Chapter 4. Autologous dendritic cell vaccine loaded ex-vivo with tumor antigens

Manglio M. Rizzo, Flavia Piccioni, Laura Alaniz and Guillermo Mazzolini.

Ex vivo generated antigen-loaded DCs have been shown to be immunogenic in patients with cancer. The load with autologous whole tumor antigens is a strategy to arm DC against tumor without human leukocyte antigen (HLA) restriction. Besides, this approach allows a presentation to immune system of a full antigen range. We describe the methods to obtain whole antigens from autologous tumoral tissues in order to load DC generated ex-vivo from patients with gastrointestinal cancer.

Key words: tumor specimen, autologous tumor tissue, immunotherapy. tumor associated antigens, colon carcinoma.

Introduction

Delivery of whole tumor lysate **into dendritic cells** for cancer vaccination

The use of denditric cell for cancer could require the ex-vivo loading with tumor in order to induce specific immune response against tumors (*1*). Antigens obtained from autologous tumor tissues could induce a broad response and cover a large variety of peptide—MHC complexes, providing enough amount of a number of tumor antigens (*2-4*).The type of tumoral antigens delivered into DCs is crucial to achieve a clinical response (*5-6*). TAA, HLA-restricted immunodominant peptides, are identified as the most widely used for tumor vaccination. However, this approach has several disadvantages: i) only patients possessing specific HLA expressions are eligible , ii) immune responses are limited to the epitopes used for immunization that might be insufficient, iii) longevity of MHC-peptide complexes in vivo is unknown (*4, 7*). A hopeful alternative is loading ex- vivo DC for

vaccination using whole tumor lysates without definite antigens. Tumoral tissues express a whole array of TAA and tumor specific antigens that are both characterized and uncharacterized. These antigens are processed by MHC II and I and promote the activation of both CD8+ cytotoxic- T and CD4+ helper T cells against tumor cells (*8*). Although whole tumor lysate may increase the risk of autoimmunity by shared epitopes clinical studies have shown its safety (*3, 9*). In our laboratory we have applied a method to prepare autologous antigens from tumor tissue by mechanical disruption, freeze and thawing to finally obtain a whole tumor lysate to load human DC.

2. Material

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

2.1 Tumoral extract

Fresh tumor specimen in sterile conditions.

100-mm petri dishes

Surgical scalpel blade #20

Saline solution (ClNa 0,9%).

Liquid nitrogen container.

Thermostated water bath.

50 ml sterile conical centrifuge tubes

Thermo Electron Centra CL3R with 243 horizontal rotor (or equivalent temperature-controlled centrifuge).

0,22 µm Millipore Express®, sterile, low binding proteins filter unit.

Coomassie (Bradford) protein assay kit.

2.2 Pulsing Dendritic Cells

Complete culture medium: Liquid RPMI-1640, 100 U/ml Peniciline, 100 U/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),

2β Mercaptoethanol (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 tumoral lysate from fresh tumor tissue

1. Take approximately 5 mm^3 fresh tumor specimen in sterile conditions and put it in a recipient with 15 ml saline solution.

2. Place tumoral tissue in a petri dish and disperse it with needle and scalpels, adding a volume of saline solution until obtain a suspension (See Note 1).

3. Collect the final suspension in a 50 ml conical centrifuge tube (See Note 2).

4. Disrupt the suspension by 5 freezing (-196 C°) and thawing (37 C°) cycles for 5 min each.

5. To remove large debris centrifuge at 600 rpm (70 g), 18° to 20°C, for 10 min.

6. Filter the sample with a 0,22 µm Millipore Express®, sterile, low binding proteins filter unit (See Note 3)

7. Quantify protein by Bradford method (Smith, 1987) (See Note 4)

8. The resulting tumor lysate could be aliquoted and stored at -40 C° until use.

3.2 Pulsing dendritic cell

1. Incubate 1-5 x 10^6 dendritic cells in 1 ml complete culture medium with 200 µg of tumoral extract for 12 hs in a humidified 37°C, 5% CO_2 incubator.
2. Centrifuge for 10 min, 18° to 20°C, at 1400 rpm (390 g).
3. Resuspend the cells, count viable cells by trypan blue and use.

1. Notes

1. Add 2-5 ml carefully, in small amounts, saline solution in order to allow the total extract dispersion. The optimal final volume of the mix depends on the tumor size and quality. In general with a tumor of 5 mm^3 size can obtain 5 ml of a solution at final concentration around to 2 mg/ml.
2. We use 50 ml conical centrige tube, although the final volume is small, given that the use of them facilitates handling of the sample in the following steps.
3. 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.
4. The Bradford assay measures only protein concentration. However, the tumor tisues lysate contains other components which cannot be measured by this method.
5. Viable dendritic cells must be around 90% in order to obtain a functional response.

2. Acknowledgements

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

1. Palucka, K., and Banchereau, J. (2012) Cancer immunotherapy via dendritic cells, *Nat Rev Cancer 12*, 265-277.
2. Nestle, F. O. (1998) Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells, *Nature Med. 4*, 328-332.

3. Geiger, J. D. (2001) Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression, *Cancer Res. 61*, 8513-8519.

4. Chiang, C. L., Benencia, F., and Coukos, G. (2010) Whole tumor antigen vaccines, *Semin Immunol 22*, 132-143.

5. Heath, W. R., and Carbone, F. R. (2001) Cross-presentation, dendritic cells, tolerance and immunity, *Annu. Rev. Immunol. 19*, 47-64.

6. Andersen, B. M., and Ohlfest, J. R. (2012) Increasing the efficacy of tumor cell vaccines by enhancing cross priming, *Cancer Letters 325*, 155-164.

7. 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.

8. Bhardwaj, N. (2001) Processing and presentation of antigens by dendritic cells: implications for vaccines, *Trends in Molecular Medicine 7*, 388-394.

9. Iwashita, Y., Tahara, K., Goto, S., Sasaki, A., Kai, S., Seike, M., Chen, C.-L., Kawano, K., and Kitano, S. (2003) A phase I study of autologous dendritic cell-based immunotherapy for patients with unresectable primary liver cancer, *Cancer Immunol Immunother 52*, 155-161.