KAPLAN MEDICAL

Blood Cells and Lymphoid Structures

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Course Objectives

To understand the following topics and how they may be tested on USMLE Step 1

- Hematopoietic system and its cell lineages
- Normal functioning of the immune system
- Lymphomas and leukemias
- Erythrocytes, hemoglobin, and various types of anemias and porphyrias
- Normal physiology and disease states of the coagulation system

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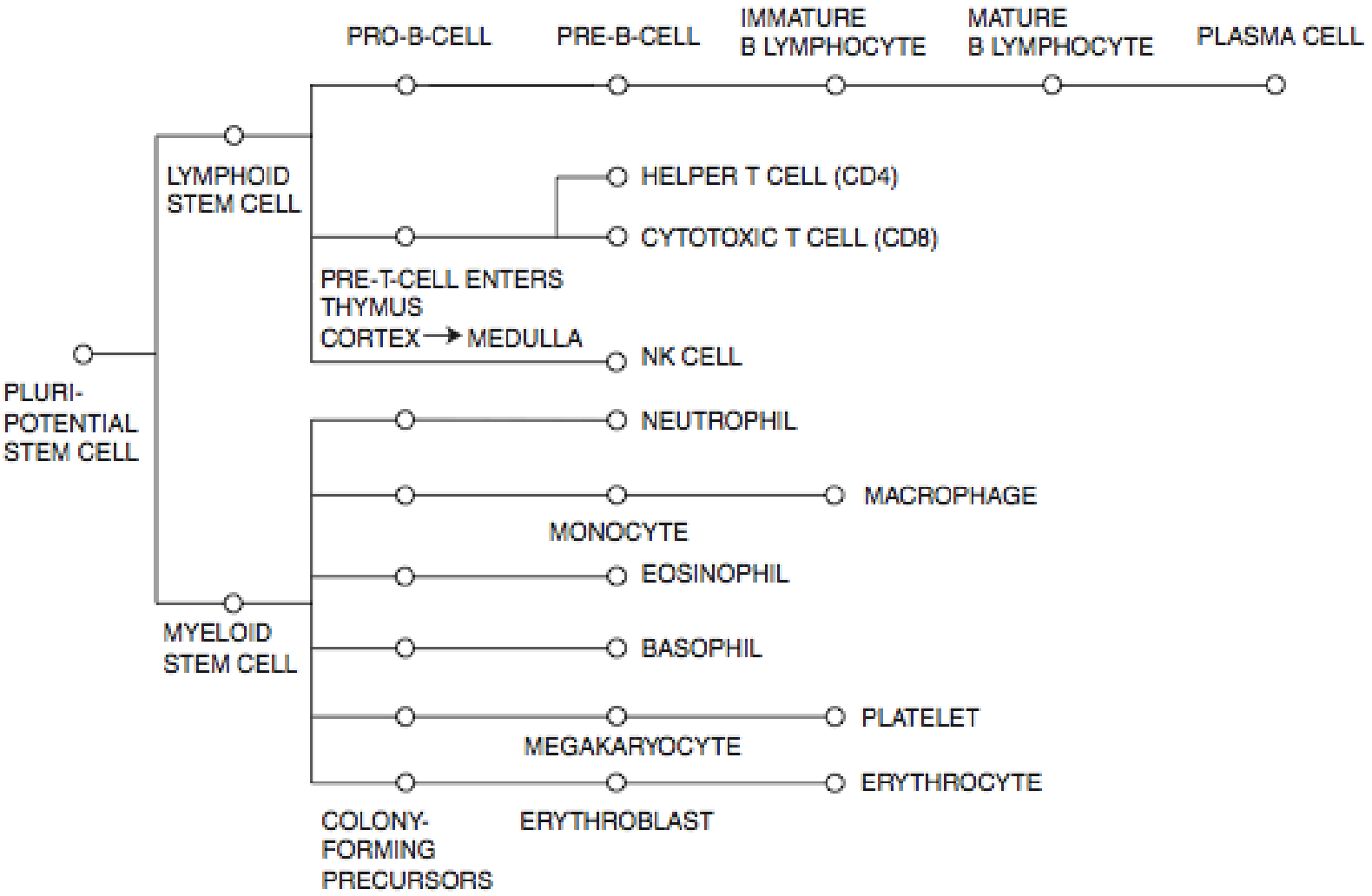
Blood Cells and Lymphoid Structures

Lecture 1

- Types of white blood cells
- Organs involved in the immune system

Stem Cell Lineages



White Blood Cell Differential

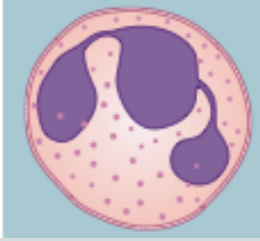
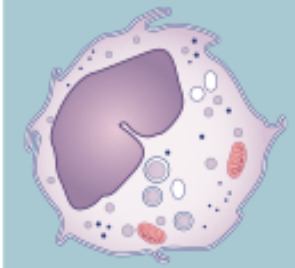
WHITE BLOOD CELLS	
Granulocyte Type	**Relative Abundance**
Neutrophils	54–62% of leukocytes Normal value: 1800–7800/μl
Eosinophils	1–3% of leukocytes Normal value: 0–450/μl
Basophils	1% of leukocytes Normal value: 0–200/μl

Normal WBC Count = 4,000–10,000 / μL

Higher = leukocytosis (infection/malignancy)

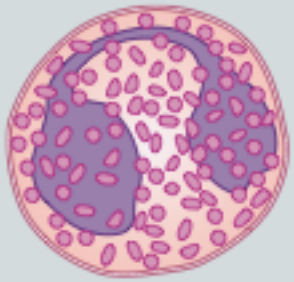
FA 2013: 343 • FA 2012: 372.1 • FA 2011: 343
ME 3e: 469 • ME 4e: 469

Neutrophils, Monocytes, and Macrophages

Myeloid Cell	Tissue Location	Identification	Function
Neutrophil 	Bloodstream, 1,800–7,800/μL	Multilobed nucleus, small pink granules Note: Hypersegmented neutrophils seen in B12/folate deficiency	Phagocytosis and activation of bactericidal mechanisms **Acute Response**
Monocyte 	Bloodstream, 0–900/μL	Kidney bean-shaped nucleus, CD14 positive	Phagocytic, differentiate into tissue macrophages
Macrophage 	Tissues	Ruffled membrane, cytoplasm with vacuoles and vesicles, CD14 positive	Phagocytosis, secretion of cytokines Spleen → RBCs Activated by: IFN-γ

Kaplan Micro-Immuno 2011 : Table I-2-1

Eosinophils

Myeloid Cell	Tissue Location	Identification	Function
Eosinophil 	Bloodstream, 0–450/μL	Bilobed nucleus, large pink granules	Killing of antibody-coated parasites Major basic protein

Causes of hypereosinophilia:

N - Neoplastic

A - Asthma

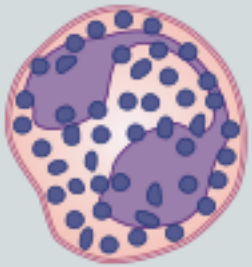
A - Allergic processes

C - Collagen vascular disease

P - Parasites

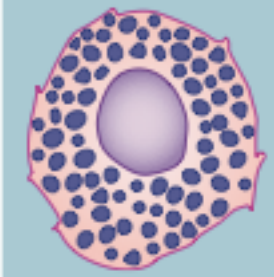
Kaplan Micro-Immuno 2011 : Table I-2-1

Basophils

Myeloid Cell	Tissue Location	Identification	Function
Basophil 	Bloodstream, 0-200/μL	Bilobed nucleus, large blue granules (e.g., with histamine LTE-4)	Nonphagocytic, release pharmacologically active substances during allergic responses

Kaplan Micro-Immuno 2011 : Table I-2-1

Mast Cells

Myeloid Cell	Tissue Location	Identification	Function
Mast cell 	Tissues, mucosa, and epithelia	Small nucleus, cytoplasm packed with large blue granules	Release of granules containing histamine, etc., during allergic responses Bind antibodies

Involved in type I hypersensitivity response

Systemic mastocytosis:

Uncontrolled proliferation of mast cells

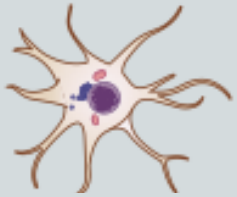
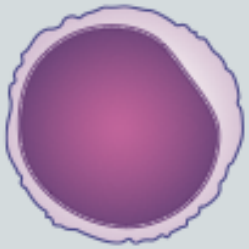
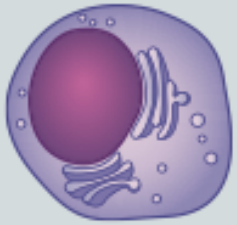
Symptoms:

Itching, flushing, abdominal cramps,

PUD (inc. histamine release à inc. gastric acid production)

Kaplan Micro-Immuno 2011 : Table I-2-1

Mature Myeloid and Lymphoid Cells

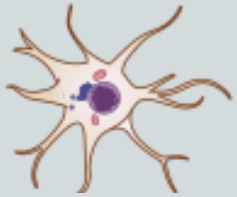
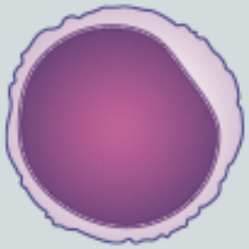
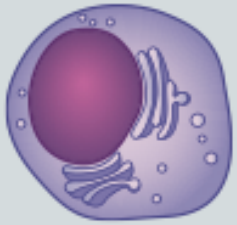
Lymphoid Cell	Location	Identification	Function
Dendritic cell 	Epithelia, tissues → Called Langerhans cells when in the skin	Long cytoplasmic arms • Express MHC class II and B7 • Langerhans cells have tennis racquet-like inclusions	Antigen capture, transport, and presentation
Lymphocyte 	Bloodstream, 1,000–4,000/μl; lymph nodes, spleen, submucosa, and epithelia	Large, dark nucleus, small rim of cytoplasm B cells – CD19, 20, 21 T cells – CD3 TH cells –CD4 CTLs – CD8	B cells produce antibody T helper cells regulate immune responses Cytotoxic T cells (CTLs) kill altered or infected cells
Plasma cell 	Lymph nodes, spleen, mucosal-associated lymphoid tissues, and bone marrow	Small dark nucleus, intensely staining Golgi apparatus	End cell of B-cell differentiation, produce antibody

Mature in:
- B cells produce antibody → Bone marrow
- T helper cells → Thymus

Kaplan Micro-Immuno 2011 : Table I-2-2

FA 2013: 345 • FA 2012: 374 • FA 2011: 344
ME 3e: 90 • ME 4e: 90

Mature Myeloid and Lymphoid Cells

Lymphoid Cell	Location	Identification	Function
Dendritic cell 	Epithelia, tissues → Called Langerhans cells when in the skin	Long cytoplasmic arms • Express MHC class II and B7 • Langerhans cells have tennis racquet-like inclusions	Antigen capture, transport, and presentation
Lymphocyte 	Bloodstream, 1,000–4,000/μl; lymph nodes, spleen, submucosa, and epithelia	Large, dark nucleus, small rim of cytoplasm B cells – CD19, 20, 21 T cells – CD3 TH cells –CD4 CTLs – CD8	B cells produce antibody T helper cells regulate immune responses Cytotoxic T cells (CTLs) kill altered or infected cells
Plasma cell 	Lymph nodes, spleen, mucosal-associated lymphoid tissues, and bone marrow	Small dark nucleus, intensely staining Golgi apparatus	End cell of B-cell differentiation, produce antibody

Mature in:
B cells produce antibody → Bone marrow
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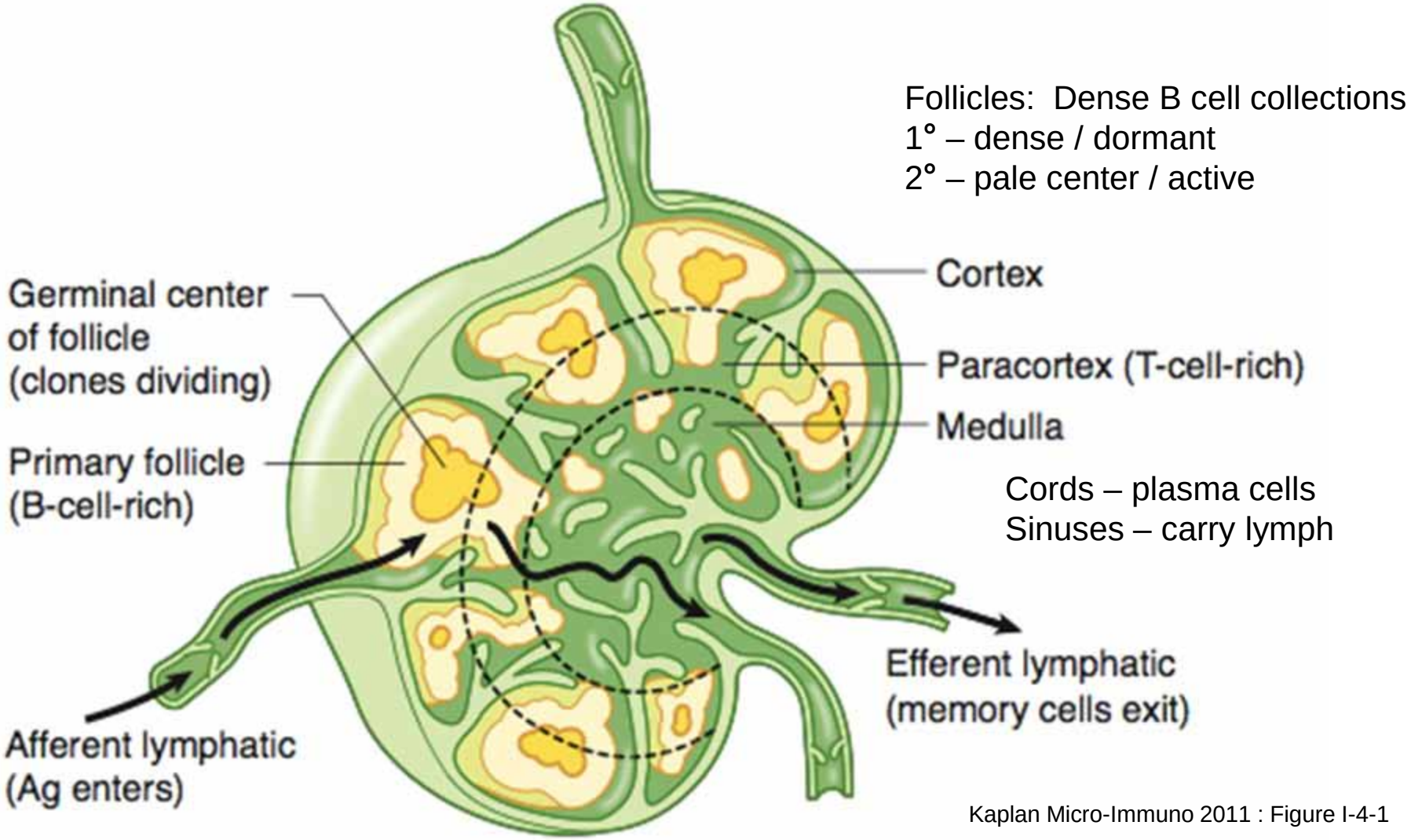
Kaplan Micro-Immuno 2011 : Table I-2-2

FA 2013: 345 • FA 2012: 374 • FA 2011: 344
ME 3e: 90 • ME 4e: 90

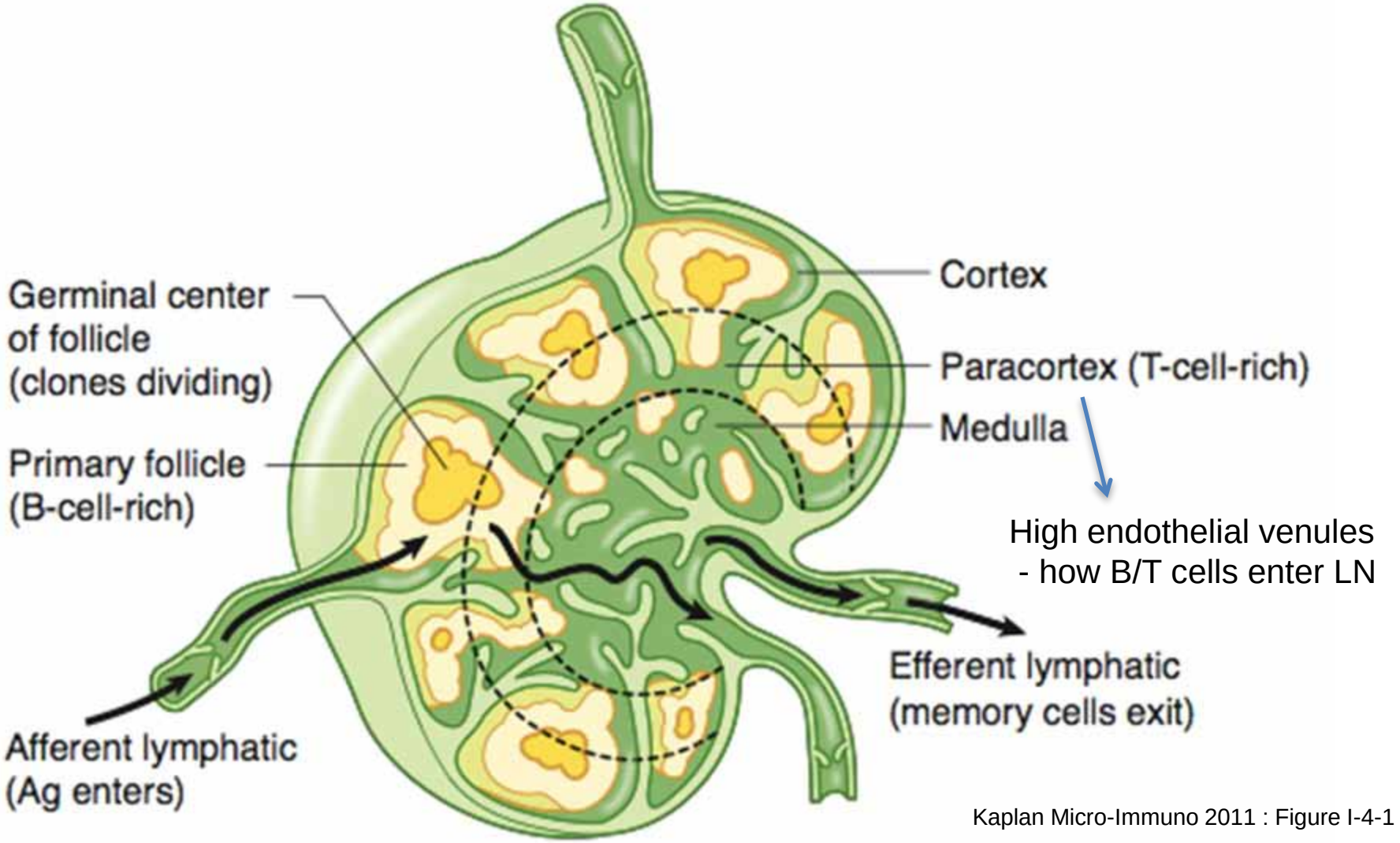
Lymph Node



Kaplan Micro-Immuno 2011 : Figure I-4-1

FA 2013: 192.1 • FA 2012: 222.1 • FA 2011: 200.1
ME 3e: 90 • ME 4e: 90

Lymph Node Circulation



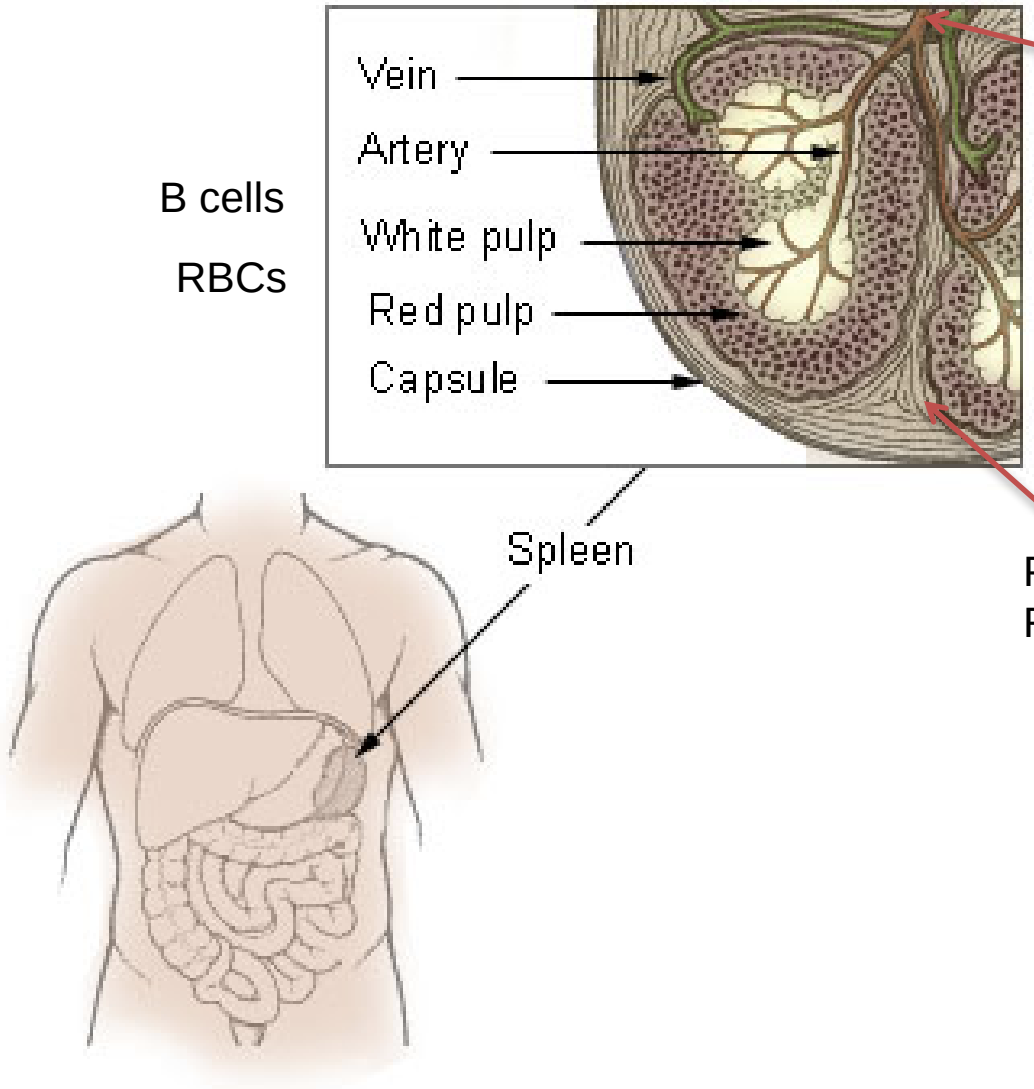
Kaplan Micro-Immuno 2011 : Figure I-4-1

FA 2013: 192.1 • FA 2012: 222.1 • FA 2011: 200.1
ME 3e: 90 • ME 4e: 90

Lymphatic Drainage

- **Some important lymphatic drainage**
  - Stomach à celiac node
  - Duodenum à superior mesenteric node
  - Colon à inferior mesenteric node
  - Rectum à above pectinate line: internal iliac node / below pectinate line: superficial inguinal node
  - Testicles à periaortic lymph nodes
  - Scrotum à superficial inguinal lymph nodes
  - Cutaneous lymph from ubilicus to feet, including external genitalia and anus below pectinate line à superficial inguinal nodes
    - Excludes the posterior calf
  - Almost all lymph drains into thoracic duct à l. subclavian vein
    - Exception: r. arm/head à r. lymphatic duct à r. subclavian vein

Spleen



B cells

RBCs

Central arteriole

PALS (periarterial lymphatic sheath):
Rich in T cells

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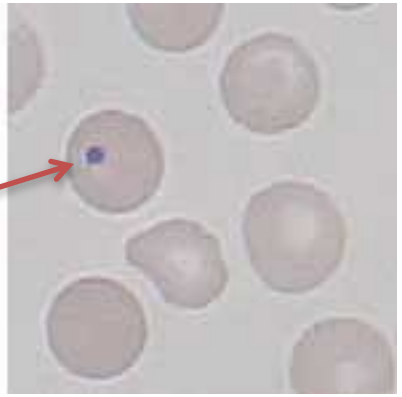
FA 2013: 193.1 • FA 2012: 223.1 • FA 2011: 201.1
ME 3e: 370 • ME 4e: 370

Asplenia

- **Asplenia**
  - Can occur due to infection or infarction (such as in sickle cell anemia)
  - Difficulty fighting encapsulated bacteria
    - *Salmonella, Streptococcus pneumoniae,*

      Haemophilus influenzae, Neisseria meningitidis
  - Patients more prone to sepsis
  - Causes lack of IgM, which is made in spleen
    - Important for initial immune response
    - Activates complement (C3b)
  - Signs of asplenia
    - Howell-Jolly bodies in RBCs
    - Thrombocytosis (increased platelet count)
    - Target cells



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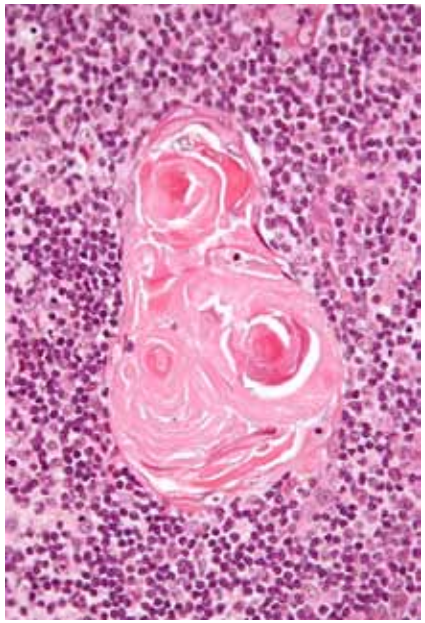
Thymus

- **Thymus**
  - Cortex: immature T cells
  - Corticomedullary junction
    - Site of T cell maturation
  - Medulla: mature T cells
    - *Hassall's corpuscles*



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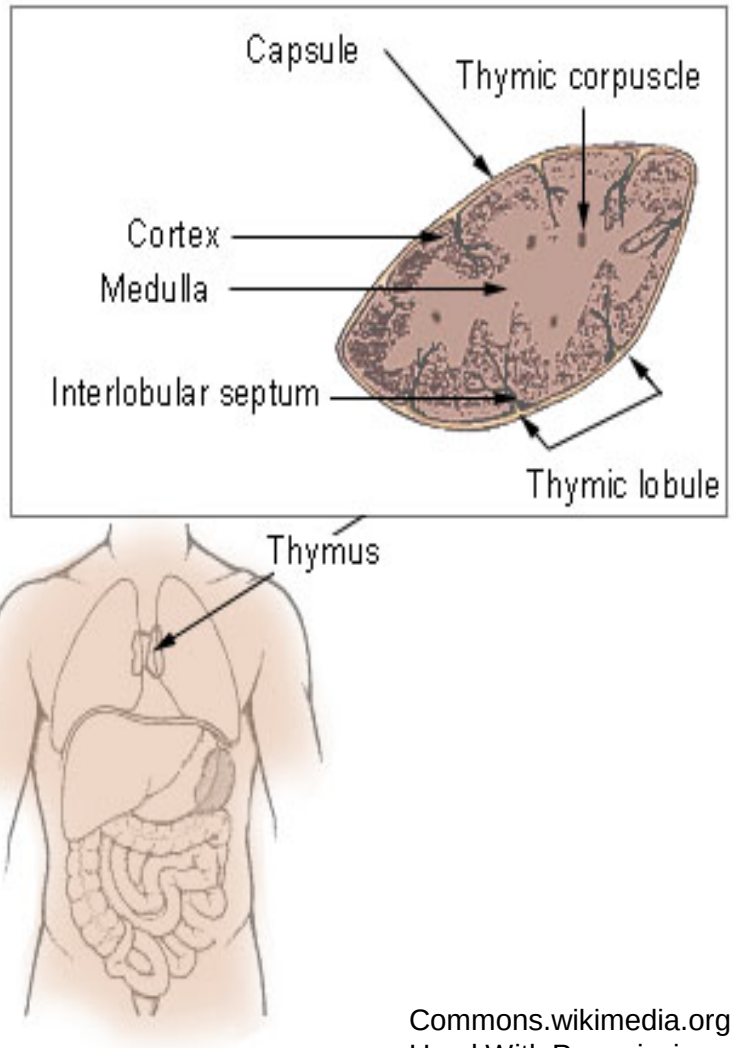

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FA 2013: 193.3 • FA 2012: 223.2 • FA 2011: 201.2
ME 3e: 470 • ME 4e: 470

Origin of Thymic Cells

- **Origin of thymic T cells**
  - Start as multipotential stem cells in fetal bone marrow and liver
    - CD4- and CD8- at this stage
  - Migrate toward anterior mediastinum
  - Undergo positive selection (is the T cell self-reactive to MHC?) first, then negative selection (does the T cell bind MHC too strongly?)
    - See Kaplan Micro-Immuno Figure I-3-5
    - CD4+ and CD8+ once in the thymus
      - After selection, they lose one or the other

Innate versus Adaptive Immunity

Components	Innate	Adaptive
Anatomic and chemical barriers	Skin, mucosa, chemicals (lysozyme, interferons α and β), temperature, pH	Lymph nodes, spleen, mucosal-associated lymphoid tissues
Blood proteins	Complement	Antibodies
Cells	Phagocytes and natural killer (NK) cells	Lymphocytes (other than NK cells)

Kaplan Micro-Immuno 2011 : Table I-1-1

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T Cell and B Cell Function

Lecture 2

- T cell and B cell differentiation
- T cell and B cell activation
- Antibody structure and function

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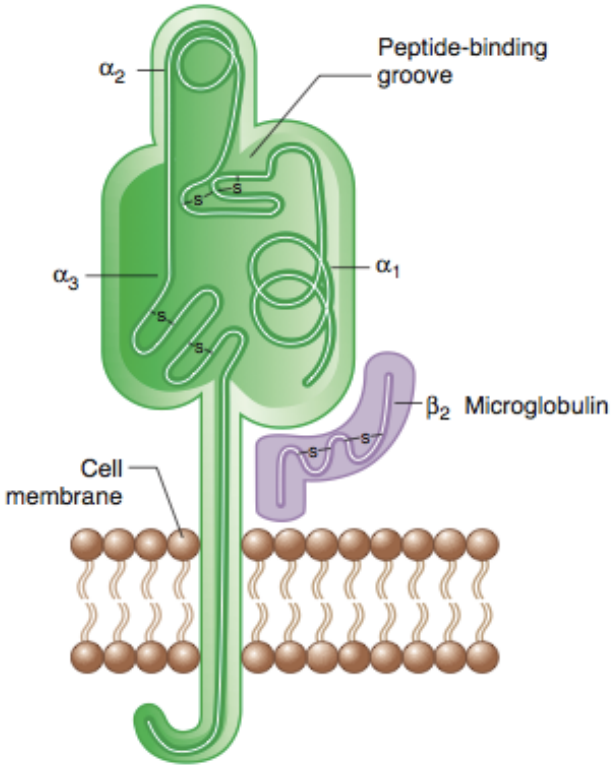
MHC I and II Features

	MHC Class I	MHC Class II
Names	HLA-A, -B, and -C	HLA-DP, -DQ, -DR
Tissue distribution	All nucleated cells, platelets	B lymphocytes, monocytes, macrophages, dendritic cells, Langerhans cells, activated T cells, activated endothelial cells
Recognized by	Cytotoxic T cells (CD8+)	TH cells (CD4+)
Peptides bound	Endogenously synthesized	Exogenously processed
Function	Elimination of abnormal (infected) host cells by cytotoxic T cells	Presentation of foreign antigen to TH cells
Invariant chain	No	Yes
β_2 microglobulin	Yes	No

Kaplan Micro-Immuno 2011 : Table I-6-1

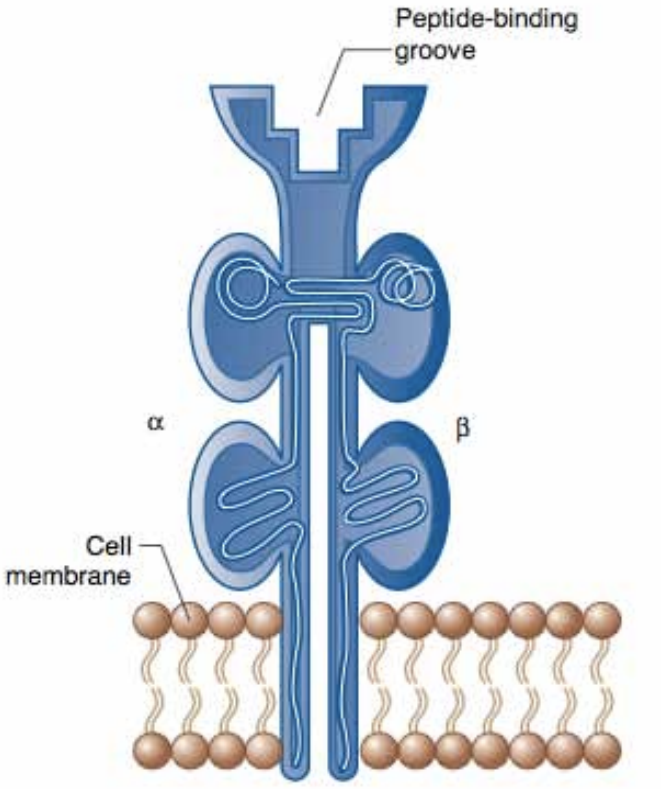
MHC I & II Structures

MHC Class I



- Antigen loaded inside RER
- Presents to CD8 cytotoxic T cells

MHC Class II



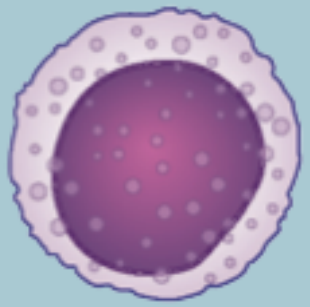
- Antigen loaded inside endosomes
- Presents to CD4 helper T cells

Kaplan Micro-Immuno 2011 : Figure I-3-3

HLA-Linked Immunologic Diseases

HLA	Associated Disease
A3	Hemochromatosis
B27	Ankylosing spondylitis, IBD, Psoriasis, Reactive arthritis
B8	Graves' Disease
DR2	SLE, MS, Goodpasture's syndrome
DR3	T1DM, Hashimoto's thyroiditis
DR4	T1DM, Rheumatoid arthritis
DR5	Hashimoto's thyroiditis, Pernicious anemia
DR7	Steroid-responsive nephrotic syndrome

Natural Killer Cells

Lymphoid Cell	Location	Identification	Function
Natural killer (NK) lymphocyte 	Bloodstream, ≤10% of lymphocytes	Lymphocytes with large cytoplasmic granules CD16 + CD56 positive	Kill tumor/virus cell targets or antibody-coated target cells

