Lookin’ at Leukocytes

BW Parry

Picture perfect

• Start with a good quality blood film: practise, practise, practise
• Then use a good quality microscope!
• Scan the feathered edge and the monolayer area at low power to ensure that leukocytes (WBC) are evenly distributed
• Perform the differential cell count at x400 if cell count is low, but always go to x1000 for the best interpretation of cell morphology
• Separate the leukocytes into:
  o Neutrophils: segmenters, bands, metamyelocytes, in severe inflammation may see myelocytes, rarely promyelocytes or myeloblasts
  o Lymphocytes (note size and shape)
  o Monocytes
  o Eosinophils
  o Basophils (rare)
• Note presence of any NRBC (nucleated erythrocytes; metarubricytes)
  o Express the number of NRBC per 100 WBC
  o NRBC are usually counted as leukocytes (nuclei) by cell counters, therefore need to ‘correct’ the leukocyte count when there are ‘significant’ numbers of NRBC present
  o Formula:
    Corrected WCC = Total nucleated cell count x 100/(100 = n)
    Where n = number of NRBC seen during the 100 cell differential count
• Use the total or corrected WCC (as appropriate) with the % values for the various leukocytes to calculate the absolute numbers of each cell type

Granulopoiesis

Neutrophils, eosinophils and basophils are all granulocytes and undergo the same stages of development (maturation) in the bone marrow

Primary (Non-specific) granules:

• These granules are common to all three tyypes of granulocyte
• They contain myeloperoxidase, lysozymes, acid hydrolases, neutral proteinases
• Numbers of these granules halve with each cell division
Review of myelopoiesis

Secondary (Specific) granules:

- The secondary granules allow neutrophils, eosinophils and basophils to be distinguished from one another
  - **Neutrophil** granules contain substances such as lysozyme, collagenase and lactoferrin
    They stain a ‘neutral’ to ‘pink pale’ colour
  - **Eosinophil** granules contain substances such as major basic protein, arylsulfatase B, histaminase and platelet activating factor
    They stain a red-orange to dark pink colour
  - **Basophil** granules contain substances such as histamine, serotonin and heparin
    They stain a pale to dark blue colour

Under “normal resting circumstances”:

- It takes about 3-5 days for a cell to mature from the myeloblast stage to the segmenter stage
- Granulocytes are released from the bone marrow in an orderly, age-related manner
- Segmented cells, released into the circulation, are incapable of further protein synthesis

Increased granulopoiesis is readily achieved by decreasing the rate of attrition of cells in the bone marrow and increasing the numbers of precursors, while shortening the transit time.
**Neutrophils**

- Neutrophil function: primarily host defense.
- Achieved by: chemotaxis, phagocytosis and phagolysosome formation (killing and digestion)

**Neutrophil "Pools"**

- Proliferation and Maturation Pool
  - Myeloblast to Myelocyte

- Maturation Pool (Bone Marrow Granulocyte Reserve)
  - Metamyelocyte to Segmenter

- Circulating Pool
  - Marginated Pool
  - Segmenter

- Tissues

- In the marginated pool neutrophils are reversibly adherent to capillary and post-capillary venule endothelium
  - In the cat this pool is about 3 times the size of the circulating pool.
  - In the dog, horse and ox the 2 pools are approximately equal in size

**Physiological leukocytosis**

- Neutrophilia resulting from mobilisation of marginated pool
- Adrenaline-mediated ("fright, fight, flight"), associated with fear, excitement, exercise
- Occurs most readily in young healthy animals and lasts about 30 minutes
- Concurrent relative polycythaemia (splenic contraction) and lymphocytosis (increased thoracic duct drainage)
- Relative frequency: Cat > Horse > Ox > Dog
- TNCC generally < 25.0 x 10⁹/l; Lymphocytosis (< 10.0 x 10⁹/l)
**Eosinophils**

- Stages and pools are similar to neutrophils, BUT shorter transit time and minimal bone marrow storage.
- Functions:
  - modulate delayed and immediate type hypersensitivity
  - parasiticidal
  - (phagocytic)

![Canine eosinophils and neutrophils](image)

**Basophils**

- Stages and pools are similar to eosinophils.
- Functions: mediation of delayed and immediate type hypersensitivity release of heparin, histamine (plasma lipoprotein lipase activator).
- NB: Basophils are NOT mast cells or mast cell precursors, although the 2 cells have similar functions.
**Monocytes**

Monocyte Pools

Proliferation and Maturation Pool  Monoblast

Maturation Pool  Promonocyte

Circulating  Marginated  Monocyte

Tissue:  Fixed  Mobile  Macrophage

NB:  Marginated pool is less well defined than for neutrophils.

