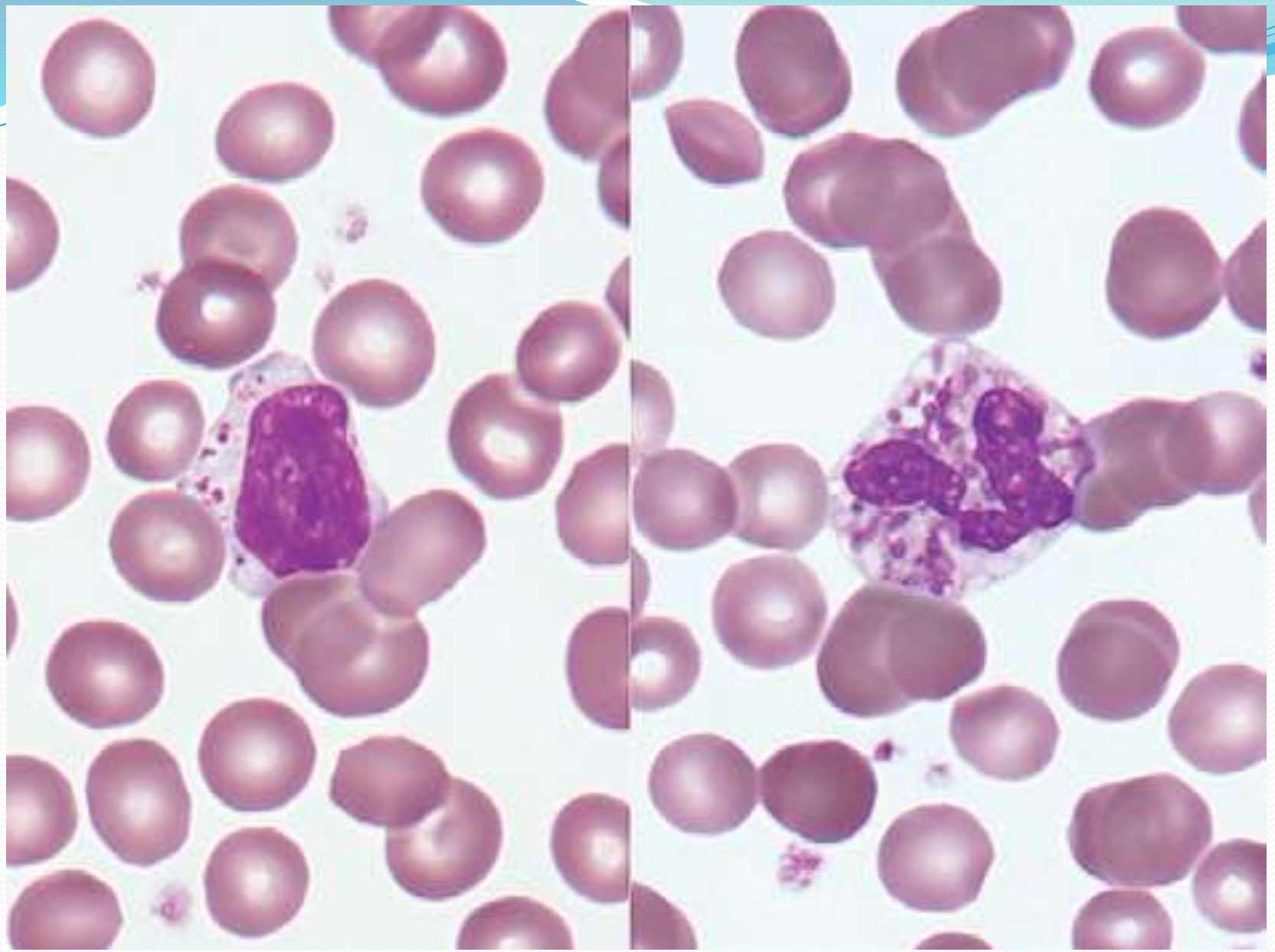


Genetic mucopolysaccharidoses often show abnormal lymphocyte granules with surrounding halos. In these cells from the same blood film, neutrophils showed similar changes

