Chapter 9
Delivery of Whole Tumor Lysate into Dendritic Cells for Cancer Vaccination

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Abstract Results from multiple human studies have continued to spur the development of dendritic cells (DCs) as therapeutic vaccines for the treatment of cancer, chronic viral infections, and autoimmune diseases. The antigen-specific activity of DCs is dependent on the ability of the DCs to take up and process tumor-associated antigens for presentation to the immune system. Although immature DCs have been shown to naturally take up tumor-associated antigens by phagocytosis, approaches that significantly affect antigen delivery need further evaluation, especially if such methodologies can be demonstrated to result in the elicitation of more robust and comprehensive immune responses. We have developed a rapid, robust, scalable, and regulatory-compliant process for loading DCs with whole tumor lysate. The use of whole tumor lysate facilitates the generation of a more robust immune response targeting multiple unique antigenic determinants in patient’s tumors and likely reduces the tumor’s potential of immune escape. We demonstrate that DCs electroloaded with tumor lysate elicit significantly stronger antitumor responses both in a tumor challenge model and in a therapeutic vaccination model for preexisting metastatic disease. These effects are observed in a processing scheme that requires 20- to 40-fold lower amounts of tumor lysate when compared with the standard coincubation/coculture methods employed in loading DCs.

Keywords: immunotherapy, tumor lysate loading, dendritic cells

1. Introduction

Electroporation has been established as an efficient method for loading a wide range of cell types (1–7), including DCs (8–10), with various bioactive molecules. Because it is a physical approach causing temporary permeability of the cell membrane, electroporation-mediated loading of macromolecules avoids virus-related complications, and it is especially important when dealing with immunotherapeutics that there will be no cross contamination with viral antigens. The use of
autologous tumor lysate allows vaccinating cancer patients regardless of their HLA haplotypes (10). Many previous studies have shown the feasibility of loading DCs with whole tumor lysate, most commonly by standard coincubation/pulsing, for treating different types of cancers, including solid carcinomas (11–13), myeloma (14), glioma (15), and melanoma (16–18). These studies demonstrate that DCs coincubated with whole tumor lysate can elicit specific antitumor T-cell responses. We hypothesized that electroloading DCs with whole tumor lysate would result in more effective antitumor responses due to active tumor antigen delivery to DCs and perhaps by forcing a class I response.

Syngeneic mouse tumor lysate was obtained by four cycles of quick freeze and thaw of cultured mouse renal carcinoma cells (RENCA) and Lewis lung carcinoma (LLC) cells. To avoid nonspecific, antitumor effects from tissue culture related materials, LLC tumor cells were subcutaneously injected into syngeneic mice. The tumor tissue that grew out was extracted and dissected into small pieces prior to the freeze and thaw procedure. After optimization of the electroloading procedure via fluorescein isothiocyanate (FITC)-conjugated dextran (250 kDa), immature DCs (imDC, $5 \times 10^7$ cells/mL) were electroloaded with the whole tumor lysate at 0.5 mg/mL, which was equivalent to the ratio of 10 imDCs to 1 tumor cell. After overnight maturation, RENCA tumor lysate loaded or incubated mDCs were subcutaneously injected into syngeneic Balb/C mice, followed by RENCA tumor challenge 10 days later. Tumor growth was measured in mice (Fig. 9.1).

For the metastases tumor model, C57BL6 mice were first injected with $5 \times 10^5$ syngeneic LLC tumor cells via tail vein. The mice later received two shots of LLC lysate electroloaded or incubated mDCs, $1 \times 10^6$ cells, at days 3 and 6 after tumor

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**Fig. 9.1** Schematic flow chart of the in vivo experiments
implantation. Metastasic lesions were measured at day 15 by weighing both lungs from the killed mice (Fig. 9.1). In addition, specific in vitro tumor killing was analyzed using splenocytes isolated from C57BL6 mice that had received LLC lysate electroloaded DCs.

2. Materials

2.1. Cell Culture

1. Renal carcinoma (RENCA) and Lewis lung carcinoma (LLC) were generously provided by EntreMed (Rockville, MD).
2. Cell culture medium: RPMI 1640 and Dulbecco’s modified Eagle’s medium (DMEM).
3. Supplemental components for cell culture medium: fetal bovine serum (FBS), L-glutamine, nonessential amino acids (NEAA) mixture, sodium pyruvate, and penicillin/streptomycin.
4. MEM vitamin mixture.

