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Plasma from some cancer patients inhibits adenoviral Ad5f35 vector transduction of dendritic cells

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Abstract

Background. Pooled AB serum is often used as a media supplement for cell culture but it has the potential to transmit infectious diseases. To avoid this risk, we used autologous plasma as a media supplement for manufacturing dendritic cells (DCs) for cancer immunotherapy. We noticed inconsistencies in the DCs and investigated their nature and cause. *Methods.* Adenovirus human epidural growth factor receptor 2 (adHER2/neu) DCs for 21 patients were manufactured from autologous peripheral blood monocytes that were treated with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 for 3 days, transduced with Ad5f35HER2ECTM and then treated with lipopolysaccharide and interferon (IFN)-γ for 1 day. The cells were cultured in RPMI-1640 supplemented with either 10% heat inactivated autologous or AB plasma. *Results.* Twenty-eight adHER2/neu DCs were manufactured for 21 patients using autologous plasma and 68 were manufactured for 20 of those patients using AB plasma. The expression of human epidural growth factor receptor 2 (HER2/neu) was less for DCs manufactured with autologous plasma (70.3 ± 33.3% versus 86.1 ± 22.8%; $P < 0.01$). Manufacturing adHER2/neu DCs using monocytes from three healthy subjects and plasma from one patient with low HER2/neu expression (18%) resulted in low HER2/neu expression by all three DCs (13%, 16% and 23%). Analysis of the levels of 1322 proteins in eight plasma samples associated with low HER2/neu expression and in 12 associated with high HER2/neu expression revealed that the levels of 14 predicted HER2/neu transduction efficiency. *Conclusion.* The manufacture of adHER2/neu DC using autologous plasma as a media supplement resulted in inconsistent HER2/neu expression. It is likely that variability in the levels of multiple proteins in autologous plasma contributed to low HER2/neu expression.

Key Words: *adHER2/neu, cancer immunotherapy, dendritic cells (DCs)*

Introduction

The manufacturing of many clinical cell therapies involves *ex vivo* culture in specialized medium that is supplemented with growth factors and other proteins. Fetal bovine serum (FBS) is often used as a culture media protein supplement, but exposure of cellular therapy recipients to FBS can result in allergic reactions or the transmission of xenogeneic infections [1–3]. Consequently, most clinical cell therapy manufacturing protocols avoid using FBS-containing media for cell culture and expansion. Defined media that is free from animal-derived proteins and human serum has been developed for the culture of some, but not all, cell types. Another alternative to FBS is AB serum prepared from pools of serum or plasma collected from many healthy subjects. Donors of blood products, including AB serum, undergo health history screening and testing for exposure to or presence of transfusion-transmitted pathogens. Despite the screening and testing of AB serum donors, their serum can still transmit infectious diseases. Exposure to

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transfusion-transmitted diseases can also be avoided by using autologous serum or plasma as a culture media protein supplement rather than FBS or pooled third-party donor AB serum.

Many cancer immunotherapies are made from autologous leukocytes. Most chimeric antigen receptor (CAR) T-cell products are made from autologous lymphocytes and many dendritic cell (DC) cancer vaccines are made from autologous monocytes [4–7]. These autologous lymphocytes and monocytes are collected by apheresis as a peripheral blood mononuclear cell (PBMC) concentrate using a blood cell separator. After the PBMC concentrate is collected, an additional 200 to 300 mL of autologous plasma can be collected, which can be used as a culture media protein supplement for manufacturing the autologous cell therapy.

We developed a protocol to manufacture an autologous DC vaccine expressing Human Epidermal Growth Factor Receptor 2 (HER2/neu) to treat patients with HER2/neu-expressing cancers. The HER2/neu (*Erb*B2) oncogene is a member of the epidermal growth factor receptor tyrosine kinase family that encodes a 185-kd transmembrane receptor that functions to regulate cell proliferation, metabolism and invasion. Overexpression of HER2/neu is associated with tumorigenesis and human cancer pathogenesis. The oncogene is overexpressed in 25–30% of all human breast and ovarian cancers, and is associated with higher recurrence and lower survival rates [8].