Morphologic Alterations in Neutrophils

Pelger-Huët anomaly—

bilobed or rounded nuclei with pince-nez shape

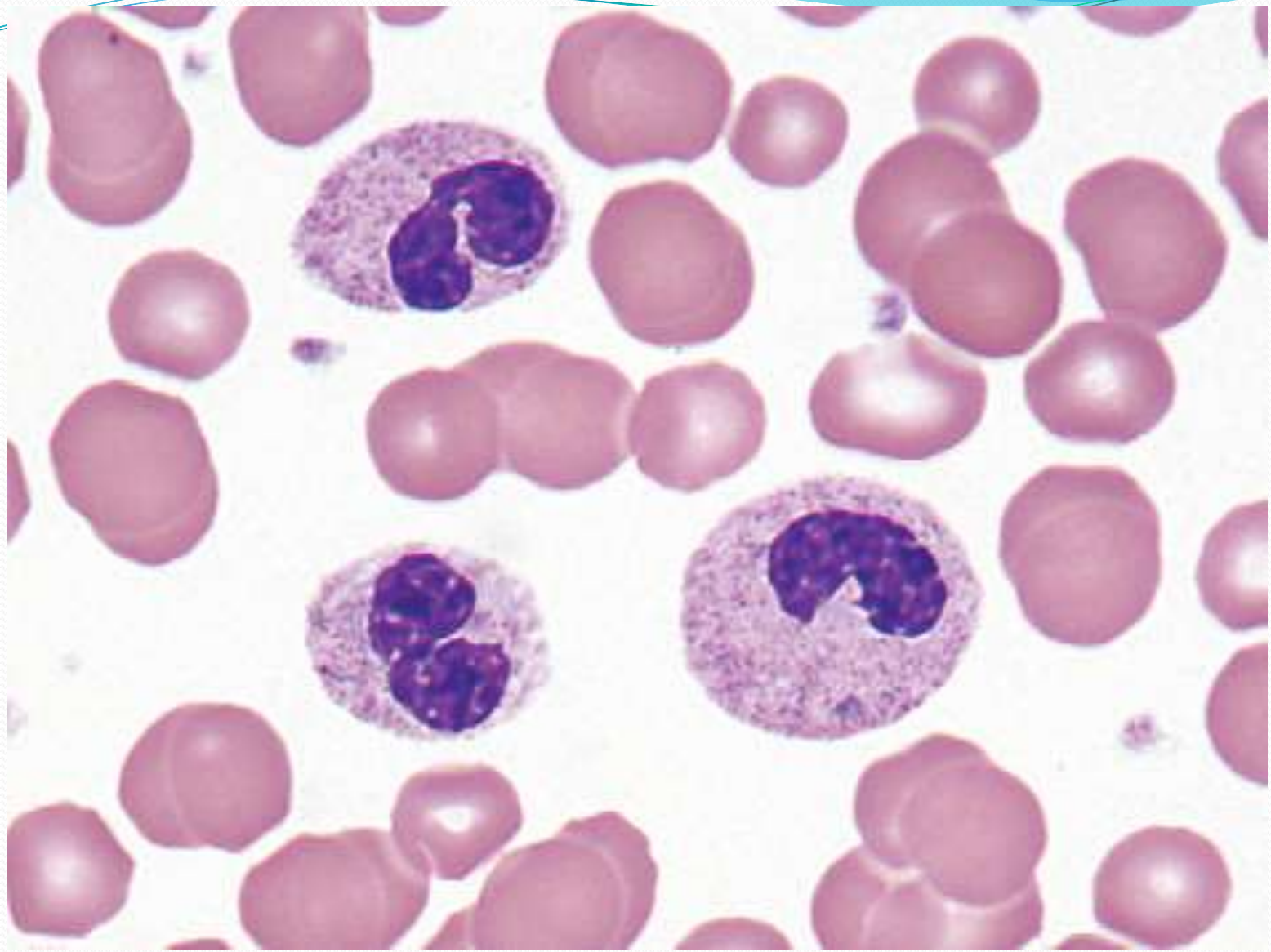
autosomal dominant condition involves failure of normal segmentation of granulocytic nuclei

Pseudo– Pelger-Huët anomaly

acquired disorder of nuclear segmentation in granulocytes may be found in cases of granulocytic leukemia, myelodysplastic and some myeloproliferative disorders, and some infections, and after exposure to certain drugs

(mature cells with round, nonsegmented nuclei and coarse

chromatin --- ring-shaped and other abnormal nuclei ---and the cytoplasm is usually hypogranular.)



Inherited Pelger-Huët anomaly

Morphologic Alterations in Neutrophils

Chédiak-Higashi syndrome—Partial oculocutaneous albinism, photophobia, immune deficiency, abnormally large granules in leukocytes and other granule-containing cells,

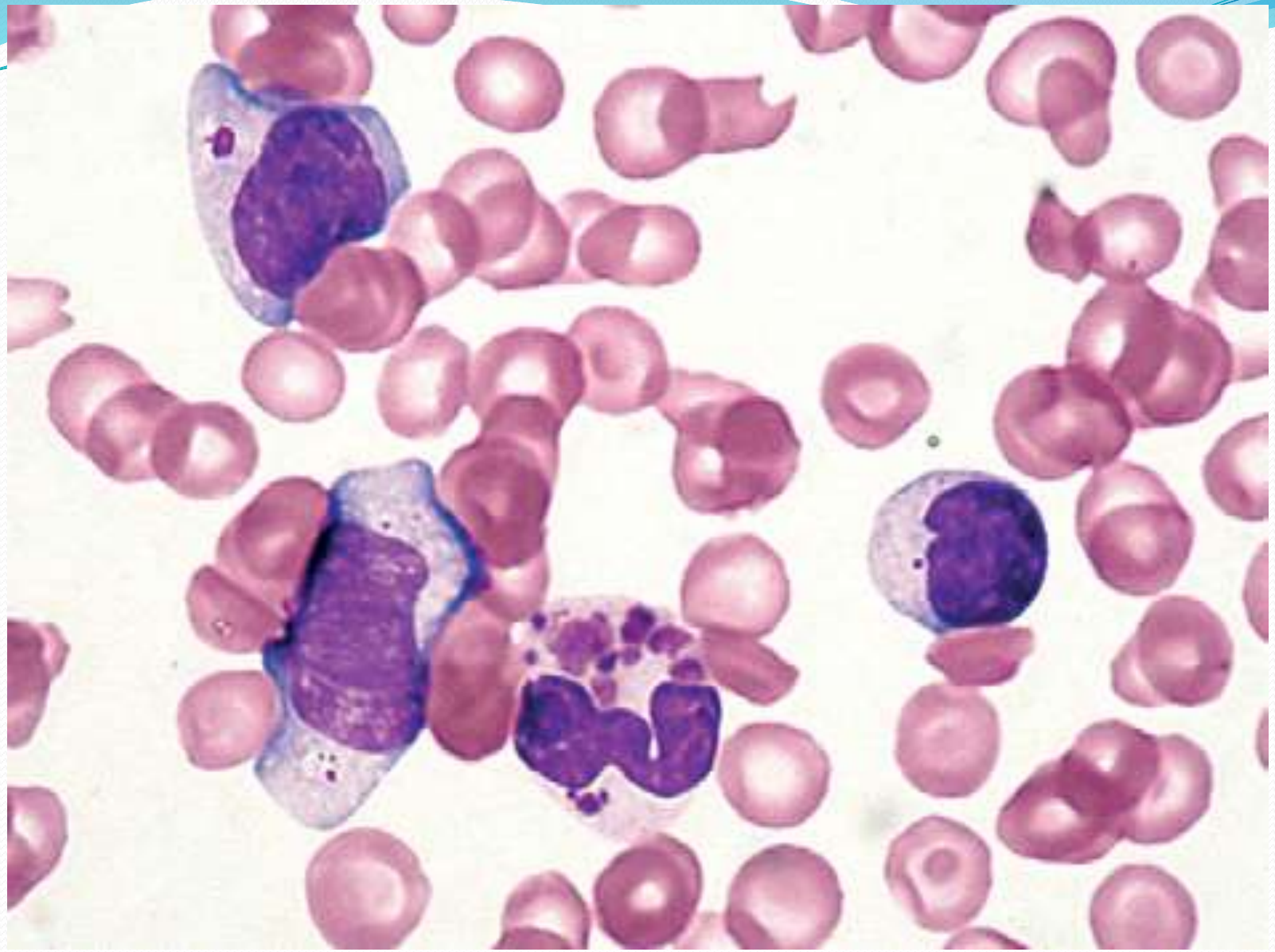
neurologic defects, and frequent pyogenic infections

