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Dendritic cell therapy with improved outcome in glioma multiforme—a case report*

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Abstract: Malignant gliomas are the most devastating tumors in clinical practice and have poorest survival. Immunological treatment of such patients may likely increase the survival and quality of life. Dendritic cells (DCs), most potent antigen presenting cells in combination with oral chemotherapeutic agents may be tried for patients giving consent to such treatment. We have successfully combined the two therapies in an adult male patient who was on downhill course after being operated on once with post operation chemotherapy and radiotherapy for glioma in the left parietal area. He received five dendritic cell therapy vaccines in combination with oral chemotherapy and responded dramatically having near normal quality of life for an additional five months with this regime, increasing the survival after operation to 11 months. This therapy is continuing with radiological betterment of the lesion. The DCs are matured with antigen extracted from wax embedded tissue at 6th day of culture. We feel that the treatment can be given to more number of patients to establish its efficacy for the dreaded cancer glioblastoma multiforme.

Key words: Dendritic cells, Glioma, Immunetherapy
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INTRODUCTION

With the enormous progress in tumor immunology, immunotherapy has reached an exciting phase in its evolution for the treatment of cancer (Hart, 2005; Onji and Akbar, 2005). Although an effective immunotherapeutic regimen has yet not been demonstrated in the clinical setting and the ultimate role of immunotherapy in the treatment of glioma is still unknown, the potential for immunotherapy as an adjunct to the current treatment of gliomas is now based on solid evidence (Yu *et al*., 2004).

Malignant gliomas, a class of CNS tumors derived from the glial lineage, are one of the most devastating tumors in clinical practice. Despite recent advances in traditional treatment options such as surgery and radiation the 5-year survival rate for glioblastoma multiforme, the most common class of gliomas, is less than 2% (Liu *et al.*, 2003).

In an effort to improve the outcome of patients with resectable brain tumors, there have been attempts to give adjunctive therapies consisting of radiation with or without chemotherapy. Thus far, research of more than three decades has failed to provide definitive evidence of improved outcome (i.e. overall survival and disease-free survival) in these patients. In general, chemotherapy is only marginally effective in the treatment of these tumors due to the difference of delivering drugs across the blood brain barrier and the development of drug resistance by the tumor. Due to the infiltrating nature of tumor into the surrounding white matter complete surgical resection is also virtually impossible (Davis *et al*., 1999).

Immune-based treatments however represent a promising new class of therapy designed to boost immune system to specifically eradicate malignant cells (Steinman and Dhodhapkar, 2001). Immuno-

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therapy with dendritic cells seems to be capable of generating a glioma-specific anti-tumor immune response (Liau *et al*., 2000). It is biologically safe, with no serious side effects noted in animal or clinical trials (Wheeler *et al*., 2004).

A combination of surgery, chemotherapy and dendritic cell therapy on a 57-year old male patient has shown successful results both clinically and radiologically. This patient, diagnosed with glioblastoma multiforme in his left brain (parietal lobe) came to us for dendritic cell therapy in July 2005. He presented change of personality, dementia, depression, off and on headache, slurred speech and swelling of right lower limb. The CT and MRI of brain revealed mass effect and contrast enhancement as well as surrounding edema. His biochemical and hematological parameters were within normal range. The patient was diagnosed with malignancy in March 2005, and was subsequently operated on with partial resection of tumor followed by chemotherapy which he was undergoing at the time of presentation. His history revealed that his general condition improved post surgery for about a month and by April 2005, he started showing signs of deterioration. He remained asleep for average of 18~20 h per day. He was slow to respond to verbal communications and during conversation also passes intermittently into sleep. His right lower limb swelling was indicative of release of pro-clotting factors commonly seen in active lesions of glioma. He had a history of occasional emesis, marked lethargy, occasional irritability, ataxia and nystagmus. Epileptic seizures were also noted but controlled by the antiepileptic treatment he was undergoing.

Dendritic cell therapy (DCT) was planned accordingly for him, duly approved by our Ethics Committee formed as per Indian Council of Medical Research (ICMR) guidelines. The treatment protocol was explained to his family members and two vaccines were given in the same month with an interval of 15 d. During vaccination he was monitored for tachycardia/bradycardia, breathlessness, change in blood pressure, signs of distress, backache and any other unexplained clinical feature. His condition remained stable after receiving these two vaccines with no further deterioration. He was called for 3rd dose of DCT in the second month of the protocol. After receiving the third vaccine his general condition improved with the following features. His swelling in lower limbs was reduced to negligible level and he was thoughtful, more alert during conversation and had no sleep hangovers. His weight remained constant during period.

