

Development of an Immunotherapy for Retinoblastoma

BY BRUCE R. KSANDER, PhD

Retinoblastoma is the most common primary eye cancer in children and an important clinical problem facing ocular oncologists. Retinoblastoma usually manifests before 3 years of age, and the tumors either grow locally within the eye, extended outside the globe migrating down the optic nerve into the central nervous system and the cerebrospinal fluid, or spread through the vasculature to form metastasis in other parts of the body, particularly the bone. Left untreated, retinoblastoma is uniformly fatal. Approximately 40% of retinoblastoma cases are hereditary, in which the child receives a single germline mutation in the retinoblastoma (Rb) gene from one parent and acquire a spontaneous Rb mutation in the other allele during retinal development. The remaining 60% of cases are sporadic, in which the child acquires spontaneous mutations in both Rb alleles during development of the retina. In hereditary retinoblastoma, a majority of the children have cancer in both eyes, and the tumors have a tendency to form earlier and grow faster. These children also have a much higher risk for developing secondary malignancies later in life, especially those of the bone (osteosarcoma). Reports indicate the rate of secondary malignancies in these patients is increased by radiation treatment and possibly by chemotherapy.

The Rb gene was the first tumor suppressor gene identified and has been studied extensively over many decades for its role in controlling cell cycle progression. Tyler Jacks, PhD, however, observed the surprising result that targeted deletion of the Rb gene in the retina of mice failed to result in tumor formation, indicating that more genes are required.¹ This issue was resolved recently in studies by Michael A. Dyer, PhD, and

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coworkers,² who produced the first gene knockout model of Rb in mice by the inducible deletion of Rb, p53, and, p107. These data suggest that in humans, a similar cascade of gene mutations is likely to be required during malignant transformation in the developing retina.

WHY IS A NEW THERAPY NEEDED?

Advances in the management of retinoblastoma have improved prognosis dramatically over the past 30 years, and the overall survival of retinoblastoma patients in the United States is currently greater than 90%. Originally, the only treatment option was enucleation of the tumor-containing eye. Today, enucleation is used only in patients with exceptionally large tumors and patients who fail to respond to treatment. Current treatments, such as radiotherapy or combining chemotherapy and radiotherapy, are successful at controlling the growth of small tumors in unilateral or bilateral retinoblastoma, respectively, and at preserving functional vision in the treated eye. The recent use of intraarterial chemotherapy has provided a new method to deliver chemotherapy directly to the tumor-containing eye. This technique has yielded some intriguing results among the early groups of

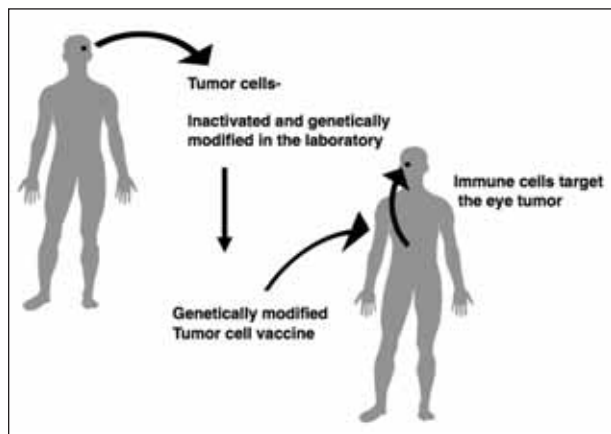


Figure 1. Tumor tissue is recovered from enucleated eyes and primary explant cultures are established in the laboratory.