autosomal recessive disorder

abnormal leukocyte function

with lymphadenopathy, hepatosplenomegaly, and pancytopenia; lymphoid infiltrates

Granulocytes, monocytes, and lymphocytes contain giant granules --- abnormal lysosomes



Chédiak-Higashi neutrophils and lymphocytes with large granules

Functional Disorders of Neutrophils

- Some inherited disorders, such as the May-Hegglin, Alder-Reilly, and Pelger-Huët anomalies, have altered morphologic appearances but apparently normal granulocytic function
- Chédiak-Higashi syndrome and specific granule deficiency (SGD), display alterations in both morphology and function

Functional Disorders of Neutrophils

- **Chronic granulomatous disease(CGD)**

lack of activity ***"phagocyte NADPH oxidase" (PHOX).***

autosomal recessive (≈66%) and X-linked (≈33%)

primary immunodeficiency

affecting neutrophils, eosinophils, macrophages, and monocytes; inability of these phagocytic cells to kill intracellular microorganisms.

Patients present with recurrent bacterial and fungal infections

(NBT) test: NBT test is '**negative**' due to the lack of NADPH oxidase activity(negative means nitroblue tetrazolium remains yellow).

- **Primary MYD- Myeloperoxidase deficiency:**normal or positive(NBT) test (positive test result means neutrophils turn blue)
- **LAD :Leukocyte adhesion deficiency**
- **Specific granule deficiency(SGD)**

Eosinophilia

≥350/μL when

Large numbers of cells are counted, as with automated instruments or direct chamber counts

≥500/μL when

The count is calculated from the 100 or 200 cell differential and the total leukocyte count.

Causes of Eosinophilia

- **Allergic**—urticaria, seasonal rhinitis (hay fever), asthma, Atopic dermatitis and eczema
- **Inflammatory**—eosinophilic fasciitis, Churg-Strauss syndrome
- **Parasitic**—parasitic worms, including nematodes, trematodes, and cestodes

  trichinosis, filariasis, schistosomiasis
- **Nonparasitic infections**—systemic fungal, scarlet fever, chlamydial pneumonia of infancy
- **Respiratory**—pulmonary eosinophilic syndromes :

Loffler's syndromes-during periods of dissemination or migration when parasites pass from the blood into the alveoli of the lung.,

Tropical pulmonary eosinophilia, *Wuchereria bancrofti (a parasite of humans that inhabits the lymphatics*