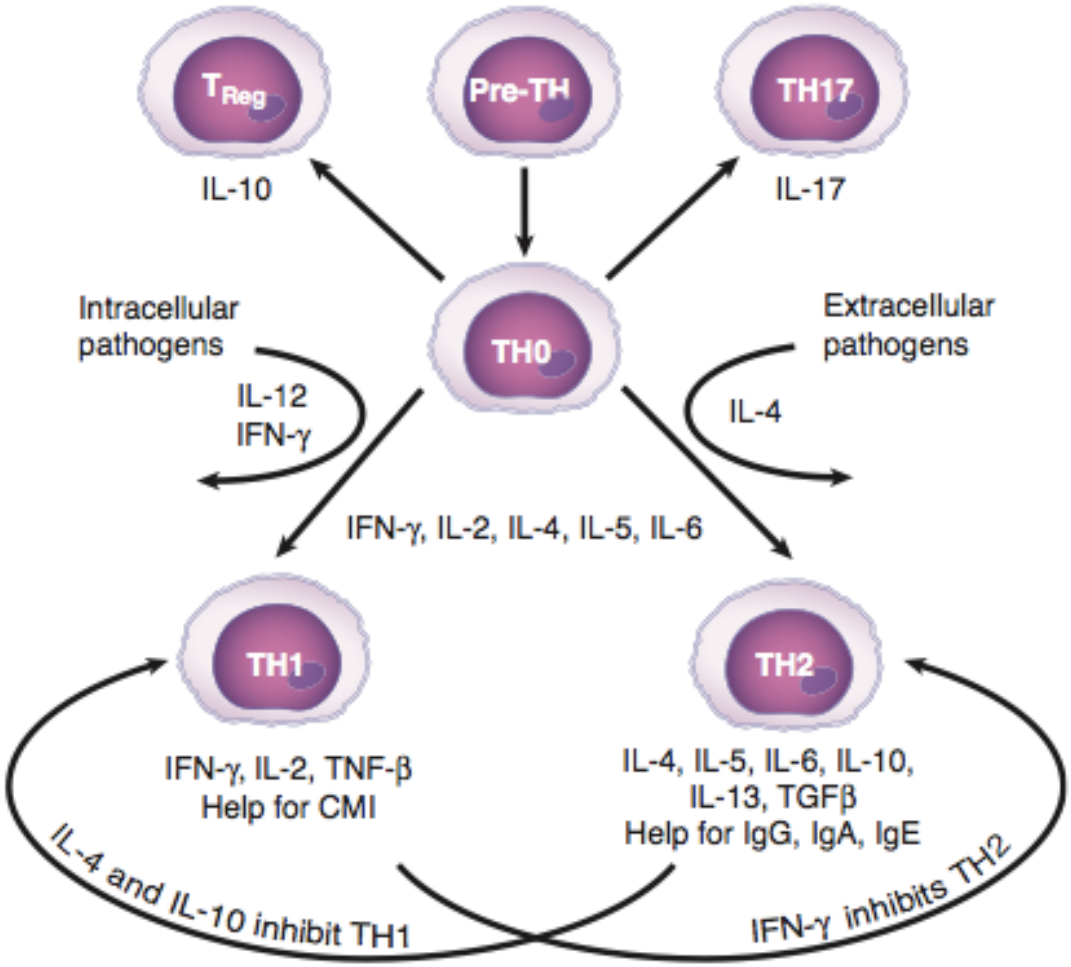
- KIR versus KAR receptors
- Attacks using perforin and granzymes
- Most prominent cells involved in graft-versus-host disease

Kaplan Micro-Immuno 2011 : Table I-2-2

B Cell and T Cell Interaction

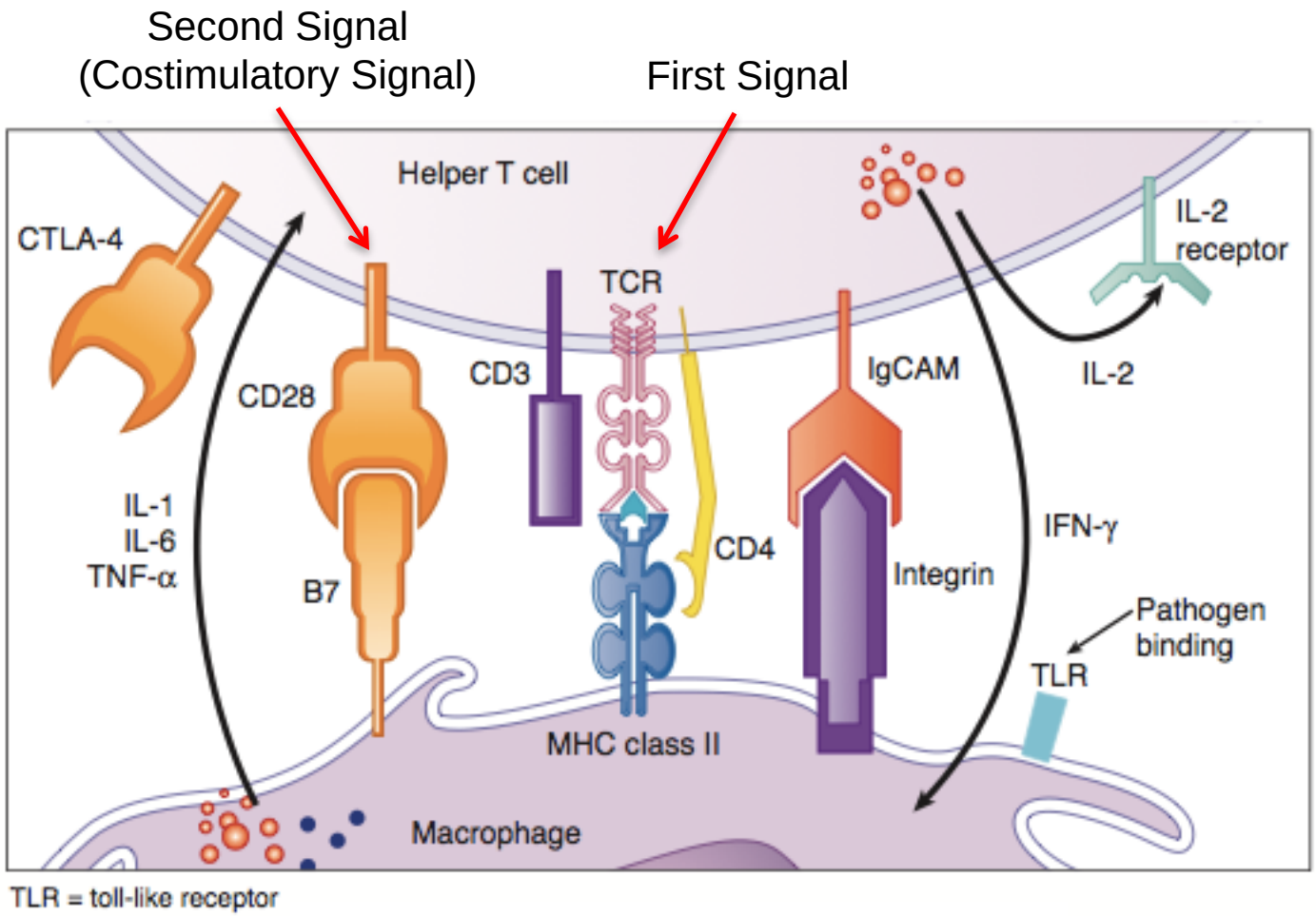
- B cells
  - Produce antibody
- T cells
  - Helper T cells (CD4)
    - Help B cells produce antibody
    - Have no cytotoxic or phagocytic activity
    - Express CD4
  - Cytotoxic T cells (CD8)
    - Kill infected cells
    - Express CD8

T Cell Differentiation



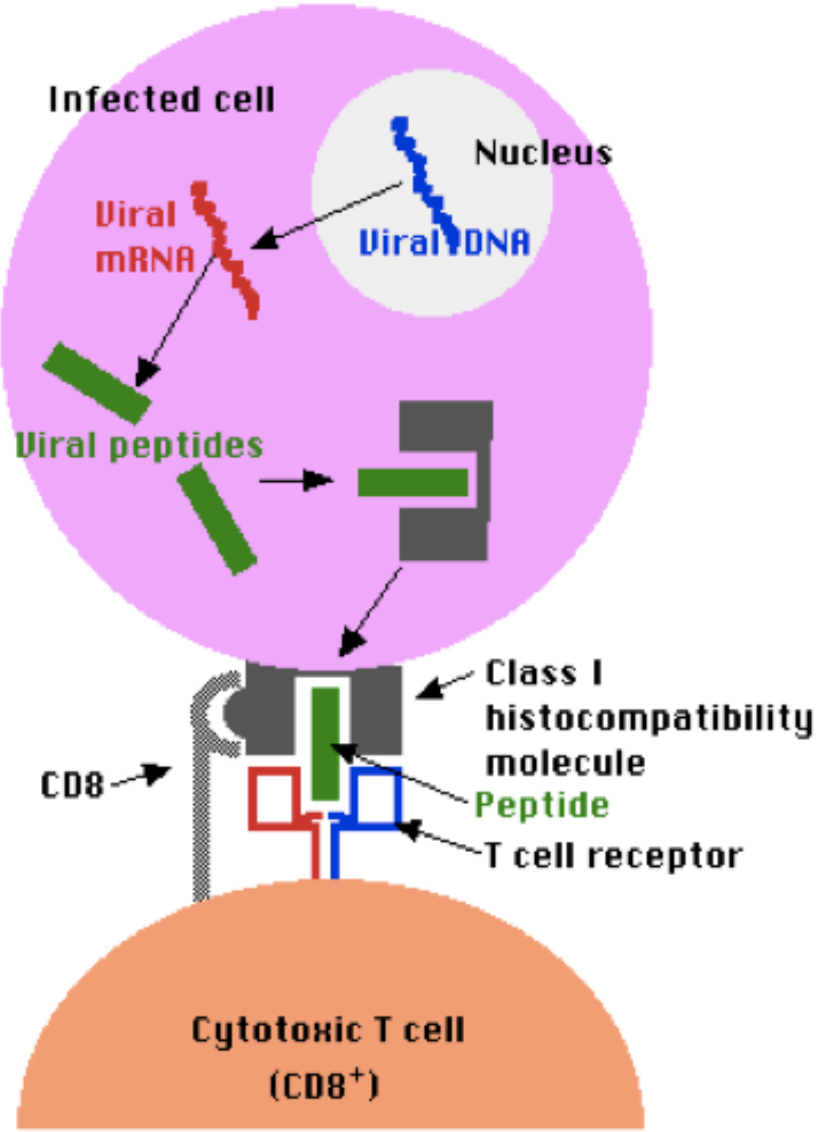
Kaplan Micro-Immuno 2011 : Figure I-6-6

Helper T Cell Activation



Kaplan Micro-Immuno 2011 : Figure I-6-4

Cytotoxic T Cell Activation



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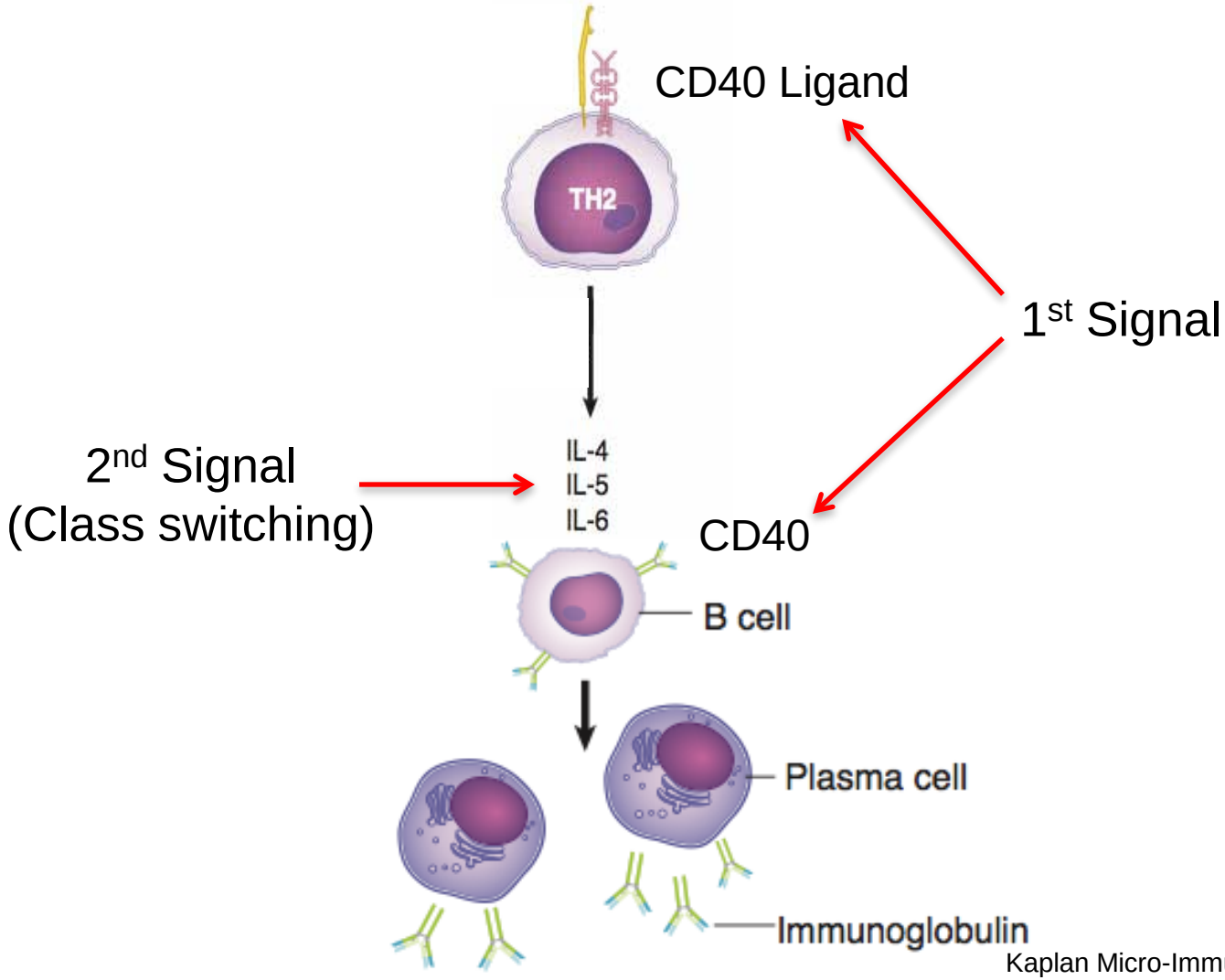
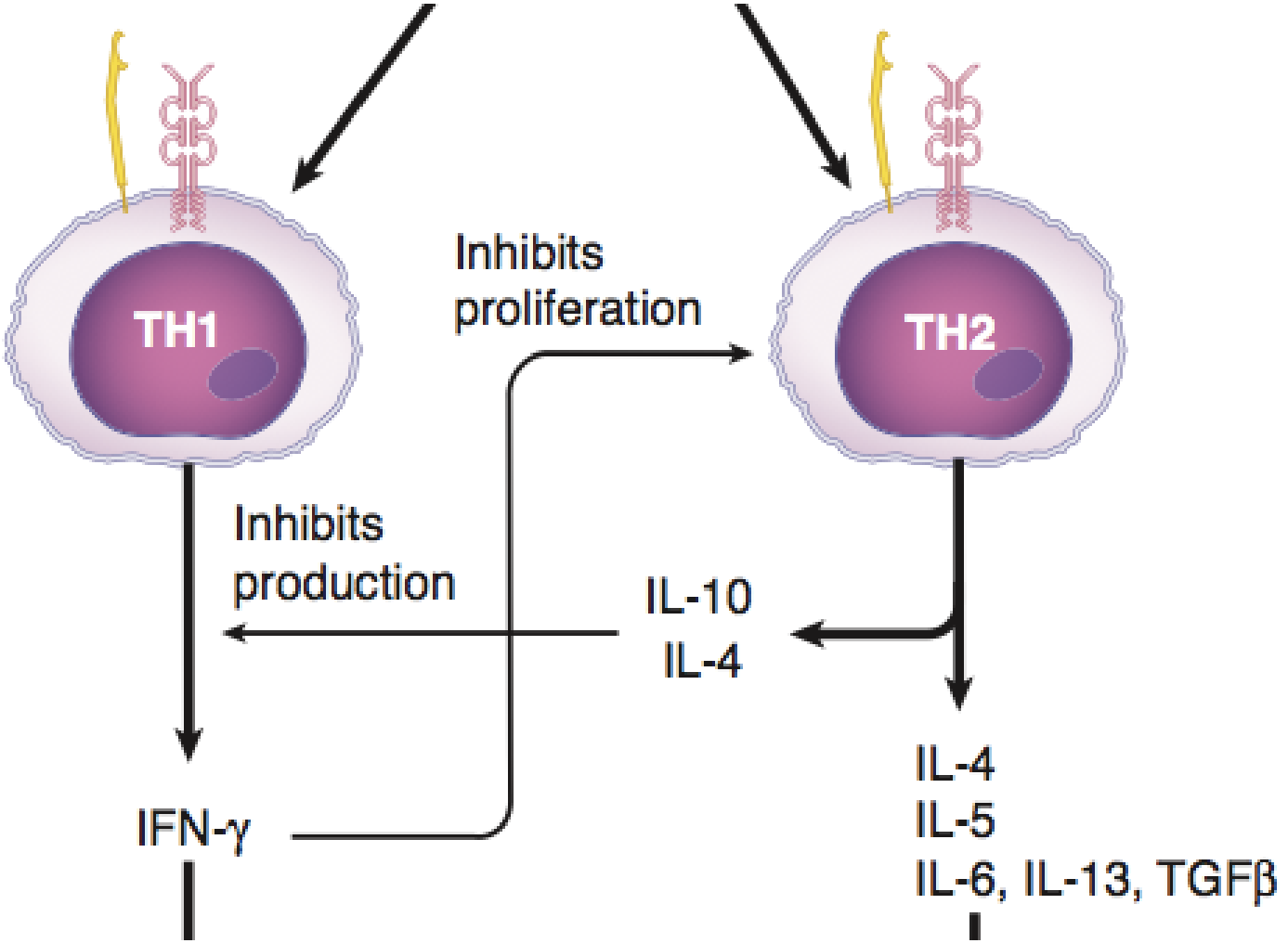
Plasma Cell Activation



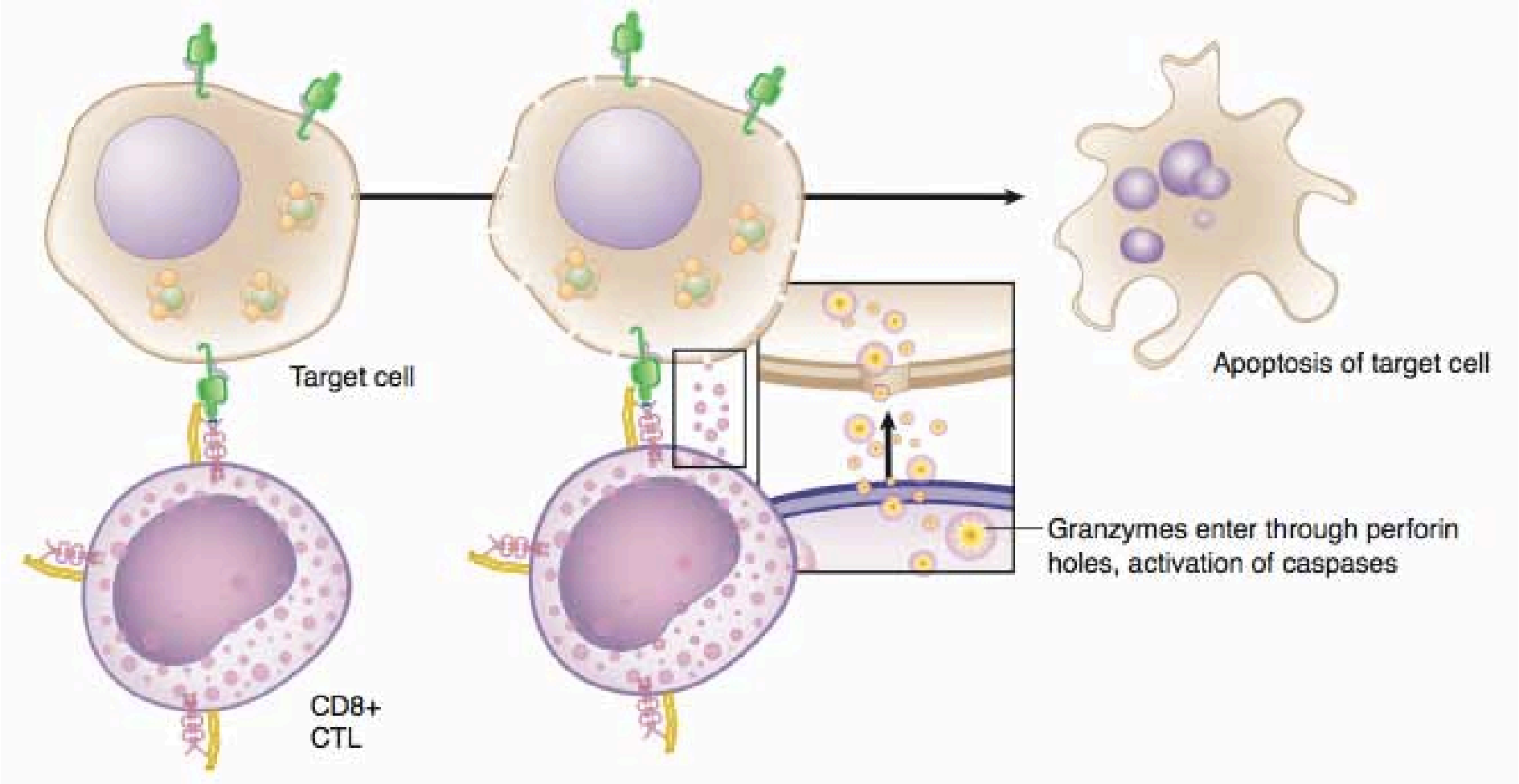
Kaplan Micro-Immuno 2011 : Figure I-6-7

Helper T Cell Inhibition



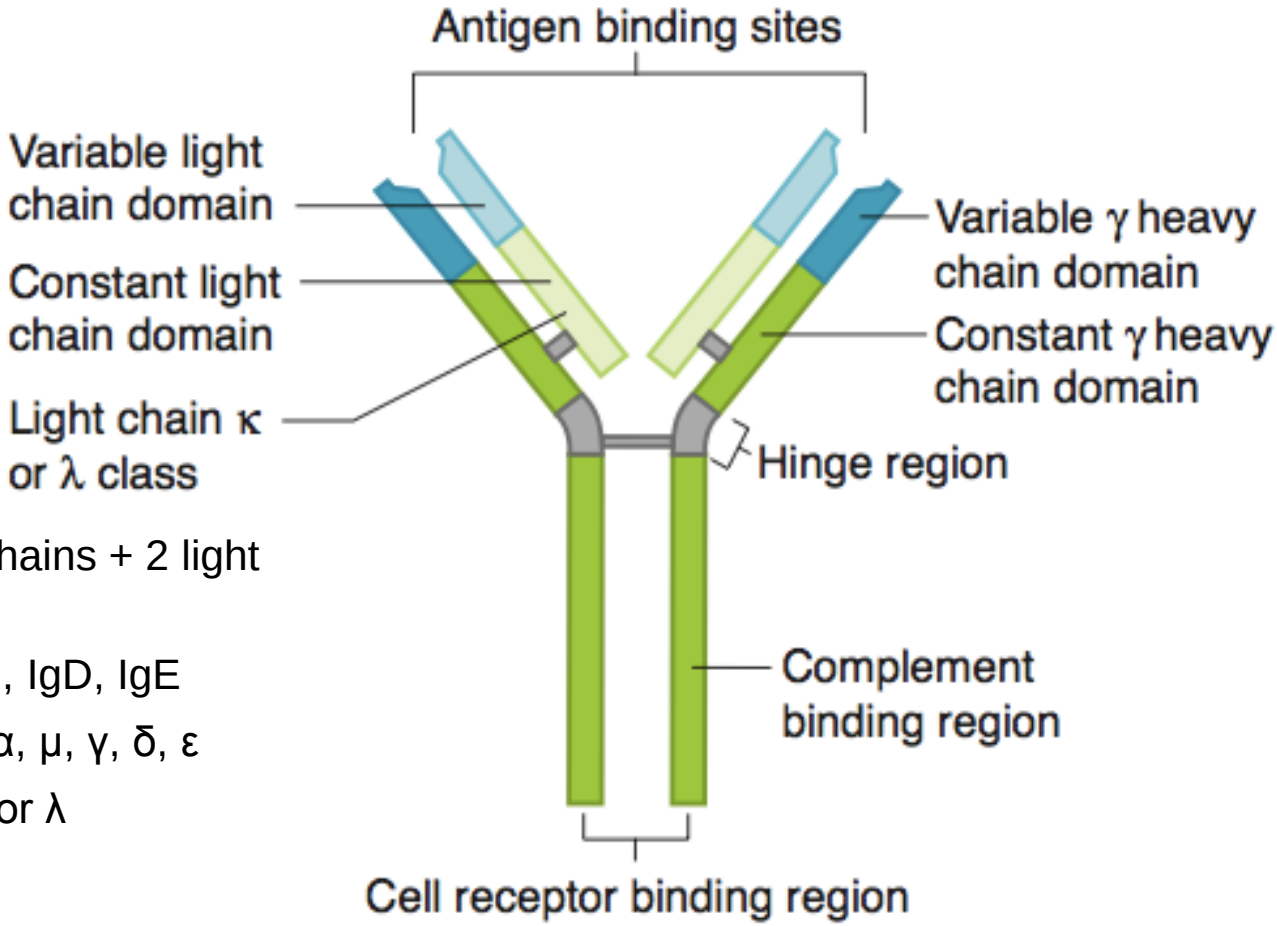
Kaplan Micro-Immuno 2011 : Figure I-6-7

Cytotoxic T Cells



Kaplan Micro-Immuno 2011 : Figure I-8-1

Antibody Structure



Antibody structure: 2 heavy chains + 2 light chains

Antibody classes: IgA, IgM, IgG, IgD, IgE

Heavy chain constant regions: α, μ, γ, δ, ε

Light chain constant regions: κ or λ

Fab (top): binds antigen

Fc (bottom/constant/not specific): binds to APC

Kaplan Micro-Immuno 2011 : Figure I-7-8

Antibody Diversity

Antibody diversity (specificity of Fab fragment)

1) Random recombination *of genes*
   a. Light chains – VJ recombination
   b. Heavy chains - VDJ recombination
2) Random recombination *of chains*
3) Somatic hypermutation (after antigen stimulation)
4) TdT nucleotide inclusions

Antibody Functions

1) Opsonization

2) Neutralization

3) Complement activation

Specific Antibody Functions

Summary of the Biologic Functions of the Antibody Isotypes

	IgM	IgG	IgA	IgD	IgE
Heavy chain	μ	γ	α	δ	ε
Adult serum levels (in mg/dL)	45–250	620–1,400	80–350	Trace	Trace
Functions					
Complement activation, classic pathway	+	+	–	–	–
Opsonization	–	+	–	–	–
Antibody-dependent cell-mediated cytotoxicity (ADCC)	–	+	–	–	–
Placental transport	–	+	–	–	–
Naive B-cell antigen receptor	+	–	–	+	–
Memory B-cell antigen receptor (one only)	–	+	+	–	+
Trigger mast cell granule release	–	–	–	–	+
Type I hypersensitivity	-	-	-	-	+

Kaplan Micro-Immuno 2011 : Table I-7-1

FA 2013: 198.1 • FA 2012: 228.1 • FA 2011: 206.1
ME 3e: 95 • ME 4e: 95

Memory Response to Antigen

- Memory response to antigen
  - IgG is produced instead of IgM
    - More rapid response to recurrent antigen exposure
  - Thymus independent antigens
    - Lack a peptide component
    - Cannot be presented by MHC to T cells
    - Example: LPS of gram-negative rod
      - Stimulates IgM à no immunologic memory
  - Thymus dependent antigens
    - **Do** contain a peptide component
      - Allow for antibody class switching
    - Example: *Haemophilus influenzae*

Notable Cytokines

Macrophage cytokines (acute response)

- IL-1 endogenous pyrogen
- IL-6 endogenous pyrogen
- TNF-α sepsis
- IL-8 neutrophil chemotaxis
- IL-12 stimulates TH1 development, activates NK cells

T cell cytokines

- IL-3 similar to GM-CSF
- TH1 IL-2 (+ CD8 T cells)

  INF-γ (+ macrophages)
- TH2 IL-4 (+ IgE & IgG production)

  IL-5 (+ IgA production)

  IL-10 (- TH1 cytokine production)

Interferons

- INF-γ
  - Secreted by TH1 cells
  - Activates macrophages
  - Inhibits the TH2 response

- INF-α and INF-β
  - Inhibit viral protein synthesis
  - INF-α used in the treatment of
    - Chronic hepatitis B and C
    - Hairy cell leukemia
    - Condyloma acuminata
    - Kaposi sarcoma
    - Adjuvant treatment for melanoma
  - INF-β used in the treatment of
    - Multiple sclerosis

Key CD Markers

- T cells
  - CD3 coreceptor for T cell receptors
  - CD28 binds B7 on APC, second signal for T cell activation
- B cells
  - CD19
  - CD20
  - CD21
- Macrophages
  - CD14 used to bind LPS (endotoxin)
- NK cells
  - CD56

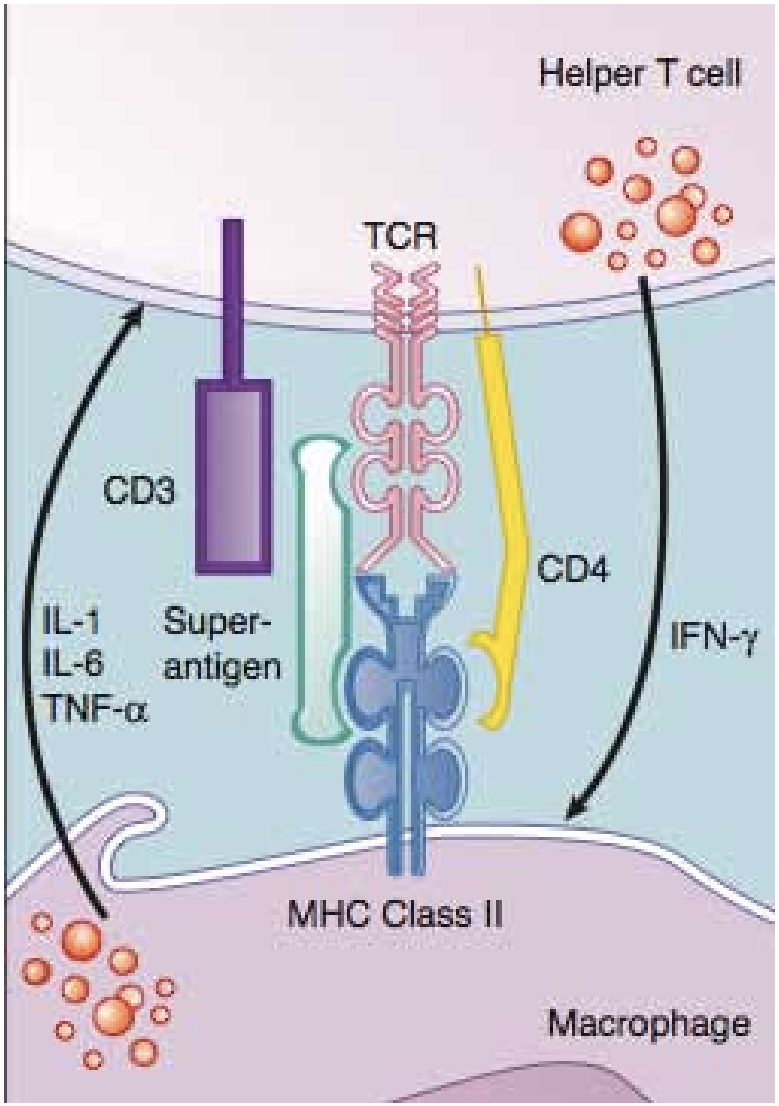
Regulation of Immune Response

Regulation of immune response

Thymus - negative selection

Anergy - self-reactive T cells interact with APC lacking costimulatory signal (B7-CD28)

Superantigens



Kaplan Micro-Immuno
2011 : Figure I-6-5

FA 2013: 201.4 • FA 2012: 231.3 • FA 2011: 209.3
ME 3e: 92 • ME 4e: 92

Antigenic Variation

Antigenic variation

Bacteria

- *Salmonella*
- *Borrelia (Lyme disease)*
- *Neisseria gonorrhoeae*

Virus

- Influenza

Parasite

- *Trypanosoma brucei rhodesiense*
- *Trypanosoma brucei gambiense*

Passive vs Active Immunity

Type of Immunity	Acquired Through	Examples
Natural	Passive means	Placental IgG transport, colostrum
Natural	Active means	Recovery from infection
Artificial	Passive means	Horse antivenin against black widow spider bite, snake bite
		Horse antitoxin against botulism, diphtheria
		Pooled human immune globulin versus hepatitis A and B, measles, rabies, varicella zoster or tetanus
		"Humanized" monoclonal antibodies versus RSV*
Artificial	Active means	Hepatitis B component vaccine
		Diphtheria, tetanus, pertussis toxoid vaccine
		Haemophilus capsular vaccine
		Polio live or inactivated vaccine
		Measles, mumps, rubella attenuated vaccine
		Varicella attenuated vaccine

*Monoclonal antibodies prepared in mice but spliced to the constant regions of human IgG

Kaplan Micro-Immuno 2011 : Table I-10-1

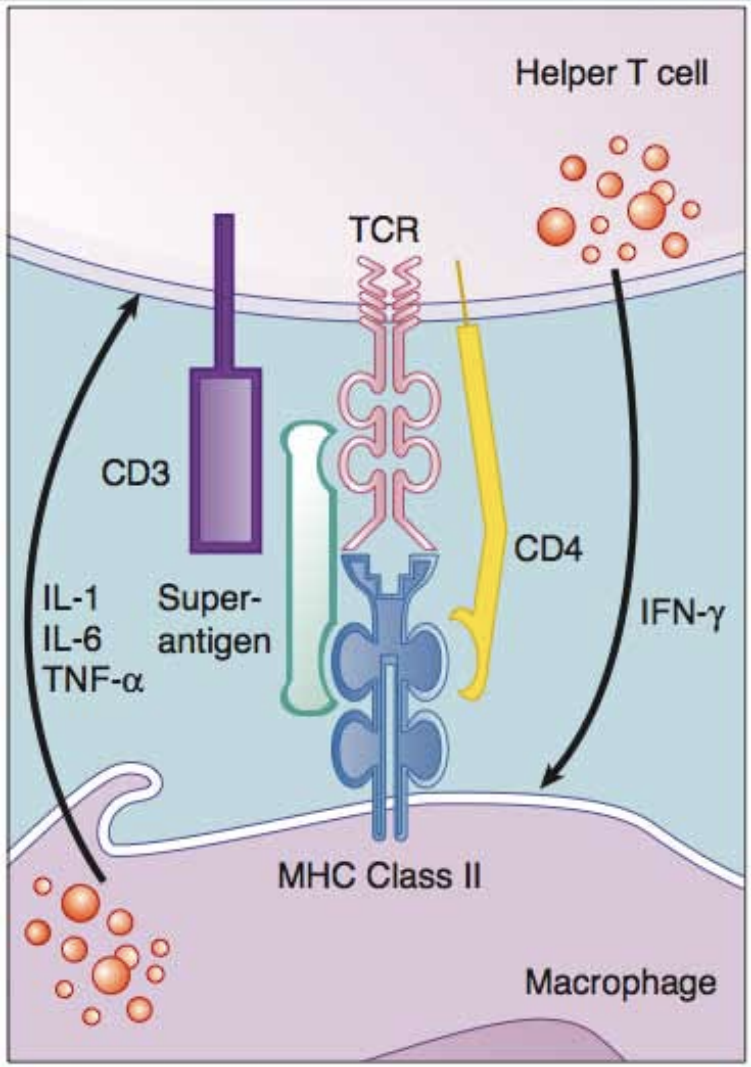
KAPLAN MEDICAL

Immunology, Hematology, and Oncology

Lecture 3

- Hypersensitivity
- Autoantibodies

Superantigens



Superantigen Activation

- Associated with:
  - *Staph. aureus*
  - *Strep. pyogenes*
- Uncontrolled T cell activation:
  - TH1 à INF-γ
  - Macrophages à IL-1, IL-6, TNF-α
- Notice that there is no complementarity between the TCR and the MHC/peptide complex
- Can lead to septic shock

