Journal of Neuroimmunology 218 (2010) 140–144

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Short communication

Intratumoral IL-7 delivery by mesenchymal stromal cells potentiates IFNγ-transduced tumor cell immunotherapy of experimental glioma

Salina Gunnarsson [a,b,*], Daniel Bexell [a,b], Andreas Svensson [a,b], Peter Siesjö [a], Anna Darabi [a], Johan Bengzon [a,b]

[a] The Rausing Laboratory, BMC D14, Division of Neurosurgery, Department of Clinical Sciences, Lund University, SE-221 84 Lund, Sweden
[b] Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, BMC B10, Lund University, SE-221 84 Lund, Sweden

ARTICLE INFO

Article history:
Received 3 September 2009
Received in revised form 23 October 2009
Accepted 23 October 2009

Keywords:
Glioma
Rats
Mesenchymal stem cells
Interleukin-7
Interferon gamma
Immunotherapy

ABSTRACT

The present study reports regression of pre-established experimental rat gliomas as a result of combining peripheral immunization using interferon gamma (IFNγ) transduced autologous tumor cells with local intratumoral delivery of interleukin 7 (IL-7) by mesenchymal stromal cells. IL-7 alone significantly decreased the tumor area and this effect was enhanced with IFNγ immunization. A higher density of intratumoral T-cells was observed in animals receiving combined therapies compared to rats receiving either cytokine alone suggesting that the therapeutic effect is dependent on a T-cell response.

1. Introduction

Experimental immunotherapy protocols directed against malignant glioma have been translated into several clinical studies (Yamanaka et al., 2003; Yu et al., 2004; Schneider et al., 2001; Steiner et al., 2004). We have earlier shown increased survival and increased numbers of intratumoral T-cells of rats peripherally immunized with IFNγ-producing tumor cells, as compared to non-immunized rats (Visse et al., 1999, 2000). The effectiveness of the IFNγ-based therapy motivated a clinical phase II glioblastoma (GBM) immunotherapy trial in which a significant clinical effect in terms of overall patient survival was seen, however no complete cures were achieved (Salford et al., in preparation), highlighting the need for improved GBM immunotherapy strategies.

Interleukin-7 (IL-7) is a multipotent growth factor that provides survival signals for T-cells, sustains lymphocyte development and provides proliferative signals that maintain peripheral naïve and memory T-cells (Kittipatarin and Khaled, 2007). The markedly lower toxicity of IL-7 compared to other cytokines (Rosenberg et al., 2006) further justifies the exploration of IL-7 as a clinically useful cytokine. In the current study we explored the antitumor immune response and therapeutic effect of intratumoral IL-7 delivery in combination with peripheral immunization with IFNγ-transduced tumor cells.

Bone marrow derived multipotent mesenchymal stromal cells (MSCs) were chosen as a vector system for intratumoral IL-7 delivery. MSCs are easily expandable, can express transgenes efficiently and for long periods of time (Caplan and Bruder, 2001) and have previously been utilized as cytokine-producing vehicles in the treatment of experimental gliomas (Nakamizo et al., 2005; Nakamura et al., 2004). We have shown that intratumorally grafted rat MSCs migrate rapidly and effectively within rat gliomas and possess the capacity to infiltrate tumor extensions without any migration into the normal brain parenchyma (Bexell et al., 2009a), implying that MSCs can be used as vehicles to deliver antitumor substances.

This is the first study to use an MSC-based vector system for intratumoral cytokine delivery in combination with peripheral immunization in order to treat experimental tumors. The results of this multi-modal treatment protocol point to synergistic antitumor effects mediated by intratumoral T-cell infiltration.

Abbreviations: AEC, 3-amino-9-ethylcarbazole; α-MEM, alpha minimum essential medium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; GBM, glioblastoma multiforme; GFP, green fluorescent protein; HPRT, hypoxanthine phosphoribosyltransferase; HTX, Mayer's Hematoxylin; IFNγ, interferon gamma; IL-7, interleukin 7; i.p., intraperitoneal; i.t., intratumoral; MNP, mononuclear phagocyte; MSC, multipotent mesenchymal stromal cell; RT-PCR, reverse transcriptase polymerase chain reaction; PBS, phosphate buffered saline; TCR, T-cell receptor.

* Corresponding author. Postal address: BMC D14, The Rausing Laboratory, Division of Neurosurgery, Department of Clinical Sciences, Lund University, SE-221 84 Lund, Sweden. Tel.: +46 46 222 14 40; fax: +46 46 222 46 06.
E-mail address: salina.gunnarsson@med.lu.se (S. Gunnarsson).
URL: http://www.stemcellcenter.se/groups/bengzon (S. Gunnarsson).

0165-5728/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2009.10.017

2. Materials and methods

2.1. Cell-lines

The rat glioma cell-line N32 was established by transplacental ethyl-N-nitrosourea (Siesjo et al., 1993). Cells were cultured as described elsewhere (Bexell et al., 2009a).

MSCs purified from adult Fischer 344 rat bone marrow and transduced to express GFP (described in Bexell et al., 2009a) were used for transduction with a retroviral vector (pLXSN) containing the IL-7 gene. Cells transduced with the empty vector were used as control. The packaging cell-line GP + E was used for transduction. Cells were centrifuged; supernatant was filtered and added to the MSC-GFP. Polybrene (Sigma-Aldrich, Milwaukee, WI, USA) (1 µl/ml) was added to increase transduction efficiency. Selection with geneticin G418 (InvivoGen, San Diego, CA, USA; 800 µg/ml) was initiated on day 5. Cells were kept in MSC medium (α-MEM, FBS and Antibiotic Antimycotic Solution (Invitrogen)) and incubated at 37 °C and 6.0% CO_2. IFNγ-transduction of N32 cells has previously been described (Visse et al., 1999).