DENDRITIC CELL CULTURE

Briefly, peripheral blood mononuclear cells (PBMCs), were extracted through Percoll (Sigma) density gradient centrifugation from 20 ml of freshly drawn blood in heparinized vials. Buffy coat was extracted and run over cell-surface treated (Nunc, Denmark) plates with added RPMI-1640 with complete medium, maintained at 37 °C. The complete medium consisted of RPMI-1640 supplemented with 2 mmol/L L-glutamine (HiMedia), 20 μg/ml gentamicin, 30 μg/ml amikacin and 10 μg/ml chloramphenicol. In addition to it, 10% autologous serum was used. These plates were incubated in humidified chamber, at incubation temperature for mononuclear cell adhesion. The plates were gently washed thrice with phosphate buffer saline (PBS), after incubation of one hour. Non-adherent cells were removed and adherent cells were cultured in 2 ml medium per well containing additionally 800 units per ml GM-CSF (R & D Systems, USA) and 500 units per ml IL-4 (R & D Systems, USA). Every other day 1 ml of complete medium and cytokines were added to replenish the cells.

PREPARATION OF CELL LYSATES FROM CANCEROUS TISSUE

Patient's Paraffin embedded tissue was separated through xylene extraction. Five micrometers thick section was cut from paraffin wax block to give 1 mg of tissue and de-waxed by xylene extraction. The sample was incubated for 10 min in xylene at room temperature (1200 μl, 2×) and 100% ethanol (1200 μl, 3×) while gently agitating the tube. After subsequent centrifugation at 13000×g for 10 min the supernatant was removed and the samples were then air-dried. For digestion of the tissues, 200 μl of lysis buffer was added. The samples were then incubated for 1 h at 56 °C and mixed continuously. A clear

lysate was obtained and the reaction was stopped by boiling the sample for 10 min. No further DNA extraction was performed (Lo *et al*., 1989). The extract was evaluated by polyacrylamide gel electrophoresis to confirm its proteinaceous nature (Chan *et al*., 2001). The protein band was eluted and mixed with cancerous cells extracted from carcinoma secondary liver from a different source. These cancerous cells were UV irradiated and confirmed non-viable through failure to grow as cell line. The cells were mixed in isotonic saline and agitated by magnetic stirrer for 30 min. This whole cell lysate antigenic mixture (WCLAM) was stored at −20 °C for further usage.

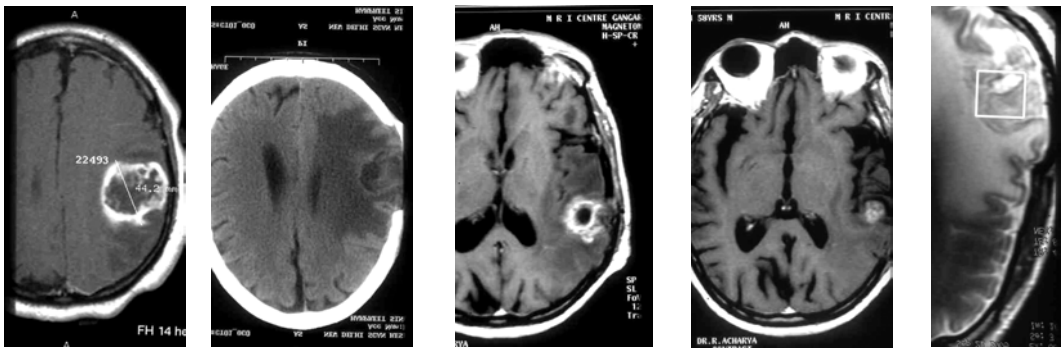
ANTIGENIC EXPOSURE

After 6 d of culture, dendritic cells (DCs) were characterized by microscopic visualization and flow cytometric analysis. Immature DCs were subjected to antigenic stimulation by whole cell lysate antigenic cocktail prepared as under. The WCLAM was mixed in DC culture medium at ratio of 15 μl:1 ml amounting to aggregate of 60 μl per well. The ratio was calculated by in-house experiments done on sheep mononuclear cells (CD14) (unpublished data). Cell culture plates were incubated further for 48 h for maturation of DCs. After completion of the incubation, cell culture medium (Mononuclear growth medium, MGM) and DCs were collected separately by gentle, washing and centrifugation. DCs were assayed for CD83 and CD86 marker study to confirm their maturation. Mycoplasma contamination was ruled out by ELISA assay. Aerobic and anaerobic bacterial cultures were used for detecting bacterial contamination. DCs were counted by trypan blue exclusion staining for cell viability and found to be 93% live (Lim *et al*., 2003).

DC VACCINATION

The patient was given monocyte growth medium and DC vaccine through intravenous route by mixing the components in 100 ml of dextrose normal saline drip with ondansetrone (2 mg) intravenous cover. Constant monitoring was done during the administration of vaccine therapy. No immediate or delayed adverse effects were observed as described earlier. Patient developed low-grade fever after 6 h of therapy which subsided with paracetamol tablet given orally. By the end of the third vaccine, there was overall improvement including better co-ordination of thoughts and mental alertness and recovery of normal sleep and appetite. There was lowering of peripheral edema indicating inactive lesion. Magnetic resonance imaging and computerized topographic scans revealed radiation-like necrosis at the rim of the resected tumor as shown in the images (Fig.1).