Kaplan Micro-Immuno 2011 : Figure I-6-5

Endotoxin

- Endotoxin
  - Lipopolysaccharides (LPS)
  - Specific to gram-negative bacteria
  - Binds directly to **CD14** on macrophages
    - Caused uncontrolled release of:

      IL-1 à Fever

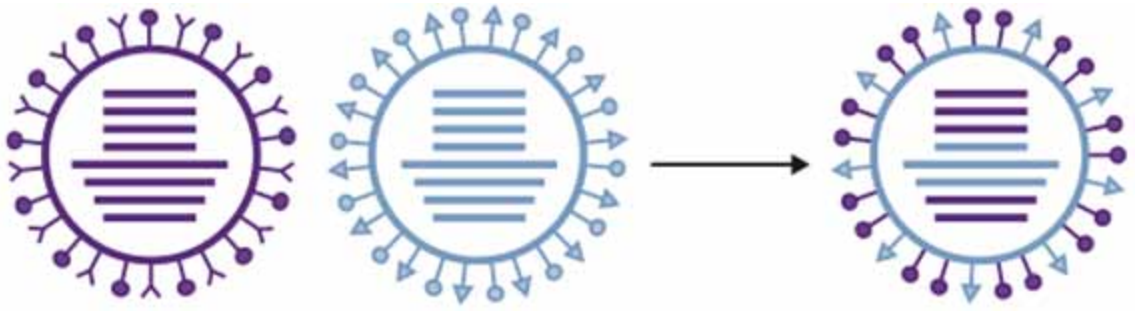
      TNF-α à Septic Shock

Antigen Variation 1

- Antigen Variation
  - Changing surface antigens to avoid immune destruction
  - Bacteria:
    - *Salmonella*: 2 different flagella
    - *Borrelia* (Lyme disease): changes surface proteins
    - *N. gonorrhoeae*: pili and outer membrane proteins
  - Virus:
    - Influenza à Can undergo genetic shifts (major)
      and genetic drifts (minor)
  - Parasites:
    - *Trypanosoma brucei rhodesiense*
    - *Trypanosoma brucei gambiense*

Antigenic Shift vs. Drift

- Antigenic shift (major)
  - *Influenza A only*
  - Rare genetic reassortment
  - Coinfection of cells with two different strains of influenza A (H5N1 and H3N2); reassortment of segments of genome
  - Production of a new agent to which population has no immunity
  - Responsible for pandemics



- Antigenic drift (minor)
  - Influenza A and B
  - Slight changes in antigenicity due to mutations in H and/or N
  - Causes epidemics

Kaplan Micro-Immuno 2011 : Figure II-4-35

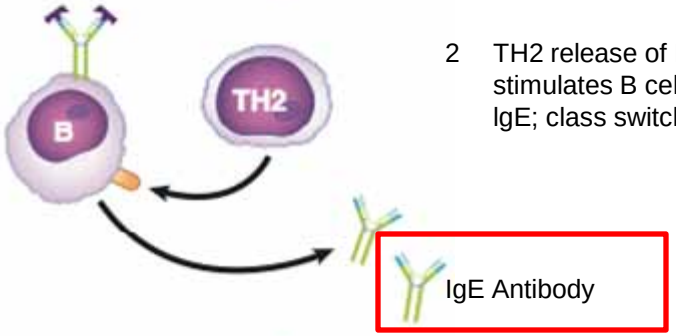
Antigen Variation 2

- Antigen Variation
  - Changing surface antigens to avoid immune destruction
  - Bacteria:
    - *Salmonella*: 2 different flagella
    - *Borrelia (Lyme disease)*: changes surface proteins
    - *N. gonorrhoeae*: pili and outer membrane proteins
  - Virus:
    - Influenza à Can undergo genetic shifts (major)
      and genetic drifts (minor)
  - Parasites:
    - *Trypanosoma brucei rhodesiense*
    - *Trypanosoma brucei gambiense*

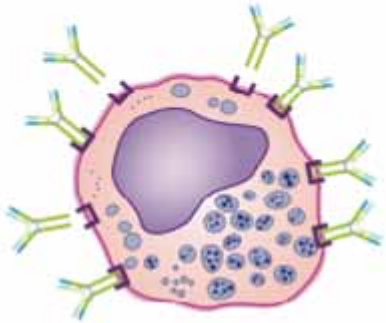
Type I Hypersensitivity

Development of Immediate Type I Hypersensitivity

1 First exposure to allergen

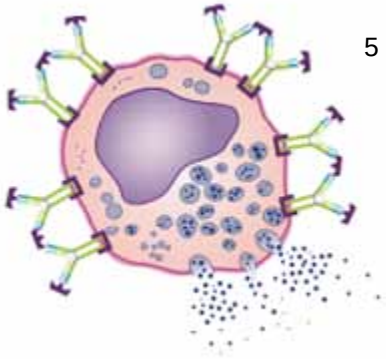


2 TH2 release of IL-4 and IL-13 stimulates B cell to produce IgE; class switching occurs



3 B cell produces IgE immunoglobulin; it attaches to Fc receptor on mast cell

4 Second exposure to allergen



5 Allergen cross-links several IgE molecules on mast cell and cell degranulates, releasing powerful chemicals

- Can cause symptoms shortly after exposure, including: flushing, itching, shock, hypotension, bronchospasm
- Can lead to anaphylactic shock

Kaplan Micro-Immuno 2011 : Figure I-13-1

FA 2013: 203.1 • FA 2012: 233.1 • FA 2011: 210.1
ME 3e: 106 • ME 4e: 106

Type II Hypersensitivity

Type II Hypersensitivity

- **Antibody**-mediated
- IgM or IgG
- Antibodies bind cells or tissue antigens, leading to:
  - Activation of complement (membrane attack complex), and/or
  - Recruitment of neutrophils
- Auto-antibodies à autoimmune disease
- Coombs test: tests for presence of antibodies
  - Direct vs. Indirect

Type III Hypersensitivity

Type III Hypersensitivity

- Antibody (IgG) binds antigen and then activates complement to form immune complexes
- Immune complexes become stuck in tissues, leading to inflammation
- **Examples:**
  - Serum sickness
    - Antibodies form against a foreign protein (horse proteins)
    - Once bound, complement cascade is activated, leading to formation of large immune complexes
    - Symptoms: 5-10 days post-exposure à fever, urticaria, arthralgias, proteinuria, lymphadenopathy
  - Arthus reaction: a local reaction (tetanus vaccine)
  - C3 deficiency: patients more prone to Type III reactions

Type IV Hypersensitivity

Type IV Hypersensitivity

- NO antibody involvement
- Delayed, cell-mediated reactions
  - CD8 and CD4 T cells
  - Takes time for these cells to activate, replicate, and spread throughout body
- **Examples:**
  - Transplant rejection
    - Hyperacute graft rejection however, is type II mediated
  - TB (PPD) skin test (granulomatous processes)
  - Contact dermatitis (poison ivy)

Examples of Type I HS Reactions

Commonly Tested Hypersensitivity Reactions

- Type I
  - IgE on basophils/mast cells binding antigen
  - **Examples:**
    - Anaphylaxis
      - » Bee stings
      - » Peanut allergies
    - Atopic disorders
      - » Allergic rhinitis (hay fever)
      - » Eczema
      - » Hives
  - **Note:** IgM is initial antibody produced, but IgE is responsible for reactions upon subsequent exposure

FA 2012: n/a • FA 2011: 211 • FA 2010: 209 • ME 3e: 106

Examples of Type II HS Reactions

Commonly Tested Hypersensitivity Reactions

- Type II
  - IgG/IgM antibody mediated
  - **Examples:**
    - Hemolytic anemia à warm antibody
    - Pernicious anemia (anti-parietal/IF antibodies à B12 deficiency)
    - Idiopathic thrombocytopenic purpura (anti-platelet antibodies)
    - Erythroblastosis fetalis
    - Acute hemolytic transfusion reaction
    - Rheumatic fever
    - Goodpasture's syndrome (anti-GBM antibodies)
    - Bullous pemphigoid (anti-hemidesmosome antibodies)
    - Pemphigus vulgaris (anti-desmosome antibodies)
    - Grave's disease (anti-TSH receptor antibodies)
    - Myasthenia Gravis (anti-Ach receptor antibodies)

Examples of Type III HS Reactions

Commonly Tested Hypersensitivity Reactions

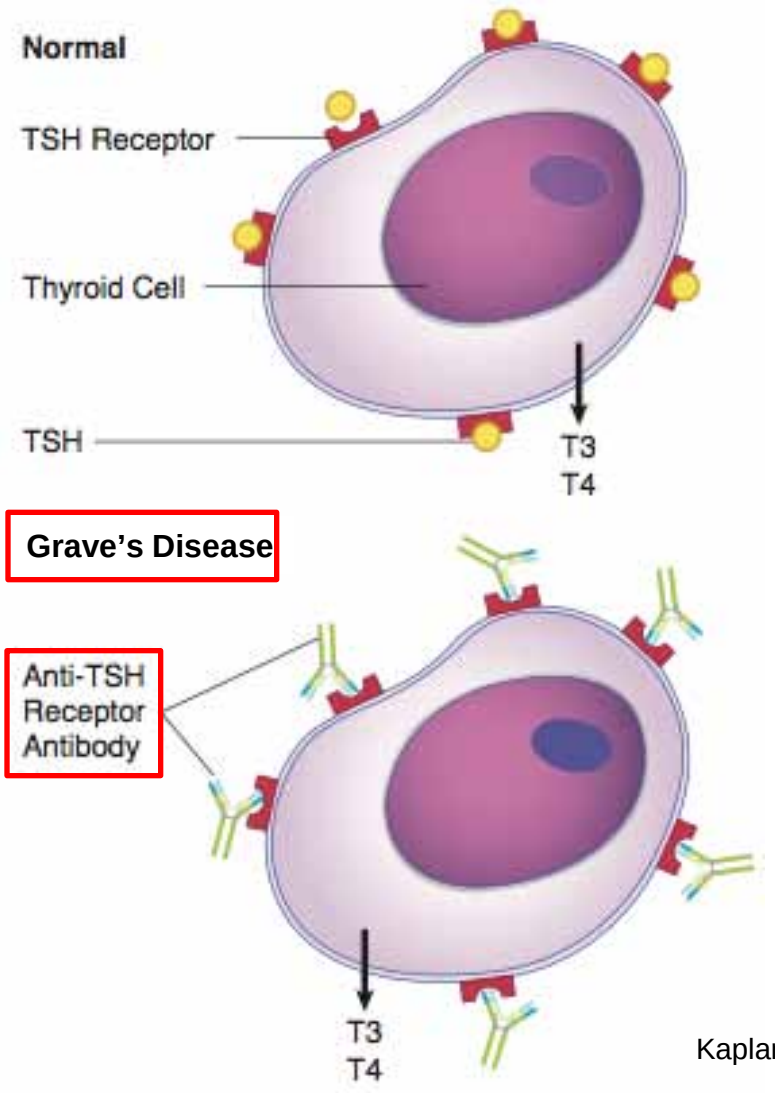
- Type III
  - Immune complexes (antibody-antigen-complement)
  - **Examples:**
    - Lupus (anti-DNA antibodies)
    - Rheumatoid arthritis (auto-antibodies in RF mediate **systemic** damage **outside** of the joints)
    - Polyarteritis nodosa (anti-HepB antibodies)
    - Poststreptococcal glomerulonephritis
    - Serum sickness
    - Arthus reaction (after tetanus vaccination)
    - Hypersensitivity pneumonitis

Examples of Type IV HS Reactions

Commonly Tested Hypersensitivity Reactions

- Type IV
  - Delayed and does not involve antibodies
  - **Examples**
    - Type 1 diabetes mellitus
      - » T cells attack β cells of pancreas
      - » Increased incidence in patients with HLA-DR3/DR4
    - Multiple sclerosis
    - Guillain-Barré syndrome
    - Hashimoto's thyroiditis (anti-thyroid peroxidase antibodies)
    - Graft versus host disease (GVHD)
    - TB (PPD) skin test
    - Contact dermatitis
    - Rheumatoid arthritis (Type IV reaction mediates **joint damage**)
  - **Note:** IL-10 inhibits Type IV hypersensitivity reactions

Autoantibodies: Grave's Disease



Kaplan Micro-Immuno 2011 : Figure I-13-4

Autoantibodies: Systemic Lupus Erythematosus

Antinuclear antibody (ANA)

- Sensitive, but nonspecific

Anti-ds DNA, anti-Smith

- Specific, but not sensitive

Autoantibodies: Rheumatoid Arthritis

- Auto-antibodies (IgM) against IgG
- Form antibody complexes that precipitate out of the blood, in the joints and vasculature, inducing compliment activation
- Complement lysis is responsible for tissue damage
- Rheumatoid factor (RF) is a serum marker used as an initial screening test

Autoantibodies: Scleroderma

Anticentromere antibodies

- Associated with CREST (a local version of scleroderma)
  - Calcinosis
  - Raynaud's syndrome
  - Esophageal dysmotility
  - Sclerodactyly
  - Telangiectasia

Anti-Scl-70 (anti-DNA topoisomerase I)

- Associated with diffuse scleroderma
  - Skin thickening
  - Pulmonary fibrosis

Autoantibodies: PBC & Celiac Disease

Primary biliary cirrhosis

- Antimitochondrial

Celiac disease

- Antigliadin
- Antiendomysial
- Antitransglutaminase

Autoantibodies 1

Goodpasture's syndrome

- Anti-basement membrane

Pemphigus vulgaris

- Anti-desmoglein

Hashimoto's thyroiditis

- Antimicrosomal
- Antithyroglobulin
- Antithyroid peroxidase

Autoantibodies 2

Polymyositis & Dermatomyositis

- Anti-Jo-1

Sjögren's syndrome ("RA + dryness")

- Anti-SS-A (anti-Ro)
- Anti-SS-B (anti-La)

Mixed connective tissue disease ("RA + SLE")

- Anti-U1 RNP (ribonucleoprotein)

Autoimmune hepatitis

- Anti-smooth muscle

Type 1 diabetes mellitus

- Anti-glutamate decarboxylase

Autoantibodies: ANCA

Wegener's granulomatosis (pulmonary + renal)

- c-ANCA (anti-neutrophil cytoplasmic antibody)

Microscopic polyangiitis & Churg-Strauss syndrome

- p-ANCA

KAPLAN MEDICAL

Immunology, Hematology, and Oncology

Lecture 4

- Immune deficiencies
- Solid organ transplantation
- Immunosuppressive drugs

B cell Disorders

1. Bruton's agammaglobulinemia

   X-linked defect in BTK; decrease in B cells & all Ig's à recurrent infections after 6 mos. of age

2. Hyper IgM syndrome

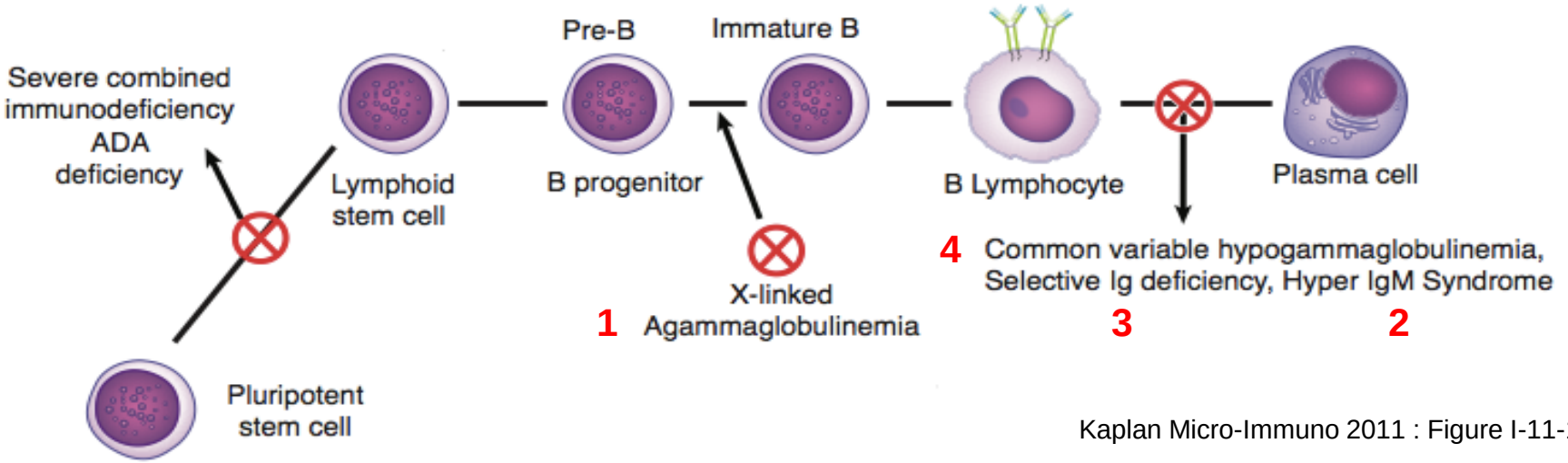
   Unable to class switch due to defective CD40L on helper T cells à recurrent pyogenic bacterial infections, lymphoid hyperplasia, sinopulmonary infections

3. Selective Ig deficiency

   Defect in isotype switching; IgA deficiency most common à GI infections (giardia), milk allergies, transfusion anaphylaxis

4. Common variable immunodeficiency (CVID) – "Acquired hypogammaglobulinemia"

   Sinopulmonary infections, autoimmune disease, lymphoma



Kaplan Micro-Immuno 2011 : Figure I-11-1

T cell Disorders – 1

- DiGeorge syndrome (thymic aplasia)
  - 22q11 deletion
  - 3rd & 4th pharyngeal pouches fail to develop à thymic aplasia
  - T cell deficiency à viral/fungal infections
  - Hypoparathyroidism à hypocalcemia à tetany
  - Characteristics: congenital heart and great vessel defects, facial abnormalities, low-set ears, depression of T-cell numbers, absence of T-cell responses
  - CXR of newborn: absent thymic shadow

- IL-12 receptor deficiency
  - Inability of macrophages to activate TH1 cells à ê INF-γ
  - Disseminated mycobacterial infections (TB, MAC)

Kaplan Micro-Immuno 2011 : Table I-11-4

T cell Disorders – 2

- Hyper-IgE syndrome
  - Job's syndrome
    - TH1 cells cannot make IFN-g
    - Neutrophils do not respond to chemotactic stimuli
    - Characterized by coarse facies, cold abscesses, retained primary teeth, increased IgE levels, and eczema

- Chronic mucocutaneous candidiasis
  - T cell dysfunction

    à Disseminated *Candida albicans* infection

Kaplan Micro-Immuno 2011 : Table I-11-1

B & T cell Disorders

- Severe combined immunodeficiency (SCID)
  - Recurrent viral, bacterial, fungal, and protozoal infections
  - Decrease in the amount & function of B & T cells
  - Causes:
    - Defective IL-2 receptor (X-linked)
      - IL-2 is secreted by TH1 cells and activates TH2 and CD8 cells
    - Adenosine deaminase deficiency
    - Failure to synthesize MHC II
- Ataxia telangiectasia
  - Defect in cell cycle kinase
  - Characteristics: ataxia, telangiectasia, deficient IgA and IgE production
- Wiskott-Aldrich Syndrome
  - X-linked defect in cytoskeletal glycoprotein
  - Defective responses to bacterial polysaccharides and depressed IgM, gradual loss of humoral and cellular responses, thrombocytopenia, and eczema; IgA and IgE may be elevated

Kaplan Micro-Immuno 2011 : Figure I-11-4

Phagocyte Disorders

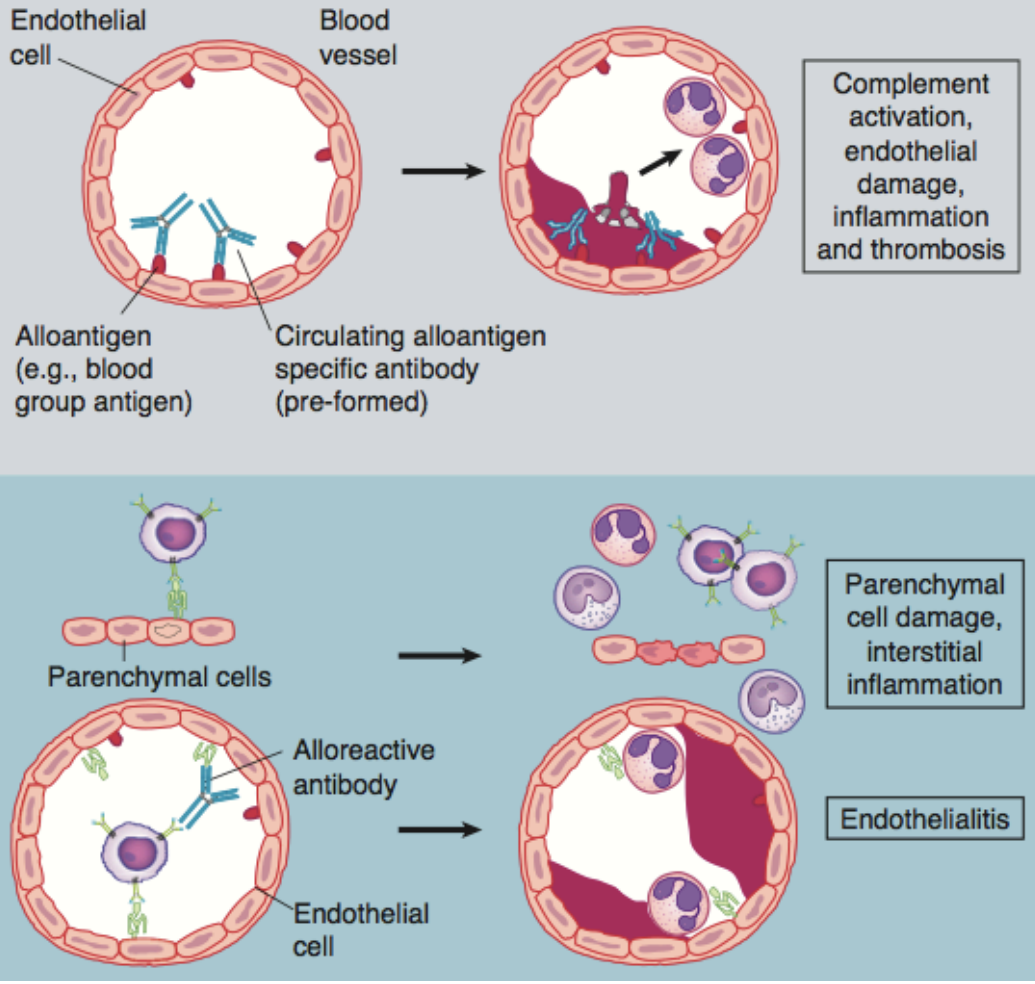
- Leukocyte adhesion deficiency
  - Absence of CD18 – common β chain of leukocyte integrins
  - Leukocytes unable to extravasate into tissues à recurrent, chronic infections; failure to form pus; no rejection of umbilical cord stump; gingivitis; periodontitis
  - Laboratory studies show neutrophilia
- Chediak-Higashi syndrome
  - Autosomal recessive defect of microtubule dysfunction à granule structural defect
  - Recurrent bacterial infections, chemotactic and degranulation defects, no NK activity, partial albinism, peripheral neuropathy, recurrent abscesses
- Chronic granulomatous disease
  - Deficiency of NADPH oxidase à no superoxide anion and other O2 radicals
  - Recurrent infections with catalase-positive bacteria and fungi
  - Abnormal giant lysosomal inclusions under light microscopy
  - Diagnosis: negative nitroblue tetrazolium dye reduction test (NO blue)

Types of Grafts

Grafts Used in Medicine	
Grafts	**Definition**
Autologous (**autografts**)	Tissue is moved from one location to another in the same individual
Isograft / Syngeneic	Transplants between genetically identical individuals (monozygotic twins)
Allograft	Transplants between genetically different members of the same species
Xenograft	Transplants between members of different species

Types of Graft Rejection – Acute

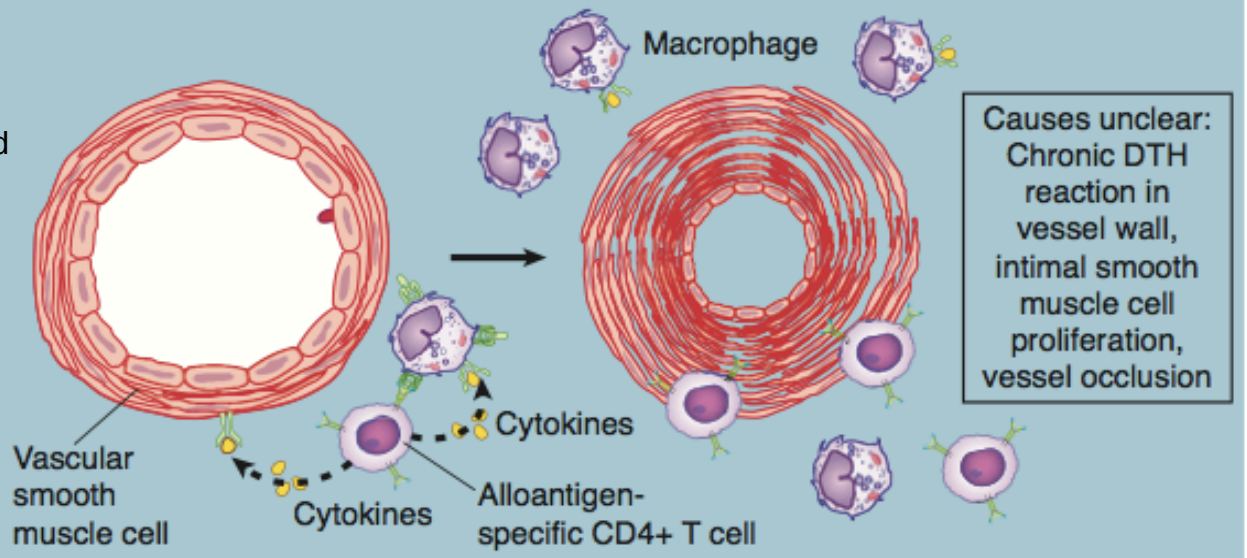
Type of Rejection	Time Taken	Mechanism & Pathogenesis
Hyperacute rejection	Minutes to hours	• Type II hypersensitivity reaction • Pre-formed antibodies to ABO antigens • Rarely occurs anymore because of screening for ABO incompatibility
Acute rejection	Days to weeks	• Cell-mediated • Cytotoxic CD8+T cells • Reversible with immunosuppressants (OKT-3)



Kaplan Micro-Immuno 2011 : Table I-14-1

Types of Graft Rejection – Chronic

Type of Rejection	Time Taken	Mechanism & Pathogenesis
Chronic rejection	Months to years	• CD4+ T cell & antibody-mediated



- Selected pathological findings:
  - Chronic rejection in the lung: bronchiolitis obliterans
    - Caused by CD8 cells
  - Chronic rejection in the kidney: injury to vascular endothelium (obliterative vascular fibrosis)
    - Mediated by antibodies

Kaplan Micro-Immuno 2011 : Table I-14-1

Types of Graft Rejection – GVHD

- Graft Versus Host Disease
  - T cells from donor organ attack recipient
  - **Symptoms:**
    - Maculopapular rash
    - Jaundice
    - HSM
    - Diarrhea
  - Seen in bone marrow and liver transplant recipients
  - Exact HLA matching in bone marrow transplant patients can prevent GVHD
  - “Graft versus tumor” effect can be useful in some cancers

Immunosuppressant Agents – 1

Immunosuppressant Agents		
Drug	**Mechanism**	**Uses**
Cyclosporine	Antibiotic that binds to cyclophilin → inhibits calcineurin (cytoplasmic phosphatase) → ↓NFAT activation of T-cell transcription factors → ↓ IL-2, IL-3, and interferon-γ (+) Cytotoxic CD+ T cells	• DOC in organ or tissue transplantation (± mycophenolate ± steroids ± cytotoxic drugs) • Side effects: peripheral neuropathy, nephrotoxicity, hyperglycemia, hypertension, hyperlipidemia, hirsutism, gingival overgrowth, cholelithiasis
Tacrolimus	Antibiotic that binds to FK-binding protein (FKBP); also inhibits calcineurin (similar to cyclosporine)	• Used alternatively to cyclosporine in renal and liver transplants • Side effects similar to cyclosporine

Immunosuppressant Agents – 2

Immunosuppressant Agents		
Drug	**Mechanism**	**Uses**
Sirolimus	• Binds to mTOR à Prevents IL-2 receptor activation à Inhibits T cell activation	• Immunosuppression after transplantation • Toxicity: hyperlipidemia, BM suppression (thrombocytopenia, leukopenia) • Minimal nephrotoxicity

Immunosuppressant Agents – 3

Monoclonal Antibodies (MABs) and Clinical Uses	
MAB	**Clinical Uses**
Daclizumab	Kidney transplants—blocks IL-2 receptors

Immunosuppressant Agents		
Drug	**Mechanism**	**Uses**
Azathioprine	Converted to mercaptopurine, whose metabolites inhibit purine metabolism Cytotoxic to proliferating lymphocytes (especially T cells)	Autoimmune diseases (e.g., SLE, rheumatoid arthritis) and immunosuppression in renal homografts Note: Lower the dose when giving to patients taking allopurinol

Monoclonal Antibodies (MABs) and Clinical Uses	
MAB	**Clinical Uses**
Muromonab (OKT3)	Allograft rejection block in renal transplants—binds the T3 (CD3) antigen on thymocytes Used for immunosuppression immediately following transplantation / Used to treat acute rejection

Immunosuppressant Agents – 4

Recombinant Cytokines and Clinical Uses	
Cytokine	**Clinical Uses**
Aldesleukin (IL-2)	↑ Lymphocyte differentiation and ↑ NKs—used in renal cell cancer and metastatic melanoma
Erythropoietin	Anemias, especially associated with renal failure
Filgrastim (G-CSF)	↑ Granulocytes—used for bone marrow recovery
Sargramostim (GM-CSF)	↑ Granulocytes and macrophages—used for bone marrow recovery
Interferon-α	Hepatitis B and C, leukemias, malignant melanoma, Kaposi sarcoma
Interferon-β	Multiple sclerosis
Interferon-γ	Chronic granulomatous disease →↑ TNF
Oprelvekin (IL-11)	↑ Platelet formation—used in thrombocytopenia
Thrombopoietin	Thrombocytopenia

Therapeutic Antibodies

Monoclonal Antibodies (MABs) and Clinical Uses	
MAB	**Clinical Uses**
Muromonab(OKT3)	Allograft rejection block in renal transplants—binds the T3 (CD3) antigen on thymocytes
Daclizumab	Kidney transplants—blocks IL-2 receptors
Digoxin Immune Fab	**Used to treat digoxin toxicity----binds digoxin**
Infliximab	Rheumatoid arthritis and Crohn disease—binds TNF-α
Adalimumab	Rheumatoid arthritis and Crohn disease **--- anti-TNF-α antibody**
Abciximab	Antiplatelet (acute coronary symptoms, post-angioplasty)—antagonist of IIb/IIIa receptors
Trastuzumab	Breast cancer—antagonist to HER2/neu receptor (*erb*-B2 monoclonal antibody)
Rituximab	Non-Hodgkin lymphoma—binds to CD20 antigen on B-cell surface protein

KAPLAN MEDICAL

Lymphoma and Multiple Myeloma

Leukemia versus Lymphoma

Leukemia

- Originates in bone marrow
- Tumor cells in peripheral blood
- **Not** a leukemoid reaction
  - Leukocytosis with left shift (increase in bands)
  - Leukemia: *Low* leukocyte alkaline phosphatase

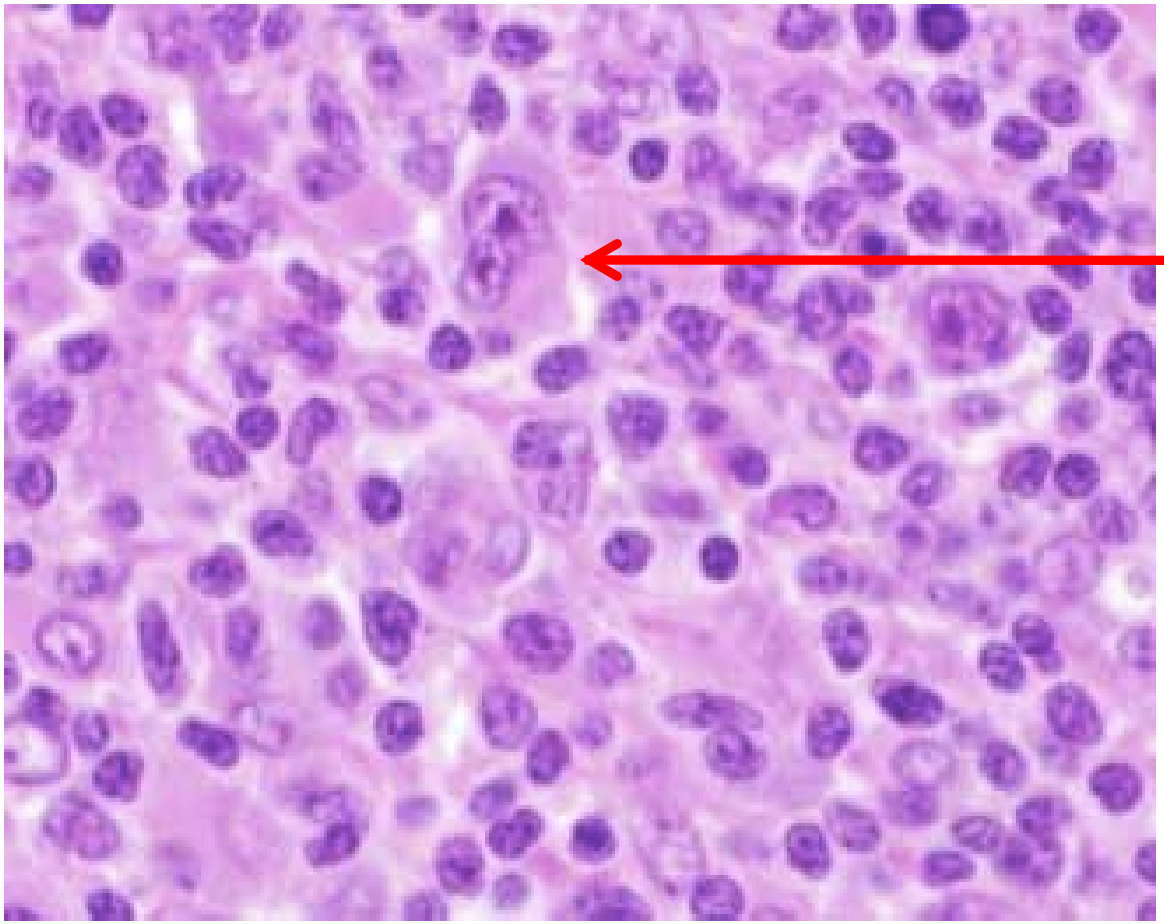
Lymphoma

- Discrete tumor mass in a lymph node

Hodgkin's versus Non-Hodgkin's Lymphoma

Hodgkin's Disease	Non-Hodgkin's Lymphoma
Reed-Sternberg cells	No characteristic cells
Local lymph nodes	Widespread lymph nodes
Constitutional symptoms (weight loss, fever, fatigue, night sweats)	Constitutional symptoms not as common
Many cases associated with EBV	May be associated with HIV
Prognosis: better with fewer Reed-Sternberg cells	Prognosis: Survival rates for NHL vary widely

Reed-Sternberg Cell



Reed-Sternberg cell

- Hodgkin's disease
- "Owl's eye"
- CD30/CD15 positive
- Somatic hypermutation

Hodgkin's Lymphoma

Four Variants of Hodgkin Disease		Prevalence
Lymphocyte predominance	Sea of lymphocytes, few RS cells, variable number of histiocytes, little fibrosis, and no necrosis	Rare subtype
Nodular sclerosis	• More common in women • Mediastinal, supraclavicular, and lower cervical nodes • Mixture of lymphocytes, histiocytes, a few eosinophils, plasma cells, and RS cells. Collagen bands create nodular pattern; RS cells called lacunar cells	Most common subtype
Mixed cellularity	• Mixture of neutrophils, lymphocytes, eosinophils, plasma cells, and histiocytes • Large number of RS cells	Common subtype
Lymphocyte depletion	• Rare lymphocytes, many RS cells with variable eosinophils, plasma cells, and histiocytes • Diffuse fibrosis may be seen	Rare subtype

Worsening Prognosis

Hodgkin’s Lymphoma Staging

Hodgkin’s lymphoma staging

1. Single lymph node (LN)

2. More than one LN / same side of diaphragm

3. More than one LN / both sides of diaphragm

4. Outside of LN system

Non-Hodgkin's Lymphoma: B Cell Types

Non-Hodgkin Lymphomas (NHL)		
Disease	**Characteristics**	**Pathology**
Burkitt undifferentiated lymphoma	Endemic in Africa (mandible or maxilla) and sporadic in the United States (abdomen) Children or young adults Tied to **Epstein-Barr virus (EBV),** especially the African form Leukemic phase is rare; prognosis is fair African Burkitt: translocations (8;14, 2;8, or 8;22) bring c-myc gene close to enhancers of heavy or light chain synthesis in B cells	Sea of moderately large lymphocytes with lipid-containing vacuoles interspersed with macrophages to produce **"starry sky pattern."**
Well-differentiated lymphocytic lymphoma (diffuse)	Older patients Generalized lymphadenopathy, hepatosplenomegaly Often seeds the blood late in the disease similar to CLL Bone marrow almost always involved Survival: 5–7 years	Lymph nodes replaced by small round lymphocytes with scant cytoplasm, dark nuclei, and rare mitoses
Mantle cell lymphoma	Median age 60; median survival 3 years t(11;14) translocation links immunoglobin heavy chain to bcl-1 Can present with lymphadenopathy, fever, night sweats, massive splenomegaly, or hepatomegaly	Expansion of the mantle zone surrounding germinal centers with small to medium atypical lymphocytes
Follicular lymphoma	Median age 60–65; median survival 8–10 years Painless adenopathy t(14;18) translocation brings bcl-2 close to heavy chain immunoglobulin gene May be associated with immunodeficiency states	Follicular or nodular pattern of growth with areas resembling germinal centers but lacking normal germinal center architecture

Non-Hodgkin's Lymphoma: T Cell Types

Non-Hodgkin Lymphomas (NHL)		
Disease	**Characteristics**	**Pathology**
Adult T-cell leukemia/ lymphoma (CD4 T cell)	• Endemic in Japan • Lymphadenopathy, hepatosplenomegaly, skin involvement, and hypercalcemia • Poor prognosis; however, many infected patients do not progress to disease	Caused by human T-cell leukemia/lymphoma virus (HTLV1); exposure to the virus may be decades earlier

Cutaneous T-Cell Lymphomas	
Mycosis fungoides	Three phases of skin lesions: inflammation, plaque, and tumor. Epidermal and dermal infiltrates by neoplastic T (CD4) cells with cerebriform nuclei. Nodules and fungating tumors may develop later in the disease. Nodal and visceral dissemination can occur.
Sézary syndrome	Rare chronic disease with progressive, pruritic erythroderma, exfoliation, and lymphadenopathy. "Sézary cells," T cells with cerebriform nuclei (similar to those seen in mycosis fungoides) infiltrate the peripheral blood. May be considered a preterminal phase of mycosis fungoides.