2.2. DC Isolation and Differentiation

1. C57BL6 and Balb/C male mice (6–8 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME).
2. PBS.
3. Scissors and forceps, kept in sterile beaker with 70% ethanol.
4. 10-mL syringe with 18-GA needle.
5. 100-mm petri dish.
6. Box or equipment for CO₂ asphyxiation.
8. ACK red blood cell lysis buffer (Quality Biological Inc.).
9. T175 tissue culture flasks.
10. Ultra low attachment (ULA) 6-well plates (Corning).
11. Mouse granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-4, tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ).
12. Prostaglandin E2 (PGE-2) and lipopolysaccharide (LPS).
13. Human serum albumin.
14. AIM-V media.
15. Enzyme-free, PBS-based cell dissociation buffer.
2.3. Electroloading

1. Electroporator: MaxCyte Gene Transfer (GT) system or electroporator from other vendors, for example, BTS EC830 (Inovio) and Gene Pulser II (BioRad).
2. Electroloading buffer (Hyclone).
3. Cell-processing cuvettes (MaxCyte or other vendors).
4. FITC-conjugated dextran (250kDa, Sigma), suspended in PBS at 10mg/mL in a 15-mL conical tube wrapped with aluminum foil and stored at 4°C.
5. Water bath set at 37°C.
6. Propidium iodine, 0.5mg/mL (Roche).
7. FACS staining buffer: PBS containing 2% FBS.
8. FACS Calibur flow cytometer and CellQuest software (BD Bioscience).

2.4. In Vivo Experiment

1. C57BL6 and Balb/C male mice (6–8 weeks old) were obtained from Jackson Laboratories.
2. 1-mL insulin syringes with various gauges of needles for injection.
4. Mouse holder for tail vein injection

2.5. In Vitro Tumor Killing Assay

1. Na$_2$[${}^{51}$CrO$_4$].
2. XVIVO-15 media (Cambrex).
3. Mouse recombinant GM-CSF, IL-2, and IL-7.
4. Scintillation counter.
5. 24-well ultra low attachment plates.
6. U-bottomed 96-well plates.
7. Triton X-100.

3. Methods

In this study, syngeneic Balb/C mice were used to demonstrate that RENCA tumor lysate electroloaded mDCs could delay tumor growth. LLC-tumor-bearing syngeneic C56BL6 mice were used to show the therapeutic effects of lysate-electroloaded mDCs on prevention of metastasis. We compared treatment groups using tumor lysate electroloaded, incubated, and control liver lysate electroloaded DCs.
3.1. Preparation of Mouse Bone-Marrow-Derived DCs

1. Six-week-old C57BL6 and Balb/C male mice were obtained from Jackson Laboratories. Maintain the mice under an approved Institutional Animal Care and Use Committee (IACUC) protocol.
2. Asphyxiate the mice in a sealed container by using CO₂ at the age of 8–12 weeks (See Note 1).
3. After cervical dislocation, use scissors and forceps to isolate both the femur and tibia bone by cleaning away as much of the surrounding muscle and fat tissue as possible and leaving the bones intact (See Note 2).
4. In a biological safety cabinet, remove one end of the bone by a pair of sterilized scissors, leaving the bone marrow exposed.
5. Flush out the bone marrow from the intact end by a 10-mL syringe with an 18-gauge needle filled with PBS into a 100-mm petri dish.
6. Carefully transfer the collected bone marrow by a pipette and filter it through a cell strainer to remove residual tissue and debris.
7. Harvest the marrow cells by centrifugation for 10 min at 400 × g.
8. Aspirate the supernatant and then wash the cells once with PBS, followed by centrifugation for 10 min at 400 × g.
9. Afterwards, discard the supernatant, then resuspend the cells with 10 mL ACK red blood cell lysis buffer and incubate the cells at room temperature for 10 min.
10. Wash the cells three times with PBS. After the final PBS wash, resuspend the cell pellet with AIM-V culture media containing 2 mM L-glutamine and 0.2% HSA, and then count the cells with a hemocytometer.
11. Seed the bone marrow cells to T-175 flasks at ~5 × 10⁶ cells/mL, 35 mL/flask in AIM-V culture media containing 2 mM L-glutamine, 0.2% HAS, 25 ng/mL of mGM-CSF, and 12.5 ng/mL of mIL-4.
12. Add fresh mGM-CSF (25 ng/mL) and mIL4 (12.5 ng/mL) to the culture every 2–3 days.
13. After 6–8 days in culture, harvest the imDCs by combining the suspension and adherent cells detached by enzyme-free, PBS-based cell dissociation buffer.
14. Process the imDCs freshly or cryopreserve them in FBS containing 10% dimethyl sulfoxide (DMSO). Use a small aliquot of the cells for surface marker analysis by flow cytometry.

