Synergy between interleukin-2 and prothymosin α for the increased generation of cytotoxic T lymphocytes against autologous human carcinomas

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Abstract Peripheral blood mononuclear cells (PBMC) from cancer patients were cultured in vitro with irradiated autologous tumor cells isolated from malignant effusions (mixed lymphocyte tumor cultures, MLTC) and low-dose (50 IU/ml) recombinant interleukin-2 (IL-2). The combination of IL-2 and prothymosin α (ProTα) resulted in a greater PBMC-induced response to the autologous tumor than that brought about by IL-2 alone. In particular, ProTα specifically enhanced the CD4+ T-cell-mediated proliferation against the autologous tumor. CD4+ T cells seemed to recognize tumor antigens presented by HLA-DR molecules expressed on the autologous monocytes, since preincubation of the latter with an anti-HLA-DR monoclonal antibody (mAb) abrogated the response. In addition, MLTC set up with IL-2 and ProTα also generated more MHC-class-I-restricted cytotoxic T lymphocytes (CTL) against the autologous tumor than did MLTC set up with IL-2 alone. The MLTC-induced CTL contained high levels of cytoplasmic perforin and their development was strictly dependent on the presence of both autologous CD4+ T cells and monocytes. In the absence of either population there was a strong impairment of both proliferative and cytotoxic responses which was not restored by the presence of ProTα. In contrast, when both cell populations were present, ProTα exerted optimal enhancement of CD4+ T cell proliferation, which was associated with potentiated CTL responses. Our data emphasize the role of ProTα for the enhancement of IL-2-induced CTL responses against autologous tumor cells. Such responses require collaborative interactions between CD4+, CD8+ T cells and monocytes as antigen-presenting cells. Our data are relevant for adoptive immunotherapeutic settings utilizing IL-2 and ProTα-induced autologous-tumor-specific CTL.

Key words Prothymosin-α · IL-2 · Autologous tumor · Cytotoxicity · CTL

Introduction

Prothymosin α (ProTα) is a highly acidic polypeptide (pI = 3.55) of 12 600 Da, first isolated from rat thymus [16] and subsequently from man. The complete primary structure of human ProTα, which consists 109 amino acid residues, was fully elucidated from simian-virus-40-transformed human fibroblasts and from clones isolated from a human spleen cDNA library [13, 14].

Although the biological role of ProTα is far from being known, its wide tissue and phylogenetic distribution suggest that it must be an important one. ProTα has been reported to enhance cell-mediated immunity and to participate in cell growth phenomena [29]. Recent reports on the detection of specific ProTα receptors on human peripheral blood mononuclear cells (PBMC) [16] may facilitate the elucidation of its immunomodulating extracellular role. Until now, ProTα has been known to participate in the in vitro enhancement of T lymphocyte proliferative responses to soluble and cellular antigens [2], regulation of interleukin-2 (IL-2) and prostaglandin E2 production in mononuclear cell cultures [4],
enhancement of MHC class II antigen expression in various cell types at both the protein and molecular level [3] and in the enhancement of natural killer (NK) cell cytotoxicity [11, 12, 15] ProTz has also been reported to be active in vivo in protecting mice against opportunistic infections, e.g. with Candida albicans [1] as well as the growth of syngeneic leukemic cells [21, 27].

Selective outgrowth and large-scale expansion of autologous tumor-reactive lymphocytes is a necessary goal for successful clinical trials of adoptive immunotherapy of cancer. In this respect several laboratories, including ours, have identified cytokine combinations favoring the outgrowth of CD8+ T lymphocytes capable of specifically lysing autologous tumors [1]. In all these studies the mechanism underlying the development of autologous-tumor-specific cytotoxicity was not explored until recently, when the specific requirement for T helper cells for optimal induction of cytotoxic T lymphocytes (CTL) against syngeneic tumors was demonstrated in animal models [22, 23, 10, 25]. In our most recent studies we used ascites as a source of T (CD4+ and CD8+) cells and dendritic cells for the generation of autologous human carcinoma-specific CTL and we demonstrated an absolute requirement for both CD4+ T cells and dendritic cells for this process [9]. Thus, it become apparent that induction of T cell help in vivo or cellular adoptive immunotherapy utilizing both tumor-specific helper and cytotoxic T cells is essential for improving clinical results in cancer immunotherapy.

Since ascitic fluid accumulates only in a minority of cancer patients, it was of interest to see whether such results can also be obtained with PBMC-derived mononuclear cells. Given the fact that ProTz increases both proliferative and cytotoxic responses of T cells [2, 4, 11, 15] we wished to investigate whether its presence in cultures would also enhance the killing of autologous tumor.