Multiple Myeloma

Multiple myeloma

Peak incidence is 50–60 years old; male = female

Multifocal plasma cell neoplasms in the bone marrow, occasionally soft tissues

Monoclonal immunoglobulin (IgG)

Signs and symptoms result from excess abnormal immunoglobulins (causing hyperviscosity) and from infiltration of various organs by neoplastic plasma cells

Proteinuria may contribute to progressive renal failure

Infiltration of bone with plasma cell neoplasms may lead to bone pain and hypercalcemia

Over 99% of patients have elevated levels of serum immunoglobulins or urine Bence-Jones proteins, or both

Serum protein electrophoresis (SPEP) shows homogeneous peak or "spike"

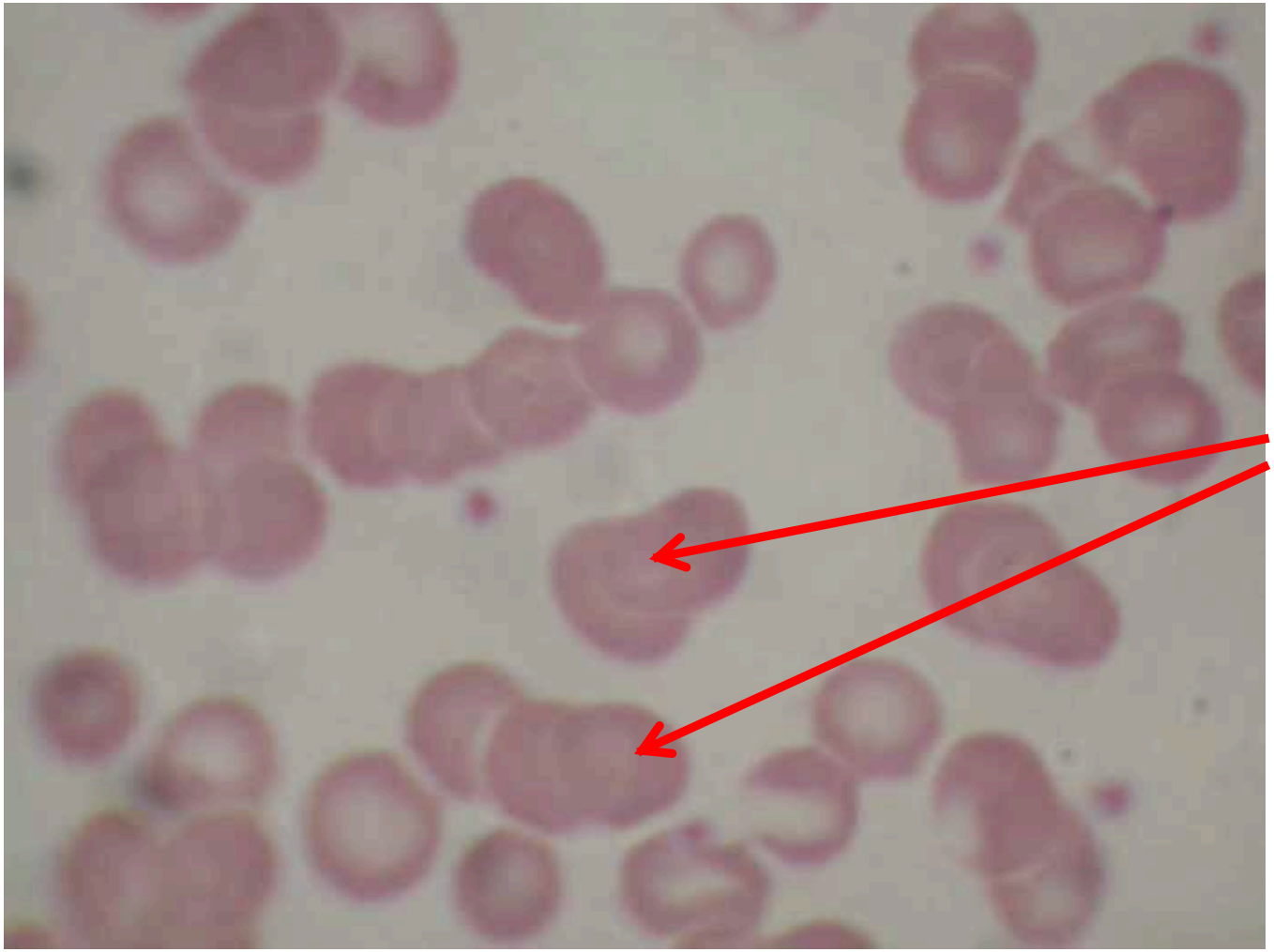
Marrow is infiltrated with plasma cells (usually over 30%) in various stages of maturation, called "myeloma cells"; contain cytoplasmic inclusions (acidophilic aggregates of immunoglobulin) called Russell bodies

Multiple osteolytic lesions throughout the skeleton; appear as "punched-out" defects on x-ray

Kidney: protein casts in distal tubules

Prognosis: less than 2-year survival without therapy; death usually results from infection, bleeding, or renal failure (**Bence-Jones proteins**)

Rouleaux Formation



Rouleaux formation of RBCs seen in multiple myeloma

Waldenström Macroglobulinemia

Waldenström macroglobulinemia

Age 60–70 years in both men and women
Monoclonal IgM resembles lymphocytic lymphoma with M-protein spike on serum protein electrophoresis
Symptoms due to hypergammaglobulinemia and tumorous infiltration
Hepatosplenomegaly, lymphadenopathy, bone pain, and hyperviscosity
Blindness and priapism due to hyperviscosity may be seen
2–5-year survival rate with chemotherapy

*** NO lytic bone lesions

Monoclonal gammopathy of undetermined significance

Asymptomatic M-protein spike on serum electrophoresis

Prognosis: initially thought benign, but approximately 2% may later develop myeloma, lymphoma, amyloidosis, or Waldenström macroglobulinemia

KAPLAN MEDICAL

Immunology, Hematology, and Oncology

Lecture 6

- Leukemia
- Myeloproliferative disorders

Leukemia vs. Lymphoma

- Leukemia
  - Lymphoid neoplasm originating in the bone marrow
  - Results in circulation of malignant lymphoid cells throughout the bloodstream

- Lymphoma
  - A discrete tumor mass arising from a lymph node

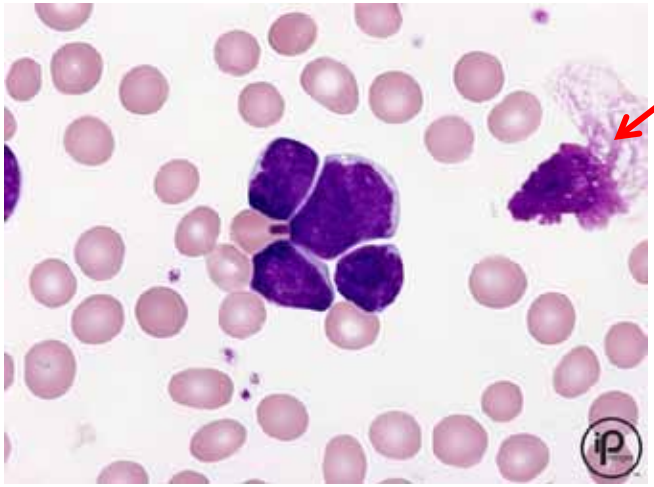
Acute Lymphocytic Leukemia

Lymphoid Leukemias		
Disease	**Characteristics**	**Pathology**
Acute lymphocytic leukemia (ALL)	• 60–70% of cases occur in childhood; peak age 4; rare over 50 • Half the children are cured; prognosis for adults is very poor • Fatigue, fever, epistaxis, gingival petechiae, ecchymoses 2° to thrombocytopenia; may have subarachnoid or cerebral hemorrhage • Present with lymphadenopathy, bone pain, hepatosplenomegaly • Most likely leukemia to involve CNS • Prognosis: death often from infection or bleed • Most cells pre-B cells; T-cell variants occur, usually affecting boys and causing a thymic mass that may compress the trachea	Smear: lymphoblasts are prominent; mature WBCs rare **CD10 (CALLA)** is the diagnostic surface marker; terminal deoxynucleotidyl transferase (TDT) positive in both B-cell and T-cell ALL and negative in AML

- **Good prognosis: CALLA+ / t(12;21)**

Chronic Lymphocytic Leukemia

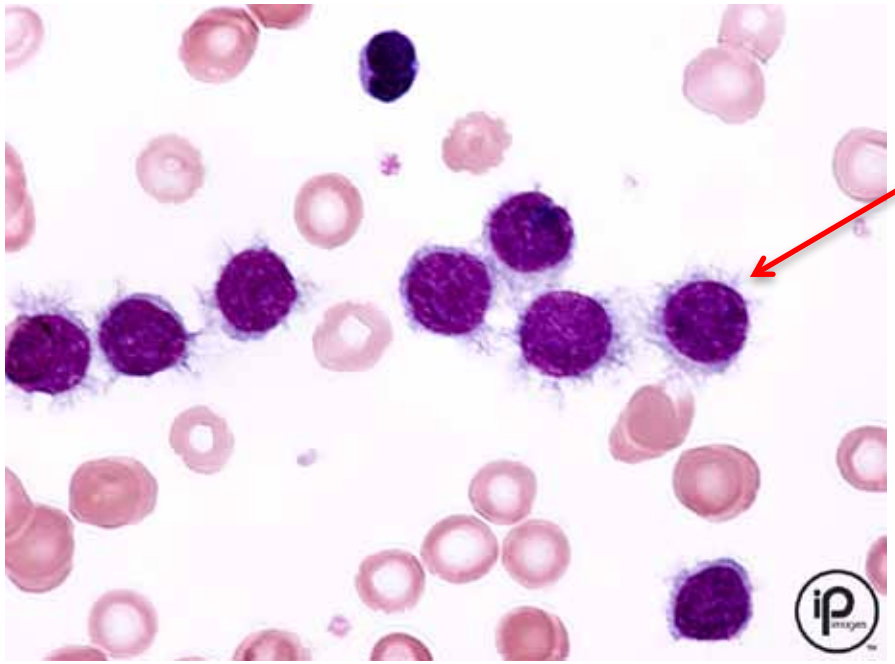
Lymphoid Leukemias		
Disease	**Characteristics**	**Pathology**
Chronic lymphocytic leukemia (CLL)	• Over 60 years of age • Asymptomatic or fatigue and weight loss; lymphadenopathy and hepatosplenomegaly later findings • Higher incidence of visceral malignancy • Median survival with treatment is 5 years but varies widely; prognostic factor is extent of disease • **Lymphocytosis** • **Hypogammaglobulinemia** • **Warm autoimmune hemolytic anemia (IgG against RBCs)**	Lymph node histology indistinguishable from diffuse, well-differentiated lymphocytic lymphoma Classic cell: CD5 B cell Cells do not undergo apoptosis **Peripheral smear:** **Smudge cells**



FA 2013: 364 • FA 2012: 392.1 • FA 2011: 359.2
ME 3e: 474 • ME 4e: 474

Hairy Cell Leukemia

Lymphoid Leukemias		
Disease	**Characteristics**	**Pathology**
Hairy cell leukemia	• Rare disease; cells express tartrate-resistant acid phosphatase • Present with hepatosplenomegaly; pancytopenia common • Prognosis: may now be cured with 2-chloro-deoxyadenosine (2CdA), an apoptosis inducer • **Mature B cell tumor** • **TRAP+ (tartrate–resistant acid phosphatase)**	Leukemic cells have "hair-like" cytoplasmic projections visible on phase-contrast microscopy Cells express some B-cell antigens



Hairy Cells

Acute Myelogenous Leukemia

Myeloid Leukemias		
Disease	**Characteristics**	**Pathology**
Acute myelogenous leukemia (AML)	20% of acute leukemia in children, most common acute leukemia in adults Signs and symptoms resemble ALL, except usually also present with lymphadenopathy or splenomegaly AML: t(15;17); acute promyelocytic leukemia: t(1;12) **M3 subtype (PML) --- responsive to Vitamin A treatment**	Primary cell type variable; see **Auer rods**

FA 2013: 365 • FA 2012: 392.1 • FA 2011: 359.2
ME 3e: 474 • ME 4e: 474

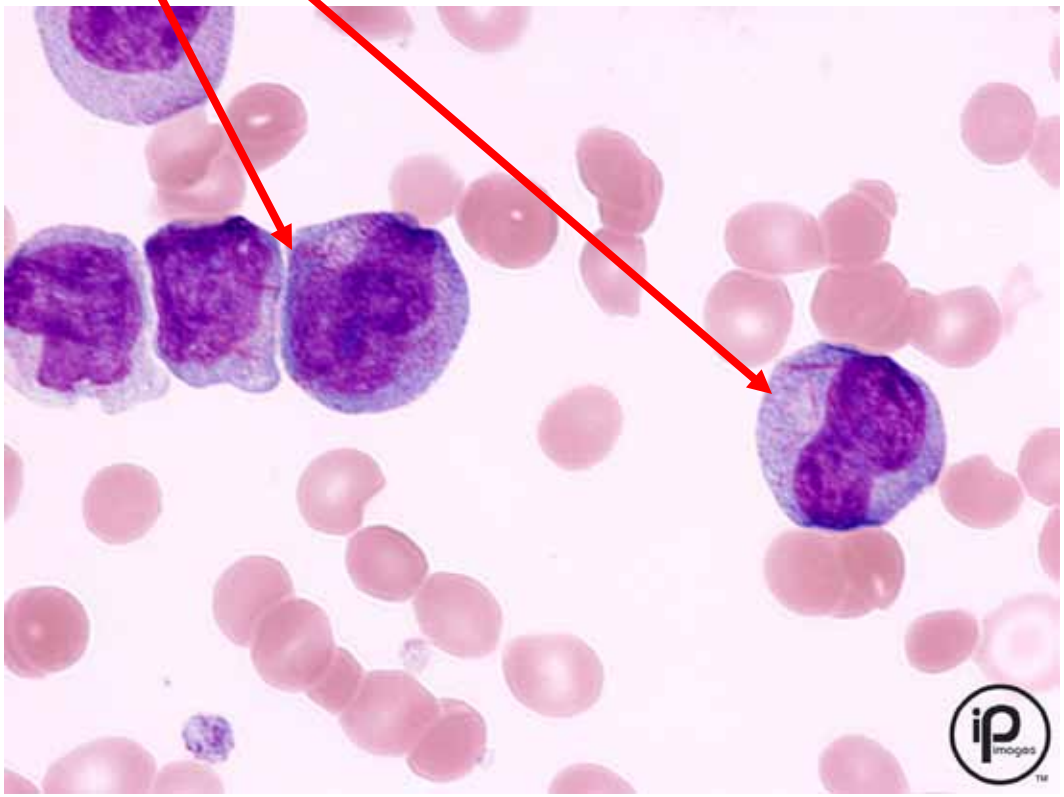
Chronic Myelogenous Leukemia

Myeloid Leukemias		
Disease	**Characteristics**	**Pathology**
Chronic myelogenous leukemia (CML)	• Middle age but may occur in children/young adults • Fatigue, fever, night sweats, and weight loss • Splenomegaly (up to 5 kg) giving abdominal discomfort • Variable remission period, may develop blast crisis • Two-thirds convert to AML; one-third to B-cell ALL • **Philadelphia chromosome (Ph1), t(9:22): *bcr:abl*** translocation is pathognomonic; present in 95% of cases • Prognosis in CML is worse in Ph1-negative patients • **Can transform into a blast crisis à ALL or AML** • **Rx: imatinib**	• Marked leukocytosis • Low-to-absent leukocyte alkaline phosphatase • Elevated serum vitamin B_{12} and vitamin B_{12}–binding proteins • High uric acid levels (due to rapid cell turnover)

Auer Rods

Auer Rods

- Peroxidase + cytoplasmic inclusions within granulocytes
- More commonly seen with promyelocytic M3 variant (APML)
- Responsive to vitamin A à can lead to DIC



FA 2013: 365.3 • FA 2012: 393.2 • FA 2011: 360.2
ME 3e: 474 • ME 4e: 474

Chromosomal Translocations

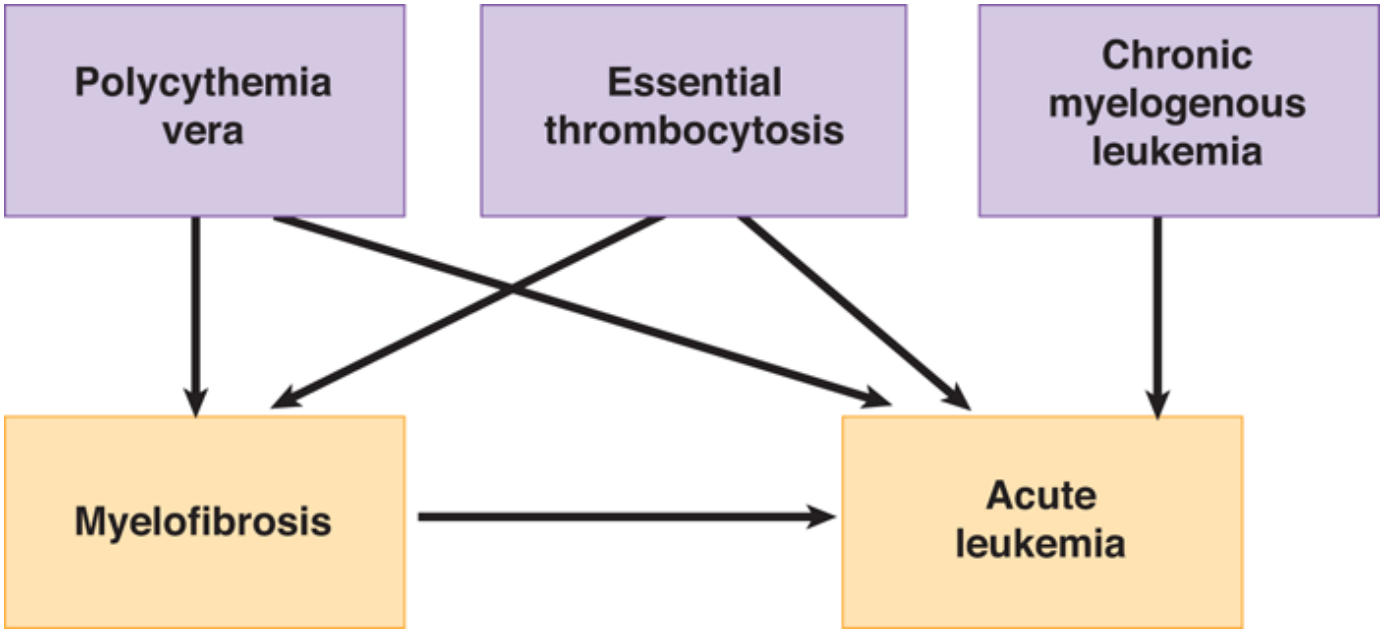
Translocation	Gene/protein association	Cause of disease	Disease association
t(9;22)	*bcr-abl* fusion protein	Activation of tyrosine kinase	CML
t(8;14)	*c-myc* activation	Transcriptional activation, proliferation	Burkitt's lymphoma
t(14;18)	*bcl-2* over-expression	Lack of normal apoptosis	Follicular lymphoma
t(15;17)	Responsive to vitamin A	Induces cellular differentiation	AML – M3
t(11;22)	*EWS/FLI* fusion protein	Master regulator of disease formation	Ewing's sarcoma
t(11;14)	cyclin D1 over-expression	Tumor cell growth	Mantle cell lymphoma

Langerhans Cell Histiocytosis

- Langerhans cell histiocytosis
  - A malignant neoplasm of Langerhans cell (skin dendritic cells)
  - Express S-100 and CD1a
  - May be characterized by erythematous papules, nodules, and/or scaling plaques, bone swelling, anemia
  - Diagnosis: Birbeck granules (tennis racquet-shaped cytoplasmic organelles)

Chronic Myeloproliferative Disorders – 1

- Myeloproliferative Disorders
  - Uncontrolled proliferation of certain cell populations



Kaplan Pathology 2011 : Figure 22-3

Myeloproliferative Disorders – 2

- Polycythemia Vera
  - EPO will be LOW
  - Abnormal clone of erythropoietic stem cells releasing increased amounts of RBCs into bloodstream
  - Results in elevated hemoglobin or hematocrit values

- Essential Thrombocytosis
  - Uncontrolled megakaryocyte production of platelets

- Myelofibrosis
  - A fibrotic obliteration of bone marrow à teardrop cells
  - Results in pancytopenia

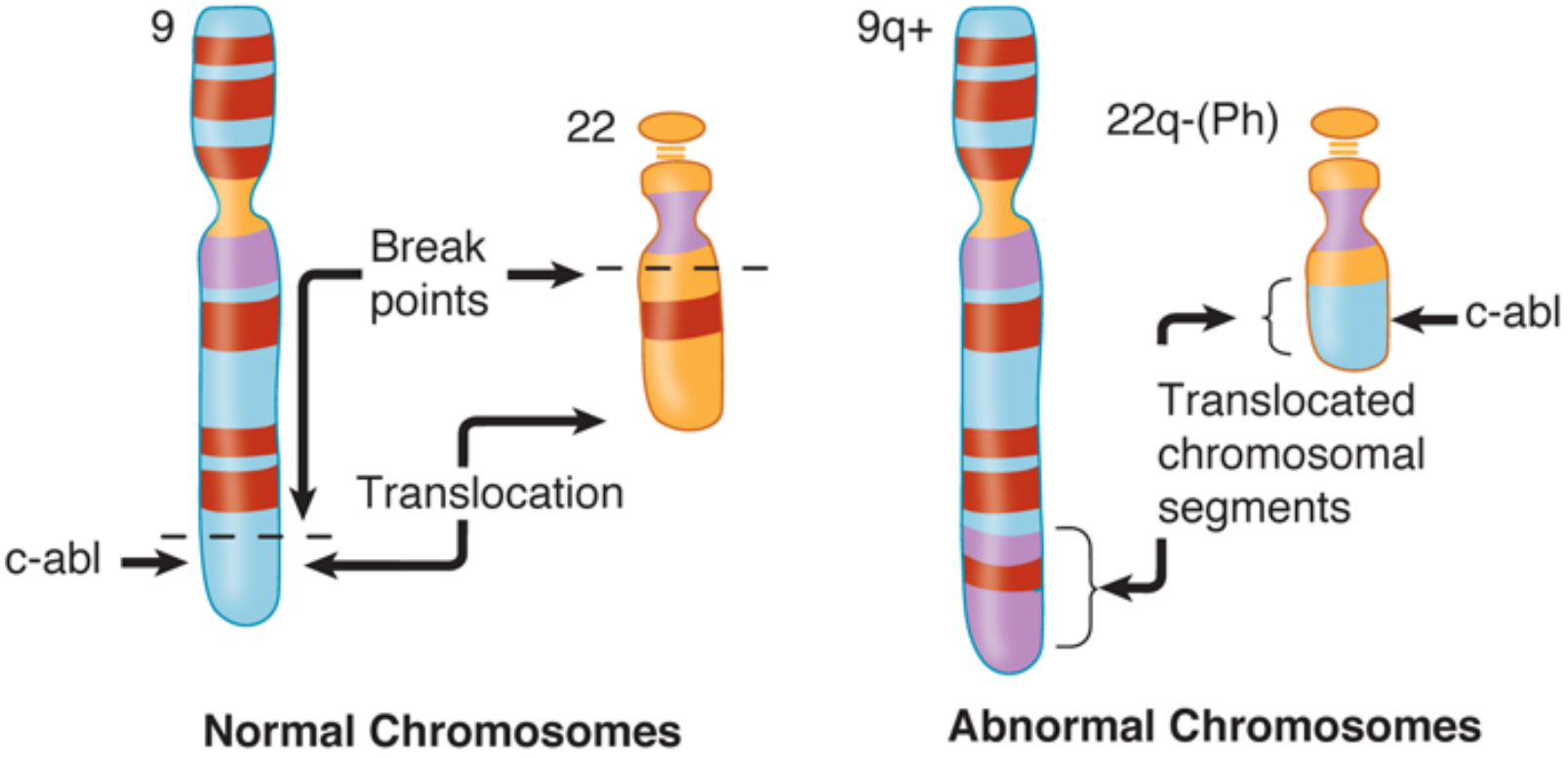
- CML
  - Secondary to bcr-abl fusion protein, t(9;22)
  - Increased cell division and inhibition of apoptosis

Myeloproliferative Disorders – 3

Disorder	RBCs	WBCs	Platelets	Phil Chrom	JAK2 mutation
Polycythemia vera				Negative	Positive
Essential thrombocytosis	--	--		Negative	Positive
Myelofibrosis	↓	↓	↓	Negative	Positive
CML	↓			Positive	Negative

- JAK2 mutation
  - Involved in hematopoietic growth factor signaling
  - Mutations can result in uncontrolled stem cell proliferation

Philadelphia Chromosome



Kaplan Pathology 2011 : Figure 22-4

FA 2013: 366.2 • FA 2012: 394.2 • FA 2011: 361.1
ME 3e: 474 • ME 4e: 474

Myeloproliferative Disorders – 4

How are myeloproliferative disorders related?

Polycythemia Vera à increase in RBCs

Essential Thrombocytosis à increase in platelets

↓

Bone marrow "burnout" à myelofibrosis

↓

Bone marrow: ↓RBC production à Liver/Spleen: ↑RBC production

↓

Myeloid Metaplasia

Evaluating Polycythemia

Polycythemia	Plasma Volume	RBC Mass	O2 Saturation	EPO	Associated Diseases
Relative	↓	--	--	--	
Appropriate absolute	--	↑	↓	↑	High altitude, Lung disease
Inappropriate absolute	--	↑	--	↑	Ectopic EPO
Polycythemia vera	↑	↑↑	--	↓	RCC, HCC, Wilm's tumor, hydronephrosis

Oncogenic Microbes

HTLV-1	Adult T cell leukemia/lymphoma
HBV, HCV	Hepatocellular carcinoma
EBV	Burkitt's lymphoma, Hodgkin's lymphoma nasopharyngeal carcinoma
HPV	Cervical carcinoma (16, 18)
HHV-8	Kaposi's sarcoma (in patients with HIV)
HIV	Primary CNS lymphoma (with CD4 < 50)
H. pylori	PUD and gastric adenocarcinoma
Schistosoma	Squamous cell carcinoma of the bladder

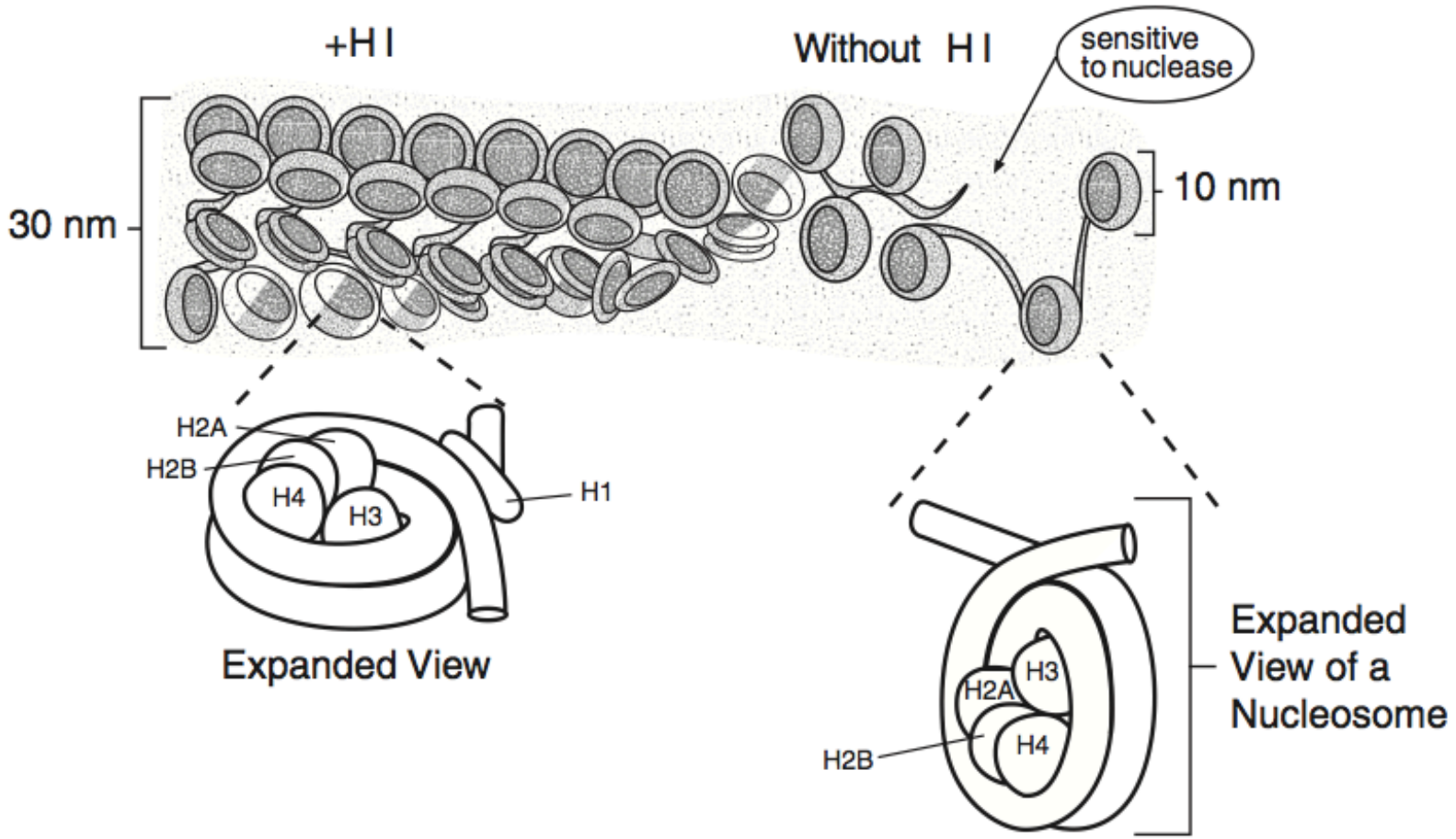
KAPLAN MEDICAL

DNA Replication and Repair

- Purine and pyrimidine synthesis
- DNA replication and repair
- Cell cycle

Structure of Chromatin

Eukaryotic Chromatin Structure



FA 2013: 64.1 • FA 2012: 68.1 • FA 2011: 66.1
ME 3e: 60 • ME 4e: 60

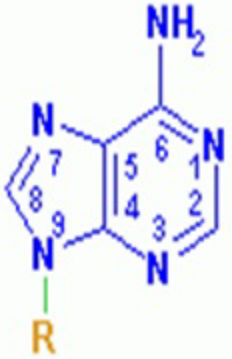
Heterochromatin versus Euchromatin

- Heterochromatin
  - Methylated DNA
  - Transcriptionally inactive

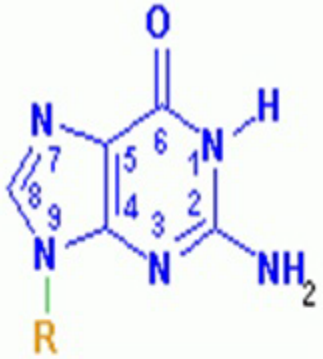
- Euchromatin
  - **A**cetylated DNA
  - Transcriptionally **A**ctive