3.2. Preparation of Whole Tumor Lysate

1. Culture murine renal carcinoma cell line (RENCA) in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 1× NEAA, 2× MEM vitamin mix, and 1× penicillin/streptomycin.
2. Culture Lewis lung carcinoma cell line (LLC) in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 2× MEM vitamin mix, 1× NEAA, and 1× penicillin/streptomycin.
3. On the day of tumor cell harvesting, after trypsinization, wash the tumor cells extensively (say three times) with PBS.
4. After counting the cell number with a hemocytometer, resuspend the tumor cells in PBS at $1 \times 10^8$ cells/mL.
5. To eliminate the potential for tissue-culture contaminants such as FBS and trypsin during lysate preparations, also prepare the whole tumor lysate from tumor tissue freshly removed from syngeneic animals with tumors established by subcutaneous injection of tumor cells. Dissect the tumors to eliminate as much normal tissue as possible, and then finely mince the tumors with razors. Resuspend the minced tissue in PBS. At the same time, also isolate the liver to prepare lysate as control.
6. Quickly freeze the cells by incubating the tube in a dry ice/alcohol bath for 5 min.
7. Quickly thaw the cells by incubating the tube in a 37°C water bath.
8. Quickly freeze and thaw the cells an additional three times.
9. Spin the lysed cells in a benchtop microcentrifuge for 10 min at the highest speed at 4°C, and then transfer the supernatants to sterilized O-ring microcentrifuge tubes.
10. Store the whole tumor lysates at −80°C until use.
11. Quantify the total protein concentration using a commercially available protein assay kit (Pierce), which approximately is 10 mg/mL.

3.3. Optimization of Electroloading imDC

1. Harvest the imDCs by combining floating suspension cells and adherent cells detached by enzyme-free, PBS-based cell dissociation buffer.
2. After centrifugation at $200 \times g$ for 10 min, resuspend the imDCs in 10 mL of electroporation buffer, followed by centrifugation at $200 \times g$ for 10 min.
3. Resuspend the imDC pellet in electroporation buffer at $5 \times 10^7$ cells/mL (See Note 3).
4. Add FITC-conjugated dextran to a final concentration of 0.5 mg/mL.
5. For electroloading, transfer 20–400 µL of imDC and FITC-dextran mixture to a MaxCyte cell-processing chamber and process using the MaxCyte ‘imDC’ protocol (See Note 4).
6. After electroporation, detach the chamber and transfer it to a biosafety cabinet. Transfer the electroporated imDCs to a sterile tube, followed by incubation in a 37°C water bath for 20 min.
7. Resuspend the processed imDCs in AIM-V media containing 2 mM l-glutamine, 0.2% HAS, 25 ng/mL mGM-CSF, and 12.5 ng/mL mIL-4 at a final cell concentration of $1 \times 10^6$ cells/mL. Plate the cells onto ULA 24-well plates at 1 mL/well.
8. For coincubation control, after finishing the electroloading process, dilute the imDC and FITC-mixture to $1 \times 10^6$ cells/mL and plate the same way as the electroporated imDCs.
3.4. Analyzing Electroloading Efficiency

1. Harvest the FITC-dextran modified DC 2–5 h after seeding by pipetting the loosely attached DCs.
2. Wash the plates once with cold PBS and pool them together with the collected DCs.
3. Centrifuge the collected cells at $200 \times g$ for 10 min.
4. After one wash with FACS staining buffer, resuspend the cells in FACS staining buffer and stain the cells with propidium iodine (PI) by adding 1 µg of PI/mL immediately prior to analysis by FACS Calibur flow cytometer and CellQuest software (See Note 5).
5. An example is shown in Fig. 9.2.

3.5. Preparation of Whole Tumor Lysate Modified mDC

1. Determine the optimal electroporation procedure by the FITC-dextran electroporation to obtain good FITC-dextran uptake and good cell viability.
2. Thaw previously prepared, frozen tumor lysate to room temperature by leaving the lysate-containing microcentrifuge tube in the biosafety cabinet for 20 min.

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**Fig. 9.2** Efficient electroporation-mediated delivery of macromolecule into DC. C57BL6 mouse bone-marrow-derived DCs were either cocultured or electroloaded with FITC-dextran (250kDa). FACS analysis was performed 3 h after electroporation. Electroporation significantly enhanced FITC-dextran uptake when compared with coculture.
3. Harvest the imDCs by combining floating suspension cells and adherent cells that detached briefly by enzyme-free, PBS-based cell dissociation buffer.

