Anemias of Inflammation, Cancer and Chronic Kidney Disease

Tomas Ganz, PhD, MD
University of California, Los Angeles
Outline

• Scope: Anemia caused by inflammation (infections, inflammatory diseases, cancer, chronic kidney diseases)
• Clinical picture
• Pathogenesis
• Simple models
  – IL-6 and hepcidin
  – Interferon-γ
• Realistic models
• Therapy
Anemia of inflammation (AI)

- Old name: “Anemia of chronic disease”
- Seen in the setting of systemic inflammation
- Usually mild to moderately severe, Hb > 7 g/dl
- Usually normal appearing erythrocytes but sometimes small and/or less hemoglobinized
- Decreased serum iron and transferrin saturation
- Bone marrow macrophages contain Fe
- Normal or increased serum ferritin
Erythrocyte morphology

Normal or AI  Iron deficiency
Anemia of inflammation

- Bacterial, fungal, parasitic infections
- **Autoimmune** inflammatory diseases (rheumatologic, inflammatory bowel diseases, kidney diseases)
- Acute forms in the **intensive care** units (sepsis, exacerbated by blood loss) and burn units (exacerbated by hemolysis)
- Many **tumors** (myeloma, lymphomas, Ca ovary)
- Similar pathogenesis also contributes to anemias of **chronic kidney disease**
Anemia of cancer

• Pathogenesis heterogeneous, depending on the disease and its treatment
  – Inflammation in response to tumor \(\Rightarrow\) if predominant, similar to AI
  – Marrow toxicity/loss of erythroid precursors from treatment
  – Infections complicating cancer or its treatment
  – Iron deficiency from bleeding tumor or due to clotting problems
  – Malnutrition and nutrient deficiencies
  – Hemolysis or loss of erythroid precursors from autoimmunity
Anemia of chronic kidney disease

- Erythropoietin deficiency caused by transdifferentiation of erythropoietin-producing cells into myofibroblasts
- Inflammation, depending on the disease process and its treatment, pathogenesis similar to anemia of inflammation
- Uremic toxins may also contribute to decreased erythropoiesis and shortened erythrocyte survival
Pathophysiology of AI

- 1950-70s: Cartwright, Wintrobe, Finch
- Inflammation causes iron sequestration in macrophages
- Iron restriction limits erythropoiesis
- Erythrocyte lifespan is mildly decreased
- Later: In some inflammatory conditions there is suppression of erythroid differentiation
Molecular mechanisms of AI

Infection
Autoimmunity
Chronic Kidney Disease
Tumor

↓ erythropoiesis
↓ erythrocyte survival

↑ Interferon-γ

↑ IL-1

↑ IL-6

↑ hepcidin
↓ plasma iron
↓ erythropoiesis

↓ uremic toxins

↓ EPO

↓ erythropoiesis

↑ PU.1
↓ GATA-1
↑ leukopoiesis
↓ erythropoiesis

↓ macrophage activation
↑ endothelial activation
↑ opsonization
↓ erythrocyte survival

↓ EPO
↓ erythropoiesis

↓ EPO
↓ erythropoiesis

↓ EPO
↓ erythropoiesis
Anemia and hypoferremia may be adaptive during infections

• Hypoferremia and particularly the prevention of NTBI formation may restrict the availability of iron to extracellular pathogens
• Shift of hematopoiesis from erythropoiesis to leukopoiesis may strengthen host defense
Siderophilic microbes

• Pathogenicity increased by iron:
• Vibrio vulnificus, Yersinia enterocolitica
• But also: K. pneumoniae, E. coli....

27th human infection in Florida is confirmed in elderly Jacksonville-area man
Flesh-eating bacteria surface as Florida's latest deadly fright

October 3, 2013
A flesh-eating bacterium that thrives in warm coastal waters has kept a low-profile for years in Florida, a state better known for shark bites, alligator attacks and python plagues. But *Vibrio vulnificus* has been grabbing headlines recently after the death of a Flagler County crabber — the ninth in the Sunshine State this year linked to the microscopic killer.
I. Inflammation and iron homeostasis

• Inflammation causes sequestration of iron in macrophages and decreased iron absorption
• These effects are largely but not exclusively mediated by hepcidin
• Hepcidin production is increased predominantly by IL-6 in inflammation and BMP-2 in myeloma
IL-6 induces hepcidin during infections

