scanning the protein for previously identified consensus tetrapeptide sequence with aspartic acic at P1. For 10 autoantigens, these sites were confirmed by mutagenesis of the P1 aspartic acid residues.

Additional information

An excellent review on caspases can be found by Los *et al* (The role of caspases in development, immunity and apoptotic signal transduction: lessons from knockout mice. *Immunity* 1999 **10**: 629-638).

References

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