4. Centrifuge the imDCs at 200 × g for 10 min, and then resuspend the imDCs in 10 mL of electroporation buffer, followed by centrifugation at 200 × g for 10 min.

5. Resuspend the imDC pellet with electroporation buffer at 5 × 10⁷ cells/mL.

6. Add the thawed whole tumor lysate to the imDC suspension at 0.5 mg/mL, which is equivalent to a ratio of 10 imDCs to 1 tumor cell lysate (See Note 6).

7. For electroloading, transfer 20–400 µL of imDC and tumor lysate mixture to a MaxCyte cell-processing chamber and process using the MaxCyte ‘imDC’ protocol.

8. After electroporation, detach the chamber and transfer it to a biosafety cabinet. Transfer the electroporated imDCs to a sterile tube, followed by incubation for 20 min in a 37°C water bath.

9. Later resuspend the processed imDCs in AIM-V media containing 2 mM l-glutamine, 0.2% HAS, 25 ng/mL each of mGM-CSF, mTNF-α, and m-IFNγ, 12.5 ng/mL mL-4, 1 µg/mL PGE-2, and 10 µg/mL LPS at 5 × 10⁶ cells/mL. Plate the cells onto ultra low attachment (ULA) 6-well plates at 2 × 10⁶ cells/mL and allow the cells to mature overnight in a 37°C, 5% CO₂ incubator.

10. For coincubation control, after all the electroloading processes, dilute the imDC and tumor lysate mixture with the maturation media to 2 × 10⁶ cells/mL and plate the same way as the electroporated imDCs for overnight maturation.

11. For negative control, electroporate the imDCs either without any lysate or with syngeneic liver lysate as described earlier.

12. Harvest the lysate-modified and matured DCs by collecting all the cells in the ULA tissue culture vessels plus a cold PBS wash of the vessels.

13. After centrifugation at 200 × g for 10 min, either directly inject the modified mDCs into animals or cryopreserve them in FBS with 10% DMSO.

### 3.6. In Vivo Experiments

#### 3.6.1. Whole Tumor Lysate Electroloaded DCs Delay Tumor Growth in a Tumor Challenge Model

1. For the RENCA tumor challenge model, subcutaneously inject Balb/C mouse with 1 × 10⁶ DCs derived from syngeneic mouse bone marrow that have been electroloaded or coincubated with RENCA lysate.

2. As controls, some mice receive no DCs at all or 1 × 10⁶ DCs that have been electroloaded with an equivalent amount of whole liver lysate.

3. Ten days later, harvest RENCA cells from tissue culture by trypsinization.

4. After three washes with PBS, resuspend the RENCA cell pellet with PBS at 2.5 × 10⁷ cells/mL.
5. Carefully inject the mouse with 200µL (5 × 10^5 cells) of the RENCA cell suspension subcutaneously at a site different from the that of DC injection.

6. Measure the primary tumor areas twice a week using a mechanical caliper (See Note 7).

7. Calculate the tumor volumes with the formula \( v = \frac{\pi ab^2}{6} \), where \( a \) is the longest diameter and \( b \) is the diameter perpendicular to \( 'a' \), and plot out as in Fig. 9.3.

### 3.6.2. Whole Tumor Lysate Electroloaded DCs Prevent Metastasis in a Therapeutic Animal Model

1. Harvest the LLC cells from tissue culture by trypsinization.

2. After three washes with PBS, resuspend the LLC cell pellet with PBS at 1 × 10^6 cells/mL.

3. Inject C57BL6 mouse with 0.5mL of the live LLC cell suspension (5 × 10^6 cells) via tail vein by using a 1-mL syringe.

4. Three days later, carefully thaw previously processed, and cryopreserved whole tumor lysate modified mDCs.

5. After a wash with PBS, resuspend the mDCs with PBS at 2 × 10^6 cells/mL.
6. Divide the LLC-cell-injected mice into four groups (8 mice/group): a) no treatment, b) treat with mDC electroloaded with control liver lysate, c) treat with mDC incubated with LLC whole tumor lysate, d) treat with mDC electroloaded with LLC whole tumor lysate.

