Macrophage primary culture

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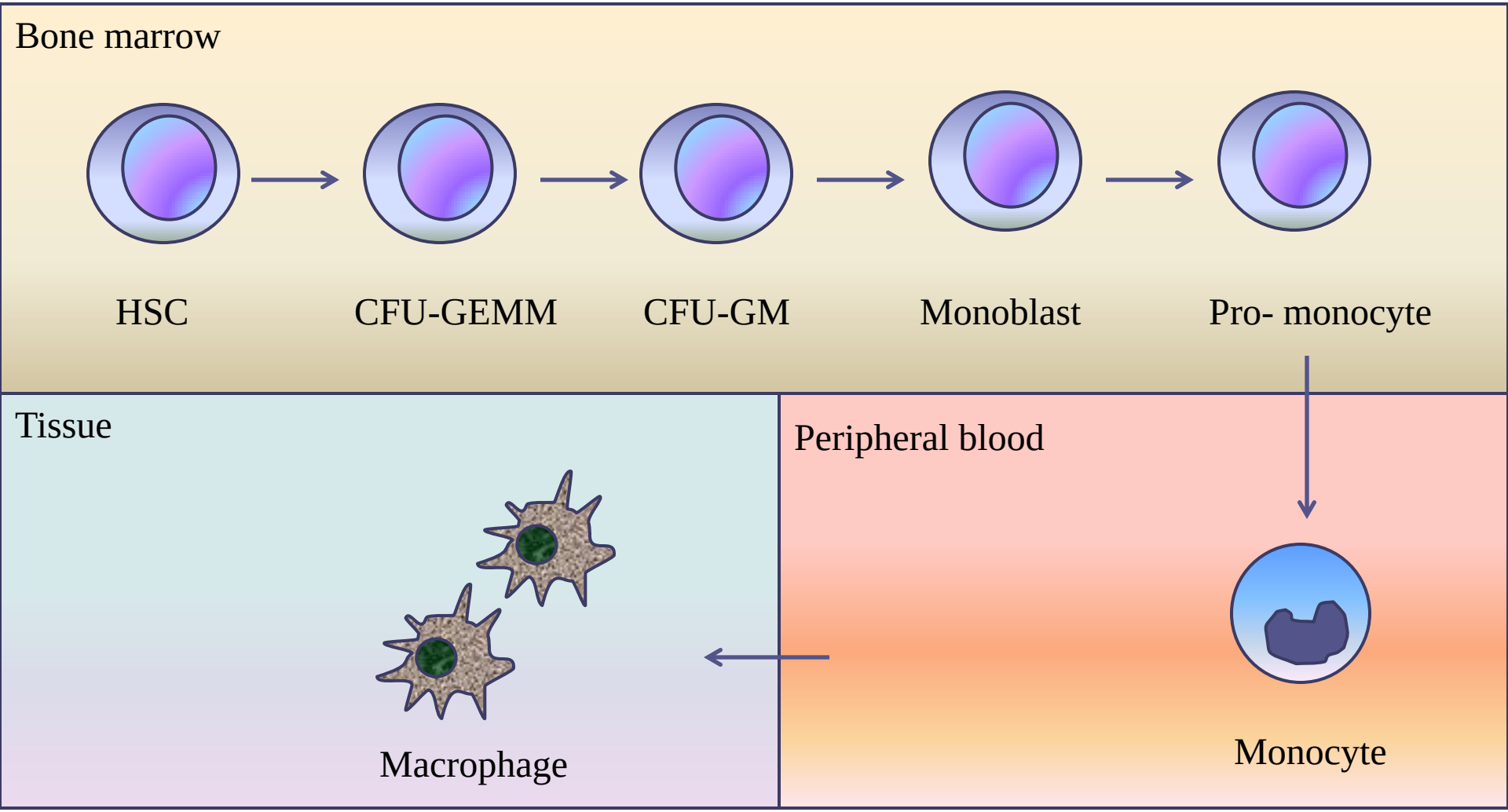
Macrophage differentiation



- In many cases, in vitro experiment with cell line is more convenient and timesaving than in vivo experiment.

How to get macrophage cell line?

- Immortalized macrophage-like myeloid cell lines
- Such as：
  - J774A.1 (*Mus musculus*)
  - RAW264.7 (*Mus musculus*)
  - P388D1 (*Mus musculus*)
  - U-937 *(Homo sapiens)*
  - THP-1 (*Homo sapiens*)

- THP-1 cell line
  - a human monocytic cell line derived from an acute monocytic leukemia patient.
  - can be differentiated into macrophage-like cells using phorbol 12-myristate 13 (PMA).

How to get macrophage cell line?

- Primary macrophages culture
  - Bone marrow-derived macrophages(BMM)
  - Peritoneal macrophages (PM)
  - Alveolar macrophages (AM)

Colony-stimulating factor (CSF)

- Include:
  - CSF1 - Macrophage colony-stimulating factor (M-CSF)
  - CSF2 - Granulocyte macrophage colony-stimulating factors (GM-CSF)
  - CSF3 - Granulocyte colony-stimulating factors (G-CSF)
  - Synthetic - Promegapoietin

- L929 cell line：
  - Murine aneuploid fibrosarcoma cell line
  - secrete a factor that is identical with murine M-CSF. Therefore conditioned medium of L929 cells is used frequently as a crude source of murine M-CSF.

Bone marrow-de phages (protocol)

Don't broke the bone; and ensure all tissues are removed.

Flush the bone immediately following the cutting of the bone.

Sacrifice mice (8-10 week old)

Isolate femur and tibia

flush the bone marrow and diluted into 10% RPMI1640 medium

Filter with 70 μm filter

Centrifugate at 500g, 8min, 4 °C. Discard supernatant.

Resuspend with complete RPMI 1640 medium (supplemented with 10%FBS, 10%L929 medium and 1%PSF)

All steps should be performed in aseptic condition

Flow chart

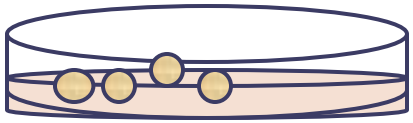
Bone marrow-derived macrophages culture (protocol)

Plate cells into six-well plate with complete medium

On the 5th day, add additional 2ml complete medium

On the 6th day, change medium with complete medium.

On the 7th day, change mediumwith RPMI 1640 medium supplemented with 10%FBS



Flow chart

Identification methods of macrophage

- Morphological observation
- Enzyme cytochemistry
- Phagocytosis Assay
- Flow cytometry or Immunocytochemistry
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Thanks for your attention!