Glioblastoma multiforme (Type IV) is known to have 5.1 months survival in spite of conventional treatment modalities including surgery, chemotherapy and radiotherapy (Sagar and Israel, 2001). Immunotherapy has proved to be effective in numerous experimental models of cancer. Mutating cells fail to get recognition through the immune vicinity and have likelihood of becoming cancerous. Failure of immunological response at five step mutations is considered



(a) (b) (c) (d) (e)

Fig.1 (a) CT before operation (44.2 mm in size); (b) MR after operation (remnants and edema); (c) MR post DC therapy (radiation like necrosis in periphery); (d) MR post DC 3rd vaccine (4.2 mm in size); (e) MR spectroscopy post DC therapy (2 mm in size)

a hallmark of cancer development. Cytotoxic T cell (CTL) response is hardly generated in cancerous tissue. Negative chemotaxis and generation of parallel immunity play an important role for establishment of solid tumors. Often, immature dendritic cells are found in and around tumor cells but they cannot elicit CTL response. The mainstay of cancer regression is due to effective CTL response that is quite possible with generation of mature DCs. In this study, DCs were matured with mixed antigen collected from paraffin embedded tissue extracted from autologous source mixed with UV irradiated and heat treated malignant cells extracted from secondary liver to present a repertoire of antigenic base. Immune treatment in poorly responding tumor with all conventional treatments was tried with good results. The patient showed remarkable overall improvement and increased survival extending to 8 months since its clinical diagnosis. Magnetic resonance scan showed radiation like necrosis at the tumor rim although no radiation was given during the DC therapy. Massive oedema was cleared from the lower extremity probably because of blockage in release of pro-clotting factors, commonly present in glioma; from tumor site, an indication of tumor repression. At this juncture, it is difficult to predict the sustained immune response as well as complete recovery from the disease in this patient but showed a new dimension in glioma treatment. In our present study we established the safety and efficacy of dendritic cell therapy for the first time in India. We are planning to have larger clinical trials combined with surgery/chemotherapy to make immune therapy with dendritic cells a standard practice in the very near future.

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References

Chan, P.K.S., Chan, D.P.C., To, K.F., Yu, M.Y., Cheung, J.L.K., Cheng, A.F., 2001. Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. *J. Clin. Pathol.*, **54**(5):401-403. [doi:10.1136/jcp.54.5.401]

Davis, F.G., McCarthy, B., Jukich, P., 1999. The descriptive epidemiology of brain tumors. *Neuroimaging Clinics of North America*, **9**:581-594.

Hart, D.N., 2005. Dendritic cell biology evolves into clinical application. *The Lancet.*, **365**(9454):102-104. [doi:10.1016/S0140-6736(05)17717-4]

Liau, L.M., Black, K.L., Martin, N., 2000. Treatment of a glioblastoma patient by vaccination with autologous dendritic cells pulsed with allogenic major histocompatibilty complex class I matched tumor peptides: case report. *Neurosurgery Focus*, **9**:1-5.

Lim, M.N., Leong, C.F., Cheong, S.K., Seow, H.F., 2003. Generation of dendritic cells from acute myeloid leukemia cells and monocytes, our experience. *Malays J. Pathol.*, **25**(2):107-112.

Liu, Y., Ng, K.Y., Lillehi, K.O., 2003. Cell-mediated Immunotherapy: a new approach to the treatment of malignant glioma. *Cancer Control*, **10**:138-147.

Lo, Y.M., Mehal, W.Z., Fleming, K.A., 1989. In-vitro amplification of hepatitis B virus sequences from liver tumor DNA and from paraffin wax-embedded tissues using the polymerase the polymerase chain reaction. *J. Clin. Pathol.*, **42**:840-846.

Onji, M., Akbar, S.M.F., 2005. On dendritic cell-based therapy for cancers. *J. Zhejiang Univ. SCI.*, **6B**(1):1-3. [doi:10.1631/jzus.2005.B0001]

Sagar, S.M., Israel, M.A., 2001. Primary and Metastatic Tumors of the Nervous System. *In*: Harrison's Principles of Internal Medicine, 15 Internationa Ed. McGraw Hill, New Delhi, p.2444.

Steinman, R.M., Dhodhapkar, M., 2001. Active immunization against cancer with dendritic cells: the near future. *Int. J. Cancer*, **94**(4):459-473. [doi:10.1002/ijc.1503]

Wheeler, C.J., Das, A., Liu, G., John, S., Black, U., Black, L.K., 2004. Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. *Clinical Cancer Research*, **10**(16):5316-5326. [doi:10.1158/1078-0432.CCR-04-0497]

Yu, J.S., Liu, G., Ying, H., Yong, W.H., Black, K.L., Wheeler, J.C., 2004. Vaccination with tumor lysate-pulsed Dendritic cells elicit antigen specific cytotoxic T-cells in patients with malignant glioma. *Cancer Research*, **64**(14):4973-4979. [doi:10.1158/0008-5472.CAN-03-3505]