Genetic Bases

Purines
"Pur as Gold"
- 2 carbon rings

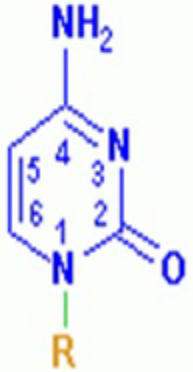


Adenine

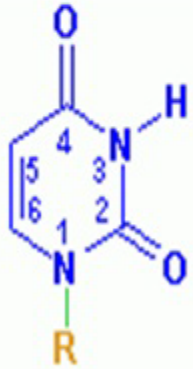


Guanine w/ Ketone

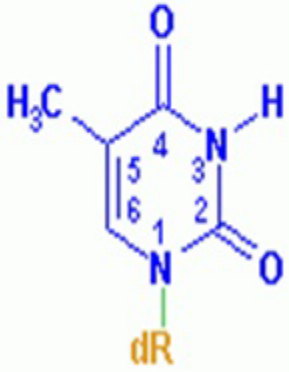
Pyrimidines
"Cut the Py"



Cytosine
w/ amino (NH2) group

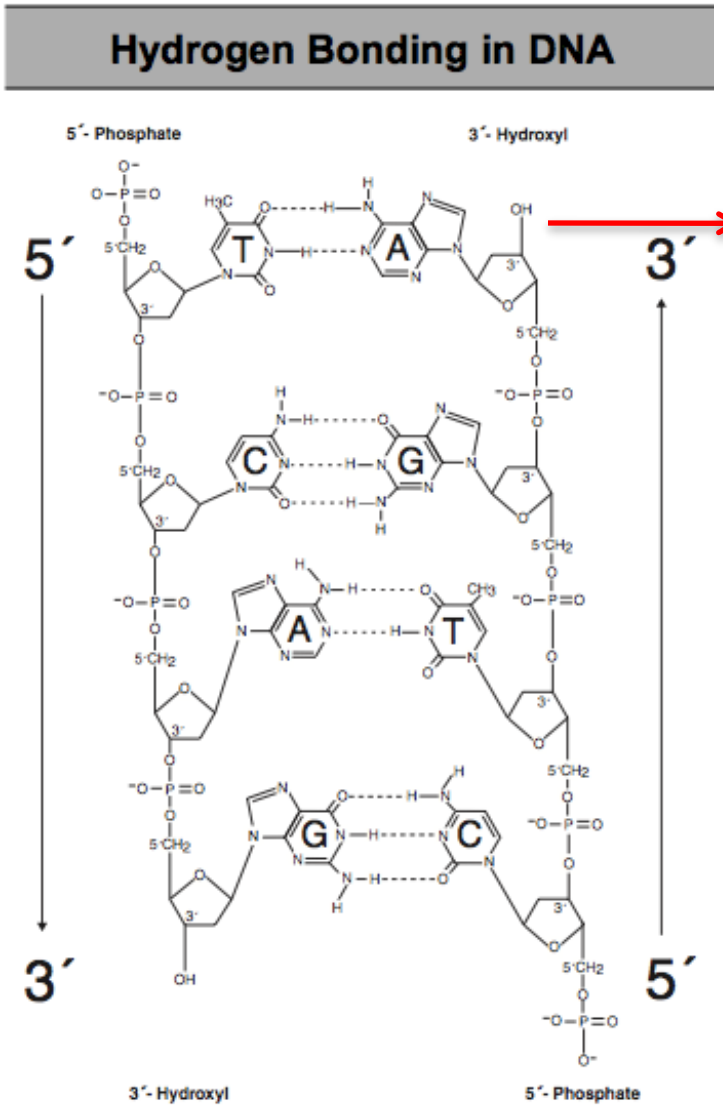


Uracil



Thymine
w/ methyl group

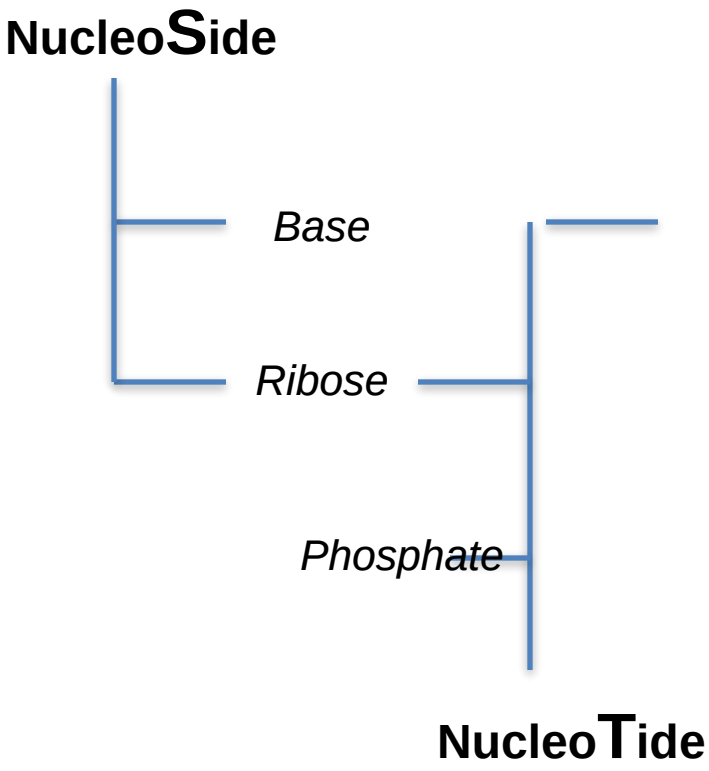
Genetic Base-Pairing



Note:
U binds to A in RNA

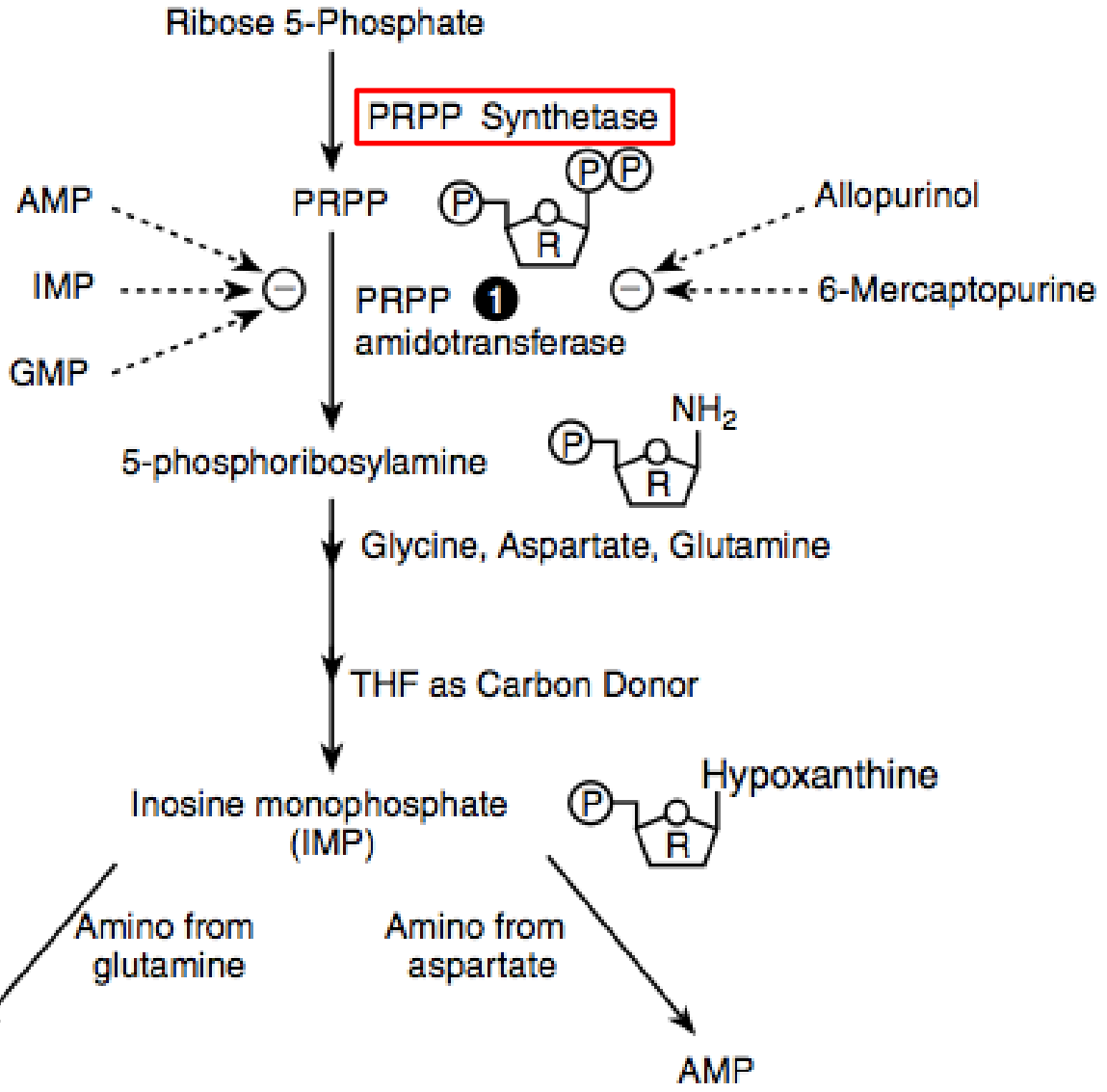
Genetic Nomenclature

Base	Nucleoside	Nucleotide
Adenine	Adenosine	Adenylic acid Adenosine monophosphate (AMP)
Guanine	Guanosine	Guanylic acid Guanosine monophosphate (GMP)
Cytosine	Cytidine	Cytidylic acid Cytidine monophosphate (CMP)
Uracil	Uridine	Uridylic acid Uridine monophosphate (UMP)



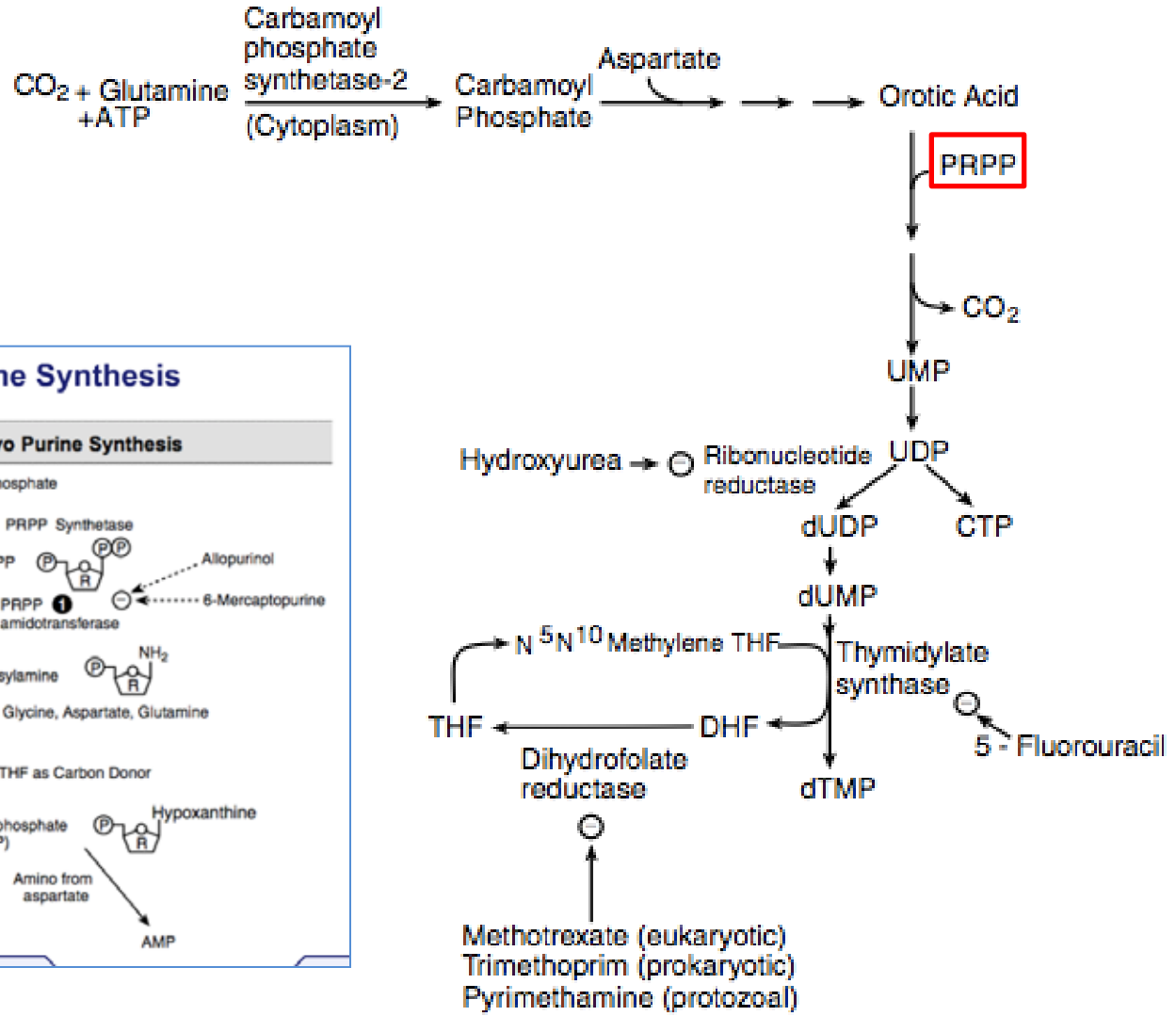
FA 2013: 64.2 • FA 2012: 68.2 • FA 2011: 67
ME 3e: 60 • ME 4e: 60

De Novo Purine Synthesis



FA 2013: 65.1 • FA 2012: 69.1 • FA 2011: 68
ME 3e: 61 • ME 4e: 61

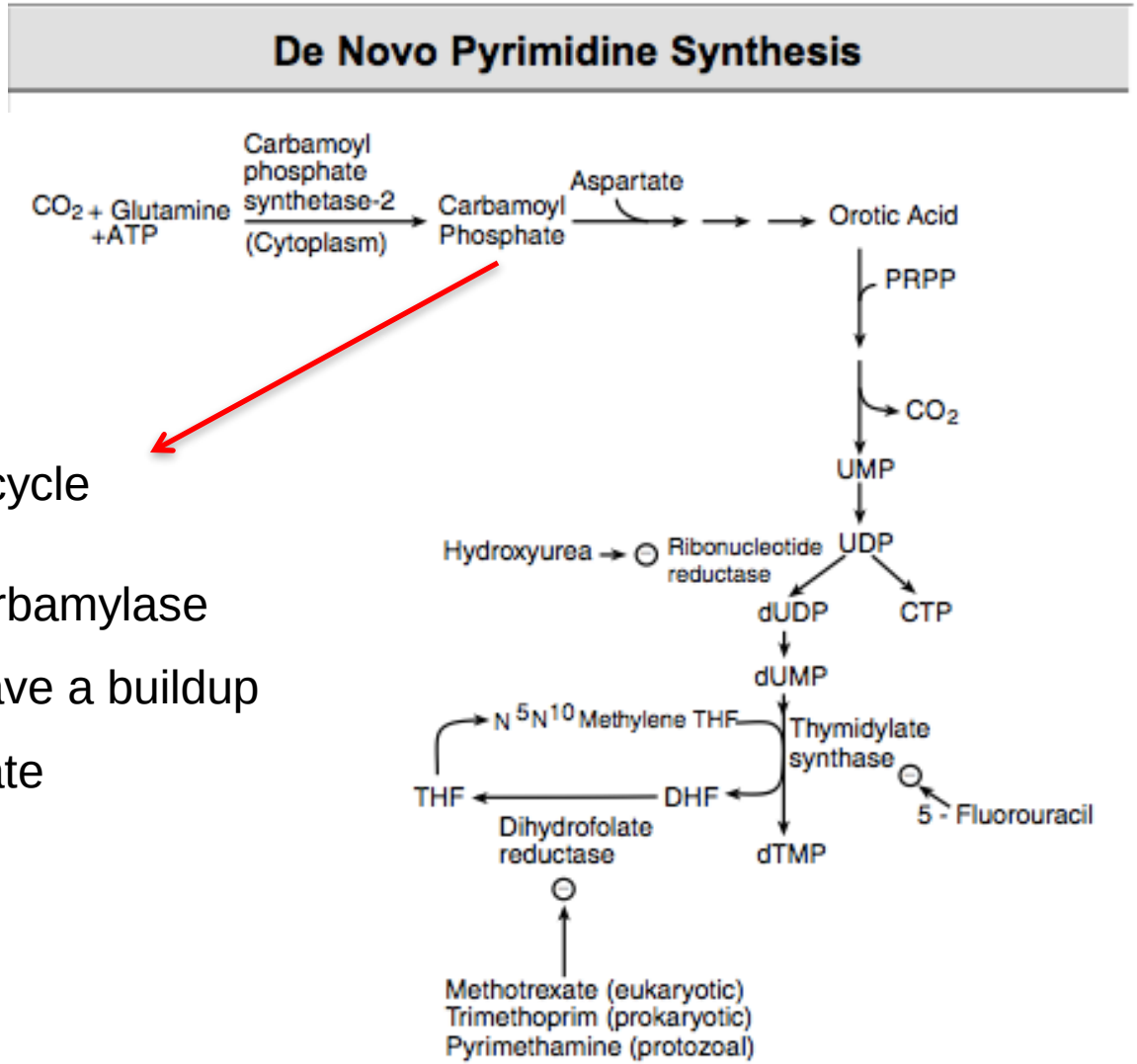
De Novo Pyrimidine Synthesis



Purine Synthesis

FA 2013: 65.1 • FA 2012: 69.1 • FA 2011: 68
ME 3e: 61 • ME 4e: 61

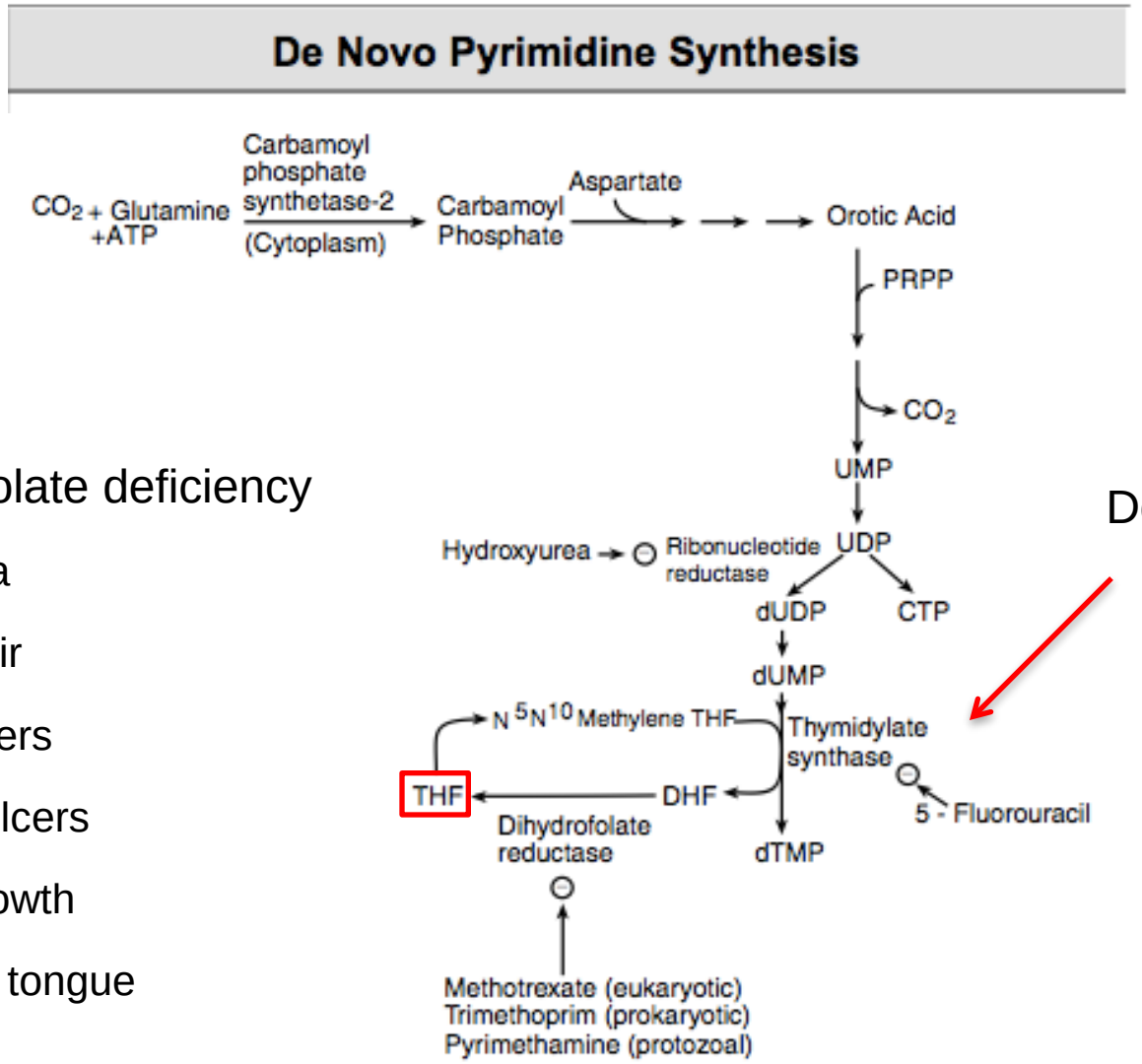
Carbamoyl Phosphate



- Also involved in urea cycle
- With ornithine transcarbamylase deficiency, patients have a buildup of carbamoyl phosphate

FA 2013: 65.1 • FA 2012: 69.1 • FA 2011: 68
ME 3e: 61 • ME 4e: 61

Thymidylate Synthase



Signs of folate deficiency

- Diarrhea
- Gray hair
- Oral ulcers
- Peptic ulcers
- Poor growth
- Swollen tongue

Dependent on folic acid

FA 2013: 65.1 • FA 2012: 69.1 • FA 2011: 68
ME 3e: 61 • ME 4e: 61

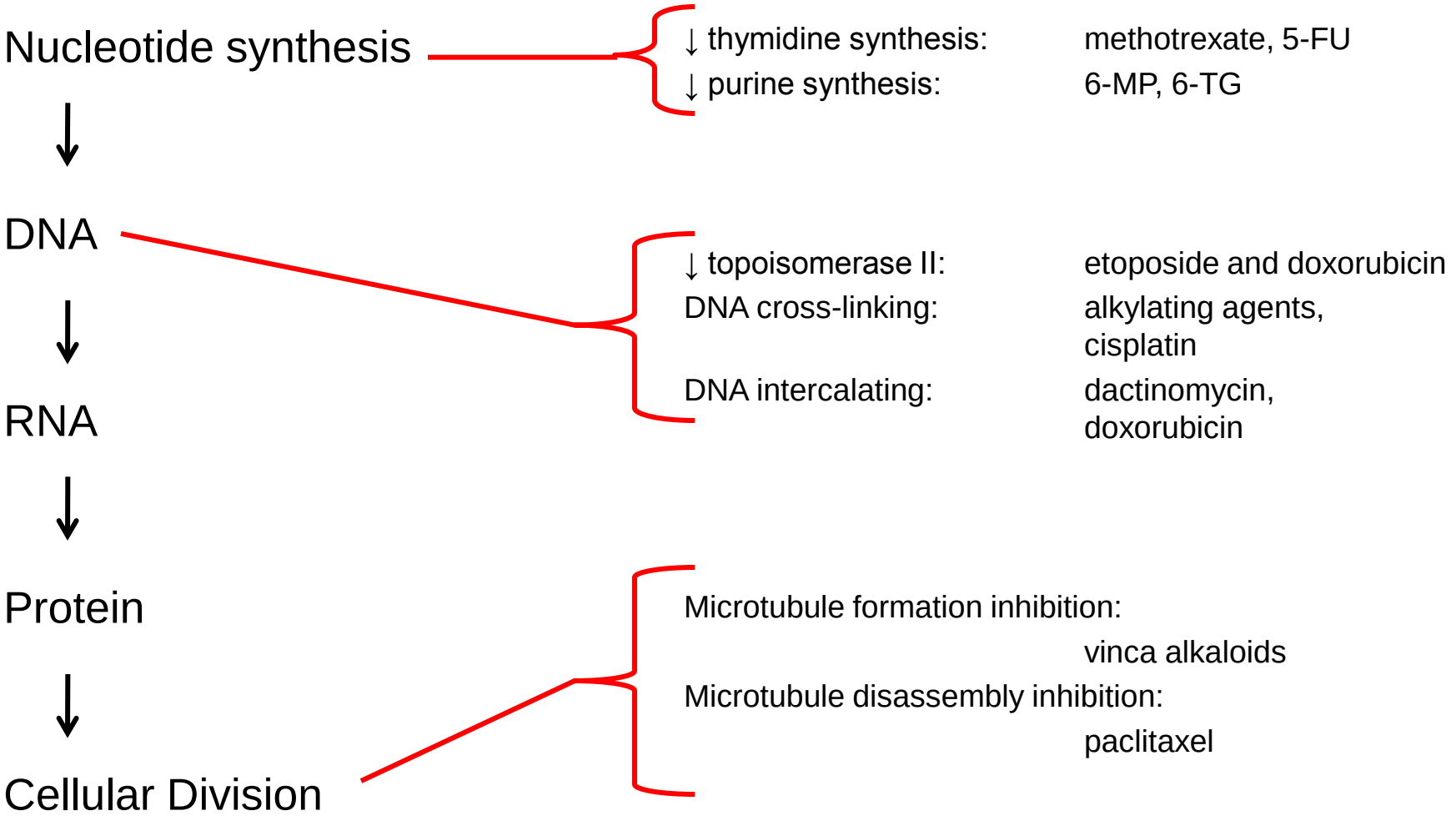
KAPLAN MEDICAL

Immunology, Hematology, and Oncology

Lecture 8

- Antineoplastic and antimetabolite drugs

Antineoplastic Drugs

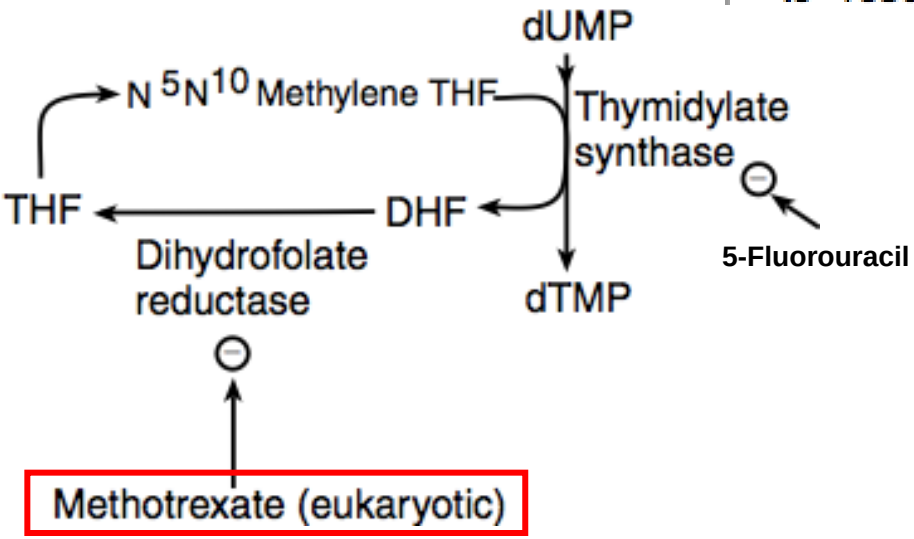


Antineoplastic Drug Resistance

- MDR-1
  - Human Multi-Drug Resistance Gene
  - P-glycoprotein
  - Inserts into plasma membrane of cancer cells
  - ATP-dependent efflux pump
  - Pumps chemotherapeutic drugs out of cell

Methotrexate

Mechanism	Indications/Toxicities
Cell-cycle specific (CCS). Inhibit synthesis of nucleic acids and thus protein synthesis.	
• A folic acid analog that inhibits dihydrofolate reductase; decreased dTMP levels hinder DNA and thus protein synthesis • **S-phase** specific	*Neoplastic indications:* leukemia, lymphomas, breast cancer, choriocarcinoma *Nonneoplastic indications:* rheumatoid arthritis, psoriasis, termination of pregnancy (e.g., ectopic) *Toxicities:* **suppresses bone marrow reversibly**; folinic acid (**leucovorin**) is used to **"rescue"**; fatty change in liver



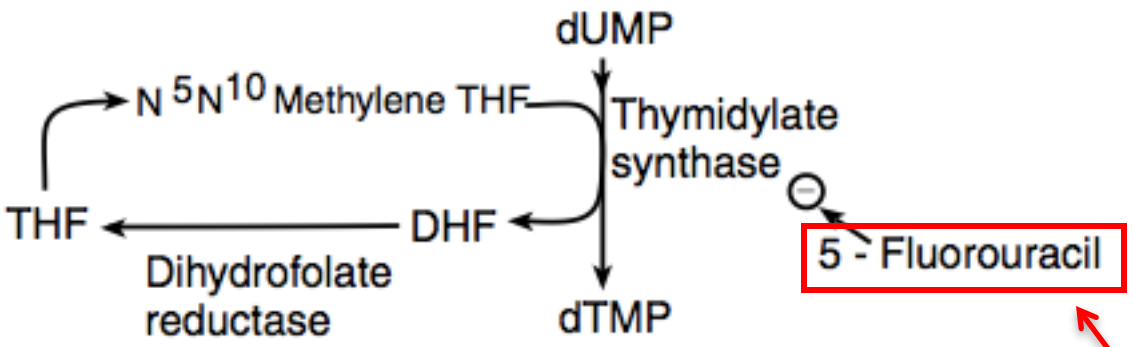
Toxicity:

- Myelosuppression (give leucovorin)
- Stomatitis
- Hepatotoxicity
- Contraindicated in pregnancy

FA 2013: 371.1 • FA 2012: 399.1 • FA 2011: 365.1
ME 3e: 176 • ME 4e: 176

5-Fluorouracil

Mechanism	Indications/Toxicities
Cell-cycle specific (CCS). Inhibit synthesis of nucleic acids and thus protein synthesis.	
• Pyrimidine antimetabolite is converted to **5-F-dUMP**, which when bound to folic acid, **inhibits thymidylate synthase**. This prevents dTMP synthesis, thus inhibiting DNA and protein synthesis. • **S-phase** specific	*Indications:* breast, ovarian, colon, head and neck cancers, basal cell carcinomas and keratoses (use topically) *Toxicities:* **irreversible myelosuppression** and **photosensitivity**, GI irritation, alopecia



Toxicity:

- Myelosuppression (give thymidine)

Dependent on folic acid

FA 2013: 371.1 • FA 2012: 399.1 • FA 2011: 365.1
ME 3e: 176 • ME 4e: 176

6-MP, 6-TG, and Cytarabine

Class	Mechanism	Indications/Toxicities
Antimetabolites	**Cell-cycle specific (CCS).** Inhibit synthesis of nucleic acids and thus protein synthesis.	
6-Mercaptopurine (6-MP)	• Activated by hypoxanthine-guanine phosphoribosyltransferase (**HGPRT**) • **Inhibits purine synthesis**, inhibiting nucleic acid synthesis • **S-phase** specific	*Indications:* acute leukemias, CML, non-Hodgkin lymphoma *Toxicities:* • BMS, hepatotoxicity—coadministration with allopurinol increases toxicity (6-MP metabolized by xanthine oxidase) • Azathioprine forms 6-MP
6-Thioguanine	• Same as 6-Mercaptopurine	*Indications:* Acute lymphoid leukemia *Toxicities:* BM suppression, liver * Can be administered to patients taking allopurinol
Cytarabine (Ara-C)	• Pyrimidine antimetabolite • Inhibits DNA polymerases • **S-phase** specific	*Indications:* acute leukemias *Toxicities:* BMS, GI irritation, ↑ doses → neurotoxicity

FA 2013: 371.1 • FA 2012: 399.1 • FA 2011: 365.1
ME 3e: 176 • ME 4e: 176

Antitumor Antibiotics

Antibiotics		
Dactinomycin (ACTinomycin D)	Intercalates DNA	*Indications:* Wilm's tumor, Ewing's sarcoma, rhabdomyosarcoma Used for childhood tumors à *children ACT out*
		Toxicity: Myelosuppression
Doxorubicin (Adriamycin)	Intercalates DNA, creating breaks. Hinders DNA replication and transcription.	*Indications:* Hodgkin lymphoma (ABVD †), breast, endometrial, lung, ovarian CAs, myeloma, sarcomas
		Toxicities: **cardiotoxic—dexrazoxane** (inhibits free radical formation may protect), BMS, alopecia, GI distress
Bleomycin	• Generates free radicals → DNA strand scission • **G_2 phase** specific	*Indications:* lymphomas, testicular, skin CA
		Toxicities: **pulmonary fibrosis,** mucocutaneous reactions (blisters, alopecia), hypersensitivity reactions
Etoposide	• Inhibits topoisomerase II, ↑ DNA degradation • **Late S/early G_2** phase	*Indications:* small cell carcinoma, prostate cancer, testicular carcinoma
		Toxicities: BMS, GI irritation, alopecia

*** All work by generating free radicals (except etoposide)**

FA 2013: 372.1 • FA 2012: 400.1 • FA 2011: 366.1
ME 3e: 177 • ME 4e: 177

Alkylating Agents

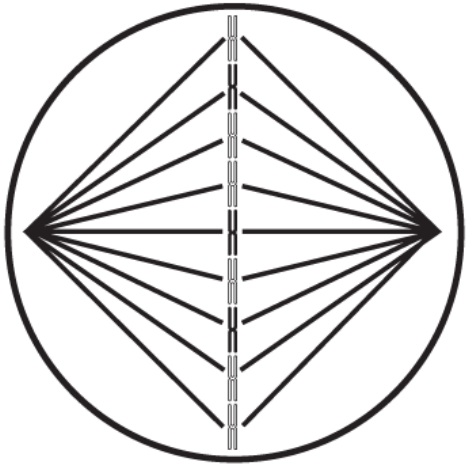
Alkylating Agents	**Cell cycle-nonspecific (CCNS).** This class of agents causes alkylation of DNA, leading to cross-linking, abnormal base pairing, or DNA strand breakage.	
Cyclophosphamide	Alkylates DNA—attacks guanine N7, induces cross-linking	*Indications:* non-Hodgkin lymphoma; ovarian and breast cancers; neuroblastoma
		Toxicities: BMS and **hemorrhagic cystitis** (can be ↓ by **mesna** which traps **acrolein**, a toxic metabolite)
Nitrosoureas (lomustine, carmustine)	• Alkylates DNA • Crosses blood–brain barrier	*Indications:* brain tumors
		Toxicities: **neurologic**
Busulfan	Alkylates DNA	*Indications:* CML
		Toxicities: **pulmonary fibrosis, hyperpigmentation**, and adrenal insufficiency
Cisplatin, carboplatin	Alkylates DNA	*Indications:* testicular, bladder, lung, and ovarian carcinomas *Toxicities:* **nephrotoxic** neurotoxicity → **Add amifostine** **(deafness, tinnitus)** Bone marrow suppresion (Carboplatin > Cisplatin)

FA 2013: 372.2 • FA 2012: 400.2 • FA 2011: 366.2
ME 3e: 176 • ME 4e: 176

Microtubule Inhibitors

Plant Alkaloids	Cell-cycle specific drugs that prevent the assembly of microtubules. Thus, mitotic spindle cannot form.	
Vinblastine	• Inhibits microtubule/spindle formation • **M-phase** specific	*Indications:* lymphoma, Wilms tumor, choriocarcinoma *Toxicities:* BMS
Vincristine	• Inhibits microtubule/spindle formation • **M-phase** specific	*Indications:* same as vinblastine, MOPP* (is Oncovin) *Toxicities:* neurotoxic, GI distress
Paclitaxel	• Stabilizes microtubules so that spindle cannot break down • **M-phase** specific	*Indications:* ovarian and breast carcinomas *Toxicities:* BMS