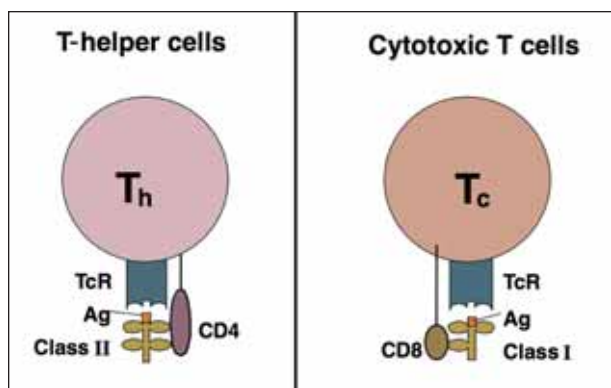


Figure 3. There are two major types of specific T cells: Cytotoxic T cells express TcR (T cell receptors) that recognize tumor antigens presented by Class I and are identified by the expression of CD8; by contrast, T-helper cells express TcR that recognize tumor antigens presented by class II and are identified by the expression of CD4.

patients receiving this treatment.³ (See “The Evolution of Treatments for Retinoblastoma” on page 56). Despite these advances, there are still numerous cases of retinoblastoma, particularly larger ones that are difficult-to-treat tumors. Moreover, there can be significant side effects related to use of radiotherapy or chemotherapy in children, some of which can be quite severe.

WHY IS AN IMMUNOTHERAPY APPROPRIATE?

Historically, it has been thought that the immune response was capable of providing the ideal protection against tumor progression because of its ability to distinguish between malignant and normal cells. Lymphocytes, specifically T cells, display a high level of specificity conveyed by receptors (TcR-T-cell receptors)

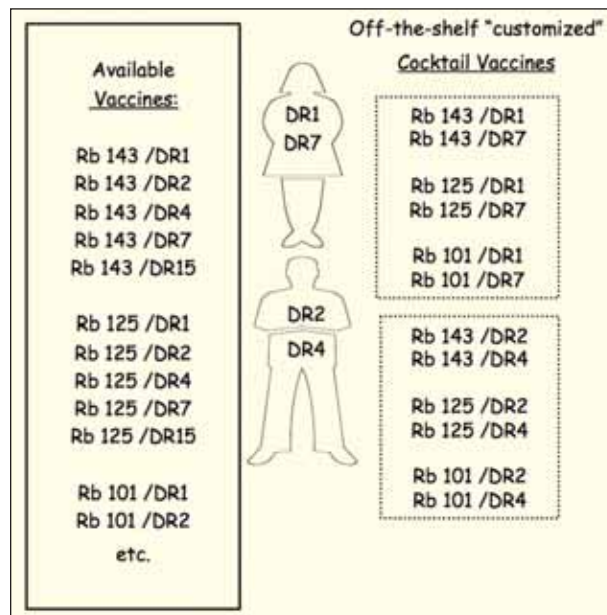


Figure 2. The available vaccines for Rb patients will contain a mixture of different Rb cell lines that together will express the widest possible array of tumor antigens.

that are capable of identifying protein fragments derived from mutated genes expressed only in tumor cells and not in normal cells. Although enthusiasm for cancer immunotherapies has waxed and waned over the years, there has been generally steady progress in this field over the past decade, particularly in the treatment of metastatic skin melanoma.

While the molecular biology of the Rb gene has been studied extensively, experiments that examine the induction and expression of tumor-specific T cells against retinoblastoma have not been conducted. This lack of information exists despite circumstantial evidence that indicates retinoblastoma may be highly immunogenic. The rate of spontaneous regression of Rb tumors has been predicted to be higher than other tumors (as high as 1.0%). Although the mechanism of Rb tumor regression is unknown, it may result from 1) ischemic necrosis, 2) formation of a benign retinoma, or 3) immune-mediated rejection. Although there is no direct evidence indicating that immune-mediated rejection of Rb occurs in patients, a local inflammatory response has been reported to accompany spontaneous regression of retinoblastoma in a few cases.