Strep. pneumoniae

Influenza virus

IL-6 causes anemia partly by inducing hepcidin

In the subchronic IL-6–induced anemia model, cynomolgus monkeys were injected with IL-6 (daily, 0.3 µg/kg) and the hepcidin antagonist NOX-H94 (daily, 10 mg/kg)

Iron restriction inhibits erythropoiesis and hemoglobin synthesis

TABLE 5. Number of nucleated erythroblasts in marrow and in spleen in IDA rats after iron repletion

<table>
<thead>
<tr>
<th>IDA</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>15 ± 1</td>
<td>19 ± 2</td>
<td>28 ± 3</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>No. of erythroblasts (× 10^-6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marrow</td>
<td>1,607 ± 283</td>
<td>2,736 ± 418</td>
<td>2,792 ± 482</td>
<td>2,913 ± 115</td>
</tr>
<tr>
<td>Spleen</td>
<td>131 ± 28.5</td>
<td>304 ± 97</td>
<td>1,011 ± 316</td>
<td>1,148 ± 220</td>
</tr>
<tr>
<td>Total (marrow + spleen)</td>
<td>1,738 ± 257</td>
<td>3,040 ± 513</td>
<td>3,801 ± 367</td>
<td>4,060 ± 335</td>
</tr>
</tbody>
</table>

*Days after iron administration. Each group consisted of four rats, except only two rats were studied on day 4.
Inflammation causes iron-restrictive anemia via IL-6 and hepcidin.
Excess hepcidin in uninflamed mice causes a microcytic anemia different from AI

<table>
<thead>
<tr>
<th>Assay</th>
<th>Hepcidin WT</th>
<th>Hepcidin +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Fe, µg/g</td>
<td>177 ± 109</td>
<td>57 ± 60</td>
</tr>
<tr>
<td>Spleen Fe, µg/g</td>
<td>206 ± 62</td>
<td>184 ± 56</td>
</tr>
<tr>
<td>Liver Fe, µg/g</td>
<td>92 ± 28</td>
<td>36 ± 9</td>
</tr>
<tr>
<td>S:L ratio</td>
<td>2.34 ± 0.71</td>
<td>5.56 ± 2.79</td>
</tr>
<tr>
<td>Hemoglobin level, g/dL</td>
<td>15.6 ± 0.5</td>
<td>13.1 ± 1.5</td>
</tr>
<tr>
<td>Mean cell volume, fL</td>
<td>50.9 ± 1.3</td>
<td>40.7 ± 4.2</td>
</tr>
<tr>
<td>Reticulocytes, × 10⁹ cells/L</td>
<td>254 ± 80</td>
<td>366 ± 98</td>
</tr>
<tr>
<td>Reticulocyte hemoglobin content, pg</td>
<td>15.8 ± 0.3</td>
<td>12.5 ± 1.5</td>
</tr>
</tbody>
</table>

Excess hepcidin in uninflamed humans causes a microcytic anemia different from AI (IRIDA)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age at evaluation</th>
<th>↓Hb (g/dl)</th>
<th>↓↓MCV (fl)</th>
<th>Retics (%)</th>
<th>↓↓Transferrin saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>6 y</td>
<td>8.8</td>
<td>58</td>
<td>n.d.</td>
<td>2</td>
</tr>
<tr>
<td>F</td>
<td>13 mo.</td>
<td>9.2</td>
<td>65</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td>M</td>
<td>17 mo.</td>
<td>7.0</td>
<td>49</td>
<td>n.d.</td>
<td>5</td>
</tr>
<tr>
<td>F</td>
<td>11 y</td>
<td>8.2</td>
<td>56</td>
<td>1.6</td>
<td>3</td>
</tr>
<tr>
<td>M</td>
<td>7 y</td>
<td>7.5</td>
<td>49</td>
<td>0.6</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>3 y</td>
<td>9.7</td>
<td>61</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>M</td>
<td>15 mo.</td>
<td>7.9</td>
<td>53</td>
<td>0.8</td>
<td>2</td>
</tr>
</tbody>
</table>