In the present study, we report that patients' PBMC cultured with autologous tumor cells in the presence of IL-2 and ProTz generated CD8+ CTL that contained higher cytoplasmic perforin levels and had a greater capacity for MHC-class-I-restricted killing of autologous tumor cells than did CTL from the same cultures set up with IL-2 alone. The killing of tumor targets by the autologous CD8+ CTL was dependent on the presence of autologous monocytes and CD4+ cells. The latter did not directly recognize the autologous tumor but there was an HLA-DR-restricted cross-priming through the monocytes. When cultured with IL-2 and ProTz, CD4+ T cells also proliferated more in response to the autologous tumor cells.

Our data support the notion that, for the development of responses against autologous tumor cells, synergistic interactions between CD4+, CD8+ cells and monocytes are required. They also demonstrate that ProTz in combination with IL-2 can render such cell-to-cell interactions more effective, resulting in increased killing of autologous tumors. Therefore, ProTz in combination with IL-2 may be useful in protocols promoting the ex vivo activation of patients' lymphocytes for further use in cellular adoptive immunotherapeutic trials.

Materials and methods

Patients

A group of 46 patients (19 male and 27 female; age 59–72 years, average: 62 years) with advanced (stage IV) breast (n = 17), ovarian (n = 10) and lung (n = 19) cancer were included in this study. All patients had been free from any kind of anticancer therapy for at least 30 days prior to sample collection. Written informed consent was required from all patients and the study was approved by the Review Board of Saint Savas Cancer Hospital.

Reagents

ProTz from bovine thymus was purified as described [16]. In brief, bovine thymus from a 15-month-old calf was cut into small pieces immediately after excision and dropped into liquid nitrogen. The frozen tissue was pulverised in a ceramic mortar chilled in solid CO2. Powder corresponding to 20 g tissue was dispersed into 400 ml boiling water and boiled for 5 min in order to prevent degradation of ProTz by endogenous proteases. The suspension was homogenized in a Sorval Omnimix blender and spun down, and the supernatant was acidified by mixing with 1/10 volume of ten-times-concentrated buffer A (1 M HCOOH/0.2 M pyridine, pH 2.9) and kept at −10 °C until further use. After thawing, the sample was spun down and the supernatant lyophilized. The syroury residue was redissolved in buffer A and applied to a 7.2 x 93-cm column of Sephacryl S-200 (Pharmacia) equilibrated with buffer A. The peptides recovered from four such gel-filtration experiments were pooled and further separated by reverse-phase high-performance liquid chromatography (RP-HPLC) on a C18 column (Lichrosorb, 5 μm; HPLC Technologies, UK). Elution was performed with a linear gradient of 10%–50% acetonitrile in 0.1% trifluoroacetic acid over 60 min. The isolation procedure yielded approximately 60 μg pure peptide (as controlled by amino acid analysis)/g fresh tissue. The endotoxin level was 0.01 ng/100 μg ProTz as measured in a standard Limulus assay.

Fluorescein-conjugated CD8, CD4 and CD14 mAb and nonlabelled HLA-A,B,C and HLA-DR mAb as well as anti-(mouse IgG) were obtained from Pharmingen (San Diego, Calif.).

Isolation of autologous tumor cells

This was performed as described [9]. Briefly, specimens of peritoneal effusions from patients with metastatic breast (n = 17) and ovarian (n = 10) cancer and pleural effusions from patients with lung adenocarcinoma (n = 19) were subjected to centrifugation at 400 g for 5 min to sediment cells that were further placed on top of 75% Ficoll/Hypaque gradients, overlayed on 100% Ficoll-Hypaque and spun at 700 g for 25 min. Tumor cells were collected from the top of the 75% Ficoll-Hypaque and cryopreserved in liquid N2 until ready for use in the cytotoxicity assays, at which time cells were carefully thawed, slowly diluted in RPMI-1640 medium (Life Technologies, New Island, N.Y.) and washed. Tumor cells were assayed only if their viability was over 80%. Phenotype analysis showed that all tumors expressed MHC class I molecules but were negative for MHC class II gene products (not shown).

Isolation of cell populations

Highly purified CD3+ T cells (more than 95% purity in all cases tested) were obtained by using an immunomagnetic isolation procedure as described previously [19]. Briefly, PBMC were incubated for 1 h at 4 °C with M-450 immunomagnetic particles...