From stem cell to blood cell: flow cytometry of the differentiation pathway
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Maturation of Blood cells

Essentials for Immunophenotyping of leukemia and lymphoma

How do you differentiate between normal and malignant populations
• Pattern recognition:
  • What is the normal pattern of expression?
  • What is the aberrant pattern of expression?

??How to Discriminate between Cells
And cell differentiation stages??

How to start??
CD45 pattern

Normal

B-CLL

Immunological differentiation based on CD45

B-cell differentiation

T-cell differentiation

Phenotyping of the Myeloid Lineage to identify the different maturation stages

Search for the myelo-monocytic progenitor cells

Phenotypic changes in the neutrophilic differentiation pathway

1. Expression pattern of CD34 / CD117
   in CD45+ population
   Normal bone marrow

Ref. Orfao et al.
Myelomonocytic differentiation/maturation share the same progenitor cells!

Phenotyping of the Myeloid Lineages

CD11b / CD13 / CD16 / CD45

2. Expression pattern of CD13, CD16, CD11b within CD45

2.a. Expression pattern CD13/CD11b/CD16 (normal BM)

2.b. Expression pattern CD13/CD11b (normal BM)

Conclusion

(myeloid differentiation pattern in CD45)
Aberrant pattern of CD13/CD16 in MDS

Aberrant pattern of CD13/CD16 in AML

3. Expression pattern

CD11b, CD15, HLA-DR

More detailed phenotyping of the Myeloid and Monocytic Lineages by CD11b / CD15 / HLA-DR / CD45

3.a. Expression pattern CD11b/CD15/HLA-DR in CD45+ population (normal BM)

Monocytic and myeloid lineage

3.b. Expression pattern of CD11b/CD15/HLA-DR in CD45+ population (normal BM)

Myeloid and Monocytic

….BUT THERE IS MORE!.... Macrophages? Erytroid cells?
MACROPHAGES are MONOCYTES that have come out of circulation and have gone into tissue.

4.a. Where are the Macrophages?
Maturation of the monocytes based on CD14 and CD16

4.b. Maturation from monocytes to macrophages (details)
4.c. Maturation from monocytes to macrophages (CD80 and CD86)

But how can we characterize these CD36+ cells

5.b. Erythroid pathway

Using CD235a/CD117/CD71

Elimination of myeloid cells from the erythroid lineage (CD36+CD33-)

Evidence of Macrophages by CD80 and CD86

Normal erythroid maturation

CD117 / CD71 / CD235a / CD36

Which lineage represents in the black population?
Summary

1. Myeloid differentiation
2. Monocytic differentiation
3. Separation point of myeloid and monocytic
4. Erythroid differentiation

How to show all the lineages in one plot??

*The Radar plots*
Erythropoiese (CD36+CD33- and CD105/CD117)

Hematopoiese (Myeloid - Monocytic - Erythroid)

Normal hematopoiese

CMML and mastocytosis

AML without differentiation and monocytic characteristics

MDS with dyserythropoiesis (increase of CD71dim/CD71CV)
MDS with dyserythropoiesis (increase CD71 CV)

MDS

Normal control

Conclusions

- CD11b/CD13/CD16 and CD11b/CD15/HLADR are essential marker combinations to study the myelo/monocytic maturation pathways
- In relatively mature myeloid stage, the SSC is expressed from dim to bright with increase in CD45 expression
- CD14/CD36/CD16 are essential marker combinations to study the monocytes and macrophages
- CD36/CD235a/CD117/CD71/CD105 are essential marker combinations to study the erythroid differentiation
- Radar plots are very useful to study
  - The differentiation pathways of the different lineages
  - The differentiation variation between normal and aberrant

He who is blind to the view of our plots, will not enjoy and see maturation as it is.

Thanks for your attention