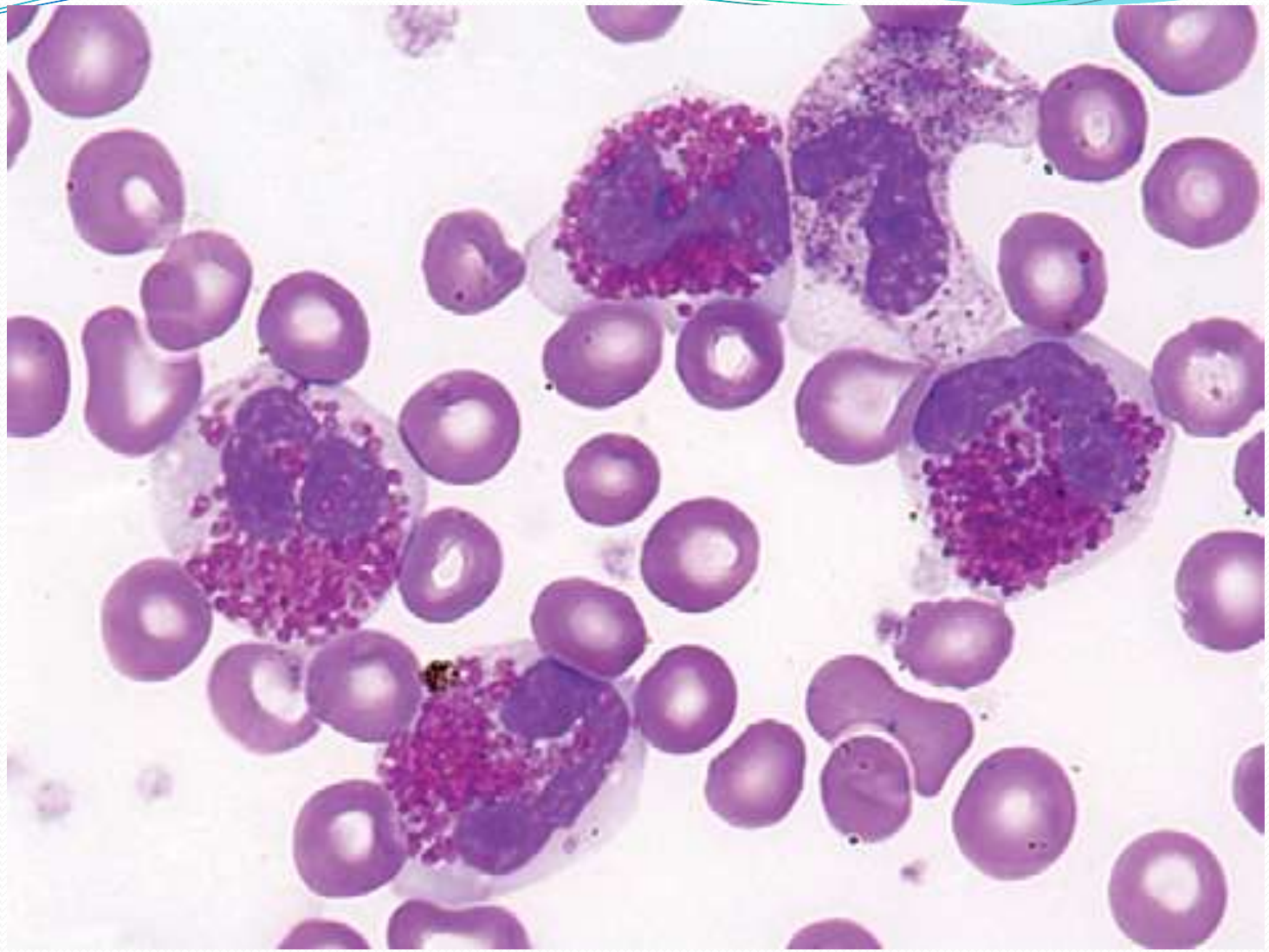
Churg-Strauss syndrome

- **Neoplastic**—CML, Hodgkin lymphoma, T cell lymphomas, acute leukemias, systemic mast cell disease, plasma cell dyscrasias, and myelodysplastic and myeloproliferative diseases, primary neoplasm originating in another organ, such as lung, soft tissue, or colon. metastatic melanoma
- **Idiopathic hypereosinophilic syndromes**—affecting heart, liver, spleen, CNS, other organs

Drugs include pilocarpine, physostigmine, digitalis,

p-aminosalicylic acid, sulfonamides, chlorpromazine, phenytoin, some antidiabetic drugs, some anticancer agents

- **Others**—GI inflammatory diseases, sarcoidosis, Wiskott-Aldrich syndrome



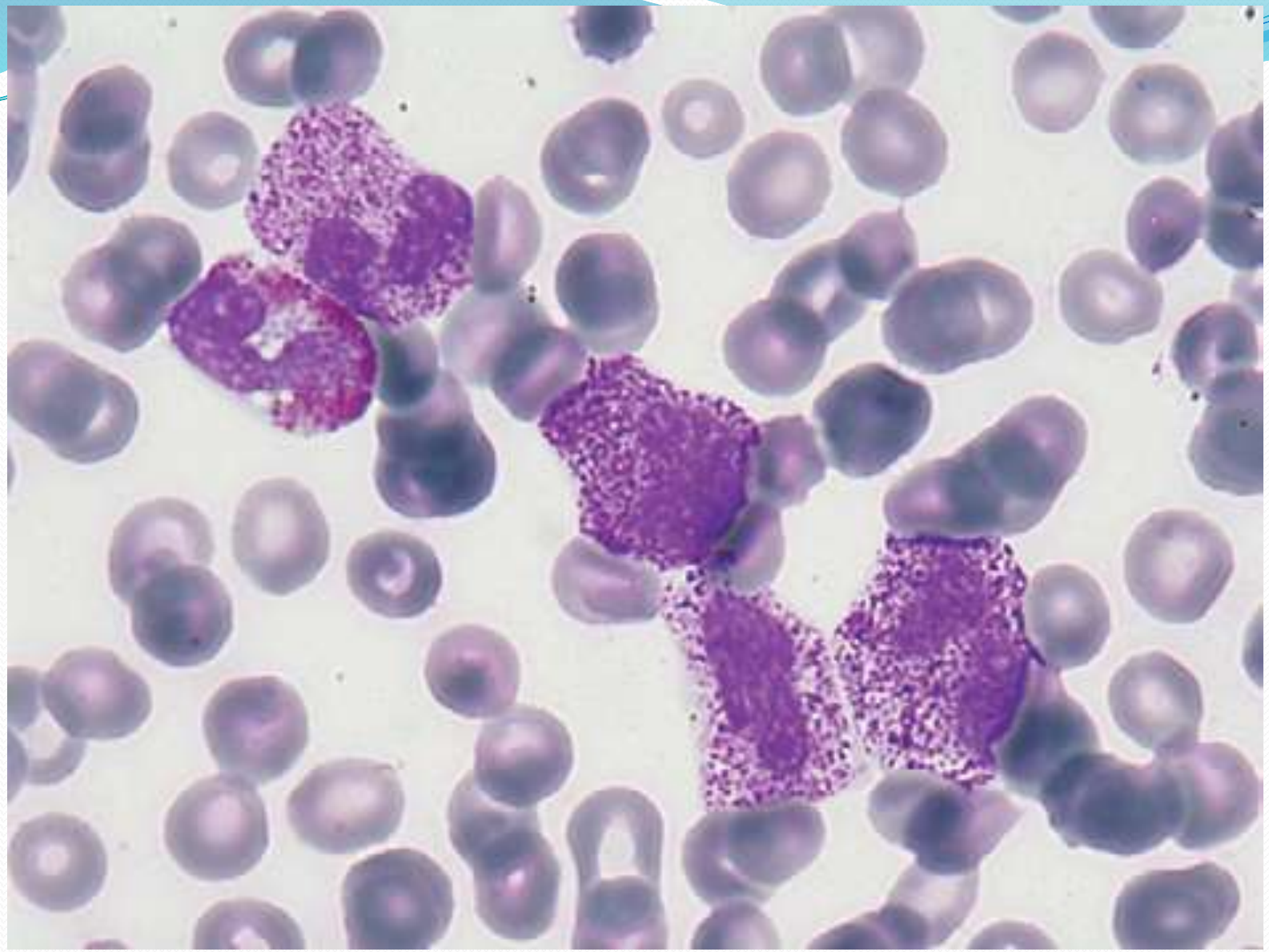
Marked eosinophilia of unknown cause, suggesting hypereosinophilic syndrome