Metaphase



FA 2013: 373.1 • FA 2012: 401.1 • FA 2011: 367.1
ME 3e: 177 • ME 4e: 177

Other Antineoplastic Agents – 1

Antineoplastic Agents (*Cont'd.*)		
Class	**Mechanism**	**Indications/Toxicities**
Hydroxyurea (HU)	An antimetabolite that inhibits ribonucleotide reductase HU reactivates HbF synthesis and increases the number of reticulocytes containing HbF in sickle cell patients	Sickle cell anemia, polycythemia vera, and chronic myelogenous leukemia
Prednisone	Induces apoptosis of lymphoid cells ↓ K^+ ↑ Na^+ ↓Ca^{2+} ←	*Indications:* chronic lymphocytic leukemia (CLL), Hodgkin lymphoma (MOPP*), autoimmune disease *Toxicities:* typical symptoms of glucocorticoid excess, including **Cushing syndrome**
Tamoxifen, raloxifene	**Selective estrogen receptor modulator (SERM)**. Prevents estrogen from binding estrogen receptor-positive breast CA cells, leading to involution of estrogen-dependent tumors.	*Indications:* breast cancer *Toxicities:* **hot flashes**, increased risk of **endometrial carcinoma** Raloxifene – no increased risk of endometrial cancer

Other Antineoplastic Agents – 2

- Trastuzumab (Herceptin)
  - Monoclonal antibody against HER-2 (erb-B2) receptors
  - Used to treat metastatic breast cancer
  - Main toxicity is cardiotoxicity

- Imatinib (Gleevec)
  - Monoclonal antibody against *bcr-abl* fusion protein
  - Used to treat CML, GI stromal tumors
  - Main toxicity is fluid retention

- Rituximab
  - Monoclonal antibody against CD20, on most B-cell neoplasms
  - Used to treat Non-Hodgkin's lymphomas, rheumatoid arthritis (with methotrexate)

KAPLAN MEDICAL

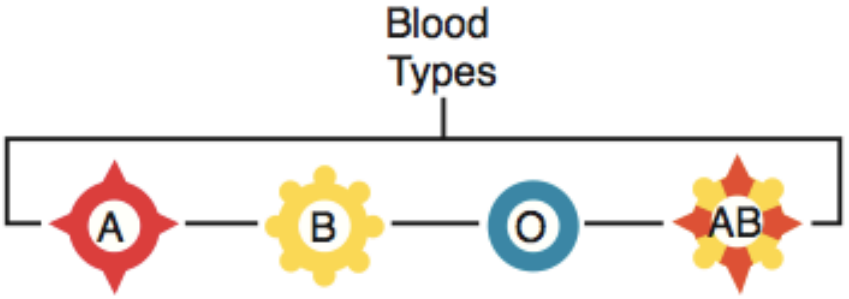
Non-hemolytic Anemia and Porphyria

- Erythrocyte
- Porphyrias
- Microcytic anemia
- Macrocytic anemia
- Nonhemolytic normocytic anemia

Red Blood Cells

- Red Blood Cells
  - Lack nuclei
  - Biconcave shape
  - Cytoplasm contains hemoglobin
    - Adult hemoglobin contains 2 α-chain and 2 β-chain subunits
  - Primary source of energy is glucose
    - Anaerboic glycolysis
    - 90% converted to lactate
    - 10% sent to Hexose Monophosphate (HMP) shunt
  - Bicarbonate chloride transporter on cell surface
  - Average lifespan of RBC is 120 days

Blood Typing Agglutination Test

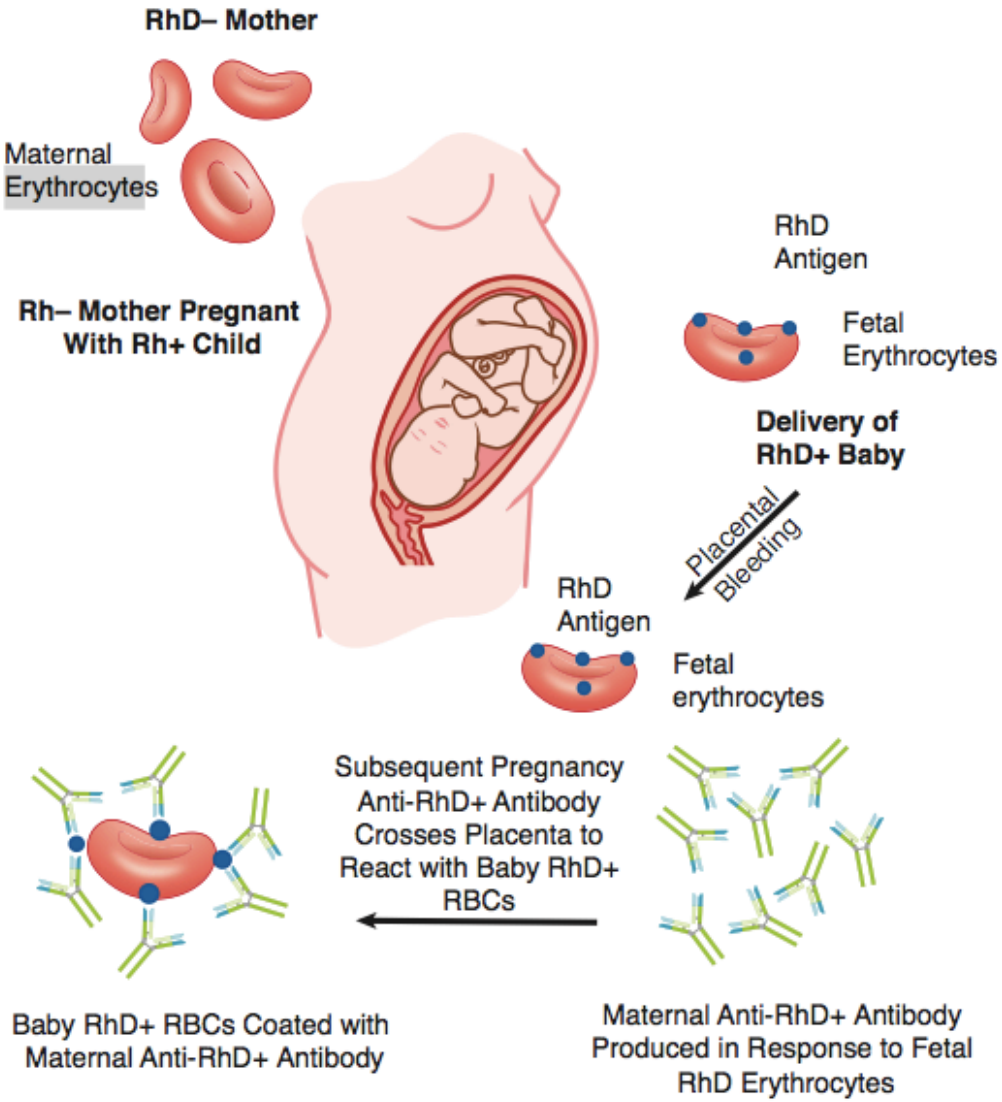


		A	B	O	AB
Serum from: Contains:	A Anti-B Antibodies	N	A	N	A
Serum from: Contains:	B Anti-A Antibodies	A	N	N	A
Serum from: Contains:	O Anti-A and Anti-B Antibodies	A	A	N	A
Serum from: Contains:	AB No Antibodies to A or B	N	N	N	N

A = Agglutination
N = No Agglutination

Kaplan Immuno-Micro 2011 : Figure I-14-1

Rh Antigen



Rhogam à given to Rh- mothers prior to delivery to prevent formation of Anti-Rh+ antibodies

Rh antigens are IgG à

Do cross placenta

A and B antigen are IgM à

Do Not cross placenta

Kaplan Immuno-Micro 2011 : Figure I-13-3

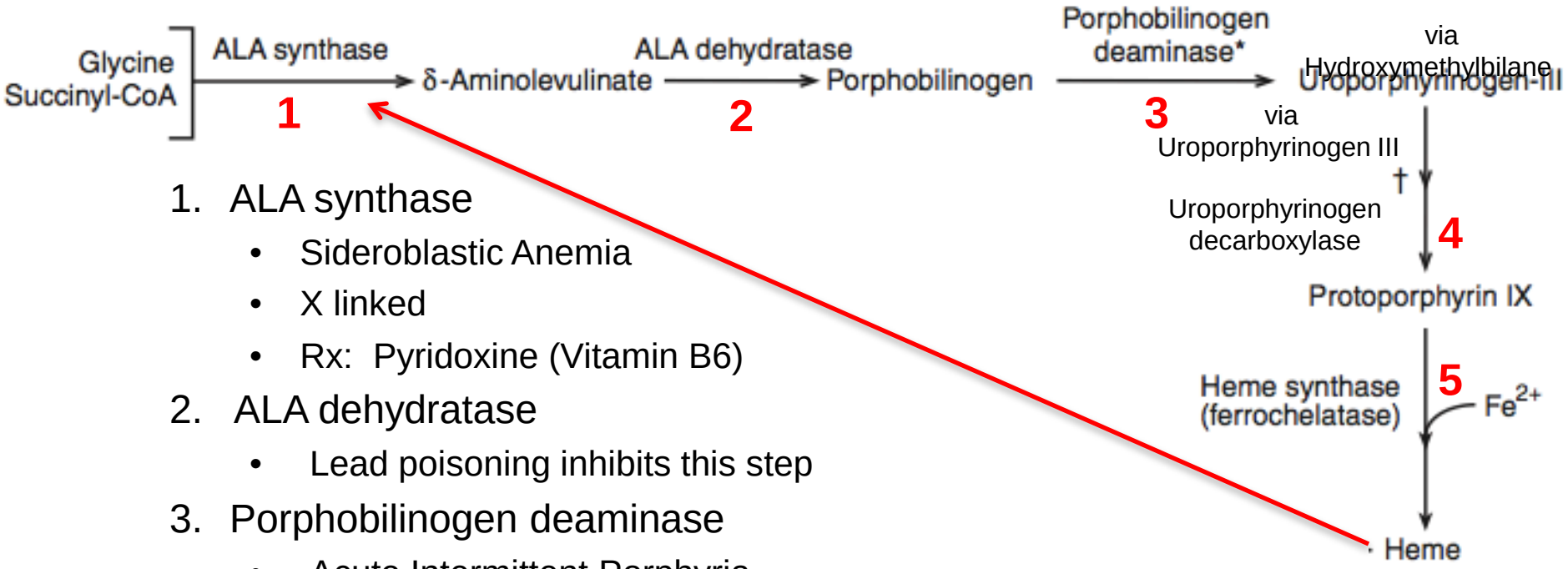
FA 2013: 347.2 • FA 2012: 375.2 • FA 2011: 345.1
ME 3e: 465 • ME 4e: 465

Heme

- Heme
  - Fe^{2+} surrounded by a porphyrin ring
  - 4 heme molecules within each hemoglobin
  - Heme is responsible for the transport of oxygen
  - Porphyrias à disorders of heme synthesis

Kaplan Immuno-Micro 2011 : Figure I-13-3

Disorders of Heme Synthesis



1. ALA synthase
   - Sideroblastic Anemia
   - X linked
   - Rx: Pyridoxine (Vitamin B6)
2. ALA dehydratase
   - Lead poisoning inhibits this step
3. Porphobilinogen deaminase
   - Acute Intermittent Porphyria
4. Uroporphyrinogen decarboxylase
   - Porphyria cutanea tarda
5. Ferrochelatase
   - Lead poisoning inhibits this step

Note: Heme is a feedback inhibitor of ALA synthase

Lead Poisoning

- Lead Poisoning
  - Inhibits ALA dehydratase and ferrochelatase
  - Inhibtion of ferrochelatase leads to a build-up of protoporphyrin in the serum
    - Causes microcytic anemia (with basophilic stipiling seen on peripheral smear)
    - Abdominal pain
    - Mental deterioration in children
    - Headache, memory loss, peripheral neuropathy are more common in adults

  Treatment: EDTA (chelating agent that binds lead)

  - Succimer or dimercaprol

Acute Intermittent Porphyria

- Acute Intermittent Porphyria
  - Porphobilinogen deaminase deficiency
  - Leads to a build-up of porphobilinogen in urine
  - Symptoms:
    - Abdominal pain
    - Red wine-colored urine
    - Polyneuropathy
    - Psychological disturbances (depression, psychosis, etc)
  - Treatment:
    - Glucose
    - Hemin

Porphyria Cutanea Tarda

- Porphyria cutanea tarda
  - Uroporphyrinogen decarboxylase deficiency
  - Leads to build-up of uroporphyrin in urine
  - Symptoms include cutaneous blisters secondary to exposure to sunlight

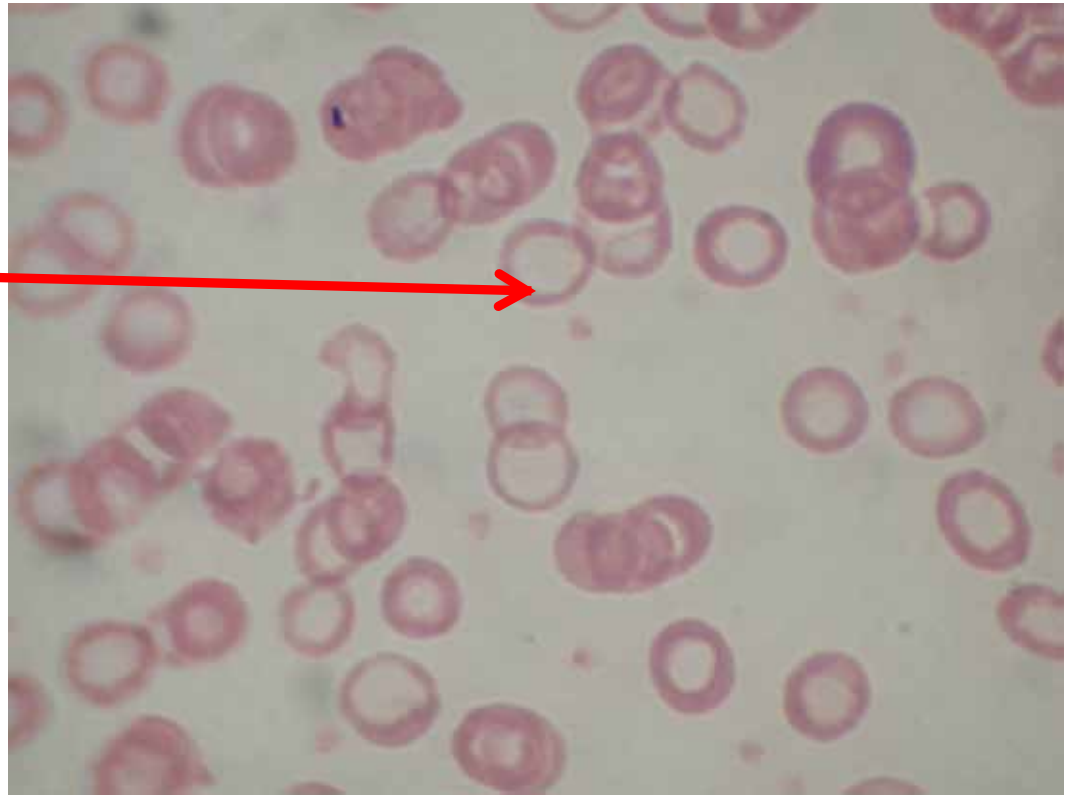
Anemias

- Microcytic anemia
  - MCV < 80
- Normocytic
  - 80 < MCV < 100
- Macrocytic
  - MCV > 100

Iron Deficiency

- Microcytic Anemia: Iron Deficiency

Microcytic, hypochromic red blood cells



α-Thalassemia

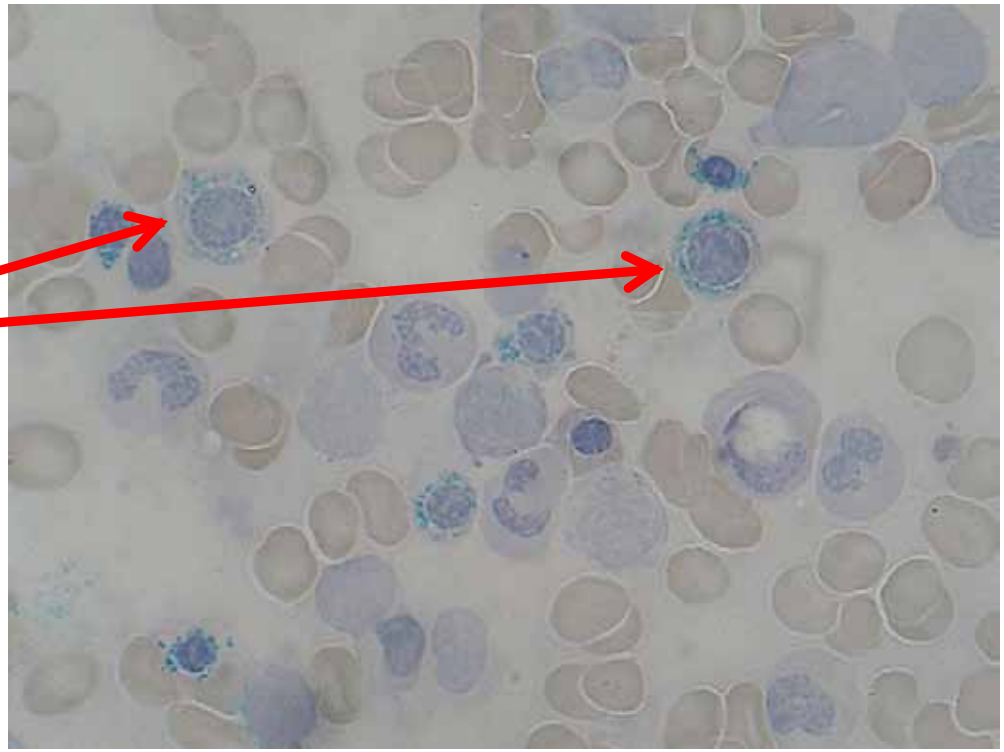
- Microcytic Anemia: α-thalassemia
  - α-globin chain defect
  - Prevalent in Asian & African populations
    - Deletion of 1-2 genes à asymptomatic or mild anemia
    - Deletion of 3 genes à HbH disease
    - Deletion of 4 genes à Hb Barts à hydrops fetalis

β-Thalassemia

- Microcytic Anemia: β-thalassemia
  - Point mutations in splicing sites and promoter sequences
  - Prevalent in Mediterranean populations
  - β-thalassemia minor
    - Heterozygotes
    - β-chain underproduced à usually asymptomatic
    - Dx: increased HbA_2 ($\alpha_2\delta_2$)
  - β-thalassemia major
    - Homozygotes
    - β-chain absent à severe anemia requiring chronic blood transfusion
    - Can present with crew cut skull XR secondary to marrow expansion

Sideroblastic Anemia

- Microcytic Anemia: Sideroblastic Anemia
  - Genetic cause: X-linked defect in ALA synthase (Rx: B6)
  - Reversible cause: alcohol, lead
  - Labs: Increased iron & ferritin, normal TIBC



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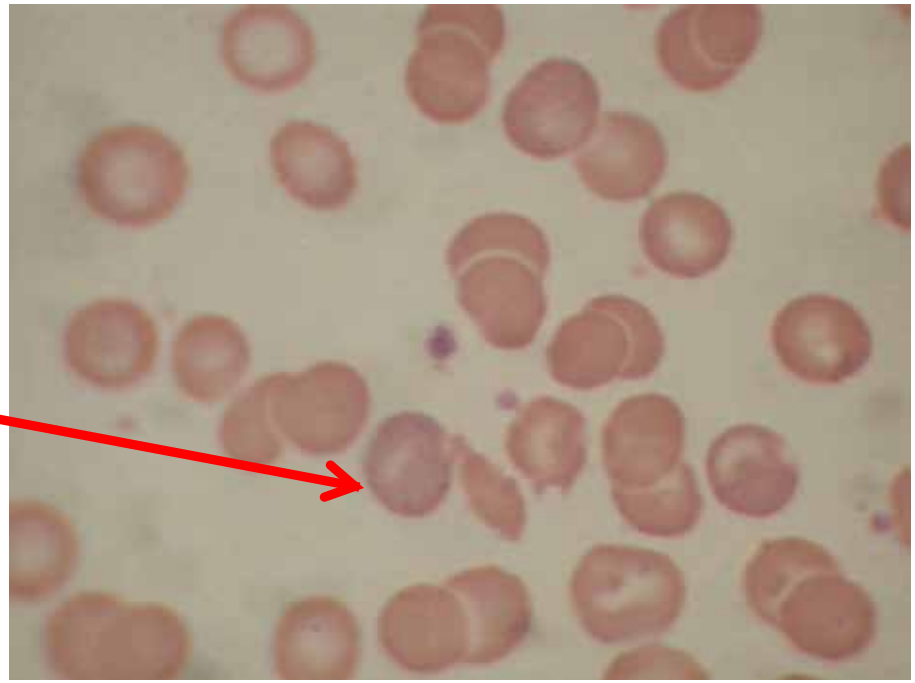
Lead Poisoning

- Microcytic Anemia: Lead Poisoning
  - Inhibits ferrochetolase and ALA dehydratase
  - Signs: lead lines on gingivae and long bone epiphyses, encephalopathy, abdominal pain, peripheral neuropathy, wrist drop, foot drop
  - Rx: EDTA, succimer or dimercaprol

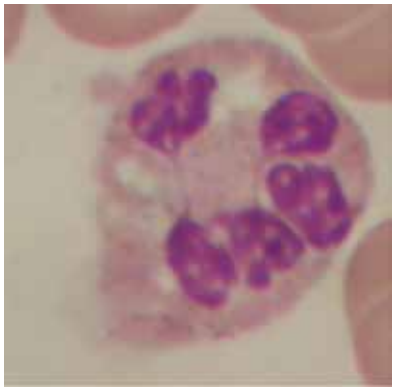
Basophilic stippling, seen in lead poisoning



Megaloblastic Anemia

- Macrocytic Anemia (MCV > 100)
  - A. Megaloblastic anemia
    - Impaired DNA synthesis à hypersegmented neutrophils
    - Folate deficiency
      - Lab Findings: ê folate, é homocysteine, normal methylmalonic acid
      - Causes: Alcoholism, malnutrition, etc
    - B12 deficiency
      - Lab Findings: ê B12, é homocysteine, é methylmalonic acid
      - Causes: Pernicious anemia, *D. latum,* Crohn's disease, ileal resection
      - Neuro symptoms: subacute combined degeneration

Hypersegmented neutrophil seen in megaloblastic anemia



Nonmegaloblastic Anemia

- Macrocytic Anemia (MCV > 100)

  B. Nonmegaloblastic anemia

  - Liver disease
  - Alcoholism
  - Reticulocytosis

Normocytic Anemia

- Normocytic Anemia (80 > MCV < 100)

  A. Anemia of chronic disease
     - Inflammation à increased hepcidin à decreased ability of macrophages and transferrin to release iron
     - Lab findings: ê iron levels, ê TIBC, é ferritin

  B. Aplastic anemia
     - Pancytopenia à petechiae, bleeding, infection, neutropenia
     - Causes: Radiation/chemotherapy, viruses, Fanconi's anemia, Idiopathic (may follow acute hepatitis)
     - Treatment: eliminate causative agent, bone marrow transplant, GM-CSF

  C. Anemia of chronic kidney disease
     - Decreased erythropoietin production à decreased hematopoiesis
     - Treatment: EPO injections

KAPLAN MEDICAL

Hemolytic Anemia and Pathologic Red Blood Cell Forms

Stephen Bagley

Hemolytic Anemia

- Hemolytic Anemia
  - Classification:
    - Intravascular vs. extravascular hemolysis
    - Intrinsic vs. extrinsic hemolysis

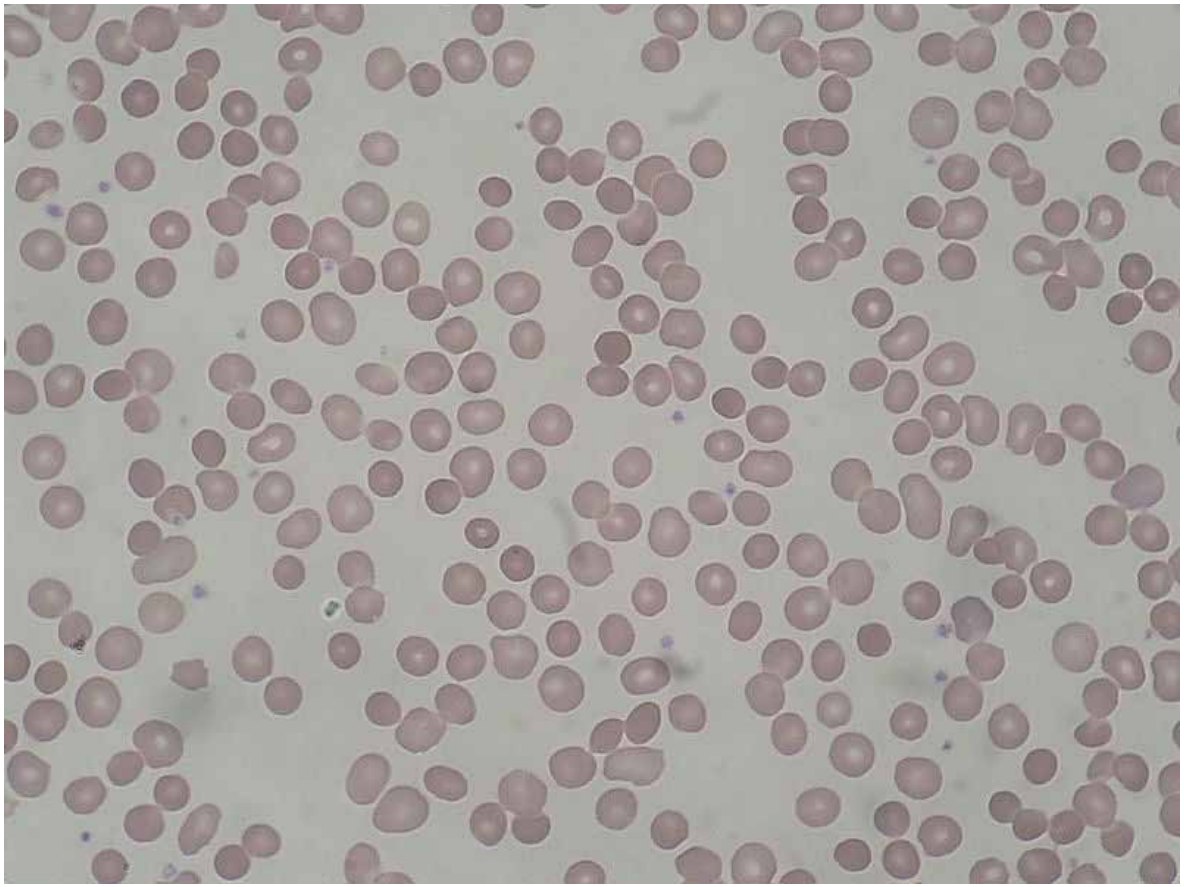
Laboratory Values

- Laboratory Values
  - Intravascular hemolysis
    - Low haptoglobin, high LDH, hemoglobinuria
  - Extravascular hemolysis
    - High LDH, high unconjugated bilirubin (blood/urine)

Hereditary Spherocytosis - 1

- Hereditary Spherocytosis
  - Defect in proteins interacting with RBC membrane
    - Ankyrin, spectrin, band 4.1
  - Cells become spherical and fragile
  - Findings:
    - Splenomegaly (Howell-Jolly bodies after splenectomy)
    - + Osmotic fragility test (rupture in hypotonic solution)
    - Aplastic crisis (Parvovirus B19)
    - Increased MCHC (mean corpuscular hemoglobin conc.)
    - Increased RDW (red blood cell distribution width)

Hereditary Spherocytosis - 2



Spherocytes seen in hereditary spherocytosis

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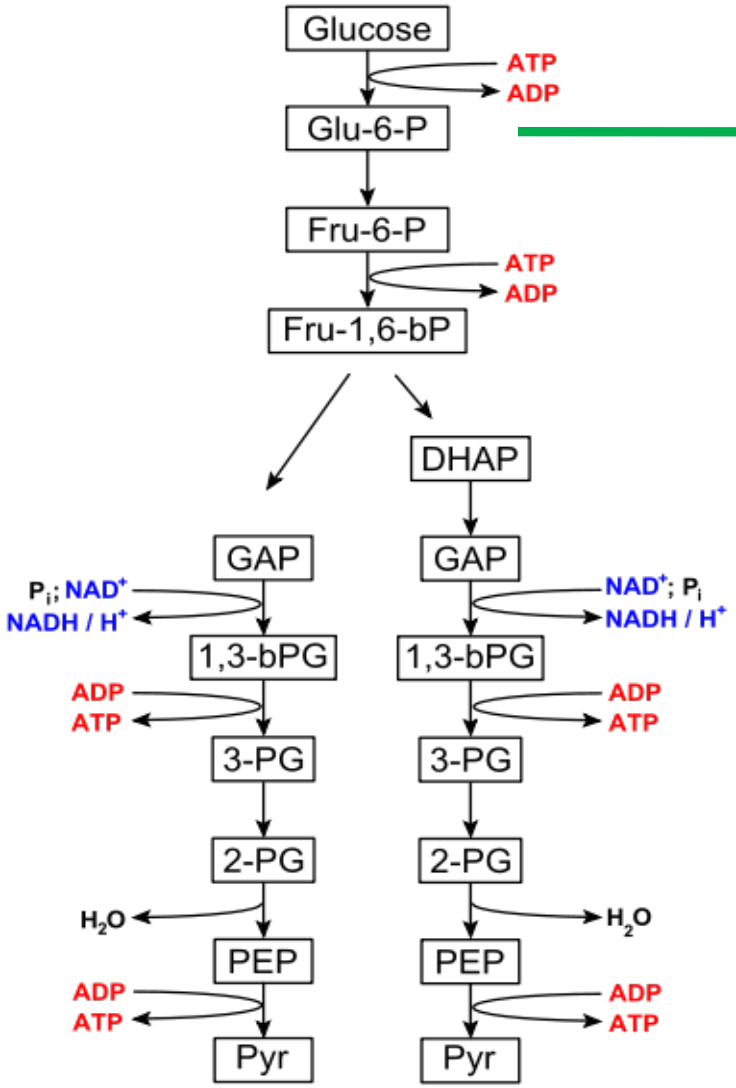
G6PD Deficiency – 1

- G6PD Deficiency
  - X-linked, more common in African populations
  - Defect in the enzyme G6PD
    - Causes low levels of glutathione in RBCs
    - Makes RBCs more susceptible to oxidative stress

Glycolysis



G6P can go into the HMP shunt (pentose phosphate pathway) instead of going into the glycolytic pathway

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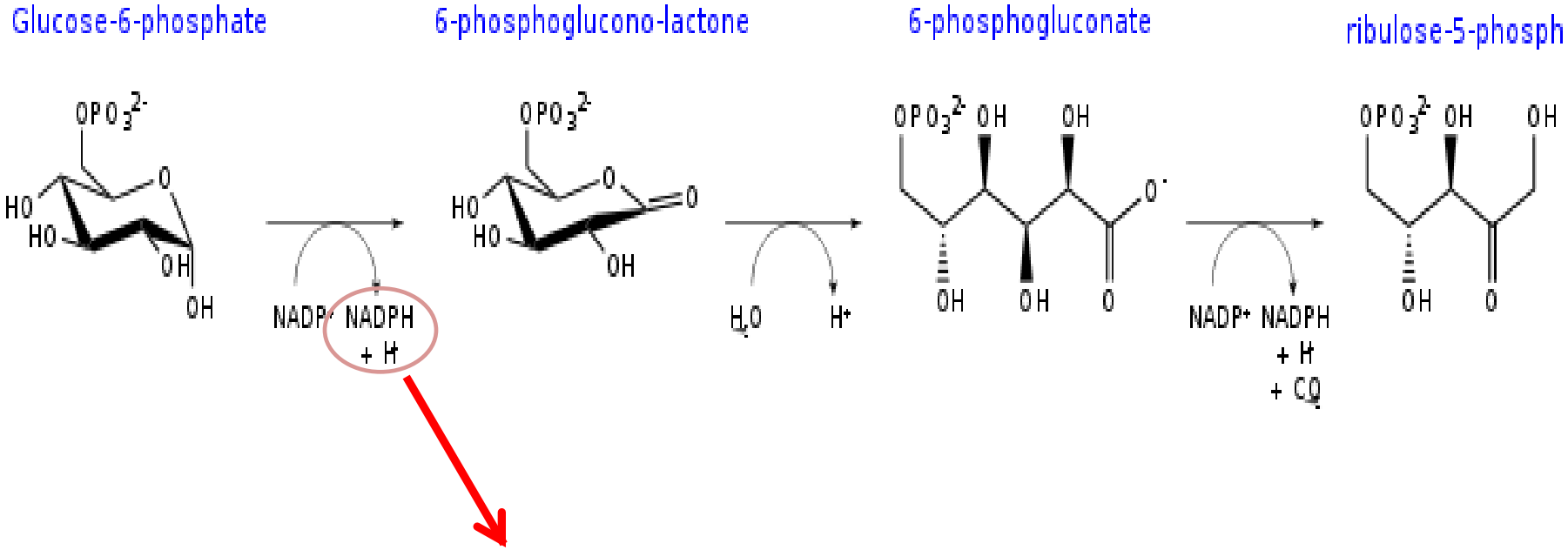
FA 2013: 103.1 • FA 2012: 107.1 • FA 2011: 101.2
ME 3e: 38 • ME 4e: 38