Tissue macrophages undergo maturation and can proliferate. Macrophages constitute the mononuclear phagocyte system (or “reticuloendothelial” system).

Functions

- Host defense
- Antigen processing in LN: optimal lymphocyte and antigen interaction
- Destruction of senescent cells, denatured proteins, lipids, etc.
- Iron metabolism
- Synthesis of:  transferrin
  Colony stimulating factor
  Interleukin 1 (IL-1), etc.

[Canine monocyte image]
**Lymphocytes**

- Seen one lymphocyte; seen them all? Not quite but almost!

- Subclasses:
  - T cells: T-helper, T-suppressor, T-cytotoxic, T-DTH
  - B cells: Become plasma cells
  - Null cells: Include NK cells (large granular lymphocytes)

- By normal light microscopy these subgroups are indistinguishable (except plasma cells and NK cells).

**Lymphopoiesis**

- Occurs in LN and other specialised lymphoid organs, e.g. thymus, spleen, GALT (gut associated lymphoid tissue; Peyer's patches), bone marrow.

- Varies with the degree and type of antigenic stimulation.

- Lymphoid hyperplasia does not necessarily cause lymphocytosis.

- Number in blood is low at birth, increases in the first weeks/months of life and then tends to decrease with age.

- Most of the lymphocytes in the circulation are T cells.

- Lymphocytes are long-lived cells.
Leukocytes in Disease

General comments

• In response to inflammation, granulocytes are released from the bone marrow in an orderly, age-related fashion.

• Increased granulopoiesis is readily achieved by decreasing the rate of attrition and increasing the numbers of precursors, while shortening the transit time in the marrow.

• NB: It is extremely important to always convert % values to absolute values before interpreting leukograms.

Leukocytosis:

• Almost always caused by a neutrophilia.
• Rarely caused by a lymphocytosis.
• Almost never caused by monocytosis, eosinophilia, basophilia.

Patterns of inflammation

Neutrophilia with a shift to the right

↑

Neutrophilia

↑ ↓

Neutrophilia with regenerative left-shift

↑ ↓

Normal neutrophil count with a left-shift

↑ ↓

Neutropenia with a degenerative left-shift

NB:

• Acute (recent onset) inflammation is usually associated with a lymphopenia and eosinopenia
• Chronic inflammation is often associated with a lymphocytosis (or a normal lymphocyte count)
• Monocytosis does NOT imply chronic inflammation
Neutrophilia

Causes of Neutrophilia

- “Stress”/Corticosteroids
- Inflammation
- Marked erythropoiesis
- Physiological (see earlier, animal should be young and healthy)

“Stress”/Corticosteroids

- “Corticosteroids” can be:
  - Endogenous, e.g. “stress”, hyperadrenocorticism
  - Exogenous, viz therapy

Effects of corticosteroids:

- Neutrophilia:
  - Decreased egress from circulation to tissues
    Consequently possible hypersegmentation
  - Increased bone marrow release of mature neutrophils
    Therefore usually without bands (i.e. no “left shift”)
  - Seen within about 8 hours; lasts 0.5 - 3 days
  - As a generalisation:
    - Dog > Cat > Horse, Ox
    - TNCC < 35, < 30, < 20 x 10^9/l respectively

- NB: Corticosteroids decrease many neutrophil functions including:
  phagocytosis, digestion, glycolysis, amoeboid movement and diapedesis.

- Lymphopenia:
  - Horse usually low normal rather than actually lymphopenic.
  - Probably caused by decreased circulation of lymphocytes,
    i.e. LN sequestration. Possibly also lympholysis (if long-term).
  - Seen within 2 hours; lasts 1 - 3 days.