7. Carefully inject 0.5 mL of mDC suspension (1 × 10^6 DC) via tail vein.

8. Three days later, at day 6, treat each mouse group with a 2nd dose of mDCs.

9. On day 17, kill all mice, including another 5 healthy mice of the same age. Isolate both lungs from each mouse. After briefly washing the lungs with PBS, lay the lungs on kleenex tissue. Weigh the lungs from each mouse.

10. Use the lung weight from the 8 healthy mice that receive no tumor cells as baseline lung weight controls and plot out all the lung weight as shown in Fig. 9.4.

![Lung Weight Graph](image.png)

Fig. 9.4 Whole tumor lysate electroloaded DCs prevented Lewis lung metastases in a therapeutic model. At the start of the experiment, 5 × 10^5 Lewis lung carcinoma (LLC) cells were administered intravenously via tail vein into syngeneic C57BL6 mice. C57BL6 mouse bone-marrow-derived mDCs were either coincubated or electroloaded with LLC whole tumor cell lysate. As a control, mDCs were electroporated with liver lysate. After maturation, the modified mDCs were cryopreserved and stored in liquid nitrogen. Three days after LLC injection, 1 × 10^6 DCs were thawed and administered by tail vein injection (8 mice/group). There were four groups: a) no control, no DCs were injected; b) liver lysate electroloaded mDCs; c) LLC tumor lysate incubated mDCs; d) LLC tumor lysate electroloaded DCs. After an additional 3 days (day 6), the mice were treated with a second dose of identically processed mDCs (1 × 10^6) by tail vein injection. On day 15 post-LLC injection, mice were killed and lungs were dissected and weighed. The no tumor control group reflects normal lung weights of mice that were not challenged with any LLC. Administration of DCs that had been electroloaded with LLC lysate caused a significant reduction in LLC lung metastases, as indicated by a significant decrease in lung weights (*p < 0.01)
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3.7. In Vitro Tumor Killing Assay

3.7.1. Preparation of Effector Cells

1. Remove spleens from syngeneic mice (See Note 8).
2. Place the spleens in a 100-mm dish with 30mL of RPMI 1640 media.
3. Using scissors and a razor blade, slice the spleens into small pieces and later mince 10–20 times using the flat end of the plunger from a 5-mL sterile syringe.
4. Carefully transfer the media, including all the splenocytes, and filter it through a cell strainer to remove residual tissue and debris.
5. Collect the splenocytes by centrifugation at 400 × g for 10 min and later wash the cells twice with PBS.
6. After the final PBS wash, resuspend the splenocytes in XVIVO-15 media and count the cells with a hemocytometer.
7. Then dilute the splenocytes to 2 × 10^7 cells/mL with XVIVO-15 media supplemented with mGM-CSF (25 ng/mL), mIL2, and mIL7 (10 ng/mL each).
8. Add 500 µL of the splenocyte suspension (1 × 10^7 cells) to one well of a 24-well, ultra low attachment plate. Divide the wells into four groups and add 0.5 mL of previously processed mDCs (1 × 10^6 cells/mL), resulting in a cell ratio of 10 responder to 1 stimulator. The four groups are as follows: a LLC cell lysate electroloaded mDC, b LLC lysate coincubated mDC, c mDC electroloaded with liver lysate, and d no mDC, just XVIVO media.
9. Restimulate the cells with appropriate mDCs two more times, 1 week apart.
10. One week after the final stimulation, collect the cells from each group via centrifugation at 400 × g for 10 min.
11. After three washes with PBS, resuspend the effector CTL in XVIVO-15.

3.7.2. Preparation of Target Cells

1. Split the LLC cells at 1:5 into T25 flasks in the DMEM complete media 2 days before the killing assay.
2. On the day of the killing experiment, harvest the LLC cells by trypsinization.
3. After a wash with complete DMEM media, count the cells with a hemocytometer.
4. Based on the cell concentration, transfer 1 × 10^6 LLC cells to a 15-mL conical tube.
5. Centrifuge the cells at 200 × g for 5 min.
6. Carefully aspirate the supernatant and leave ~200µL of complete media in the tube.
7. Add 20 µL of heat-activated FBS and 100µL of Na_2[^51]CrO_4 (1µCi/µL) to the tube (See Note 9).
8. After carefully resuspending the cell pellet, place the tube in a 37°C, CO_2 incubator with the cap loosely attached to the tube.
9. Incubate the LLC cells with ^51Cr for 1 h.
10. Then, add 5 mL of XVIVO-15 complete media to the $^{51}$Cr-labeling tube.
11. Centrifuge the $^{51}$Cr-labeled LLC cells at 200 × $g$ for 5 min.
12. Carefully remove and discard the $^{51}$Cr-containing supernatant into a radioisotope waste container.
13. Wash the $^{51}$Cr-labeled LLC cells four times with 5 mL of complete media via centrifugation.
14. After the final wash, resuspend the cells in XVIVO-15 complete media at $1 \times 10^5$ cells/mL.

