Cytotoxic Drugs, Programmed Cell Death, and the Immune System: Defining New Roles in an Old Play

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Since the first use of aminopterin to treat childhood leukemia by Sidney Farber almost 50 years ago, chemotherapy for leukemias and solid tumors has come a long way (1). With combinations of several drugs in the 1960s and early 1970s and the development of repetitive cycle protocols, long-term remission of some cancers has been achieved in a considerable number of patients. Dose-intensification strategies and more sophisticated protocols developed primarily in pediatric oncology in the early 1980s have now established that the majority of children and adolescents with leukemias and solid tumors (neuroblastoma and sarcomas) can be cured from these otherwise fatal diseases. In addition, adjuvant chemotherapy has become an important treatment option for tumors such as those of the colon or breast. However, the widespread use of chemotherapy also has shown that certain tumors are chemosensitive, whereas others are chemoresistant. Most malignant disorders in children and adolescents are chemosensitive, whereas most of the malignant tumors in later life are relatively chemoresistant. This situation has been explained by the “immature” state of tumors early in life, with fewer genetic mutations, in contrast to the “mature” tumors found with increasing age or by the different origin of the tissue involved (e.g., sarcomas versus carcinomas).

Why is chemotherapy effective? Anticancer drugs have not been designed for a particular function or molecular target, but they have been found in assays based on inhibition of cell proliferation and clonogenicity. Early concepts on how chemotherapy may kill tumor cells have focused on interference with cellular metabolism and DNA synthesis. This view reflected the idea that tumors are caused by metabolic “alterations.” However, the biochemical characterization of drug-mediated inhibition of cell proliferation has shown that most drugs hit various targets. Drugs found to be efficacious in the treatment of cancer include diverse chemical compounds, such as antimetabolites (e.g., methotrexate and fluorouracil), DNA-damaging agents (e.g., cyclophosphamide, cisplatin, and doxorubicin), mitotic inhibitors (e.g., vincristine), nucleotide analogues (e.g., 6-mercaptopurine), or inhibitors of topoisomerases involved in DNA repair (e.g., etoposide). While cell death induced by anticancer agents has been considered to be a consequence of a block in cell proliferation or simply “toxicity,” recent studies (2,3) have shown that most anticancer agents kill cells by a common death program called apoptosis. Apoptosis is the usual form of physiologic cell death in eukaryotic cells directed by a machinery that consists of molecular pathways discovered during the past several years. One of the best defined apoptosis pathways is mediated by the CD95 (APO-1/Fas) system (4–6). The CD95 cell surface receptor originally defined by two apoptosis-inducing antibodies mediates death in many cell types following binding of the natural ligand (CD95L). CD95 and CD95L are expressed on many tissues throughout the body. Generally, either the receptor or the ligand is expressed, and co-expression of both is not found under normal conditions. Most of the key molecules involved in the execution of the apoptotic death program are involved in the CD95 pathway (4,7). Following receptor multimerization, a signal is generated that activates the caspase (ICE/Ced-3) family of cysteine proteases, effector molecules probably involved in all forms of apoptotic cell death (8). The CD95 system plays a pivotal role in growth regulation, especially in that of lymphohematopoietic cells. CD95 or CD95L is also involved in the interaction of various tissues with the immune system, leading to tissue destruction (liver damage and hematopoietic failure). While induction of apoptosis has been shown following treatment of tumor cells with cytotoxic drugs and gamma irradiation (2,3), a molecular mechanism other than “toxicity” has not been defined so far. During the past few years, the major focus has been on p53, the magic “guardian of the genome,” which is mutated in many human tumors. Experimental systems using p53 knockout mice have shown that the lack of p53 contributes to resistance of cells to DNA-damaging agents and gamma irradiation. Thus, it was proposed that p53 may be a master switch in mediating a cellular apoptotic response following anticancer treatment.