Basophilia

absolute basophil count above 200/μL

Causes of Basophilia

- Myeloproliferative disease
- Allergic—food, drugs, foreign proteins
- Infectious—variola, varicella
- Chronic hemolytic anemia—especially post splenectomy
- Inflammatory—collagen vascular disease, ulcerative colitis



Marked basophilia and an eosinophil in a patient with Philadelphia chromosome positivity

Monocytosis

Monocytosis is an increase in monocytes above the upper reference value,

especially when greater than 1000/µL.

- Monocytosis is present during the recovery stage from acute infection and from agranulocytosis, in which it is considered a favorable sign.

Causes of Monocytosis

- **Infectious**—tuberculosis, subacute bacterial endocarditis, syphilis, protozoan, rickettsial
- **Recovery from neutropenia**
- **Hematologic**—leukemias, myeloproliferative disorders, lymphomas, multiple myeloma
- **Inflammatory**—collagen vascular disease, chronic ulcerative colitis, sprue, myositis, polyarteritis, temporal arteritis
- **Others**—solid tumor, immune thrombocytopenic purpura, sarcoidosis

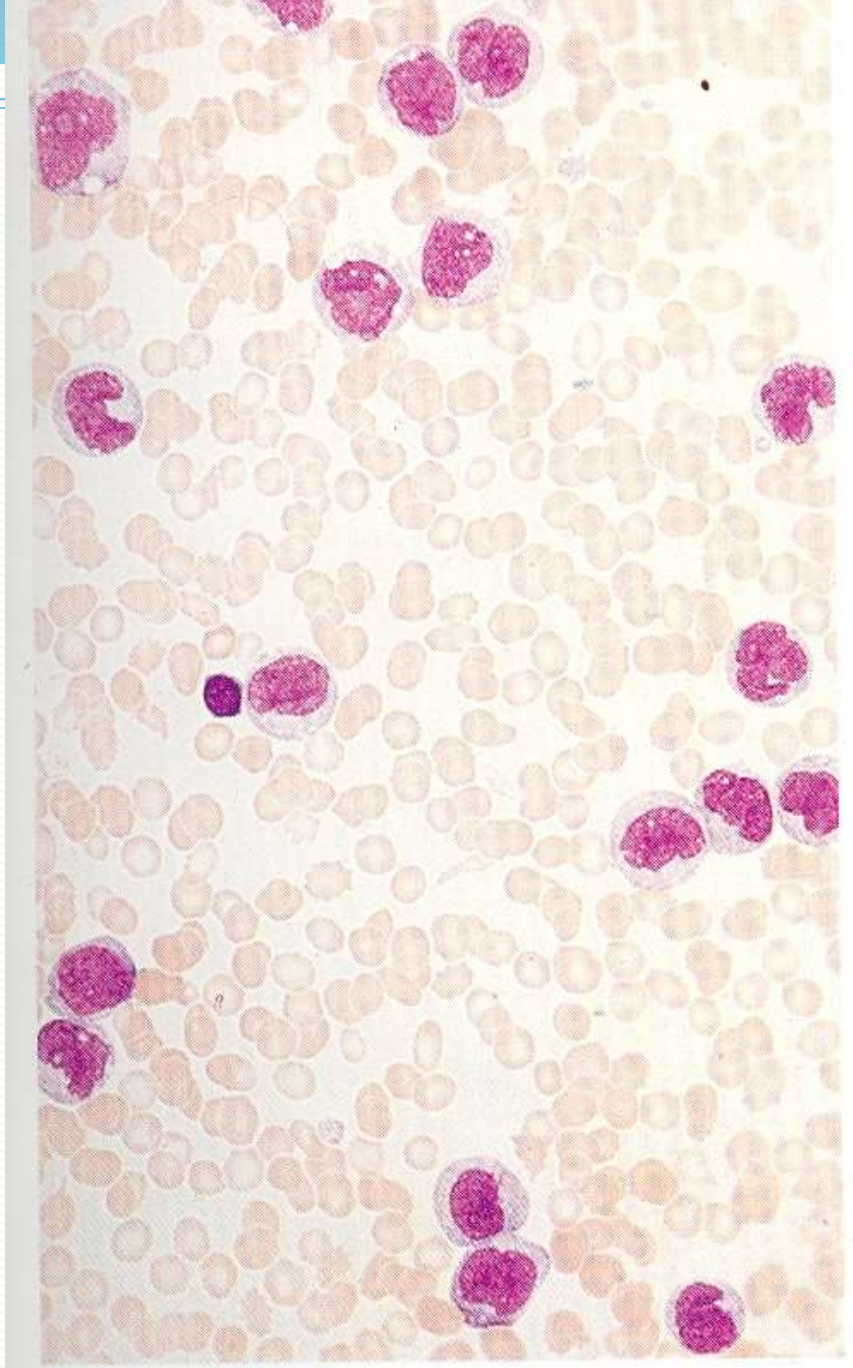


Fig. 7.15 Monocytosis: in this peripheral blood

Monocytopenia

Monocytopenia is a decrease in circulating monocytes below the lower reference value of 200/µL

- During therapy with **prednisone**, monocytes fall during the first few hours after the first dose but return to above original levels by 12 hours.
- Monocytopenia has also been observed in **hairy cell leukemia**

LYMPHOCYTIC AND PLASMACYTIC DISORDERS

Lymphocytes in Normal Individuals

- Percentage of lymphocytes in the blood is normally **up to about 50% for the first 5 years**.
- During the **first decade** of life, the absolute lymphocyte count and the **absolute number of T cells decrease**