 Serum and urinary hepcidin high normal to mildly elevated

II. Iron-independent effects of inflammation

- Suppression of erythropoiesis
- Decreased erythrocyte survival
Hepcidin-independent mediators of anemia of inflammation

• Interferon-γ
  – Secreted by activated T-cells and NK-cells during inflammation
  – GATA-1 to PU.1 switch promotes leukopoiesis (especially monocytes) at the expense of erythropoiesis (Libregts et al. Blood 2011)
  – Increased erythrophagocytosis, shorter erythrocyte survival

• HMGB-1: suppression of erythropoiesis
  – High-mobility group protein 1 (HMG-1)
  – Activated macrophages and monocytes secrete HMGB1 as a cytokine
Heat-killed Brucella abortus (BA): inflammation, erythropoietin suppression, iron restriction and shorted erythrocyte lifespan

200 µL (5x10^8 particles)

Time course: 0 (no injection), 3h, 6h, 12h, 1d, 2d, 3d, 7d, 14d, 21d, 28d

III. Realistic models

Kim et al. Blood 2014
**Inflammation**

### SAA-1

- **Time:** 0, 3h, 6h, 1d, 7d, 14d, 21d, 28d
- **SAA-1 mRNA ddCT:**
  - Saline
  - BA

### WBC

- **Time:** 0, 3h, 6h, 12h, 1d, 2d, 3d, 7d, 14d, 21d, 28d
- **WBC (K/µL):**
  - Saline
  - BA

*Kim et al. Blood 2014*
Erythropoietic suppression

Hgb

Epo

serum Fe

retics

Kim et al. Blood 2014
Iron restriction

Time
0  3h  6h  12h  1d  2d  3d  7d  14d  21d  28d

Hepcidin mRNA ddCT
-6  -4  -2  0  2  4  6  8  10

Saline
BA

Hemoglobin (g/dL)
4  6  8  10  12  14  16  18

Saline
BA

Reticulocyte production index
0  10  20  30  40

Saline
BA

Hgb retics

Hepcidin

Fe restriction

ZPP

Kim et al. Blood 2014

Hepcidin suppression by epo

Recovery

Suppression
Shortened erythrocyte lifespan

- Macrophage activation
- Opsonization of erythrocytes
- Endothelial activation

Kim et al. Blood 2014
Without hepcidin, inflammation increases plasma iron

- Erythropoietic suppression = ↓utilization of Fe from plasma
- ↑destruction of erythrocytes and other cells = ↑delivery of Fe to plasma

Kim et al. Blood 2014
Anemia of inflammation induced by heat-killed *Brucella abortus* is partly hepcidin-dependent

Kim et al. Blood 2014
Other models of anemia of inflammation

- **Lewis rats** treated with Group A Streptococcal Peptidoglycan-Polysaccharide

- Mice with chronic **turpentine** abscess

- Septic peritonitis after **cecal ligation and puncture**
Anemia of chronic kidney disease from adenine diet is hepcidin-dependent

Hanudel et al. Haematologica 2017
Anemia of chronic kidney disease from adenine diet is hepcidin-dependent
Pathogenesis of anemia of inflammation

• Multifactorial
  – Iron restriction due to hepcidin
  – Suppression of erythropoiesis
  – Shortened erythrocyte lifespan
  – Erythropoietin deficiency in chronic kidney disease
Treatment of anemia of inflammation

• Treat underlying disease if possible

• Is specific treatment of anemia necessary?
  – anemia may be mild or not limiting in the context of underlying disease
  – treatment may involve small risks that exceed small benefits

• Treatment may sometimes be warranted
  – anemia may be severe or performance-limiting
  – ESA + IV iron makes sense in view of pathogenesis: ESAs stimulate erythroid progenitors and suppress hepcidin, iron relieves iron restriction
  – concerns about short and long term risks
  – experimental therapies under development
Experimental therapy of anemias of inflammation

- **Anti-cytokine** therapies (anti-IL-6 or IL-6R, anti-TNF-α, ...) may also treat underlying disease

- **Anti-hepcidin** therapies (under development)
  - hepcidin binders
  - antagonists of hepcidin synthesis or of its upstream regulators

- Blocking the action of hepcidin on **ferroportin (antibodies)**

- Antagonize the **suppression of erythropoiesis**
  - Isocitrate
  - Activin traps