Adoptive immunotherapy of cancer with pharmacologically activated lymph node lymphocytes: a pilot clinical trial

Abstract Adoptive immunotherapy (AIT) of cancer with T lymphocytes may be limited by the need to activate tumor antigen-sensitized cells in vitro. In murine models, we have shown that AIT with tumor-sensitized T cells that have been pharmacologically activated with bryostatin 1 and ionomycin plus interleukin-2 can induce tumor regression. A Phase I clinical trial was carried out to assess the feasibility and toxicity associated with using tumor- or vaccine-draining lymph node cells, activated pharmacologically and expanded in culture with low-dose interleukin-2 and infused intravenously, followed by IL-2 infusion. Nine patients were entered into the trial, and six were treated as planned. Average expansion of cell numbers over 13 to 27 days in culture was 118-fold. No patient’s cells reached the target cell number \(2.5 \times 10^{10}\). Infusion of these cells did not result in any unexpected toxicities. The toxicities observed were related to IL-2 infusion, and conformed to the expected range of side-effects. Based on these Phase I results, additional trials, with tumor antigen vaccine-sensitized DLN and technical modifications of the culture technique, are planned.

Key words Immunotherapy · Lymphocytes · Bryostatin 1 · Ionomycin · Interleukin-2

Introduction

Adoptive immunotherapy (AIT) with T lymphocytes has been demonstrated to induce regression of established tumors in several different animal models [7, 14, 24, 29, 31]. The adoptive transfer of tumor-sensitized T cells that have been activated in vitro prior to infusion may well be more effective than active immunization with defined tumor antigens or altered tumor cells [15, 22, 28]. In human patients with cancer, however, only limited success has been achieved with this approach. A major obstacle in the development of clinical AIT for cancer has been a lack of effective methods for activating and expanding tumor-reactive effector cells. Tumor-specific T lymphocytes can be isolated from tumors and tumor-draining lymph nodes (DLN) or lymph nodes draining sites of vaccination with tumor antigen [7, 14, 24, 29, 31]. In most models, these cells are not therapeutically active immediately after harvest, but must be activated in vitro and usually are expanded to increase the number of tumor-reactive cells transferred. Optimal activation and expansion of these cells in low concentration interleukin-2 (IL-2) appears to require stimulation of both the T cell receptor complex and the IL-2 receptor. Antigenic ligand binding to the T cell receptor results in activation of phospholipase C, which, in turn, cleaves phosphatidylinositol bisphosphate into diacylglycerol (DG) and inositol triphosphate (IP3). DG causes activation and translocation to the cell membrane of protein kinase C (PKC), an effect that can be pharmacologically induced by bryostatin 1 (Bryo) [3]. IP3 induces an increase in free intracellular \(Ca^{2+}\), an effect that can be pharmacologically induced by the calcium ionophore, ionomycin (Io) [6,36]. This suggested to us that tumor-reactive, tumor-specific T lymphocytes harvested from DLN might be pharmacologically activated and expanded in vitro for AIT with Bryo, Io, and IL-2.

The feasibility of this approach has been investigated in our laboratory in a series of preclinical investigations [32–35]. DLN cells harvested from the popliteal lymph
nodes of mice previously inoculated in the hind footpad with cells of a sarcoma cell line could be activated and expanded in vitro after exposure to Bryo, Io, and IL-2. Infusion of this expanded cell population into a second tumor-bearing host followed by a short course of IL-2 induced regression of established lung metastases [32–35]. This activity was dependent upon pharmacological activation (DLN cells not treated with Bryo/Io/IL-2 were ineffective), and on prior sensitization of DLN with tumor antigen (AIT with Bryo/Io/IL-2-activated cells from non-DLN was ineffective). The anti-tumor effect of these cells was tumor-specific (ineffective against a tumor cell line other than that used to sensitize DLN). AIT with Bryo/Io/IL-2-activated DLN cells also induced regression of hepatic and intradermal tumor implants, indicating homing to tumor sites via the systemic circulation, and AIT with these pharmacologically activated lymphocytes conferred long-lived tumor immunity.

Human DLN cells can also be pharmacologically activated and expanded in a manner analogous to murine DLN cells [18]. Average expansion for breast cancer patients’ axillary DLN cells was approximately 2,000-fold within 17 days after Bryo/Io activation. Expanded human DLN cells were >90% CD3+ T cells, mostly CD8+, with some CD4+ (4 to 30%). These cells were not cytolytic for autologous tumor cells; but, in 5 out of 6 patients tested, they released significant amounts of IFN-γ and TNF-α in response to autologous tumor cells in a tumor-specific manner.