- Absolute number of **B lymphocytes** remains **stable** during all stages of life
- Normal **CD4/CD8 ratio** is between **1.0 and 3.4**, but we have seen values **as high as 12** in reactive conditions.

Lymphocytosis

- In adolescence and **adulthood**, lymphocytes constitute about **20%–40%** of all leukocytes, or **1500-4000 /µL--** More than 4000: **lymphocytosis**
- **1500-8800 /µL** in the child
- More than 10000 (less than 2 years) : lymphocytosis
- More than 7000 (2-10 years) : lymphocytosis
- The **'absolute lymphocytosis**' pattern applies when the lymphocytosis is composed of cells with normal-appearing morphology.

- **Relative lymphocytosis** (an increase in the percentage of lymphocytes) is especially prominent in disorders with neutropenia

Causes of Lymphocytosis

- **Infectious**—many viral (Epstein-Barr virus [EBV], hepatitis), pertussis, tuberculosis, toxoplasmosis, rickettsial
- **Chronic inflammatory**—ulcerative colitis, Crohn's
- **Immune mediated**—drug sensitivity, vasculitis, graft rejection, Graves',Sjo͡gren's
- **Hematologic**—ALL, CLL, lymphoma
- **Stress**—acute, transient

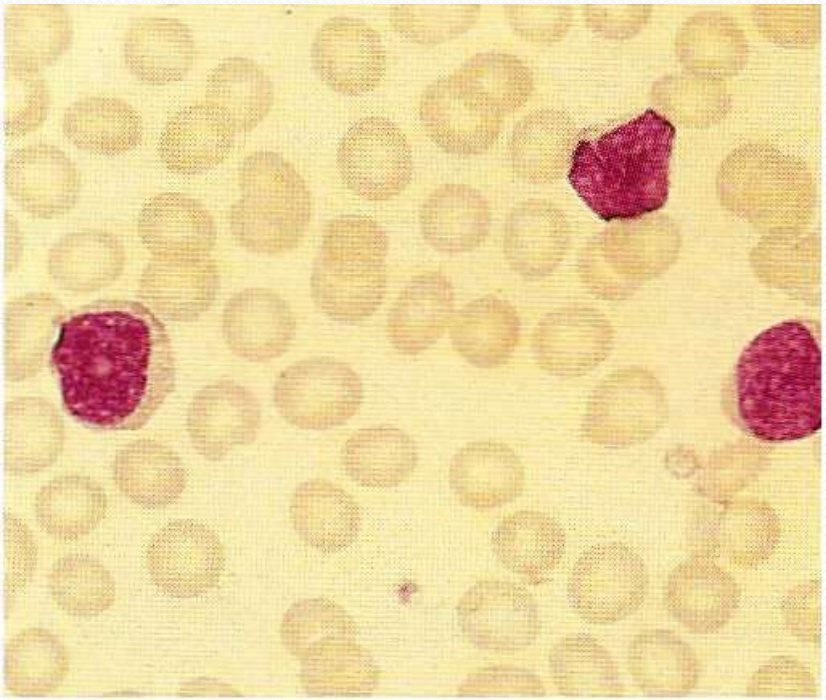
Acute infectious lymphocytosis (AIL)

- Contagious condition characterized by lymphocytosis mainly in children
- coxsackievirus A, coxsackievirus B6, echoviruses, and adenovirus type 12
- Symptoms such as vomiting, fever, abdominal discomfort, signs suggesting involvement of the nervous system, cutaneous rashes, upper respiratory infection, and diarrhea.
- Leukocytosis 20000 ----60%–95% of blood leukocytes are small mature lymphocytes ---- **In contrast to infectious mononucleosis, atypical lymphocytes are uncommon.**