HMP Shunt



This oxidative phase of the HMP shunt (pentose phosphate pathway) produces NADPH

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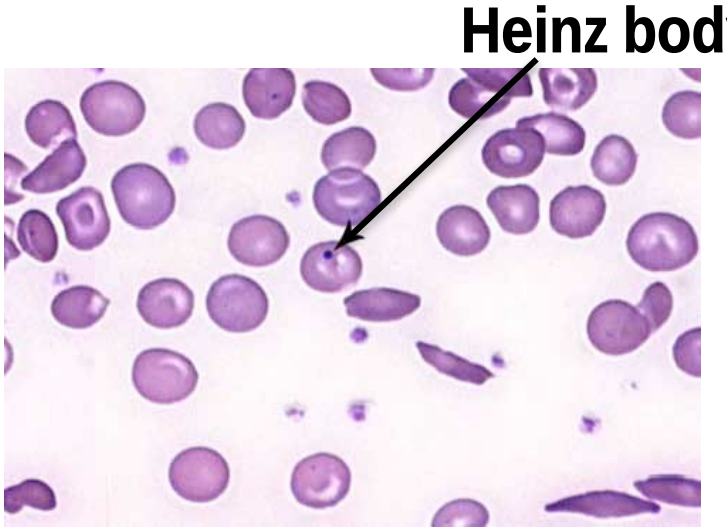
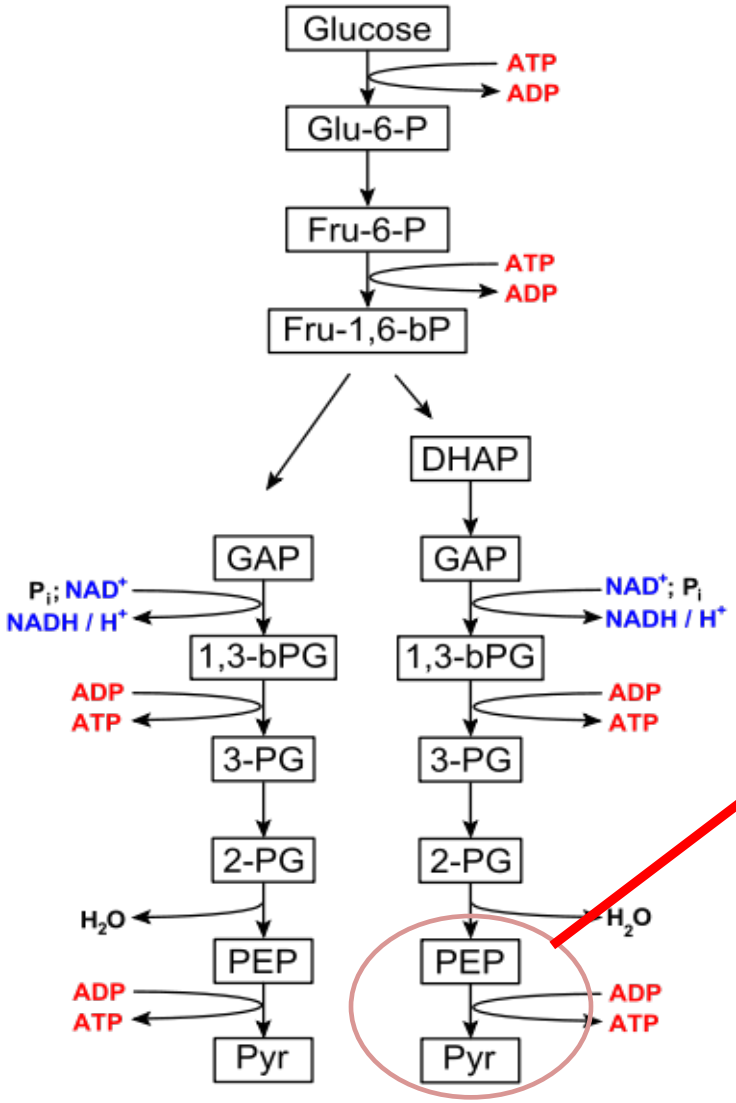
G6PD Deficiency – 2

- G6PD Deficiency (cont'd)
  - X-linked, more common in African populations
  - Defect in the enzyme G6PD
    - Causes low levels of glutathione in RBC's
    - Makes RBC's more susceptible to oxidative stress
    - Oxidative stress leads to hemolysis
    - On peripheral smear, may see Heinz bodies and/or bite cells
  - Precipitating factors:
    - Fava beans
    - Sulfonamide drugs
    - Primaquine (anti-malarial)
    - Anti-TB drugs



Pyruvate Kinase Deficiency



This step is catalyzed by
PYRUVATE KINASE

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FA 2013: 104.1 • FA 2012: 108.1 • FA 2011: 102.2
ME 3e: 38 • ME 4e: 38

Sickle Cell Anemia – 1

- Sickle Cell Anemia
  - Most common in African populations
  - Defect:
    - HbS occurs due to a single amino acid replacement in the β chain
    - Valine replaces glutamic acid
    - HbS: 2 normal α chains and 2 abnormal β chains
  - Pathogenesis:
    - HbS precipitates during hypoxic episodes à RBC sickling
    - Sickled RBCs cause vaso-occlusive crises à infarction, pain
  - Complications:
    - Aplastic crisis (Parvovirus B19), autosplenectomy, *salmonella* osteomyelitis, painful crisis, renal papillary necrosis

Sickle Cell Anemia – 2

- Sickle Cell Anemia (cont'd)
  - Complications:
    - Aplastic crisis (Parvovirus B19)
    - Autosplenectomy
    - *Salmonella* osteomyelitis
    - Painful crisis
    - Renal papillary necrosis
    - Avascular necrosis of the hip
  - Treatment:
    - Hydroxyurea (increases HbF)

HbC Defect

- HbC Defect
  - Defect:
    - Lysine replaces glutamic acid at position 6 of hemoglobin chain
    - Less severe than HbS
    - Patients can have both HbS/HbC

Paroxysmal Nocturnal Hemoglobinuria

- Paroxysmal Nocturnal Hemoglobinuria
  - PIGA enzyme makes GPI protein
    - GPI is an RBC membrane anchor that binds to DAF protein (decay-accelerating factor...also known as CD55), thus inhibiting complement
    - With mutated GPI, DAF is not able to inhibit complement à hemolysis
  - Diagnosis:
    - Worse at night due to acidosis precipating hemolytic episodes à morning hemoglobinuria
    - Lab: increased urine hemosiderin
    - Flow cytometry: CD59 negative

Complement System

- Complement System
  - Innate response
    - Alternative pathway
    - Triggered by microbial surfaces, endotoxins
  - Adaptive response
    - Classic pathway
    - Triggered by antigen-antibody complexes
  - Both pathways result in production of:
    - C3 à C5 à MAC (membrane attack complex)
    - MAC binds to cell surfaces to cause lysis

Complement Deficiencies

- Complement Deficiencies
  - C3b deficiency
    - Prone to infection with encapsulated bacteria
  - C3a and C5a deficiency
    - Associated with anaphylactic shock
    - C5a is important for neutrophil chemotaxis
  - Hereditary angioedema
    - C1 esterase inhibitor deficiency
    - Uncontrolled complement activation

Autoimmune Hemolytic Anemia – 1

- Autoimmune Hemolytic Anemia
  - Warm agglutinin
    - IgG antibodies
    - SLE, CLL, drugs (alpha methyldopa)
  - Cold agglutinin
    - IgM antibodies
    - CLL, mycoplasma pneumonia, infectious mononucleosis

Autoimmune Hemolytic Anemia – 2

- Autoimmune Hemolytic Anemia (cont'd)
  - Erythroblastosis fetalis
    - An Rh negative mother creates antibodies against an Rh positive fetus
    - Causes severe hemolytic anemia in the newborn
    - Accumulation of bilirubin can collect in the basal ganglia (kernicterus)

Coombs' test

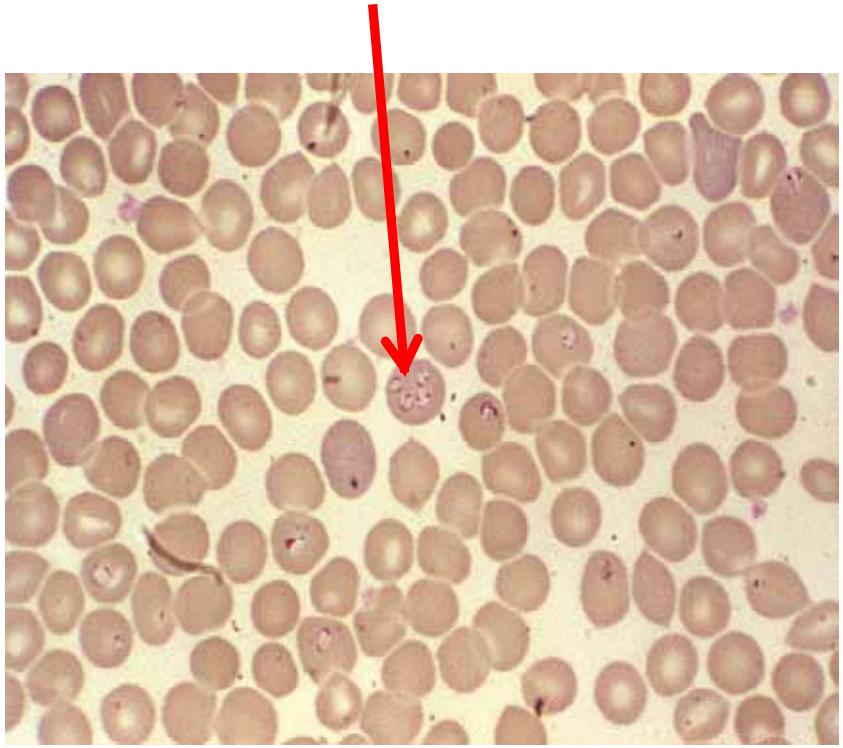
- Coombs' test
  - All automimmune hemolytic anemias are Coombs' +
  - Direct:
    - anti-IgG antibody added to patient's blood
    - Agglutination if RBCs are coated with IgG
    - Used to test newborns for risk of hemolytic disease
  - Indirect:
    - Normal RBCs added to patient's serum
    - Agglutination if serum has IgG that bind the RBCs
    - Used to test mothers for anti-Rh anitbodies

Microangiopathic Anemia

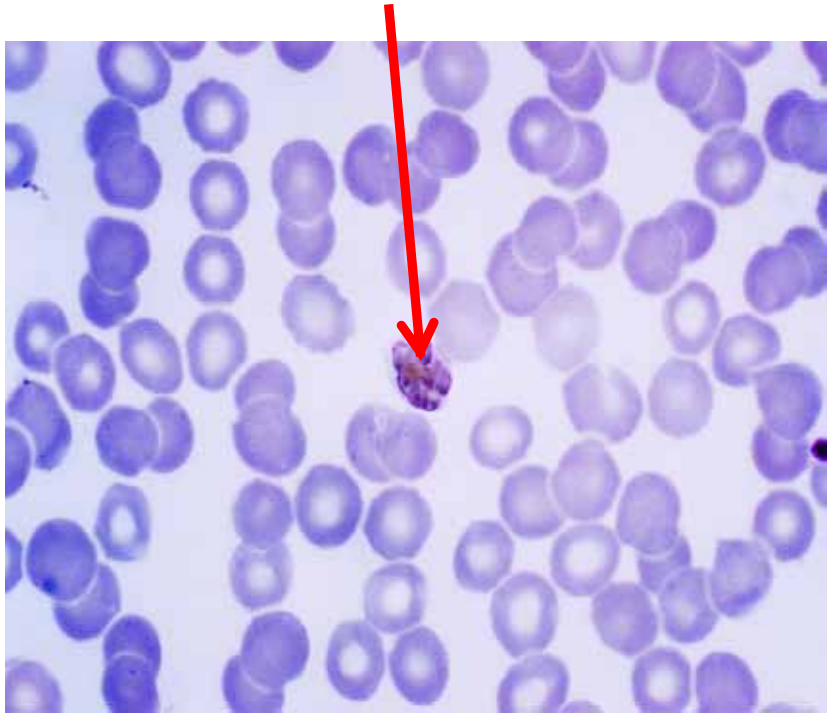
- Microangiopathic Anemia
    - RBCs damaged when passing through narrowed vessels
    - Associated with DIC, TTP, SLE, malignant HTN
    - Blood smear: schistocytes
- Macroangiopathic Anemia
    - Mechanical damage to RBCs occuring in larger vessels
    - Seen with prosthetic heart valves

Infections

Maltese cross in RBC secondary to babesiosis



RBC schizont secondary to malarial infection



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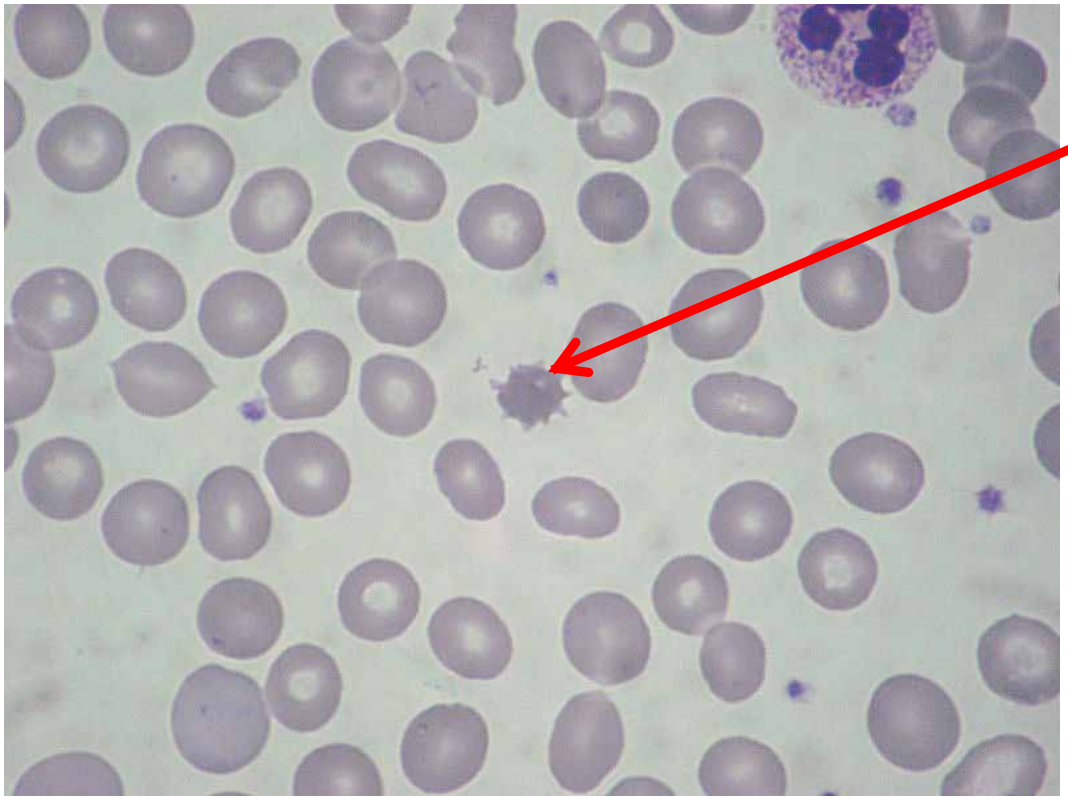
Acanthocytes

Pathologic Red Blood Cell Forms:

- Acanthocytes



Acanthocyte
(aka spur cell)

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Basophilic stippling

Basophilic stippling

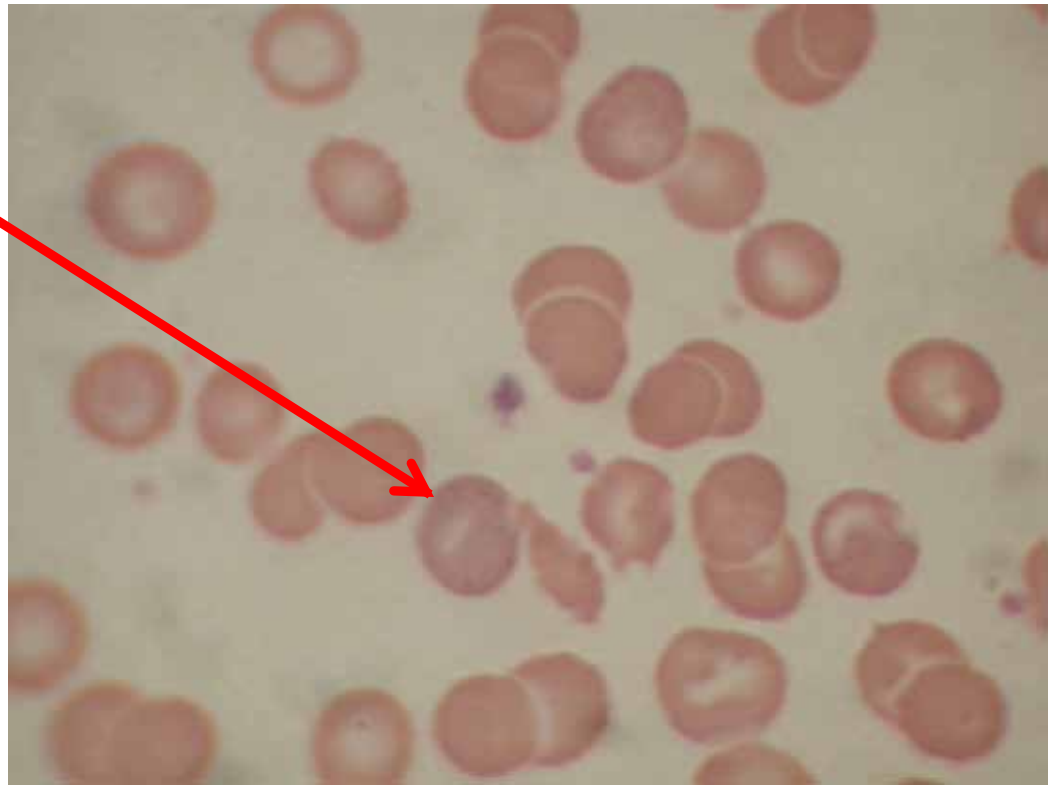
Basophilic stippling, seen in:

Thalassemias

Anemia of chronic disease

Iron deficiency

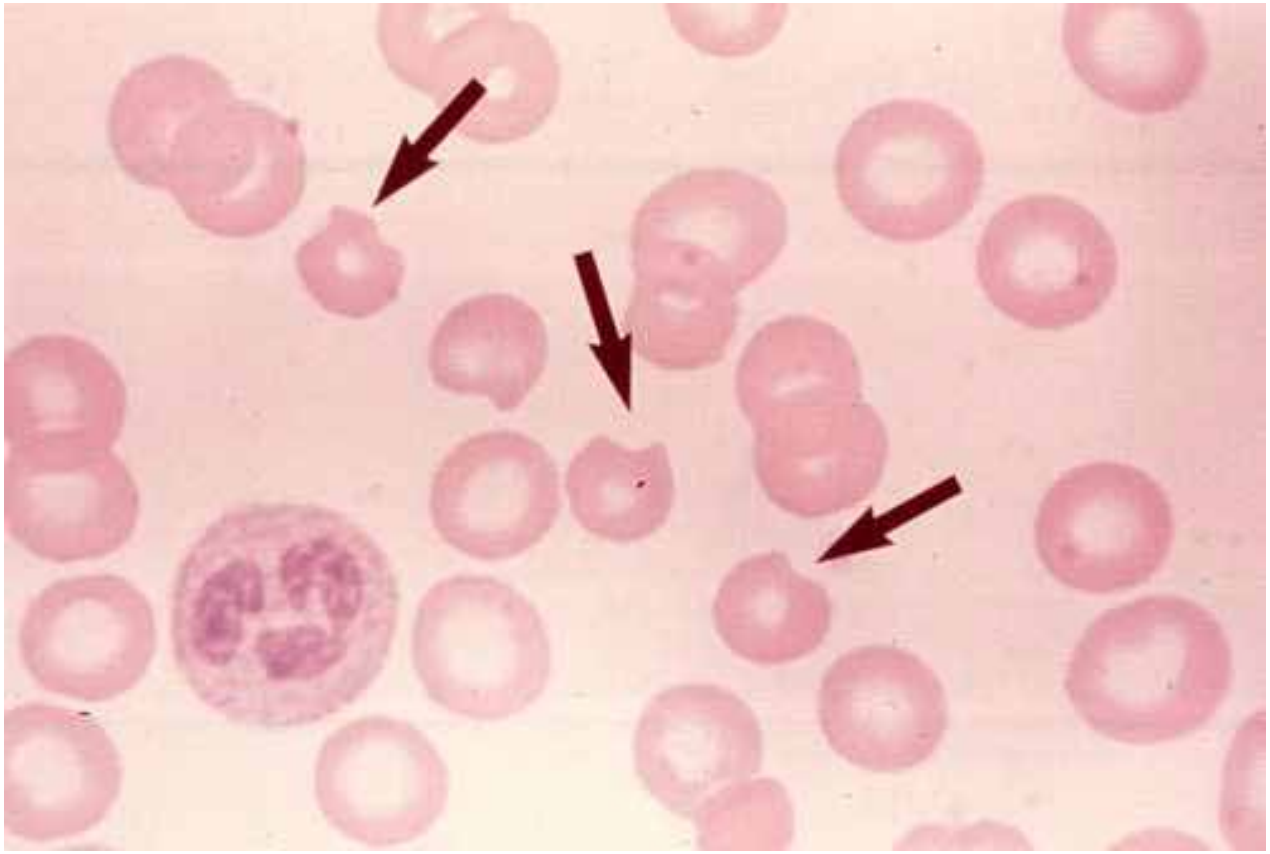
Lead Poisoning



Bite Cells

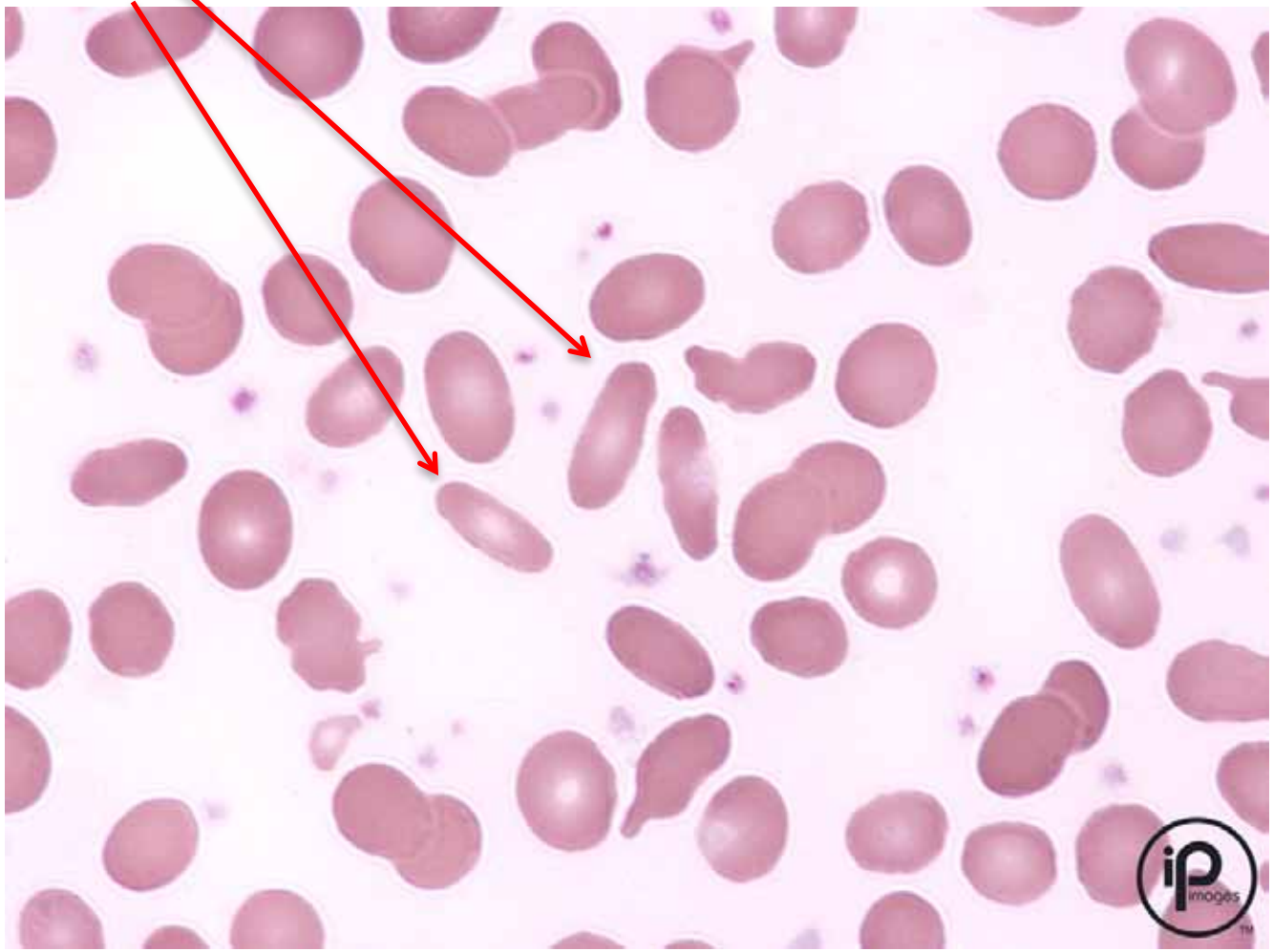
Bite cells

- Seen in G6PD deficiency



Elliptocytes

Elliptocytes

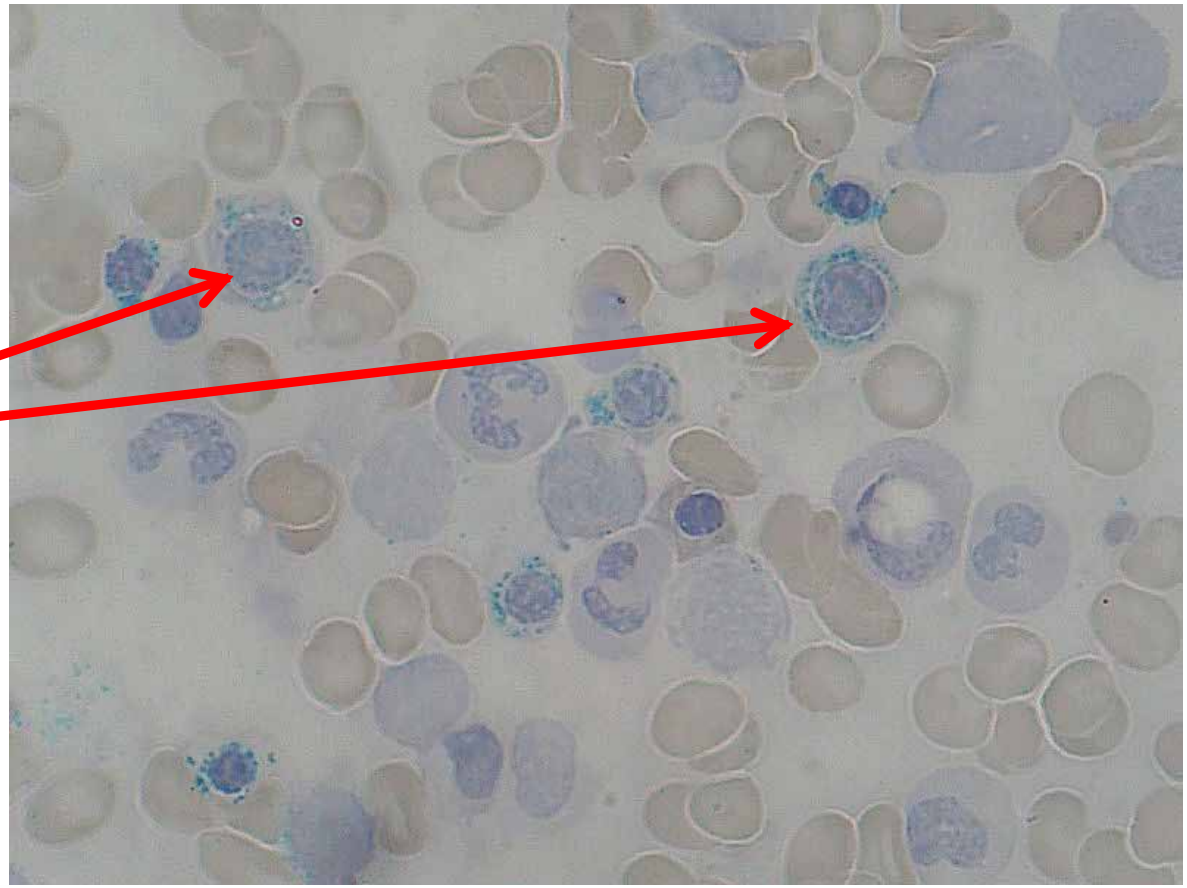


FA 2013: 350 • FA 2012: 378 • FA 2011: 348
ME 3e: 498 • ME 4e: 498

Ringed Sideroblasts

Ringed sideroblasts

Ringed sideroblasts
(DIC, TTP, HUS)



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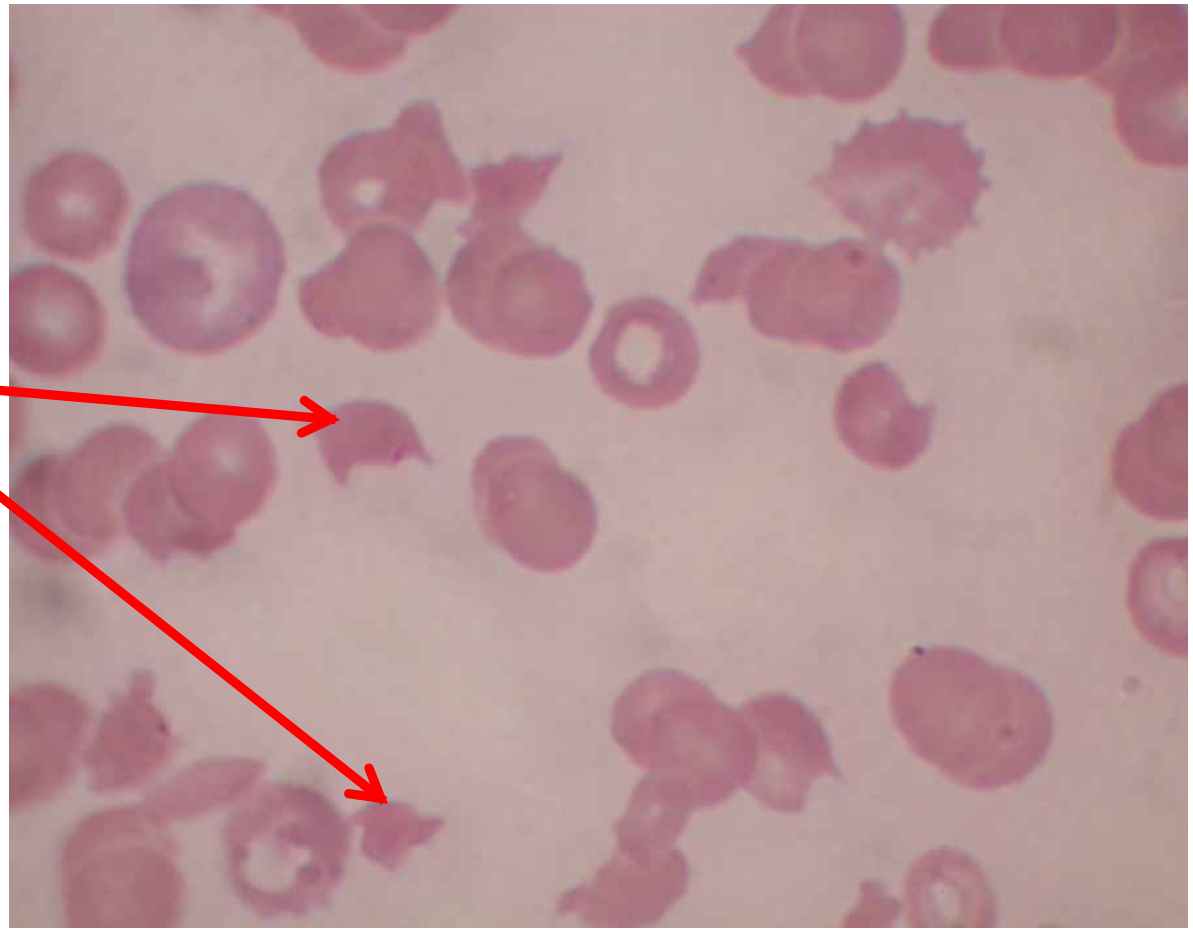
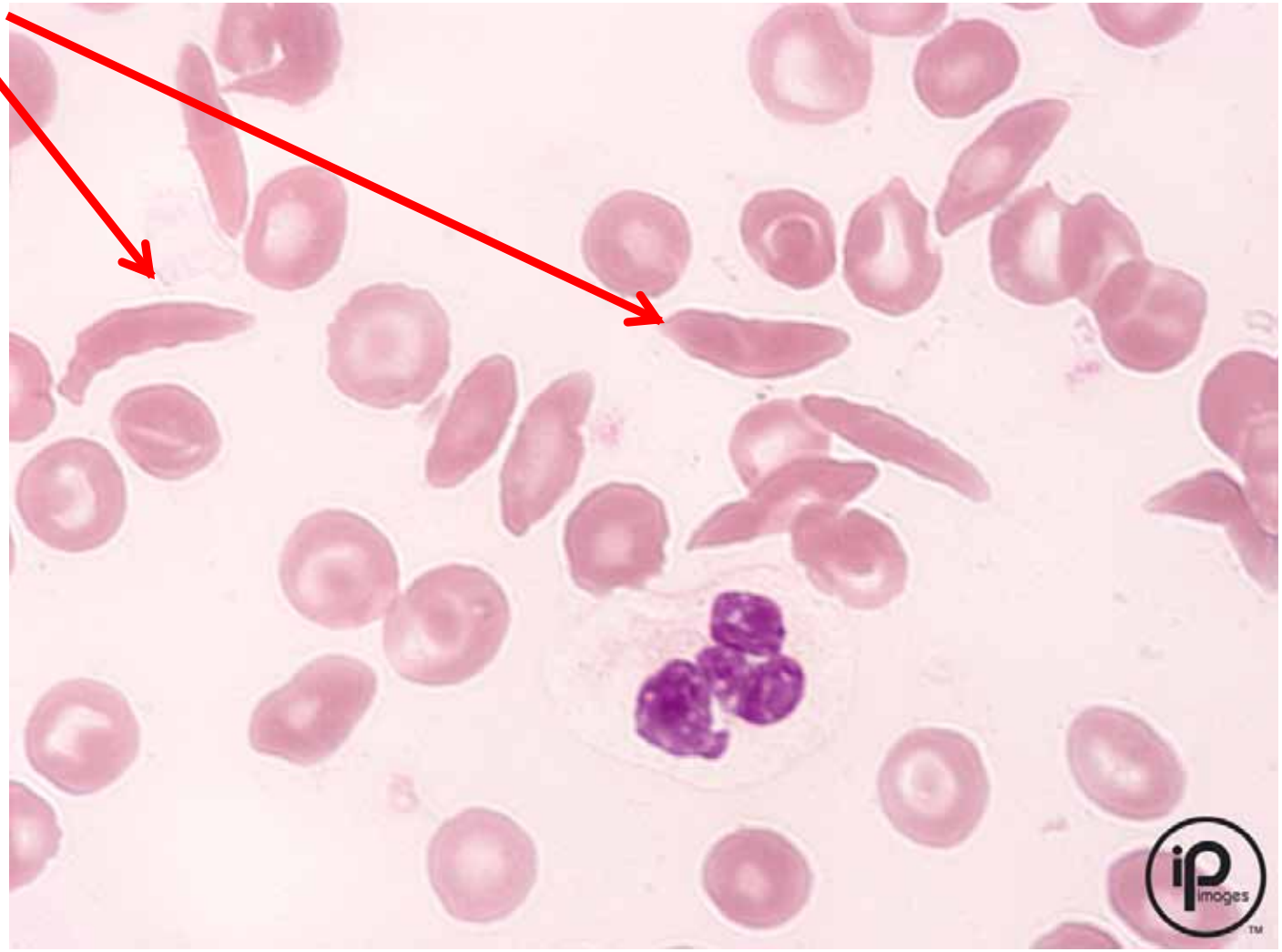
Schistocytes