GENERAL CONCEPT OF A TUMOR CELL VACCINE

Our laboratory has investigated the creation of tumor cell vaccines. Tumor tissue is recovered from enucleated

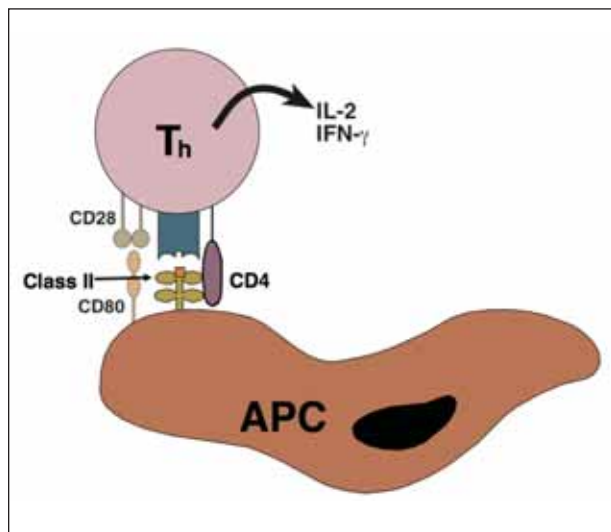


Figure 4. Successful activation of T-helper cells is normally triggered by antigen presenting cells (APC), but occurs only when APCs express two signals: 1) an antigen presented by class II, and 2) a costimulatory signal provided by CD80.

eyes, and primary explant cultures are established in the laboratory (Figure 1). Cultures that yield tumor cell lines are analyzed for Rb gene mutations and expression of Rb protein. These data are compared with sections from the original tumor specimen to validate the tumor cell line. Tumor cells are genetically modified to express two genes (CD80 and MHC class II, as described below) using lentiviral vectors. The tumor cells are tested in the laboratory to demonstrate that they effectively activate tumor-specific T cells and are inactivated to prevent proliferation. The vaccine can be used via either direct immunization of the patient or in vitro activation of the patients, in which lymphocytes are then adoptively transferred back into the patient. Activated T cells then migrate systemically and eliminate malignant cells.

Although our initial studies examined the T-cell response to specific tumor cell lines, we do not plan to make individual tumor cell vaccines for each patient. This would not be feasible for many reasons. Most important, it is not possible to obtain tumor samples unless the patient's eye is enucleated. Moreover, even if we were able to obtain tumor samples, Rb cell lines are produced from only a small percentage of samples. Therefore, an individualized tumor cell vaccine is absolutely impossible for Rb patients.

In order to solve this problem, we are producing a group of stably transfected Rb cell lines that express CD80 plus different Class II alleles. How we will use these cell lines to vaccinate Rb patients is illustrated in Figure 2. The available vaccines will contain a mixture of

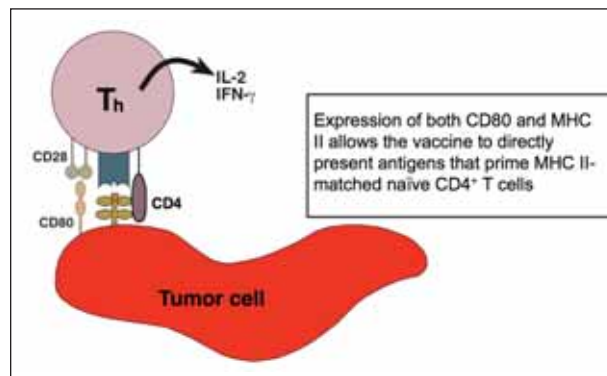


Figure 5. To activate T-helper cells against tumor antigens, our approach is to convert tumor cells into APCs by genetically altering the tumor cells to express CD80 and class II.

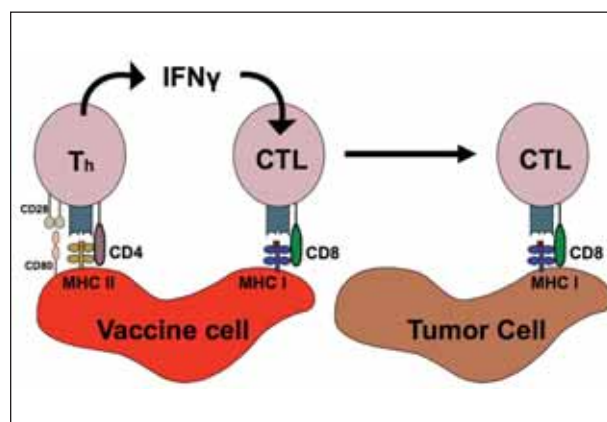


Figure 6. T-helper cells that are activated by the tumor cell vaccine provide the necessary "helper" lymphokines that are needed to activate CD8 cytotoxic T cells that are capable of eliminating the tumor cells.

