Cancer Vaccines:
The Molecular Basis for T Cell Killing of Tumor Cells

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This issue of The Oncologist provides the reader with two important reviews on the subject of cancer vaccines. In these reviews, the authors address the rationale and biology supporting the use of immune-response enhancing cancer vaccines in human clinical trials. Although the titles of the manuscripts are similar, the subject is large and these two well-known research groups provide unique perspectives with little overlap of content. As with so many areas of human biology and disease, the field of tumor immunology has benefited tremendously by the application of molecular genetic technologies to the study of the critical mechanisms of antigen recognition, antigen processing and effector cell generation. This progress in elucidating the molecular events operative in the generation of an anti-tumor response justifies the translation of the many animal models of tumor vaccine therapy into clinical trials, especially for those tumors known to be unusually susceptible to immunotherapy.

As more and more information has been forthcoming regarding the generation of specific anti-tumor cytotoxic T lymphocytes (CTL) and how the population of these cells can be expanded, it has become increasingly important to understand the genetic mechanisms involved in the destruction of the targeted tumor cell [1, 2]. During the past two decades, very exciting progress has resulted from the observation that this cell-mediated destruction, as well as other methods of cell killing, including ionizing radiation, occur by signaling a pre-programmed path of senescence within the cell [3, 4].

The destruction of targeted cancer cells by antigen-specific, MHC-restricted CTL occurs by two independent effector pathways that induce genetically programmed cell death, termed apoptosis [5-12]. The first pathway involves the release (exocytosis) of granules by the CTL upon appropriate contact and antigenic recognition of the target cell. The second pathway is nonsecretory and requires the interaction of the CD95L molecule (Fas ligand) on the CTL with the CD95 (Fas) molecule present on the target tumor cell. As both pathways culminate in apoptotic cell death, it has been easy to speculate that the intracellular processes are common to both.

In the secretory pathway, granules disperse their contents into the intercellular space between the CTL and the cancer cell. The lymphocyte-specific granules release two principal proteins—perforin and serine protease(s) [6]. This mechanism of tumor cell destruction by CTL is often referred to as the perforin-based pathway because it is dependent on the presence of the pore-forming activity of perforin for the function of the serine protease, granzyme B [6, 8, 10, 13]. Perforin induces complement-like holes in the cancer cell membrane [14]. Perforin appears to accomplish this by polymerizing in the cell’s plasma membrane when ionized calcium is present [15-18]. It has been estimated that up to 20 perforin monomers are required to form a functional transmembrane tubular pore [19-21]. While these pores seriously damage the cell membrane, perforin by itself, even at high concentrations, is not capable of apoptosis, as it requires the presence of granzyme B. Similarly, granzyme B cannot by itself produce cell killing. It has been shown that granzyme B can enter the cytoplasm of the target cell without perforin being present, but requires perforin for activity and for translocation to the nucleus [22-25].

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Granzyme B has some unique features. For example, its proteolytic activity is specific for aspartic acid in the P1 position [26-29]. This observation is important when it is placed in the context of activation of a second family of cellular proteases, the cysteine protease interleukin-1β-converting enzyme (ICE) family [30, 31]. This family of proteases, called the caspase family, is involved in the process of apoptosis [32]. Caspase-1, interestingly, is a homologue of the CED-3 cell death gene first discovered in nematodes (Caenorhabditis elegans) [33]. Members of the caspase family identified to date (caspases 1-10) include: ICE, ICH-1, CPP32, ICE-2, IAREl-III, Mch2, Mch3, MACH, Mch6, and Mch4 [32].

Although the first “cell death” gene to be extensively characterized at the molecular level was the mammalian gene bcl-2, the discovery of a family of genes responsible for cell death in C. elegans served as a major stimulus for our current understanding of apoptosis [35-37]. Analysis of the C. elegans genes showed remarkable overall homology with the caspase family of genes in humans, providing an excellent model for study [34]. Specific mutants of the C. elegans genes demonstrated that apoptosis required activity of two genes, ced-3 and ced-4 [35]. The ced-3 gene encodes a cysteine protease with aspartic acid specificity for its activation and ced-4 appears to be essential for this activation [38-41]. Specifically, the ced-3 protein is present in its inactive form and is activated when its regulatory prodomain is cleaved at aspartic acid, permitting the active domain to assemble itself as a heteromeric protease [41, 42]. Initially, it was thought that granzyme B functioned to activate the ced-3-equivalent caspase-3, but more recent evidence suggests that granzyme B activates an upstream caspase called Apaf-1 similar to ced-4 in the C. elegans model [41-47].