3.7.3. Determining the CTL Activity by Coincubation of Target and Effector Cells

1. Seed 100 µL of the $^{51}$Cr-labeled LLC cell suspension into each well on a U-bottomed 96-well plate (1 × 10⁴ cells/well).
2. Add the LLC-specific CTL generated from the in vitro stimulation to the $^{51}$Cr-labeled LLC cells at various ratios: 50:1, 10:1, and 1:1. Each ratio includes triplicate wells.
3. As complete cell lysing positive control, add 100 µL of 2% Triton X-100 to the $^{51}$Cr-labeled target LLC cell suspension on the U-bottomed 96-well plate (4 wells).
4. As a negative control that measures spontaneous cell lysis (the background), add 100 µL of XVIVO-15 complete media to the $^{51}$Cr-labeled target LLC cell suspension on the U-bottomed 96-well plate (4 wells).
5. Spin the 96-well plate briefly at 200 × $g$ for 1 min. For the wells containing LLC-specific CTL, after carefully aspirating the supernatant from the wells, add 200 µL of XVIVO-15 complete media.
6. Place the 96-well plate in a 37°C, CO₂ incubator and incubate the plate for 4 h, followed by brief centrifugation of the plate at 200 × $g$ for 1 min.
7. Then, carefully transfer 100 µL of the supernatant from each well to scintillation tubes containing 2 mL of scintillation fluid.
8. Measure the amount of $^{51}$Cr in each tube by a scintillation counter.
9. Average the counts from the triplicate wells.
10. Calculate the LLC tumor specific killing using the following equation: CTL activity = \left[ \text{radiation cpm from experimental wells} - \text{cpm from spontaneous $^{51}$Cr release from tumor cell alone wells} \right]/\left[ \text{cpm from LLC treated with Triton X-100} \right]. An example is shown in Fig. 9.5.

3.8. Statistical Analysis

A two-tailed Student’s $t$ test was performed to compare tumor growth, lung weight, and CTL activity among treatment groups.
Acknowledgments The authors thank Nicholas Chopas for instrumentation assistance.

4. Notes

1. Older mice (older than 3 months, younger than 1 year) can also be used for bone marrow isolation.
2. Freshly isolated bones need to be kept in PBS to prevent from being air dried. Harvest bone marrow promptly. We usually kill 20 mice at a time. The work is divided between two sites: bone isolation in the animal room and marrow harvesting in the tissue culture room. We process bones from 5 mice each time.
3. imDC concentration of 0.5 × 10^8 to 1 × 10^8 cells/mL is used during electroloading.
4. The MaxCyte electroporation instrument system allows for flexible configuration and processing utilizing a sterile closed fluid path (19, 20). The instrumentation and cell processing assemblies are described in MaxCyte’s Master File on record with Center for Biologics Evaluation and Research (CBER), the United States Food and Drug Administration (FDA), and Health Canada. The FDA Master File (no. BBMF10702) has been referenced in four instances of review for Investigational New Drug (IND) applications and is currently in use for clinical manufacturing of biological products. MaxCyte’s systems are scalable from R&D to clinical/commercial scale and accommodate sample volumes in the 20-µL (1 × 10^6 cells) to 1,000-mL (1 × 10^11 cells) range. The instruments are capable of delivering an optimal amount of energy (40 J/mL) for efficiently electroloading imDCs (with > 90% cells loaded) with multiple molecules without significant
observable loss in cell viability (>90% as assayed by trypan blue exclusion) and with high cell recovery (~80 to 90%), which results in enhanced biological activity for imDCs and in the clinical implementation of such optimized procedures in a current good manufacturing practices (cGMP) environment.

5. FACS analysis is used to identify optimal electroloading conditions to obtain efficient loading while the cells maintain good cell viability measured by FITC-dextran uptake and PI staining exclusion respectively.

6. Various ratios of imDC vs. tumor cells can be tested.

7. One person should be responsible for measuring the tumors from the beginning to the end of the experiment to avoid investigator variation among measurements.

8. We used spleens from the C57BL6 mice that received treatment with LLC tumor lysate electroloaded DCs.

9. All liquid and solid waste starting from this step shall be treated cautiously and discarded in appropriate containers for radioactive materials.

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