different Rb cell lines that together will express the widest possible array of tumor antigens. Therefore, when a potential Rb patient is considered for vaccination, we would customize a combined vaccine from a number of tumor cell lines that each match the class II alleles of the patient. Because a majority of people in the general population expresses class II (either DR1, DR2, DR4, DR7, or DR15), it is very likely that we will have the appropriate matching class II vaccines already produced.

ACTIVATION OF A TUMOR-SPECIFIC IMMUNE RESPONSE

There are two major types of specific T cells (Figure 3): Cytotoxic T cells express TcR (T cell receptors) that recognize tumor antigens presented by class I and are identified by the expression of CD8; by contrast, T-helper cells express TcR that recognize tumor anti-

Cytotoxic T cells eliminate tumor cells by lysing the target cells, and T-helper cells release a variety of lymphokines that activate cytotoxic T cells and macrophages.

gens presented by class II and are identified by the expression of CD4. The function of these two populations of lymphocytes is distinct; cytotoxic T cells eliminate tumor cells by lysing the target cells, and T-helper cells release a variety of lymphokines that activate cytotoxic T cells and macrophages. Successful activation of T-helper cells is normally triggered by antigen-presenting cells (APC), but this occurs only when APCs express two signals: 1) an antigen presented by class II, and 2) a costimulatory signal provided by CD80 (Figure 4). The TcR on the T-helper cell recognizes the antigen, and a second receptor (CD28) recognizes CD80. When the T cell receives both of these signals, it is activated to release a variety of lymphokines including interferon gamma (IFN- γ) and interleukin-2 (IL-2). This pathway of activating T-helper cells in cancer patients is blocked by factors secreted by tumor cells that inhibit the two signals provided by APCs. To activate T-helper cells against tumor antigens, our approach is to convert tumor cells into APCs by genetically altering the tumor cells to express CD80 and Class II (Figure 5). This allows tumor cells to express the two signals required to activate tumor-specific T-helper cells.

T-helper cells that are activated by the tumor cell vaccine provide the necessary "helper" lymphokines that are needed to activate CD8 cytotoxic T cells (Figure 6) capable of eliminating the tumor cells. Once activated, cytotoxic lymphocytes no longer require two signals in order to trigger lysis of target cells. They require only one signal: the expression of the tumor-specific antigen. This allows the vaccine to activate specific T cells that circulate systemically throughout the body searching for malignant cells. One critical factor in activating protective anti-tumor immunity is the development of a sustained T cell response. Past attempts at tumor cell vaccines focused on activation of cytotoxic T cells in the absence of T-helper cells. These vaccines were capable of triggering a T-cell-mediated cytotoxic T-cell response in patients, but the duration of the response was short-lived and unable to sustain protective immunity. We predict that activation of T-helper cells is critical for a sustained immune response against the tumor.

RISKS/BENEFITS OF A TUMOR CELL VACCINE FOR RB

The most significant and common side effect of a protective anti-tumor immune response is the development of autoimmunity. For example, the most recent immunotherapy for metastatic skin melanoma uses a Gp-100 (tumor antigen) vaccine combined with an antibody (ipilimumab or tremelimumab) that promotes T-cell activation. A recently completed randomized double-blind phase 3 trial demonstrated a benefit in overall survival in the treated population.^{4,5} However, patients who responded displayed a variety of secondary inflammatory complications including colitis/diarrhea, dermatitis, hepatitis, endocrinopathy, nephritis, and uveitis. Although these complications were successfully treated in most patients, their manifestation illustrates the close connection between protective anti-tumor immunity and potentially damaging autoimmunity. The potential benefit of immunotherapy, however, is also illustrated by the limited success of the immunotherapy clinical trials. Currently, the only documented cure for metastatic melanoma is restricted to therapies that stimulate anti-tumor immunity. ■

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