Protocol 202_Preparation of bone marrow-derived mouse DC

I. MATERIALS:

1. RPMI 1640 medium (GIBCO-BRL, Gaithersburg, MD): complete RPMI 1640 medium contains 10% FCS (GIBCO-BRL), 50 μM b-mercaptoethanol and 100 U/ml penicillin/streptomycin (GIBCO-BRL).
2. Recombinant mouse GM-CSF (rmGM-CSF): 200 U/ml in complete medium.
3. 10 cm tissue culture dishes
4. Sterile scissors and pincers for mouse bone marrow preparation.
5. Disposable 1 ml syringes and 23G needles.

II. METHODS:

1. Prepare the bones of the hind legs (femurs and tibia) of 8-12 weeks old mice, remove all muscle tissue and leave bones in complete medium until processed further. Bones can be disinfected for 2 min in 70% ethanol, but this is not mandatory if all manipulations are done under aseptic conditions.
2. Cut both ends of bones with sterile scissors/ scalpel. Put 1.5ml R10 into a sterile 5ml polypropylene tube and flush out bone marrow using a 1 ml syringe with a 23G needle, by first sucking medium up through the bone, then expelling it back into the tube. Pool bone marrow of hind legs from one mouse.
3. Strain the cells through a 70μm filter into 12.5ml R10. Transfer supernatant to 10 cm tissue culture dish. Incubate cells 30 min at 37 °C and 5% CO_2. (This step can be omitted but final CD11c% is lower)
4. Recover non-adherent and loosely adherent cells from the dish by carefully resuspending them and transfer cells to a 50 ml Falcon.
5. Adjust volume to 24 ml f.v. with complete medium with GM-CSF. Seed 4 ml cell suspension per well into a 6-well-plate (1 x plates/mouse). Culture cells at 37 °C and 5% CO_2 (see Note 2).
6. On day 2 of culture perform a partial medium change by removing 2.5 ml (6-well-plate) culture medium per well and adding 3 ml new complete medium. The freshly added medium should contain 200 U/ml rmGM-CSF calculate for the whole volume per well.
7. On day 3 of culture remove all non-adherent cells and wash off all loosely-adherent cells with culture medium and discard (see Note 1). The remaining cells consist of an

adherent macrophage-like cell population and small round progenitor cells attached to them. Add 4 ml new complete medium per well plus 200 U/ml rmGM-CSF.

8. On day 4 or 5 DC are still in an immature stage (low B7.1, B7.2 and CD40) and should be used for experiments. Expect 1-2 x 10^7 cells on day 5

III NOTES:

1. For generation of mouse DC of good purity, it is on one hand crucial to remove all non-adherent cells at day 3, but on the other hand one should avoid loosing the small round progenitor cells that are attached to the adherent cell layer. Loss of progenitor cells at day 3 results in decreased DC yield. Therefore non-adherent cells should not be removed before day 3, since some progenitor cell will not attach to the adherent cell layer before day 3.
2. To ensure that high numbers of functional mouse DC are generated, it is crucial to maintain sufficiently high levels of rmGM-CSF. New rmGM-CSF should be at least added every second day calculated for the entire volume of culture medium.