To determine IL-7 RNA levels, RT-PCR was performed (primers from TAG A/S Symbion, Copenhagen, Denmark). A 1 kb DNA ladder (Fermentas, Ontario, Canada) was used as a molecular weight marker and HPRT housekeeping gene cDNA was blotted in parallel to amplified RT-PCR products. TRIZOL Reagent was used to extract RNA according to product protocol (Invitrogen, Carlsbad, CA, USA).

IL-7 protein production was assessed in supernatants of cultured cells using ELISA (R&D Systems, Minneapolis, MN, USA) from two time-points, one week apart. 1×10^5 cells were transferred into 1 ml MSC medium in a 24-well plate and incubated for 48 h before the supernatant was collected.

2.2. Surgical procedure and study setup

On day 1, 3000 N32 tumor cells were inoculated in adult male Fisher 344 rats as previously described (Bexell et al., 2009a). Animal procedures were approved by the Ethical Committee for Use of Laboratory Animals at Lund University, Sweden. The study included the following groups; 1: MSC-GFP i.t. + sham-immunization i.p ($n = 8$), 2: MSC-GFP-IL7 i.t. + sham-immunization i.p. ($n = 8$), 3: MSC-GFP i.t. + N32-IFNγ-immunization i.p ($n = 11$), 4: MSC-GFP-IL7 i.t. + N32-IFNγ-immunization i.p ($n = 11$) (A). On days 5 and 15, 3×10^6 irradiated IFNγ-producing tumor cells or medium were injected i.p. to glioma-bearing animals. On days 8 and 12, all animals received intratumoral grafting of 2.5×10^5 MSCs with or without IL-7. Time-points of MSC grafting were chosen in order to optimize tumor IL-7 levels at the time of maximum immunization-induced intratumoral T-cell inflow. T-cell infiltration starts on day 10 following tumor inoculation (Visse et al., 2000) and MSCs require 4–8 days to efficiently infiltrate the tumor following transplantation (Bexell et al., in preparation). Coordinates for MSC grafting into striatum were identical to those used for tumor cell inoculation. All of the therapies were well tolerated, without any signs of toxicity evident during the study.

2.3. Immunohistochemistry

On day 25 following tumor inoculation, all animals were sacrificed; the brains were dissected, freeze-fixed and cut in 6 µm sections. Thawed sections were fixed in acetone for 10 min. Endogenous peroxidase was blocked using Peroxidase blocking solution (Dako, Glostrup, Denmark) before 5% donkey serum (Jackson ImmunoResearch, West Grove, PA, USA) was added (20 min), followed by incubation with the primary antibody anti-rat-TCRαβ (BD Biosciences, San Jose, CA, USA) for 60 min. Incubation with the secondary antibody, donkey anti-mouse-biotin, was followed by streptavidine-peroxidase (30 min each) (Jackson ImmunoResearch). As a negative control, the primary antibody was omitted. Sections were stained with AEC for 5 min before being counterstained with Mayer's Hematoxylin (HTX) for 30 s. The glass-slides were rinsed and then mounted using Faramount mounting medium (Dako). Sections were rinsed in PBS between every step.

For fluorescence staining sections were fixed as described above, blocked with 5% goat serum, incubated with primary antibody chicken-anti-GFP (Chemicon, Temecula, CA, USA) for 60 min. The secondary antibody, Alexa488 goat-anti-chicken (Molecular Probes, Eugene, OR, USA) was added after rinsing and 30 min later sections were washed and mounted with Pro-Long Gold anti-fading reagent (Molecular Probes) containing nuclear staining (DAPI).

2.4. Digital analysis of tissue sections

Tumor sections were analyzed using a light microscope equipped with filters for fluorescence (BX-60, Olympus America, Melville, NY, USA). Images were taken at 10× (immunohistochemistry) or 4× (area determination) magnification using an Olympus ColorView digital camera and captured using the analySIS® Image-analysis software (Olympus). The ratio of stained area compared to area of interest was calculated and expressed as percent stained cells/tumor area. Area was measured on three sections, at predetermined rostro-caudal levels through the tumor mass.

2.5. Statistics

Differences in tumor size as well as TCR immunoreactivity between groups were analyzed using the Mann–Whitney *U* test, using GraphPad Prism Software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. IL-7 expression in MSCs

MSCs express IL-7 endogenously (Namen et al., 1988). Retroviral-mediated transfer of the gene encoding IL-7 into MSC-GFP markedly and stably enhanced mRNA and protein levels of this cytokine. Analysis of MSC-GFP-IL7 using RT-PCR showed a pronounced increase in IL-7 mRNA expression in transduced compared to non-transduced cells (Fig. 1). To determine the stability of IL-7 production ELISA was performed on cell supernatants at two time-points. Levels were 57.2 pg/ml and 60.2 pg/ml respectively for the

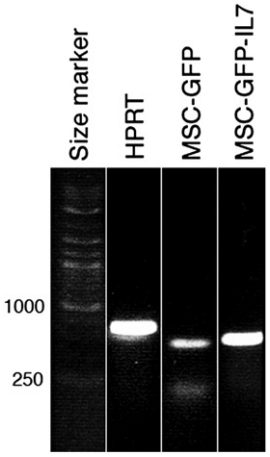

Fig. 1. IL-7 expression in MSCs. IL-7 mRNA was detectable by RT-PCR in non-transduced MSC-GFP (third lane); however, there was a marked increase in expression in the transduced cells (fourth lane). A 1 kb DNA ladder was used as molecular weight marker (first lane) and the HPRT housekeeping gene cDNA (second lane) was blotted in parallel to amplified RT-PCR products.