Cancer Vaccines Methods and Protocols.

Chapter 3: Pulsing dendritic cells with whole tumor cell lysate

Title

Pulsing dendritic cells with whole tumor cell lysate…. o Tumor lysate-pulsed dendritic cells

Laura Alaniz, Manglio M. Rizzo and Guillermo Mazzolini.

Summary

One of the strategies employed in immunotherapy of cancer is to use ex-vivo generated dendritic cells pulsed with tumoral antigens. Several approaches have been used to obtain tumor antigens. One of them is the use of whole tumor cell lysate from one or more tumor cell lines of the tumor type to be treated. The advantage of applying this method is that it provides a large spectrum of specific tumoral antigens. Here, we describe the steps to obtain a whole tumor cell lysate by repetitive freeze–thaw cycles from human tumor cell lines in sufficient amount and quality to pulse dendritic cells.

Key words: tumor associated antigens, hepatocellular carcinoma cell lines, tumoral lysates, necrotic cells, immunotherapy.

1. Introduction

The efficiency of using dendritic cells (DC) as a tool to treat cancer depends on its capability to activate specific host`s T cells against tumor antigens (*1*). Despite tumor associated antigens (TAA)

or tumor specific antigens (TSA) have been identified for several tumors (*2*), it is still necessary to perform a full characterization of their capability to induce an active immune response. A method to cover a plentiful range of antigens is through the use of allogeneic tumoral cell lines. The mix obtained contains not only proteins, but other molecules which are able to induce a potent immune response, such as RNA, DNA and cellular components (*3*). Tumor cell lines that share one or even several tumor antigens provide a simple source of delivering antigens in tumor immunotherapy. Another advantage that offers this method is the possibility to obtain standardized and controlled preparations to be used clinically under good manufacturing practice (GMP) facility (*4-5*). Here, we describe the principal steps to obtain a whole tumor cell lysate by repetitive freeze–thaw cycles from human tumor cell lines, growing as monolayer, in adequate concentration and sterility to pulse ex-vivo generated blood monocytes- derived dendritic cells.

2. Materials

Use all reagents and culture medium at room temperature and in sterile conditions.

2.1 *Tumoral extract*

HCC Tumor cell lines: Hep3B and Huh7, growing in 80-90% of confluence and viability over 90% (see Note 1).

Saline solution (ClNa 0,9%).

Scrapers.

Liquid Nitrogen container.

Water bath thermostated.

Conical centrifuge tubes (50 ml).

Filters 0,22 µm (Millipore Express®), low binding proteins membrane.

Temperature-controlled centrifuge (ThermoElectron Centra CL3R with 243 horizontal rotor or equivalent).

Bradford protein assay kit.

2.2 *Pulsing Dendritic Cells*

Dendritic cell suspension (monocyte-derived dendritic cells, cita paper de Guillermo o Schuler G, Brang D, Romani N. Production and properties of large numbers of dendritic cells from human blood. Adv Exp Med Biol 1995;378:43-52.)

Complete culture medium: serum-free AIMV medium (GIBCO BRL), 100 U/ml peniciline, 100 µg/ml streptomycin, human recombinant GM-CSF (Bioprofarma, Growgen, 350 ng/ml or Leukine GM-CSF 1000 UI/ml), human recombinant IL-4 (IL-4 peprotech 20 ng/ml or IL-4 R&D Systems 500 UI/ml), 2-βMercaptoethanol (0,05 M).

Humidified 37°C, 5% CO2 incubator.

2.3 *Counting viable cells*

Neubauer chamber

Trypan Blue solution

3. Methods

Work all the time in sterile conditions under biosafety level II.

3.1 Necrotic tumor cell lysate from tumor cell lines.

1. Discard culture medium. Wash cells twice with saline. Place the culture dishes on ice (see Note 2).

2. Scrap the cells growing at confluence and harvest. Collect the cell suspension in a 50 ml conical centrifuge tube (see Note 3). Wash cells twice and resuspend in 2,5 ml of saline per 5 x 10^7 cells.

3. Disrupt cell suspension by 5 freezing (-196 C°) and thawing (37 C°) cycles of 5 min each one.

4. Centrifuge at 97 x g, 18° to 20°C, for 10 min to remove large debris. Filter the supernatant with a 0,22 µm pore, sterile, low binding proteins filter unit (See Note 4).

5. Measure protein concentration by Bradford method (6).(See Note 5).

6. Aliquot and store at -80 C° until use.

3.2 Pulsing dendritic cell

1. Incubate 1-5 x 10^6 dendritic cells in 1 ml of complete culture medium with 500 µg of tumor cell extract for 12 h in a humidified incubator at 37°C and 5% CO_2, before check viability by tripan blue dye exclusion test.

2. Wash the cell twice with saline solution by centrifugation for 10 min at 18° to 20°C, 530 x g.. Resuspend the cells in saline solution and check viability by trypan blue exclusion test (see Note 6). These cells are ready to use for subsequent immune assays.

4. Notes

1. For cells growing in suspension, take into account the cellular density and viability.
2. Ice helps to easily harvest the cells using scrapers. Avoid the use of trypsine because it could completely degrade some proteins or peptides.
3. Although the final volume is 5ml, we use 50 ml conical centrifuge tube given that it facilitates sample handling in the following steps.
4. Carry out the filtration gently to prevent filter getting clogged, given that final suspension has a high density of proteins and other cellular components. We use **polyethersulfone filter** which has a low-protein binding capability to prevent protein loss.
5. The Bradford assay measures only protein concentration, but the lysate contains other components which cannot be measured by this method.Yield of proteins depends on the tumor cell quantity and type. We obtain ≈ 5 mg of total protein from ≈5 x 10^7 tumor cells.
6. Viable dendritic cells must be around 90% in order to obtain a functional response.

5. Acknowledgements

This work was supported by grants from Austral University, Agencia Nacional de Promoción Científica y Tecnológica, Argentina.

6. References

1. Palucka, K., and Banchereau, J. (2012) Cancer immunotherapy via dendritic cells, *Nat Rev Cancer 12*, 265-277.
2. Novellino, L., Castelli, C., and Parmiani, G. (2005) A listing of human tumor antigens recognized by T cells: March 2004 update, *Cancer Immunol Immunother 54*, 187-207.
3. Chiang, C. L., Benencia, F., and Coukos, G. (2010) Whole tumor antigen vaccines, *Semin Immunol 22*, 132-143.
4. Ribas, A., Camacho, L., Lee, S., Hersh, E., Brown, C., Richards, J., Rodriguez, M., Prieto, V., Glaspy, J., Oseguera, D., Hernandez, J., Villanueva, A., Chmielowski, B., Mitsky, P., Bercovici, N., Wasserman, E., Landais, D., and Ross, M. (2010) Multicenter phase II study of matured dendritic cells pulsed with melanoma cell line lysates in patients with advanced melanoma, *Journal of Translational Medicine 8*, 89.
5. Prins, R. M., Soto, H., Konkankit, V., Odesa, S. K., Eskin, A., Yong, W. H., Nelson, S. F., and Liau, L. M. (2011) Gene Expression Profile Correlates with T-Cell Infiltration and Relative Survival in Glioblastoma Patients Vaccinated with Dendritic Cell Immunotherapy, *Clinical Cancer Research 17*, 1603-1615.
6. Smith, J. A. (1987) Colorimetric methods, *In Current Protocols in Molecular Biology*, pp. 10.11.11-10.11.13.