Having demonstrated in several different murine models that T cells activated with Bryo + Io could mediate tumor regression, we initiated a Phase I clinical trial to test this same approach in patients with cancer. The primary objective of this study was to assess the feasibility, safety and toxicity of AIT using three dose levels of tumor-sensitized lymph node cells (TSLNC) activated with Bryo, Io and IL-2, and expanded in low dose IL-2. Initially, the study was designed to use tumor-draining lymph nodes as a source of tumor-sensitized T lymphocytes. However, during the first half-year of the study it became apparent that patients with incurable solid tumors and accessible, non-tumor-replaced, tumor-draining lymph nodes were uncommon, and, as a consequence, patient accrual was slow. Although no cancer vaccines that had been adequately tested were available for use at the time the study was initiated, subsequent to the inception of the study, a number of tumor vaccines completed Phase I testing. It was then considered appropriate to expand the eligible patient population by vaccination of patients with appropriate tumor vaccines of known safety and toxicity, followed by harvest of vaccine-draining lymph nodes.

**Study design and methods**

**Eligibility**

Patients with histologic or cytologic evidence of a solid tumor malignancy for which there was no known cure with conventional therapies and with tumor-draining lymph nodes (DLN) that were surgically accessible, or DLN that had been previously harvested, frozen, and stored appropriately for AIT, were eligible for the initiation of the trial. Later in the trial, eligibility was expanded to include melanoma patients with metastatic disease who were appropriate for vaccination with a specific tumor vaccine [Melacine (Corixa, Seattle, Wash.)] and subsequent vaccine-sensitized lymph node harvest [1, 19]. Other eligibility criteria included the following: age 18 years or older; Zubrod performance status 0–2; WBC > 3.5 × 10⁷/μL; platelets > 130 × 10⁹/μL; Hgb > 10 g/dL; prothrombin time within normal limits; creatinine < 1.5 × ULN (upper limit of normal); total bilirubin < 1.5 mg/dL; AST normal, unless an abnormality was presumed due to metastatic disease, in which case it had to be < 3 × ULN. This study was approved by the Cancer Treatment and Evaluation Program (CTEP) of the National Cancer Institute (NCI), the Massey Cancer Center Clinical Trials Review Committee and the VCU Committee on the Conduct of Human Research. All subjects gave informed consent and were informed that appropriate precautions to avoid conception should be taken during and for 3 months following treatment. Criteria for ineligibility were: active second malignancy; previous severe reactions to any blood product; chemotherapy, radiation therapy, hormonal therapy (excluding megestrol acetate for cancer cachexia), species or nonspecific cytokine or corticosteroids within four weeks; brain metastatic disease, unless controlled with surgery and/or radiation; active infection requiring treatment or unexplained febrile illness; any autoimmune process; significant infectious, congestive, or arrhythmic cardiac disease; significant respiratory impairment; HIV or hepatitis infection; malignant disease likely to require intervention with conventional therapies within 3 months.

**Lymphocyte preparation**

**Harvest/thawing of tumor-sensitized lymph nodes**

Tumor-draining lymph nodes (DLN) obtained specifically for AIT (one to three nodes per patient) were harvested surgically, usually under local anesthesia. For patients with melanoma, whose DLN were to be sensitized by vaccination, Melacine was inoculated in one thigh weekly, four times, and ipsilateral inguinal nodes were harvested one to three weeks after the last injection. Each inoculation contained Melanoma Lystate, 20 × 10⁶ tumor cell equivalents, in 0.625 ml Detox PC, which contains 300 μg Cell Wall Skeleton (30 μg of Mycobacterium phlei, 30 μg of Monophosphoryl Lipid A (MPL) from Salmonella minnesota Re595, 4.5 mg squalene, 0.6 mg TWEEN 80, 1.8 mg egg phosphatidylcholine and 60 μg θ-tocopherol per 1.4 ml [1, 19].

All lymph node samples were obtained and processed with continuous adherence to sterile technique. DLN were transported in cold (4 °C) Hanks Balanced Salt Solution (HBSS) for immediate processing. DLN suspended in HBSS were minced with scissors into fragments approximately 2 mm² in size, and the fragments were pressed through a stainless steel mesh using the blunt end of a 10 ml plastic syringe plunger. Cell suspensions were filtered and washed with HBSS × 3, and then either immediately resuspended in medium for activation or resuspended for cryopreservation in heat-inactivated 45% human AB serum, plus 45% X-VIVO 15 or 20 (Whittaker Biologicals, Walkersville, Md.) medium, plus 10% DMSO or 90% human AB serum, plus 10% DMSO. DLN cells were used fresh or after thawing frozen cells stored in liquid nitrogen.

**Activation, growth and testing of lymphocytes for adoptive transfer**

DLN cells were resuspended at 1–5 × 10⁶/ml in warm (37 °C) X-VIVO containing 10% autologous or heat-inactivated human AB serum with 50 nM bryostatin 1, 1 μM Io, and 80 IU/ml of IL-2 (Chiron Therapeutics, Emeryville, Calif.). The cell suspension was incubated for 18 h at 37 °C in humidified air with 5% CO₂. Activated tumor-sensitized lymph node cells were then washed in warm HBSS × 2 and resuspended in X-VIVO 15 or 20 with 10% heat-