Monocyte subsets in health and disease

Marion Frankenberger
Composition of whole blood

main cellular components:
- Leukocytes
- Erythrocytes

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Buffy Coat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 92% by weight</td>
<td>Platelets 120–300 thousand per cubic mm</td>
</tr>
<tr>
<td>Proteins 7% by weight</td>
<td>Leukocytes 5–10 thousand per cubic mm</td>
</tr>
<tr>
<td>- Albumins 58%</td>
<td>- Neutrophils 60–70%</td>
</tr>
<tr>
<td>- Globulins 37%</td>
<td>- Lymphocytes 20–25%</td>
</tr>
<tr>
<td>- Fibrinogen 4%</td>
<td>- Monocytes 3–8%</td>
</tr>
<tr>
<td>- Regulatory proteins 1%</td>
<td>- Eosinophils 2–4%</td>
</tr>
<tr>
<td>- Electrolytes</td>
<td>- Basophils 0.5–1%</td>
</tr>
<tr>
<td>- Nutrients</td>
<td></td>
</tr>
<tr>
<td>- Respiratory gases</td>
<td></td>
</tr>
<tr>
<td>- Waste products</td>
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</tbody>
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Erythrocytes
- Erythrocytes 4.2–6.2 million per cubic mm

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Monocytes belong to the cellular components of peripheral blood.
Properties of monocytes

As part of the innate immune system monocytes are responsible for

- phagocytosis  bacteria, viruses, particles
- antigen presentation
- cytokine production  TNF, IL-1, IL-6, IL-8, IL-10
Cells of the immune system (Haematopoiesis):

all blood cells derive from bone marrow

From: Robert G. Ramsay & Thomas J. Gonda
No further differentiation possible by microscopy techniques

Monocyte → Monocyte subsets??

Lymphozyte

T-Zelle ???
B-Zelle ???
NK-Zelle ???
Immunofluorescence
Analysis of cells by flow cytometry
FACS = fluorescense activated cell sorter

Becton Dickinson BD LSR II Flow Cytometer
Principals of flow cytometry

- single cell suspension
- droplets with single cells

From: http://flow.csc.mrc.ac.uk
Forward scatter FSC  
(cell size)

Side scatter SSC  
(granularity)
Scatter properties of whole blood

- Granulocytes
- Monocytes
- Lymphocytes

PBMC* - peripheral blood mononuclear cells
CD markers for staining of immune cells
CD = cluster of differentiation

• Lineage markers:
  • CD3 T-cells
  • CD14 monocytes
  • CD15 neutrophils
  • CD19 B-cells
  • CD56 NK-cells

Used to identify cell populations by a single cell surface marker
CD14 staining on blood monocytes

at least two different monocyte subsets can be detected with CD14-FITC
Other markers used for further monocyte differentiation in human blood

<table>
<thead>
<tr>
<th>Antigen</th>
<th>function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>LPS receptor</td>
</tr>
<tr>
<td>CD16</td>
<td>Low affinity Fcγ-receptor</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Class II antigen</td>
</tr>
<tr>
<td>CD45</td>
<td>Pan leukocyte antigen</td>
</tr>
</tbody>
</table>
CD45 / CD14 / CD16 / HLA-DR

3 monocyte subsets can be distinguished:

- **Classical**: CD14++CD16-
- **Intermediate**: CD14++ CD16+
- **Non-classical**: CD14+CD16++
Nomenclature of monocytes in blood

From: Ziegler-Heitbrock et al
Blood (2010)
LPS-induced TNF expression in human blood monocyte subpopulations

Intracellular TNF-production

non-classical

classical

Belge et al. (2002) J Immunol
Properties of CD16-positive non-classical monocytes

*expanded in inflammatory processes (sepsis, HIV, RA)
*expanded in solid tumors
*main source of LPS-induced TNF
*no production of IL-10
Additional marker to further characterize the CD16-positive monocytes

6-sulfo LacNAc (slan) carbohydrate residue that is O-linked via a 6-O-sulfotransferase to the P-selectin glycoprotein ligand (PSGL-1) on the surface of blood leukocytes
Donor 2059 healthy control 26.11.2013: Classical 417.6/uL; intermediates 16.8/uL; non-classical 39.3/uL
SLAN-positive monocytes 28.1/uL; SLAN-positive non-classical monocytes 26.3/uL; SLAN-negative intermediate monocytes 31.2 /uL
with slan it is possible to differentiate between intermediate and non-classical monocytes
Sarcoidosis

- Slan-positive non-classical monocytes
- Slan-negative intermediate monocytes
- Classical monocytes
- Intermediate monocytes
- Non-classical monocytes

increase
Increased intermediate monocytes in Sarcoidosis

![Graph showing increased intermediate monocytes in Sarcoidosis](image)

Figure 6


HDLS decrease

CD14 PE-Cy5-A

CD16(3G8) PE-Cy7-A

CD14 PE-Cy5-A

non-classical monocytes

intermediate monocytes

classical monocytes

slan-positive non-classical monocytes

slan-negative intermediate monocytes

Depletion of slan-positive non-classical monocytes in hereditary diffuse leukodystrophy with axonal spheroids (HDLS)

HLDS
Neurological white matter disease that is clinically dominated by behavioural change, depression and dementia.
On molecular base HDLS is associated with mutations in the M-CSFR gene.
Lack of CD16+ monocytes can occur without disease

From: Frankenberger et al. Immunobiology (2013)
Depletion of non-classical monocytes under GC-therapy

<table>
<thead>
<tr>
<th></th>
<th>Control donor [cells/µl]</th>
<th>Patient under GC-therapy [cells/µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>classical</td>
<td>344 / µl</td>
<td>687 / µl</td>
</tr>
<tr>
<td>intermediates</td>
<td>12 / µl</td>
<td>18 / µl</td>
</tr>
<tr>
<td>non-classical</td>
<td>62 / µl</td>
<td>4 / µl</td>
</tr>
</tbody>
</table>
## Monocyte subsets in mice

<table>
<thead>
<tr>
<th>Human monocytes</th>
<th>Mouse monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical CD14^{++}CD16^{-}</td>
<td>Classical Ly6C^{++}CD43^{+}</td>
</tr>
<tr>
<td>Intermediate CD14^{++}CD16^{+}</td>
<td>Intermediate Ly6C^{++}CD43^{++}</td>
</tr>
<tr>
<td>Nonclassical CD14^{+}CD16^{++}</td>
<td>Nonclassical Ly6C^{+}CD43^{++}</td>
</tr>
</tbody>
</table>

**TNF production:**

- **Nonclassical**
  - CD43^{+} isotype, 71%
- **Classical**
  - CD43^{+} isotype, 32%
Conclusions

- Monocytes in human blood can be subdivided in three subsets
  - Classical
  - Intermediate (slan-negative)
  - Non-classical (slan-positive)

- Increase of CD16+ monocytes in sepsis, solid tumors, HIV, RA
- Increase of intermediate monocytes in sarcoidosis
- Depletion of non-classical monocytes in HDLS
- Depletion of non-classical monocytes under GC-therapy
Human monocytic system

Myelo-Monocytic Stem Cell

Monoblast

Classical monocyte

Intermediate monocyte
Non-classical monocyte

Macrophage

Alveolar Macrophage
Microglia Cell
Kupffer Cell
Synovial Macrophage
Langerhans Cell
Osteoclast
Peritoneal Macrophage

Inflammatory Macrophage
Small sputum Macrophage
Tumor-associated Macrophage
even larger heterogeneity of tissue macrophages

...but this would be topic of a further lecture...!
Thank you for your attention!