- “Eosinopenia”
  - Count varies with “previous” eosinophil count.
  - Mechanism: capillary sequestration and decreased bone marrow release
    (short-term); decreased bone marrow production (long-term)
  - Seen within 2 hours; lasts 1 - 3 days
• **Monocytosis**
  - Not always present
  - Frequency: Dog > Cat Others

**Inflammation**

• Neutrophilia will result if tissue demand is exceeded.
• Neutropenia will result if tissue demand is not met.
• More immature neutrophils are released as tissue demand increases or persists at a high level; usually “back” to the band stage. Called a “shift to the left” (or “left-shift”) if numbers are greater than “normal”.
• Magnitude of neutrophilia varies with:
  - Species Dog Cat Horse Ox
    - TNCC 60 40 30 20 x $10^9$/l
  - Type of inflammation: localised > generalized
  - Type of bacteria: pyogenic > non-pyogenic
• Inflammation is “stressful”, therefore frequently concurrent lymphopenia and eosinopenia. [Cortisol] may be normal; it is “never” measured in such situations.
• If there is a normal lymphocyte count or lymphocytosis, the inflammation is likely to be more ‘chronic’ in nature.
• Monocytosis is often concurrent, but does not imply chronicity.

**Marked erythropoiesis**

• Neutrophilia, possibly with a left-shift, can be seen in some cases with a markedly regenerative anaemia.
• Usually see concurrent thrombocytosis.

**Neutropenia**

• Leukopenia is most frequently due to neutropenia.
• Exceptions are animals with a low blood N:L ratio, e.g. pigs and ruminants. In these species lymphopenia very significantly contributes to leukopenia.
Causes of neutropenia:

- Severe inflammation
- Decreased production
- Ineffective production
- Margination

Severe inflammation

- Animals with a high N:L ratio (dog, cat) generally have a large bone marrow granulocyte reserve (BMGR). Consequently it takes a very severe acute inflammatory reaction to cause a neutropenia in such species.

- In contrast, animals with a low N:L ratio generally have a smaller BMGR and neutropenia may develop more easily with severe inflammatory demand. Therefore, in ruminants, this is not as poor a prognostic finding as other species, UNLESS it persists or develops after an observed neutrophilia.

- Typical N:L ratios (adults) are:

<table>
<thead>
<tr>
<th>Dog</th>
<th>Cat</th>
<th>Horse</th>
<th>Pig</th>
<th>Ruminant</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5:1</td>
<td>2:1</td>
<td>1:1</td>
<td>1:1.5</td>
<td>1:2</td>
</tr>
</tbody>
</table>

Decreased production

- Possibly with thrombocytopenia
- Possibly without anaemia

Causes include:

  - Infectious agents; especially those which attack the stem cells, e.g. FePLV, canine parvovirus, FeLV, toxoplasmosis.
  - Toxicity, e.g. oestrogens (exogenous - bitches, endogenous - ferrets), chemotherapy.
  - Myelophthisis
  - Cyclic haematopoiesis of grey Collies

Ineffective granulopoiesis

- FeLV infection

Margination

- Anaphylaxis
- Endotoxaemia: early
Adequacy of the bone marrow response to inflammation

This can be gleaned from the leukogram:

- Mature neutrophilia (i.e. without a left-shift) = good bone marrow response
- Neutrophilia with a “regenerative” left-shift = good bone marrow response
- Marked neutrophilia with a marked left-shift (in number of cells and “degree to the left”) is called a “leukemoid” reaction = good bone marrow response
- DDx Chronic granulocytic leukaemia
- Normal neutrophil count with a left-shift = questionable bone marrow response; repeat leukogram in 1-2 days (sooner if clinical situation deteriorates).
- Neutropenia with a “degenerative” left-shift = poor (“inappropriate”) bone marrow response
- Neutrophilia with a “shift to the right” may be seen in cases where a localised pyogenic focus is abruptly removed and the bone marrow is in a state of myeloid hyperplasia. For example: after ovariohysterectomy for pyometra in the bitch. Neutrophils become hypersegmented (age) in the circulating because of ablated tissue demand.