Pertussis

- Unimmunized children and adults with immunity reduced since childhood vaccination
- ***Bordetella pertussis***
- Paroxysms of coughing productive of thick sputum. The paroxysms typically end with a **“whooping”** sound
- Significant lymphocytosis with counts higher than 30000 recorded.The lymphocytes are **small,mature T**
- lymphocytosis is due to redistribution of lymphocytes into the peripheral circulation **without increased lymphopoiesis**

Lymphocytosis in an 8-year-old child with whooping cough



Chronic Lymphocytosis/ Persistent Polyclonal B Cell Lymphocytosis

- Uncommon event in **adults**,
- **Suspicion of neoplastic disease**, such as chronic lymphocyte leukemia.
- Coexistence of neutropenia or classic Felty's syndrome suggests **LGL leukemia**
- **Mononucleosis** or lymphocytosis due to other **viruses** occasionally presents in later life
- In adults, predominantly in **female smokers**, and in the **postsplenectomy** state
- **Immunophenotyping** of peripheral blood benign **polyclonal B cell** proliferation
- **Binucleated atypical lymphocytes** in the peripheral blood.
- **Polyclonal serum IgM** is usually increased

Retrovirus-Associated Diseases and Conditions

- Human T lymphotropic virus type 1 **(HTLV-1),** associated with adult **T cell leukemia/ lymphoma** (ATL)
- **HTLV-2**, found in a patient with **HCL**
- HTLV-1 and -2 also infect and transform **T cells**, primarily **CD4+ cells with HTLV-1** and **CD8+ cells with HTLV-2.**
- Most (**90%– 95%**) patients with antibodies against HTLV-1 are **symptom free**. Most individuals exhibit only a **viral-like syndrome**; however,
- others manifest a **chronic progressive leukemia**, and still others develop tropical **spastic paraparesis**
- HTLV-1 immortalizes CD4+ lymphoblasts in some patients, producing monoclonal T cell lymphocytosis and ATL
- ATL typically develops decades after initial infection, and thus is usually seen in older patients

Infectious Mononucleosis and Epstein-Barr Virus Infection

- Epstein-Barr Virus (Human Herpesvirus-4)
- Self-limited infectious disease
- Sore throat, prolonged malaise, atypical lymphocytosis
- Presence of large transformed lymphocytes
- Lymphadenopathy (most often posterior cervical), and often splenomegaly
- Immunocompromised patients : benign B cell hyperplasia, malignant lymphoma, and posttransplantation lymphoproliferative disease

Pathophysiology

- Virus enters through **oropharyngeal epithelial** and lymphoid cells
- Virus attaches to **CD21 on B cells**
- Viral antigens—**viral capsid antigen (VCA), early antigen (EA), Epstein-Barr nuclear antigen (EBNA)—**are produced and elicit antibody production
- **IgM against VCA** rises during incubation and prodrome, falls over few weeks to months
- **IgG against VCA** rises during incubation, decreases during convalescence, remains detectable for life

Pathophysiology

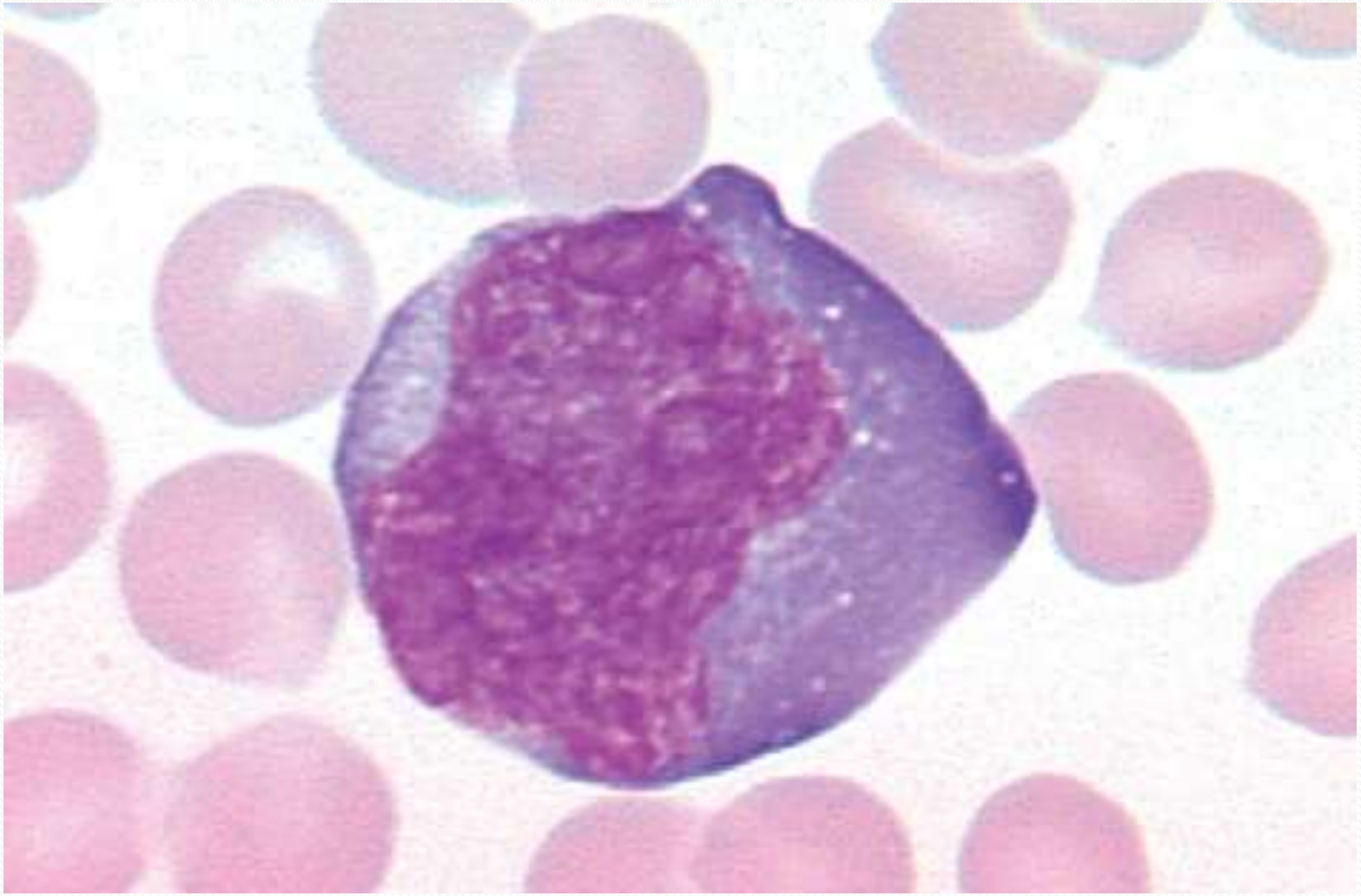
- **T cells activated** during **second week** of illness
- **CD8-positive cytotoxic T cells** kill infected B cells
- **Natural killer cells** kill infected B cells
- Some resting **memory B cells** remain **latently infected**
- An atypical lymphocytosis of **at least 20 percent** or atypical lymphocytosis of **at least 10 percent plus lymphocytosis of at least 50** percent