Why are tumors chemosensitive? Resistance to anticancer drugs has been considered mainly to be a consequence of increased drug efflux by overexpression of membrane pumps such as the multidrug-resistant genes (MDR1 and MDR2). The p53 data, however, without providing downstream molecular clues, indicated that cells need an intrinsic capacity to die in response to anticancer treatment. Recent data from different laboratories (9,10) now demonstrate that death induced in tumor cells by anticancer treatment is in fact an active program of the cell that involves key systems of the physiologic apoptosis program. Drugs widely used in effective chemotherapy of such cancers as leukemias strongly induce CD95L expression in CD95-positive tumor cells following drug treatment. CD95L is produced, expressed in a membrane form, or secreted by the tumor cells exposed to the drug. Binding of CD95L to the receptor then initiates the apoptosis cascade in chemosensitive cells. This scenario reflects the activation-driven death that occurs in activated T cells following T-cell receptor stimulation. Originally described for leukemia cells, this basic finding has been extended to other tumor types, such as hepatoblastoma and neuroblastoma, or other chemosensitive pediatric tumors. In addition to the expression of CD95L, which may follow the rules of cellular

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“stress” responses, cells treated with anticancer drugs also up-regulate (i.e., increase) CD95 receptor expression in some cases. In this respect, the up-regulation of CD95 expression seems to be the crossroad at which DNA damage, p53 accumulation, and the apoptosis response meet. At least for some drugs, DNA damage is considered to be an important contribution to the cytotoxic effect. The p53 protein may function by sensing damaged DNA and activating the expression of genes encoding apoptosis promoting molecules such as CD95. In addition, in some tumors, p53-independent expression of CD95L that binds to the receptor and mediates the drug-induced death is induced. The up-regulation of CD95 and the induction of CD95L expression in tumor cells by anticancer drugs give a new twist to the explanation of how chemotherapy may actually work.

However, there is another side to the coin, and that is the interaction of the tumor with the immune system, since cytotoxic T cells use the CD95 system as one of the key mechanisms to kill their target cells (11). The presence of CD95 and/or CD95L in tumor cells and attacking T cells generates a scenario of reciprocal interactions. For example, constitutive expression of CD95L in certain tissues and tumors may provide an immune privilege: Invading T cells, engaged in destruction of the tissue, may be eliminated through CD95L produced by the target cells (12–14). On the other hand, tumor cells that express the CD95 receptor may be turned into highly susceptible targets for killers. Along this line, the article by Micheau et al. (15) in this issue of the Journal adds new information. Micheau et al. report that clinically relevant concentrations of diverse anticancer drugs, such as cisplatin, doxorubicin, mitomycin C, fluorouracil, or camptothecin, induce CD95 expression in colon carcinoma cell lines and leukemia cell lines, thereby strongly increasing the sensitivity for CD95-induced apoptosis by an agonistic antibody, CD95L, or activated killer cells. This important finding may explain why low-dose chemotherapy is effective in certain tumors (16). For example, “maintenance” therapy is an indispensable element in the treatment of acute leukemias. The doses of methotrexate and 6-mercaptopurine used may be too low to mediate a direct cytotoxic effect, but they may be sufficient to sensitize the tumor cells for physiologic apoptosis signals by up-regulating expression of death regulators such as CD95. Likewise, fluorouracil is successfully used in adjuvant therapy for colon carcinomas. The new findings would suggest that chemotherapy not only has an immunosuppressive effect on the effector side but also may be immunomodulating at the side of the target cell. Reciprocal interactions between the tumor cell and the T cell may also provide other mechanisms involved in induction of tumor regression. Thus, it is conceivable that cytokines produced by activated T cells induce CD95L expression in CD95-positive tumor cells, thereby initiating suicide in the target without using its own cell-killing machinery (“death by clean hands”). This would correspond to the situation recently described for Hashimoto’s thyroiditis, where T cells, without direct contact, induce CD95L in CD95-positive thyrocytes (17). The new findings on the active role of the key players in the apoptosis pathway in the killing of tumor cells by anticancer drugs (and by γ irradiation?) entirely change our view on the sensitivity and resistance of malignant (and normal?) cells to chemotherapy and interaction with the immune system.

References