Video Article

Experimental Metastasis and CTL Adoptive Transfer Immunotherapy Mouse Model

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Abstract

Experimental metastasis mouse model is a simple and yet physiologically relevant metastasis model. The tumor cells are injected intravenously (i.v) into mouse tail veins and colonize in the lungs, thereby, resembling the last steps of tumor cell spontaneous metastasis: survival in the circulation, extravasation and colonization in the distal organs. From a therapeutic point of view, the experimental metastasis model is the simplest and ideal model since the target of therapies is often the end point of metastasis: established metastatic tumor in the distal organ. In this model, tumor cells are injected i.v into mouse tail veins and allowed to colonize and grow in the lungs. Tumor-specific CTLs are then injected i.v into the metastases-bearing mouse. The number and size of the lung metastases can be controlled by the number of tumor cells to be injected and the time of tumor growth. Therefore, various stages of metastasis, from minimal metastasis to extensive metastasis, can be modeled. Lung metastases are analyzed by inflation with ink, thus allowing easier visual observation and quantification.

Protocol

1. Experimental Metastasis Mouse Model

1. On the day before the tumor cell injections, seed one T75 flask with up to 1×10^7 CMS4-Met cells in 10 mL of RPMI medium containing 10% serum to obtain fast-growing tumor cells. Incubate overnight at 37°C.
2. On the day of the injection, remove the medium and rinse the cells once with PBS, then harvest the tumor cells with 0.05% trypsin-EDTA at 37°C for 5 minutes. Stop the reaction with 10 mL of RPMI medium containing 10% serum. Transfer cells to a conical tube.
3. Spin down the cells at 1300 rpm in a Sorvall Legend RT centrifuge for 3 minutes at room temperature. Remove the supernatant. Resuspend the cells in 10 mL fresh 1X HBSS to wash, then spin down again and resuspend in the same manner.
4. Count the tumor cells on a hemocytometer. Dilute the cells in HBSS, so that the cells required for each injection are resuspended in a total volume of 100 μ L.
5. Warm the 6-8 week old BALB/c mice in a beaker immersed in warm water to dilate the tail vein. Place the mouse on a tail vein restrainer on the bench. Use a microsyringe to inject 100 μ L of tumor cells into the lateral tail vein. Use aseptic technique to avoid infection. After injection, apply slight pressure to the injection site until bleeding has stopped.
6. Return the mouse to the cage, and allow the tumor to grow to the desired stage.

2. Cytotoxic T-Lymphocyte (CTL) Adoptive Transfer Immunotherapy

1. On the day of the transfer, pipette up and down to resuspend purified cytotoxic T-lymphocytes, or CTLs, prepared as described in the text. Transfer all of the cells from one plate to a 15 mL conical tube, not letting the volume exceed more than 2/3 of the tube.
2. Insert a sterile Pasteur pipette into the conical tube and lay Lymphocyte Separation Medium, or LSM, under the cells until the total volume nears 14 mL.
3. Be careful not to disturb the layers. Centrifuge at 2000 rpm for 20 minutes at room temperature. Do not use the brake to slow the rotor after spinning.
4. Transfer the CTLs to a new 15 mL conical tube. Add HBSS to a volume of approximately 10 mL to wash.
5. Count the cells. Spin down at 1300 rpm for 5 minutes at room temperature. Resuspend in HBSS to the required cell density for injections, keeping the total volume per injection at 100 μ L.
6. Use the same tail injection technique as seen earlier in this video to inject the CTLs into tumor-bearing mice.
7. Return the mouse to the cage, and allow the CTLs to interact with the tumor. Mice are usually sacrificed for analysis 14-21 days after CTL treatment.

3. Visualization of Lung Metastases

1. To visualize lung metastases, place a sacrificed mouse on its back on a Styrofoam board. Pin the legs to ensure unobstructed access to the trachea. Spray the ventral side with 70% ethanol.
2. Starting from the mid-abdomen, use scissors to cut along the midline, through the ribcage, and up toward the salivary glands. Expose the trachea. Use forceps to remove tissues surrounding the trachea.
3. Thread a 200 μ L pipette tip underneath the trachea. Holding the tip with one hand, gently lift the trachea up and away from the body.
4. Rotate the platform 180°. Use a 50 mL syringe to inject India Ink into the lungs via the trachea. Completely inflate the lungs with ink until you feel a strong resistance.
5. Use scissors to cut the trachea. Slide a pair of forceps under the lungs and lift them out of the mouse. Rinse the lungs briefly in a 1L beaker of water.

6. In a chemical fume hood, transfer the lungs to a glass scintillation vial containing 5 mL of Fekete's solution. The tumor tissue will emerge as white nodules on the black lungs after a few minutes. The tumors can now be counted and stored in Fekete's solution indefinitely.

4. Representative Metastases in Visualized Lungs

1. Here, lungs from mice that were injected with mammary carcinoma cell line 4T1 show white spots indicating tumors. Mice that were injected with 4T1 cells transfected with an IRF8 dominant-negative mutant K79E show the enhanced metastatic potential of tumor cells.
2. These images show a histological analysis of efficacy of CTL adoptive transfer immunotherapy. Tumor-bearing mice injected with saline showed multiple tumors 6 days later, indicated by yellow arrows (A). Mice injected with tumor-specific T cells, on the other hand, showed a reduction of tumors (B).
3. In the same experiment, India ink treatment gives a simpler way to measure the efficacy of CTL adoptive transfer. Here, the white tumor nodules on tumor-bearing lungs clearly distinguish them from lungs that have successfully undergone CTL adoptive transfer, and counting the nodules allows for a degree of quantitation not possible with histology.

5. Representative Results

Figure 1. Experimental scheme for experimental tumor metastasis and CTL adoptive transfer immunotherapy mouse model. Red dots indicate tumor cells and green dots indicate CTLs.

Figure 2. Disruption of IRF8 function enhanced the metastatic potential of tumor cells. Mouse mammary carcinoma cell line 4T1 was stably transfected with vector (4T1.Vector) or vector expressing an IRF8 dominant-negative mutant K79E (4T1.IRF8K79E) (15, 16). Tumor cells were injected i.v. into mouse lateral tail veins. Tumor-bearing lungs were inflated with India ink to visualize tumor nodules. Tumor nodules are easily seen as white spots on the black lung tissue background.

Figure 3. Histological analysis of efficacy of CTL adoptive transfer immunotherapy. Mouse sarcoma cell line CMS4-Met was injected i.v. into mouse lateral tail veins. Three days later, saline (A) or tumor-specific T cells (B) were injected into the tumor-bearing mice. Lungs were analyzed six days after CTL treatment by conventional H&E histological staining. Tumor nodules are indicated by arrows.

Figure 4. Visualization of tumor nodules by India ink inflation. Mouse sarcoma cell line CMS4-Met was injected i.v. into mouse lateral tail veins. Three days later, saline (Control) or tumor-specific T cells (+CTL) were injected into the tumor-bearing mice. Lungs were analyzed fourteen days after CTL treatment. The white spots, which are tumor nodules, allow for easy quantification of the efficacy of CTL treatment.

Disclosures

Mice were purchased from the National Cancer Institute (Frederick, MD) and housed in the Medical College of Georgia animal facility. Experiments and care/welfare were in agreement with federal regulations and an approved protocol by the Medical College of Georgia IACUC committee.

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