While it has been frequently speculated that both the secretory (perforin-based) and nonsecretory (Fas) pathways of CTL-mediated target cell death share common downstream caspase activation events for the induction of apoptosis, this does not appear to be the case. Perhaps this should be expected, since both pathways have been preserved. In the Fas-based nonsecretory pathway, the FasL on the CTL cross-links with CD95 Fas on the target cell. Trimerization of CD95 occurs as a result of the cross-linking stimulus, causing an intracellular attraction of signaling molecules around the cytoplasmic tail of CD95 [25]. The CD95 cytoplasmic tail contains a “death domain” which interacts with a corresponding domain on a Fas-associated protein (FADD). The recruitment of FADD to the plasma membrane and its binding to the Fas tail activates FADD so that it can interact with caspase FLICE [40, 48, 49]. These interactions occur through specific motifs termed “death effector domains” found at the amino terminus of FLICE and the carboxyl terminus of FADD [50].

What is common to these two pathways is the ability to activate the caspase system which is ultimately responsible for nuclear fragmentation (implosion and endonuclease activation). Nuclear fragmentation is the oft-used hallmark of apoptotic cell death. Recent experiments, however, remind us that target-cell lysis is more complex and can certainly occur without nuclear damage. For example, Nakajima and colleagues showed that neither of the two CTL-induced apoptotic pathways require the presence of target cell nuclei for cell destruction [51]. Both secretory and nonsecretory pathways are active in nuclear-free target cell preparations. Furthermore, when caspase-specific inhibitors such as peptide-based fluoromethyl ketones and baculovirus protein p53 were used in vitro, cell death occurred by the perforin-granzyme B pathway but not by the FasL-Fas path [52, 53].

In these experiments, nuclear damage was effectively blocked for both secretory and nonsecretory pathways. This suggests that while CTL granular exocytosis-induced target cell lysis (estimated to account for approximately two-thirds of the destruction of target cells) can clearly activate the nuclear caspases, cell lysis can occur independent of the caspase system and without nuclear fragmentation. The downstream non-nuclear molecular targets for granzyme B remain to be identified. These experimental observations emphasize the need to recognize that apoptotic cell death has more than one intracellular process and, on occasion, occurs in the absence of activation of the caspase family of cell death genes.

If these are the genes and gene products functioning to effect apoptosis, are there specific molecules functioning as inhibitors of apoptosis? To answer this question, one needs only to look to certain viruses known to carry genes whose protein products are able to prevent the infected cell from dying so that the virus can replicate [54]. A family of proteins capable of inhibiting apoptosis has been identified in a number of γ-herpes viruses and have been termed γ-FLIPS for viral FLICE inhibitory proteins. These γ-FLIP proteins can be shown to interact with FADD through the

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death-effector domains effectively blocking the interaction of FLICE and FADD in the intracellular signaling pathway induced by Fas stimulation [53]. Some of these viral anti-apoptotic genes resemble the bcl-2 gene, and this gene is similar to an anti-cell death gene ced-9 of C. elegans [37]. When the ced-9 protein is activated, it prevents the activation of ced-3 [54]. The development of strategies for generating effective numbers of antigen-specific CTL in an immune response against a tumor is a major goal of investigators hoping to add an anti-tumor immune response to a patient’s therapeutic options. As the two reviews in this issue of The Oncologist indicate, many recent advances hold considerable promise for reaching this goal. The mechanisms by which antigen-specific MHC-restricted CTL effectively cause tumor cell death are of potentially similar value, as they provide additional opportunities at the genetic level and protein function level by which the efficacy of the anti-tumor immune response can be further enhanced.

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