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Cancer immunogene therapy. Anti - gene anti IGF-I approach. Case of glioblastoma.
Ed. Lambert Academic Publisher, Germany, 2017, pp 92 - 96;
ISBN-13: 978-3-330-06345-7; ISBN-10: 3330063459

Chapter 4

Immune response in anti IGF-I clinical therapy

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Introduction

The strategy based on antisense, antigene or triple helix targeting IGF-I had been reported in animal models with glioblastoma, teratocarcinoma, hepatoma as well as melanoma (1-6). It was shown that significant response controlling tumor proliferation was observed in animals vaccinated with antisense anti-IGF-I modified

tumor cells. In the present work, we reported results relevant to application of antisense anti-IGF-I strategy in phase I of clinical trial to patients with glioblastoma. The strategy was also extended to patients with colon, ovary as well as prostate cancer.

Results and Discussion

The IGF-I antisense strategy suppressing IGF-I expression was applied as previously described (1,2,4), essentially with the use of episomal based plasmid expressing IGF-I RNA antisense. The cassette contains the Epstein-Barr virus origin of replication and the gene encoding nuclear antigen I which, together, drive extrachromosomal replication.The established cancer cells were originated from biopsies of patients with malignant glioma (glioblastoma multiforme), colon carcinoma, ovary carcinoma (cystadeno-carcinoma), prostate adenocarcinoma (cytologic malignancy, grade III) (University Hospital of Bromberg, Poland; Case Western Reserve University, Ohio, USA; Paul Brousse University Hospital, Villejuif, France). Cell lines were established during 3-4 weeks in culture with conventional protocol in DMEM (Gibco-BRL, Invitrogen, UK) supplemented with 10% FCS, 2mM L-glutamine, 100 U/ml penicillin and 100 micrograms/ml streptomycin at 37°C and 5% CO2. Established primary cells were transfected by Ca++/Ph or FuGENE 6 transfection reagent technique (Boehringer Mannheim, Ingelheim, Germany). 48 hours after transfection, hygromycin B was added up to 150ug/ml. Two weeks after transfection, hygromycin resistant cells were verified for expression of IGF-I by immunohistochemical (1,4) but also by RT-PCR (reverse transcriptase polymerase chain reaction) using RNA isolation kit from Roche Diagnostics (Basel, Switzerland) and application according to Reverse Transcription System from Promega Corporation (Charbonnières-les-Bains, France) with the following primers: forward, GCATCTCTTCTACCTGGCGCTG; reverse, CAGGCTTGAGGGGTGCGCAATA. Thereafter, established transfected cell lines were examined in flow cytometry with fluorescence-activated cell sorter (FACS) equipment FACSCAN and/or FACScalibur (Becton Dickinson, Le Pont-De-Claix, France), using specific monoclonal antibodies (mAbs) to major histocompatibility complex (MHC) I and II, CD80, CD86, namely W632, BU26, MEM-233 and BU63, respectively (AbD Serotec, Düsseldorf, Germany). The secondary antibody used

was Goat F(ab')2 anti-mouse Ig conjugated to FITC (fluorescein isothiocyanate) (Tebu, Le Perray-en-Yvelines, France). Thus, IGF-I antisense transfected cells that were positive in expressing MHC-I molecules were essentially selected for preparation of vaccines which were either membrane preparations (7) or whole cell ones. The latter were submitted to 5000 CGy gamma irradiation with 60Co or 137Cs.

The first clinical trial concerned 4 patients with glioblastoma, two patients with colon carcinoma, two patients with ovary cystadeno-carcinoma and two patients with prostate adeno-carcinoma. Patients received 3 successive subcutaneous injections. Only the group of patients with glioblastoma was divided in two groups, the first one was treated with cellular membrane preparations, the second group received after the first injection of membrane preparations two successive injections of irradiated antisense whole cell preparations. The second protocol was applied to patients with colon, ovary and prostate cancer. All vaccine preparations were injected subcutaneously, generally into the left arm of cancer patients. Blood samples were collected before and after vaccinations which corresponded to 5-6 weeks after the vaccine injections. PBMCs were isolated from blood red cells using ficoll-hypaque centrifugation. Flow cytometry analyses were performed as above but using mAbs conjugated to FITC and directed to cell surface markers, namely CD3, CD4, CD8, CD19, CD56. Particularly, dual staining were performed using mAbs specific to CD8-FITC and CD11b-PE (phycoerythrin), CD8-FITC and CD28-PE.

It was observed in the group of patients with glioblastoma that the two patients vaccinated with membrane preparations have only survived 9,5 and 10 months respectively while the two other patients receiving injections of whole cell preparations in addition to the first membrane preparation injections had prolonged survival up to 19 and 24 months respectively. The case relevant to the vaccinated patients with colon, ovary and prostate cancer was monitoring onward. All treated patients presented a slight increase of temperature 24-36 hours after vaccination that lasted a couple of days. A clear cut change was observed concerning PBMCs' cell surface expression of vaccinated patients essentially relevant to the CD8+ CD28+ cell subpopulation which showed a significant increase in its percentage after vaccination (Table 1). No difference was noticed about cell populations with surface markers CD3, CD4, CD19, CD56, or CD8+ CD11b+ in PBMCs harvested before and after vaccination. Several encouraging aspects were observed. The vaccinated patients with whole cell preparations had a prolonged survival time (19 and 24

months, respectively) compared to the range of 12-15 months in conventional therapy. The interesting observation concerned the PBMC subpopulation with CD8+ CD28+ phenotype in vaccinated patients. The increased percentage of these cells might indicate a stimulation of immune effectors in patients vaccinated with antisense anti-IGF-I modified tumor cells, and might thus explain their improved survival time.

Table 1 **% of PBMC from patients**

	Before vaccination	After vaccination
CD8+ CD11b+	25 ± 3 (a)	21 ± 2 (a)*
	26 ± 5 (b)	22 ± 3 (b)
	24 ± 2 (c)	20 ± 5 (c)
	25 ± 5 (d)	22 ±4 (d)
CD8+ CD28+	14 ± 1 (a)	25 ± 4 (a)
	10 ± 3 (b)	26 ± 2 (a)
	12 ± 3 (c)	24 ± 5 (c)
	12 ± 4 (d)	25 ± 2 (d)

* (a): patients with glioblastoma; (b): patients with colon carcinoma; (c): patients with ovary cystadno-carcinoma; (d): patients with prostate adeno-carcinoma. Difference in percentage of CD8+ CD28+ subpopulation before and after vaccination was significant with a range of p from 0.001 to 0.02 according to the Student's t test, and not significant concerning the subpopulation CD8+ CD11+ from the relevant patients.

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Acknowledgement

The text of this chapter is based on published articles: Biomed & Pharmacother 64(8): 576-8; 2010, and Rev Cien, 2 (25); 2016, doi: 10.14483/udistrital.jour.RC.2016.25.a6