“Toxic change”:

- Mature neutrophils have a clear to slightly pinkish cytoplasm. Such cells have ‘lost’ virtually all of the cytoplasmic machinery for production of proteins etc., which is one of the reasons for the cells’ short life-span.
- Younger cells may have some evidence of cytoplasmic immaturity. These ‘signs’ are referred to as “toxic change”. The latter reflects strong inflammatory demand and shortened bone marrow retention, rather than real ‘toxic insult’ to the cells. (These cells were originally described in severe inflammation associated with toxaeamias, hence the misnomer.)
- Signs of "toxic change" (listed in order of increasing ‘significance’) are:
  - Dohle (Doehle) bodies: Remnants of rough endoplasmic reticulum; stain as bluish round to angular structures.
  - Diffuse cytoplasmic basophilia: Increased cytoplasmic RNA and ribosome concentration
  - “Toxic granulation”: Obvious primary granules (represents skipped cell divisions)
  - Cytoplasmic vacuolation
- Species differences: toxic change in dogs suggests a ‘guarded’ prognosis, whereas it is relatively common in cats (and ‘intermediate’ in horses and cattle).
Hypersegmentation of neutrophils may be seen with:
- Rapidly ablated inflammatory demand
- Corticosteroid therapy
- Delayed sample processing.

Monocytosis
- Monocyte numbers seldom increase to a point where they cause a leukocytosis per se.
- Causes of monocytosis
  - Inflammation
  - Haemolytic anaemia
  - Corticosteroids

Monocytopenia
- Monocytopenia “doesn’t exist”
  ‘Normal’ animals often have “no” circulating monocytes (on a differential cell count of 100 cells).

Eosinophilia
- Eosinophil numbers seldom increase to a point where they cause a leukocytosis per se.
- Causes of eosinophilia
  - Allergic diseases
  - Parasitism
  - (Severe) tissue necrosis
  - Adrenal insufficiency (Addison’s disease)

Eosinopenia
- Although ‘normal’ animals may have “no” circulating eosinophils, we still often refer to an animal in which none are seen (on a differential cell count of 100 cells) as having an eosinopenia!
- Causes of eosinopenia
  - Normal
  - “Stress”
  - Corticosteroids: hyperadrenocorticism, therapy
**Lymphocytosis**

**Causes of lymphocytosis:**
- Physiological (see earlier)
- "Chronic" inflammation
- Lymphoid leukaemia
- Adrenal insufficiency

**“Chronic” inflammation**
- Usually concurrent neutrophilia, possibly with a left-shift, and monocytosis.
- Possibly anaemia (of chronic inflammatory disease)
- Possibly hypergammaglobulinaemia and hyperfibrinogenaemia (especially in ruminants, horses)

**Adrenal insufficiency**
- Haematology often unremarkable, but some ‘classic’ changes are sometimes seen, these may include:
  - Anaemia (non-regenerative); unless dehydrated
  - Eosinophilia
  - Lymphocytosis

**Lymphopenia**

**Causes of lymphopenia:**
- "Stress"/Corticosteroids (see earlier)
- Some (viral) infections
- Lymph loss
- Impaired lymphopoiesis
- Primary severe combined immunodeficiency (PSCID)

**Infections**
- For example:
  - Canine distemper
  - Canine parvovirus
  - FeLV
  - Canine ehrlichiosis
Lymph loss
- Thoracic duct rupture (chylothorax); especially if repeated thoracocentesis. Also loss of protein, with potential hypoproteinaemia/hypoalbuminaemia.
- Lymphangiectasia.
- Protein-losing enteropathy.

Impaired lymphopoiesis
- Corticosteroids
- Chemotherapy
- Irradiation
- Athymic kittens (FeLV)

Primary severe combined immunodeficiency disease
- Arabian foals (Part-bred Arabians; Appaloosa)
- T and B cell deficiency
- Diagnosis:
  - Absence of presuckle IgM
  - or absence of IgM after about 2 - 5 weeks if foal has been nursed
  - Lymphopenia is a healthy foal; <1.0 x 10⁹/l
- Treatment: None. Could do histocompatible bone marrow transplant, but only useful for experimental purposes.
- Outcome: Invariably fatal; usually within 6 months. Opportunistic pathogens: EAdV pneumonia, Pneumocystis carini pneumonia, Cryptosporidiosis.
- PM: Thymic hypoplasia, LN hypoplasia.