DCs for this clinical trial were manufactured from autologous monocytes that were isolated from PBMC concentrates by counter-flow elutriation. The monocytes were cultured with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 to produce immature DCs and then with interferon-gamma (IFN-γ) and lipopolysaccharide (LPS) to produce mature DCs. The immature DCs were transduced with a chimeric adenoviral vector, Ad5 serotype with the knob and fiber of the Ad35 serotype (Ad5f35), which expressed the extracellular and transmembrane (ECTM) domains of human HER2 (Ad5f35HER2ECTM). This chimeric adenovirus is less susceptible to anti-Ad5 neutralizing antibodies and is effective at transducing human DCs. We initially manufactured the adHER2/neu DCs in media supplemented with autologous plasma; however, we found that the expression of HER2/neu was highly variable. We then changed the manufacturing protocol and used third-party AB plasma collected from a single donor rather than autologous plasma as a media supplement. This study compared the variability in expression of HER2/neu among DCs manufactured with autologous serum and DCs manufactured with AB plasma and investigated the source of this variability.

Material and methods

DC manufacturing process

DCs were manufactured according to a standard procedure established in the Cell Processing Section (CPS), Department of Transfusion Medicine (DTM), Clinical Center (CC), National Institutes of Health (NIH), Bethesda, MD. Briefly, PBMC concentrates were collected using apheresis. For the 21 patients treated with adHER2/neu that were studied, PBMC concentrates were collected with the Cobe Spectra (Terumo BCT) blood cell separator and an additional 100 to 200 mL of autologous plasma was collected. For some mechanistic studies PBMC concentrates were collected using apheresis from healthy subjects using the Amicus Separator (Baxter Healthcare, Fenwal Division). All donors signed an informed consent approved by a NIH institutional review board. Monocytes were enriched directly from the PBMC concentrates by elutriation using the Elutra (Gambro BCT) automatic mode according to the manufacturer's recommendations and the monocytes were cryopreserved in aliquots of 100×10^6 cells each.

On day 0, donor monocytes were thawed and manufactured in RPMI-1640 supplemented with either 10% autologous plasma or 10% healthy donor AB plasma that had been heat inactivated at 56°C for 120 min. The media was also supplemented with GM-CSF (Leukine Sargramostin, 2000 IU/mL; Genzyme), 10 μg/mL gentamicin and IL-4 (United States Pharmacopeia grade recombinant human IL-4, 2000 IU/mL; CellGenix, Gmbh) at a final concentration of 1.5 x 10^6 cells/mL in T75 flasks (Corning Incorporated Life Sciences). The flask was incubated at 37°C in 5% CO_2. On day 2, fresh cytokine IL-4 (2000 IU/mL) and GM-CSF (2000 IU/mL) were added in addition to Keyhole limpet hemocyanin (KLH; 10 μg/mL; Stellar Biotechnology). Day 3, immature DCs were transduced with Vector Ad5f35HER2ECTM (Center for Cell and Gene Therapy, CACT, Baylor Medical College) at a 1 monocyte to 3000 physical viral particles (measured by optical density) ratio. After transduction, the cells were incubated at 37°C in 5% CO_2 for 4 h then maturation cocktail containing LPS (30 ng/mL, CTEP, NIH) and interferon gamma 1b (Actimmune interferon gamma-1b, 1000 IU/mL; Intermune) were added to the culture. On day 4, 24 h after the addition of the adenoviral vector, the cells were washed, harvested and resuspended in cold Hank's Balanced Salt Solution (HBSS).

Gene expression profiling

Total RNA was extracted from the DCs, using a miRNeasy kit (Qiagen). Universal Human Reference RNA (Stratagene) was used as reference. Test