Schistocytes

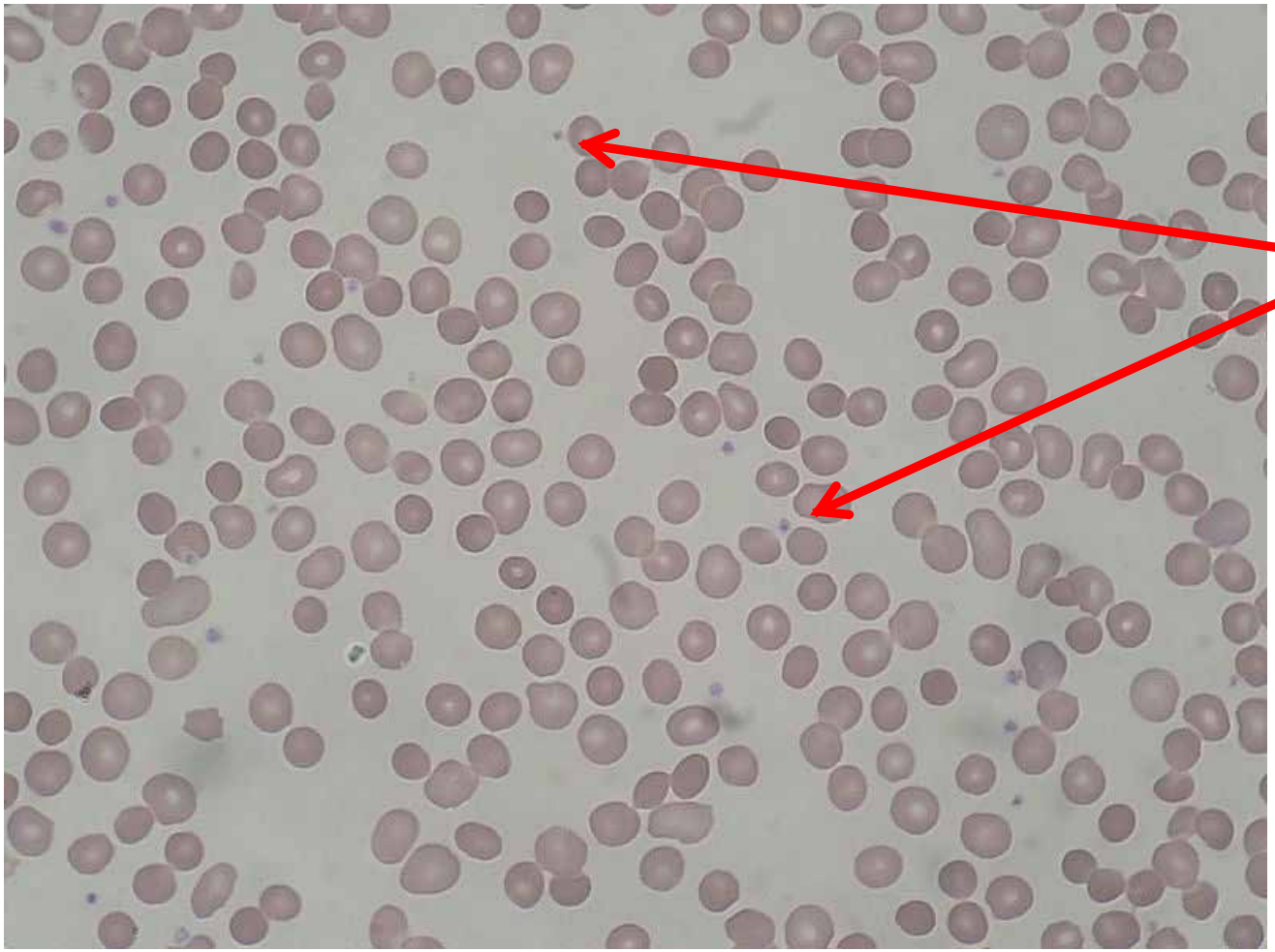


Sickle cells



Spherocytes

Spherocytes



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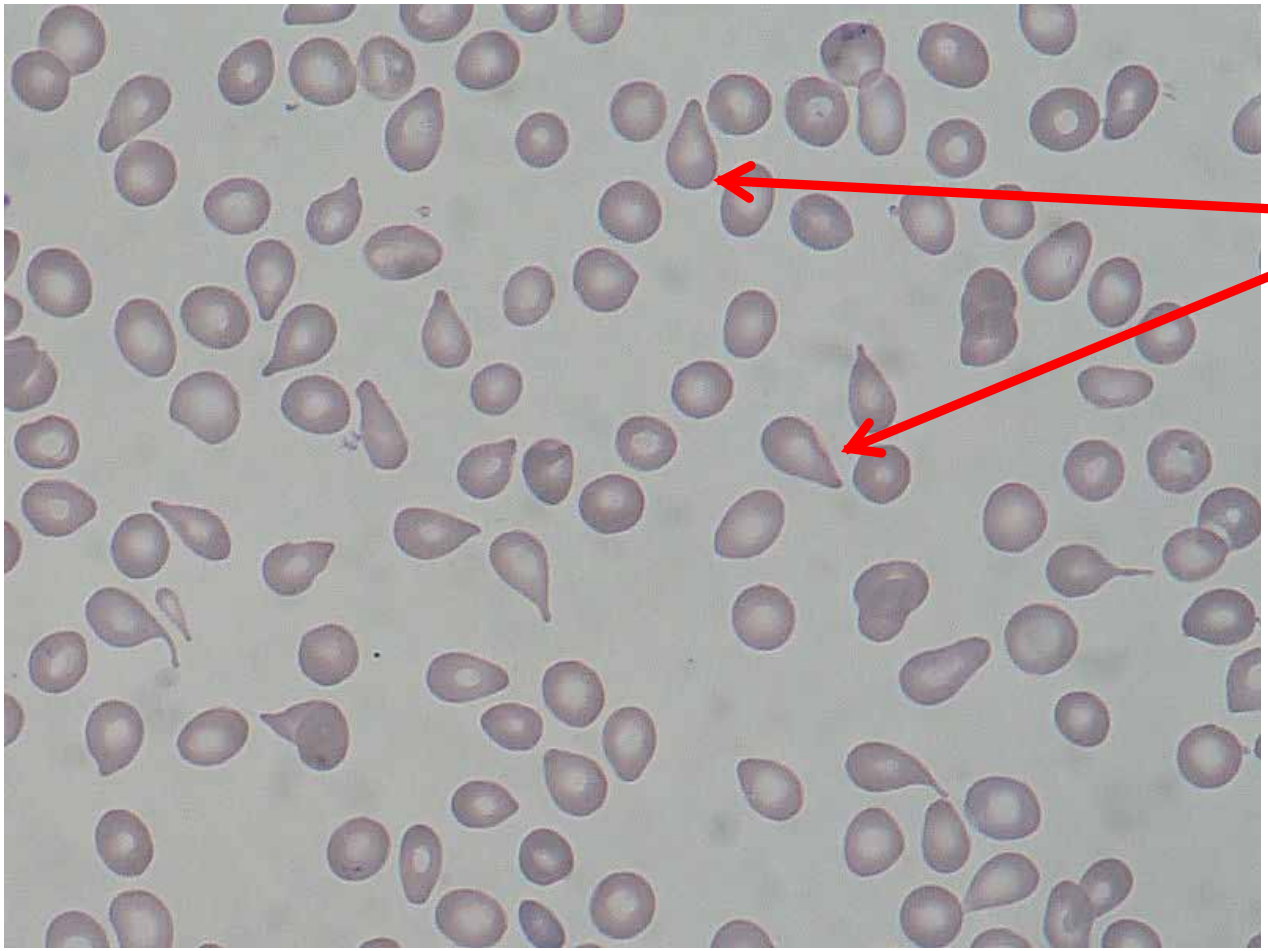
Teardrop cells

Teardrop cells



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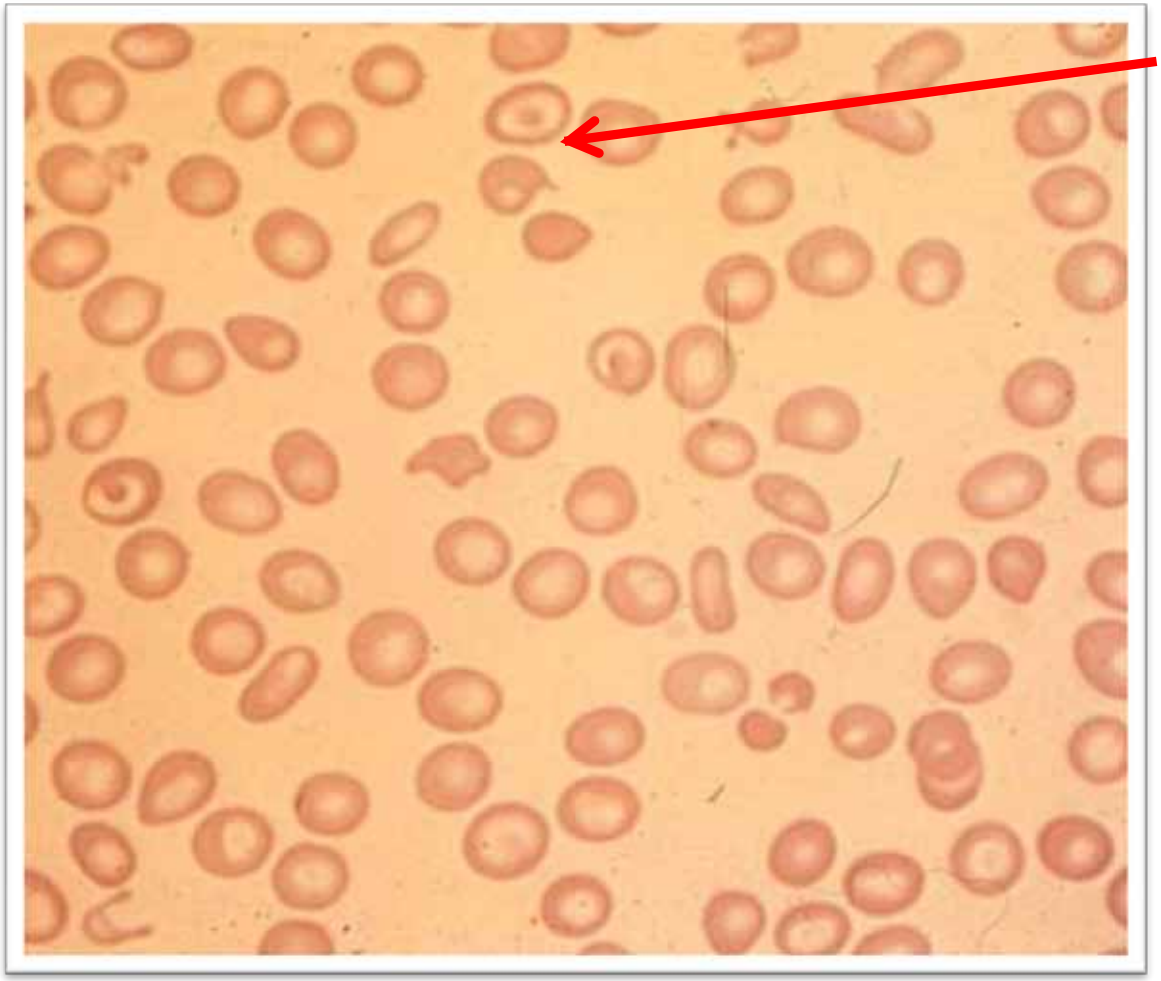
Target cells

Target cells



Target cells:

- HbC
- Asplenia
- Liver disease
- Thalassemias

Lab Values in Anemia

Anemia	Serum Iron	Transferrin (TIBC)	Ferritin (Iron storage)
Iron deficiency	Low	High	Low
Anemia of chronic disease	Can be low	Very low	High
Hemochromatosis	High	Low	High

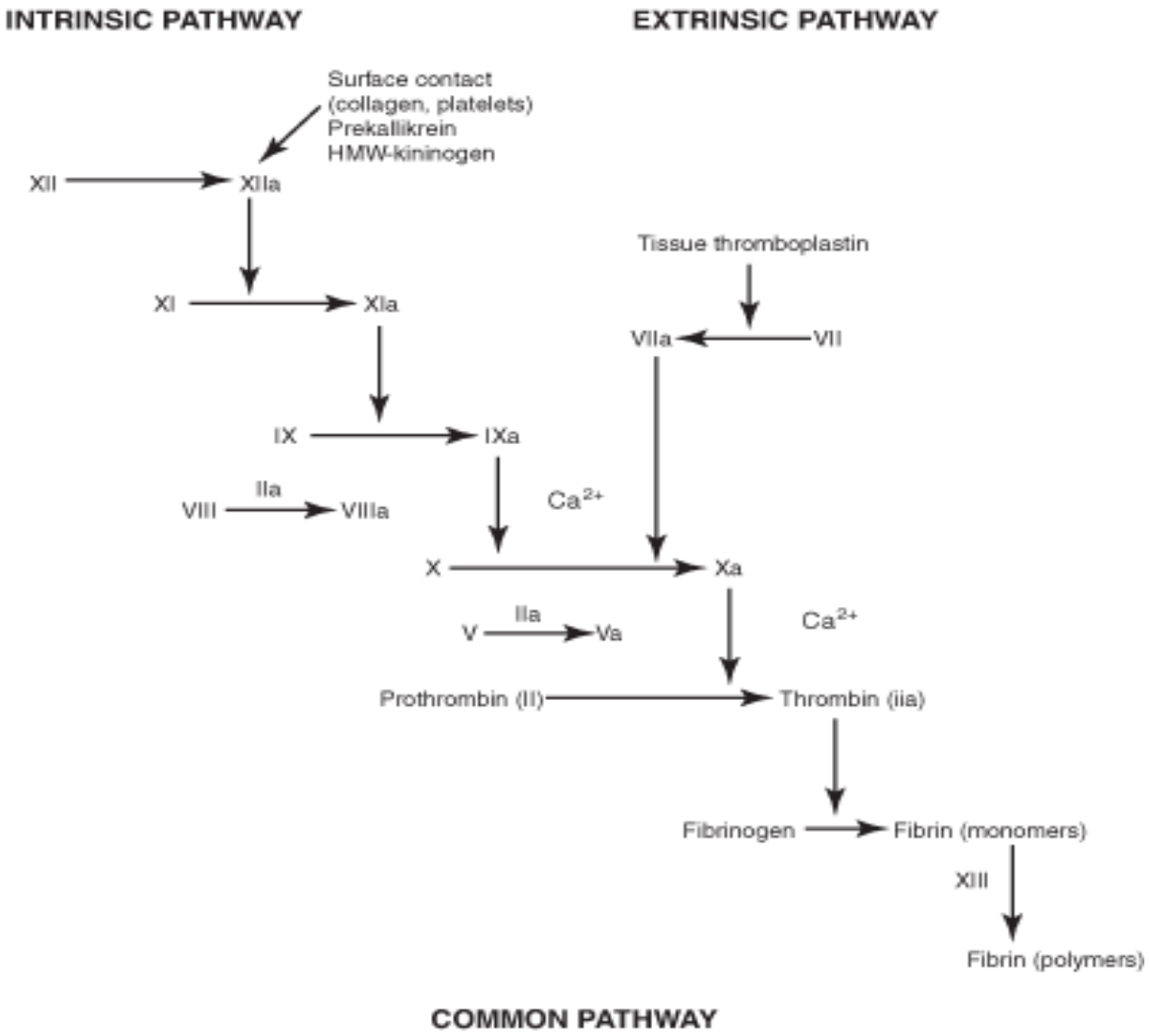
KAPLAN MEDICAL

Immunology, Hematology, and Oncology

Lecture 11

- The coagulation system
- The platelet plug and thrombogenesis

Coagulation Pathways

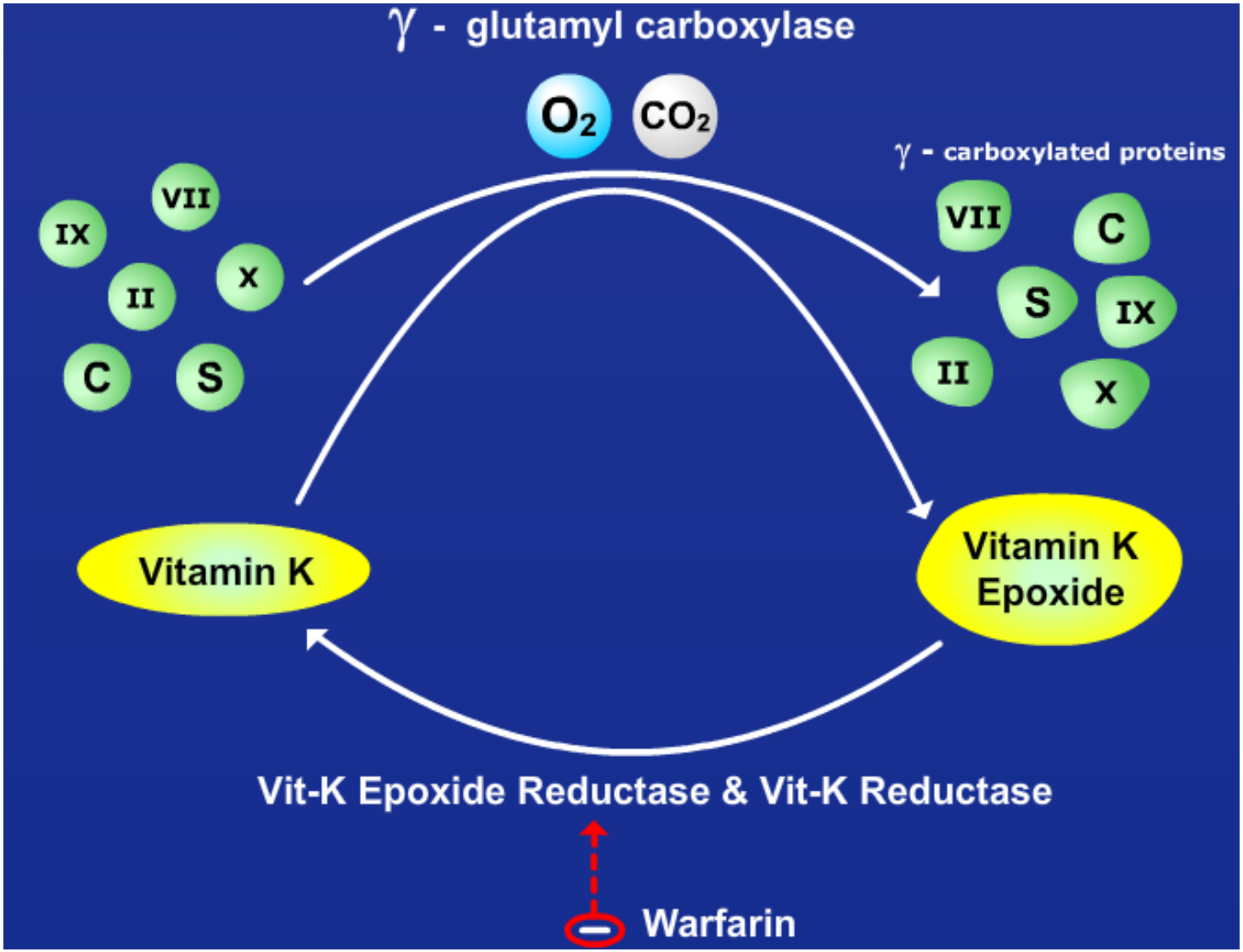


FA 2013: 348.1 • FA 2012: 376.1 • FA 2011: 345.2
ME 3e: 462 • ME 4e: 462

Kinin Cascade

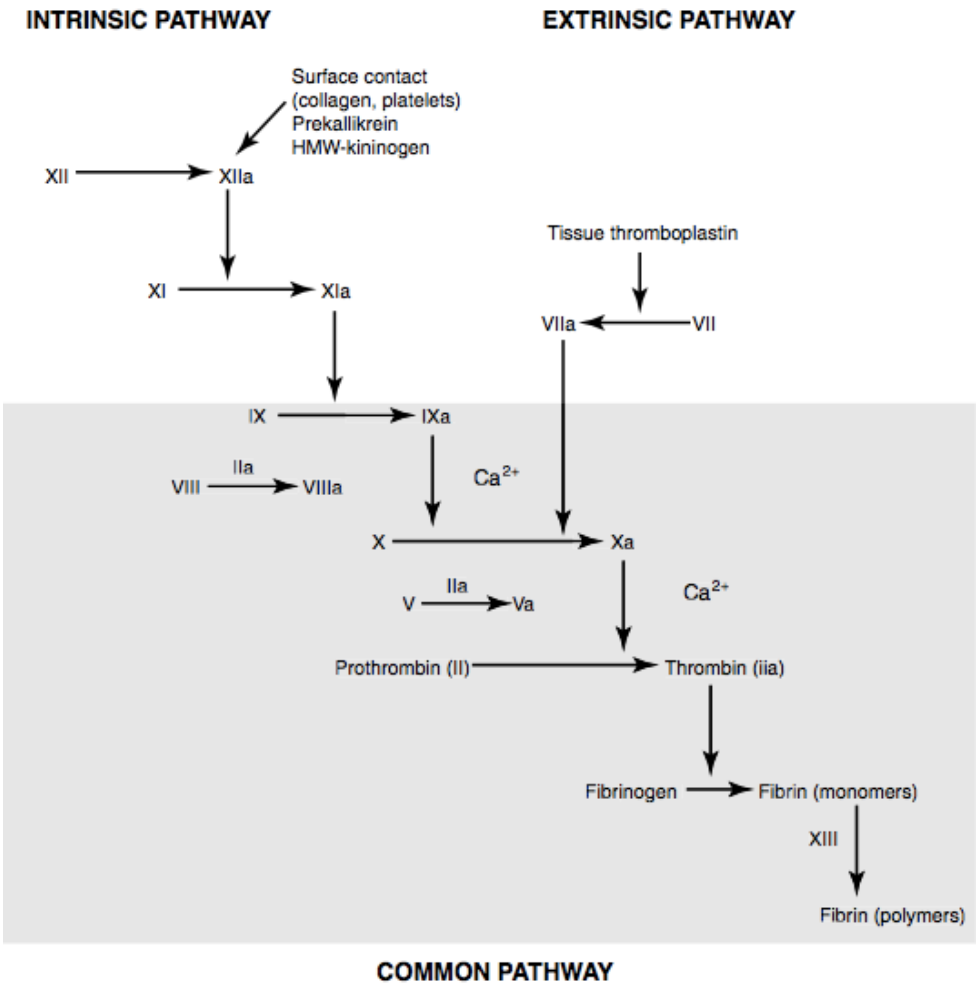
- HMWK converts factor XII into XIIa
- Factor XIIa converts prekallikrein into kallikrein
- Kallikren converts HMWK into bradykinin
- Bradykinin induces: vasodilation, permeability, and pain
- Kallikrein converts plasminogen into plasmin
- Plasmin cleaves fibrin mesh & activates C3 to become C3a
- ACE degrades bradykinin

Vitamin K


γ - glutamyl carboxylase
O2
CO2
γ - carboxylated proteins
VII
IX
X
II
C
S
VII
C
S
IX
II
X
Vitamin K
Vitamin K Epoxide
Vit-K Epoxide Reductase & Vit-K Reductase
Warfarin

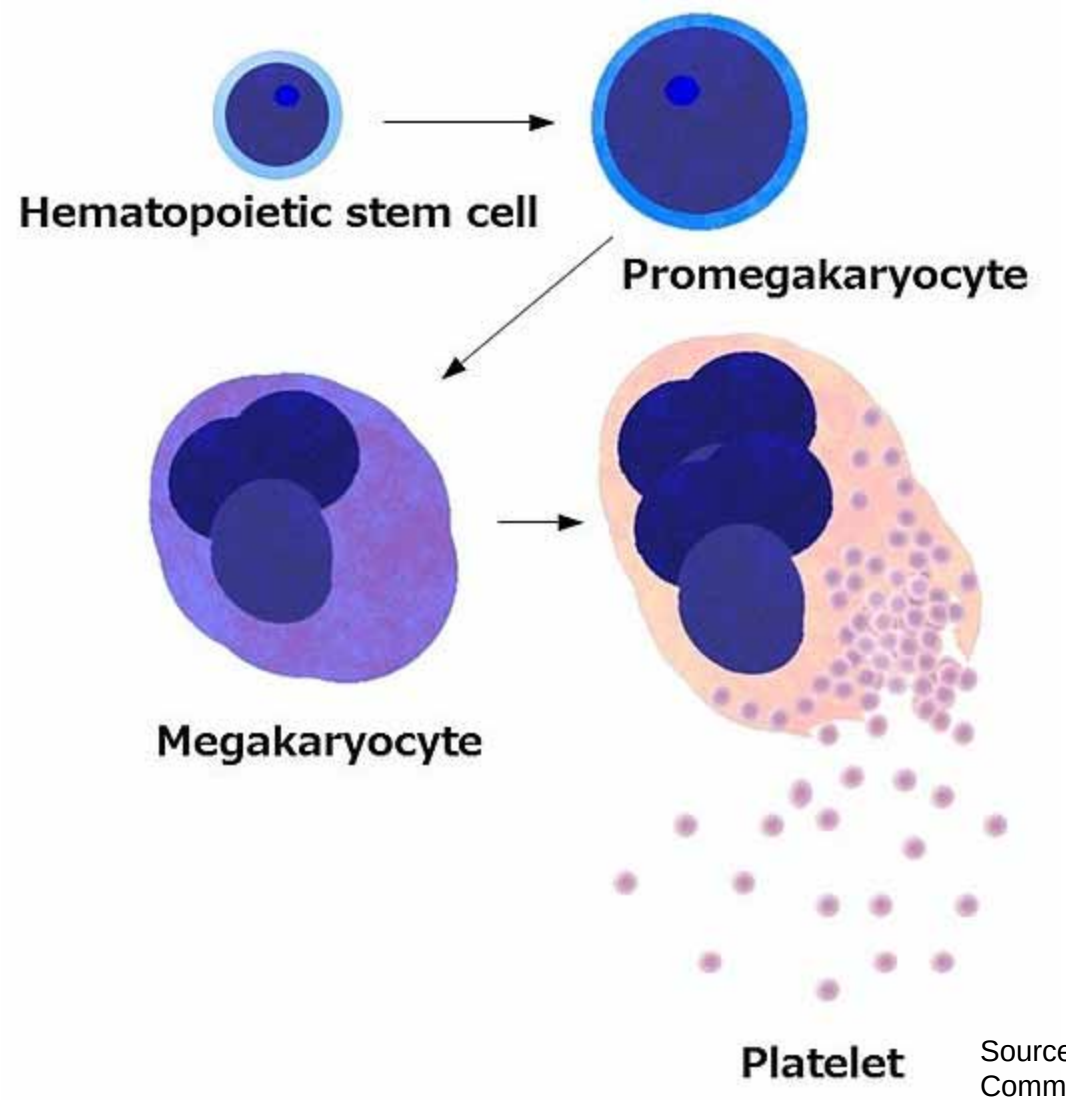
Anti-coagulation

- Heparin
  - Activates antithrombin
  - Antithrombin inactivates factors II, VII, IX, X, XI, XII
- Protein S activates protein C, which inactivates factors Va and VIIIa
  - Factor V Leiden: mutation in V, making it resistant to inactivation by protein C
- Tissue plasminogen activator (tPA) cleaves fibrin mesh



FA 2013: 348.2 • FA 2012: 376.2 • FA 2011: 346.1
ME 3e: 463 • ME 4e: 463

Platelets – General Characteristics



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FA 2013: 349.1 • FA 2012: 377.1 • FA 2011: 346.2
ME 3e: 460 • ME 4e: 460

Formation of the Platelet Plug

FORMATION OF THE PLATELET PLUG	
Vascular wall injury	• Injury causes exposure of subendothelial extracellular collagen • Arteriolar contraction due to reflex neurogenic mechanisms, and the local release of endothelin occurs
Adhesion	• von Willebrand factor (vWF) binds exposed collagen fibers in the basement membrane • Platelets adhere to vWF via glycoprotein Ib and become activated (shape change, degranulation, synthesis of thromboxane A2, TxA2)
Release reaction	• Release contents of platelet dense bodies (e.g., **ADP, calcium, serotonin, histamine, epinephrine) and** alpha granules (fibrinogen, fibronectin, factor V, vWF, platelet-derived growth factor) • Membrane expression of phospholipid complexes
Aggregation	• ADP and thromboxane A2 (TxA2) are released by platelets and promote aggregation (TXA2 production is inhibited by aspirin) • Cross-linking of platelets by fibrinogen requires the GpIIb/IIIa receptor, which is deficient in Glanzmann thrombasthenia • Decreased endothelial synthesis of antithrombogenic substances (e.g., prostacyclin, nitric oxide, tissue plasminogen activator, thrombomodulin)

Bleeding Disorders

DISORDERS OF PLATELET FUNCTION LEADING TO INCREASED BLEEDING	
Bernard-Soulier disease	Defective platelet plug formation secondary to decreased Gp1b, which causes impaired platelet-to-collagen aggregation
Glanzmann thrombasthenia	Defective platelet plug formation secondary to decreased GpIIb/IIIa, which causes impaired platelet-to-platelet aggregation
von Willebrand disease	Defective platelet plug formation due to an autosomal dominant defect in quantity or quality of von Willebrand factor (vWF); increased bleeding time and increased PTT (because vWF stabilizes factor VIII)

- Aspirin inhibits COX 1 and 2, irreversibly inhibits platelet aggregation
- Clopidogrel and ticlopidine block ADP receptor
- Abciximab inhibits GpIIb/IIIa receptor

KAPLAN MEDICAL

Immunology, Hematology, and Oncology

Lecture 12

- Disorders of the coagulation system
- Disorders of platelets
- Mixed disorders of both coagulation and platelets
- Treatment of clotting disorders

Laboratory Tests of Coagulation System

LABORATORY TESTS OF COAGULATION SYSTEM		
Test	**Measures**	**Specific Coagulation Factors Involved**
Prothrombin time (PT)	Extrinsic and common coagulation pathways	VII, X, V, prothrombin, fibrinogen
Partial thromboplastin time (PTT)	Intrinsic and common coagulation pathways	XII, XI, IX, VIII, X, V, prothrombin, fibrinogen

Causes of Failure to Clot

Causes of Failure to Clot	
Factor VIII deficiency (hemophilia A)	X-linked Severe cases bleed in infancy at circumcision or have multiple hemarthrosis Moderate cases have occasional hemarthrosis Mild cases may be missed until dental or surgical procedures Bleeding may require treatment with cryoprecipitate or lyophilized factor VIII
Factor IX deficiency (Christmas disease, hemophilia B)	X-linked recessive Signs and symptoms same as hemophilia A
Vitamin K deficiency	Vitamin K is fat-soluble, produced by gut flora Essential in the posttranslational modification of factors II, VII, IX, and X, as well as proteins C and S Vitamin K deficiency may result from fat malabsorption, diarrhea, antibiotics

Disorders of Platelets 1

- PT, PTT – not affected
- Bleeding time – increased
- Manifestation: gum bleeding, epistaxis, petechiae, purpura

DISORDERS OF PLATELET FUNCTION LEADING TO INCREASED BLEEDING	
Bernard-Soulier disease	Defective platelet plug formation secondary to decreased Gp1b, which causes impaired platelet-to-collagen aggregation
Glanzmann thrombasthenia	Defective platelet plug formation secondary to decreased GpIIb/IIIa, which causes impaired platelet-to-platelet aggregation

Disorders of Platelets 2

DISORDERS OF PLATELET NUMBERS	
Idiopathic thrombocytopenic purpura (ITP)	• Spleen makes antibodies against platelet antigens (e.g., GpIIb/IIIa, GpIb/IX); platelets destroyed in the spleen by macrophages • Acute form (children): self-limited, postviral • Chronic form (adults): ITP may be primary or secondary to another disorder (e.g., HIV, SLE) • Smear shows enlarged, immature platelets; normal PT and PTT • Treatment: corticosteroids, immunoglobulin therapy, splenectomy
Thrombotic thrombocytopenic purpura (TTP)	• Clinical features: pentad (thrombocytopenic purpura, fever, renal failure, neurologic changes, microangiopathic hemolytic anemia); usually in young women • Smear shows few platelets, schistocytes, and helmet cells • Hemolytic uremic syndrome (HUS): mostly in children after gastroenteritis with bloody diarrhea; organism: verotoxin-producing *E. coli O157:H7;* similar clinical triad

Von Willebrand Disease

- Defective platelet plug formation due to a defect in quantity or quality of von Willebrand factor (vWF)
- Autosomal dominant
- Increased bleeding time and increased PTT (because vWF stabilizes factor VIII)
- Treatment: desmopressin

DIC

- Massive, persistent activation of both coagulation system and fibrinolytic system
- Consumption deficiency of clotting factors and platelets
- Etiologies: amniotic fluid embolism, infections (particularly gram-negative sepsis), malignancy, and major traumas, particularly head injury
- Diagnosis: low platelets, low fibrinogen, increased PT, increased PTT, presence of fibrin degradation products (increased D-dimer), schistocytes

Causes of Excessive Thrombosis

Causes of Excessive Thrombosis	
Protein C or S deficiency	Deficiency of these factors decreases the ability to inactivate factors V and VIII, leading to increased risk of deep vein thrombosis and pulmonary embolism, cerebral venous thrombosis, and warfarin-induced skin necrosis.
Factor V Leiden deficiency	Mutant factor V cannot be degraded by protein C, leading to increased risks of deep vein thrombosis with pulmonary embolism, and possibly increased risk of miscarriage.
Prothrombin gene mutation	Prothrombin gene mutation in the 3' untranslated region causes increased circulating thrombin and venous clots.
ATIII deficiency	Antithrombin III is a potent inhibitor of the clotting cascade, and its deficiency leads to increased venous clots.

Anticoagulants

Class	Mechanism	Comments/Agents
Anticoagulants	Decrease fibrin clot formation. Differ in pharmacokinetics/pharmacodynamics. **Heparin** is used when immediate anticoagulation is necessary (acute MI, DVT, pulmonary embolism, stroke, beginning therapy); **warfarin** is used chronically. LMWHs have a longer half-life than does heparin.	
Heparin (IV, SC) LMWHs	Binds **AT-III**; this complex inactivates **thrombin**, factors IXa, **Xa**, and XIIa	Acts in **seconds**; used acutely (days) a PTT used to monitor heparin, not LMWHs **Protamine** reverses heparin and LMWHs Used in pregnancy **LMWHs** (ardeparin, dalteparin, enoxaparin) inhibit **factor Xa** more and thrombin less than heparin LMWH preferred for long-term use due to risk of HIT
Direct thrombin inhibitors	Bind directly to thrombin substrates and/or thrombin (ATIII not required) Bind to soluble thrombin and clot-bound thrombin	**Lepirudin**, bivalirudin, argatroban, hirudin
Warfarin **(PO)**	Interferes with the synthesis of the vitamin K-dependent clotting factors (II, VII, IX, X)	Takes 2-5 **days** to fully work; chronic use **PT** or **INR** used to monitor **Vitamin K** reverses effect **Contraindicated** in pregnancy **Cytochrome P450**-inducing drugs ↓ effect; cytochrome P450 inhibitors ↑ effect

Thrombolytics

- Mechanism: convert plasminogen into plasmin
- Agents: Streptokinase, alteplase, urokinase
- Indications: MI, DVT, PE, ischemic stroke (t-PA)
- Contraindications:
  - Active bleeding
  - History of intracranial bleeding
  - Recent surgery
  - Severe hypertension
- Overdose – treat with aminocaproic acid

Antiplatelets

Antiplatelets	Platelets adhere to site of vascular injury, where they are activated by various factors to express a glycoprotein to which fibrogen binds, resulting in platelet aggregation and formation of a platelet plug. Antiplatelet drugs inhibit this process, thus reducing the chances of thrombus formation.	
COX inhibitors	Block COX-1 and COX-2, thereby inhibiting **thromboxane A_2**-mediated platelet aggregation	**Aspirin**—also antipyretic, antiinflammatory, analgesic Affected platelets are impaired for their lifespan (9-12 days) Side effects—tinnitus, ↓ renal function, GI ulceration/bleeding, Reye syndrome (in children with viral syndromes)
ADP antagonists	Irreversibly inhibit ADP-mediated platelet aggregation	**Ticlopidine, clopidogrel**
Glycoprotein IIb/IIIa inhibitors	Reversibly inhibit binding of fibrinogen to platelet glycoprotein IIb/IIIa, preventing platelet cross-linking	**Abciximab**, eptifibatide, tirofiban