Laboratory Features

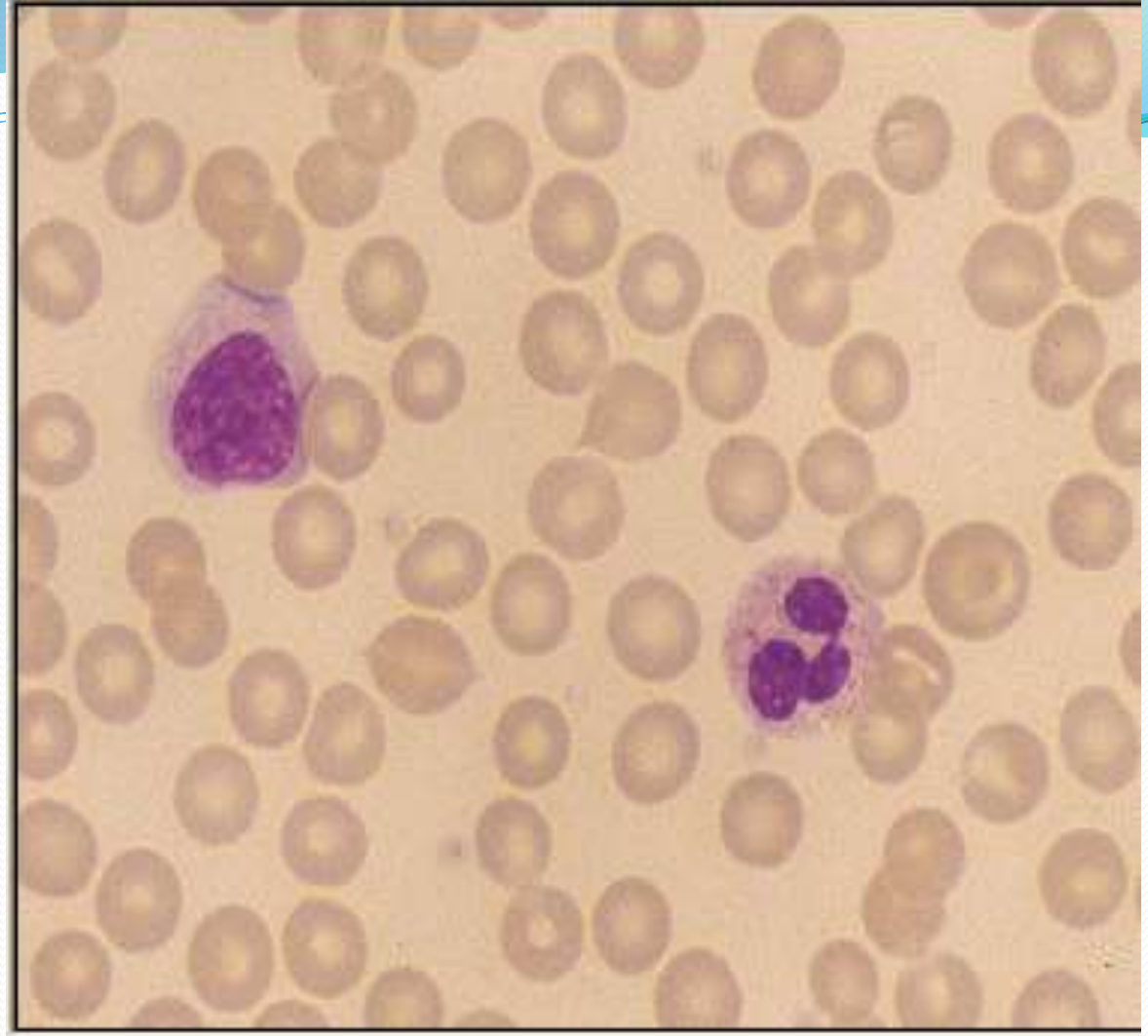
- **Leukocytosis 12000-25000** with

  absolute lymphocytosis 60-90% and **atypical lymphocytes**

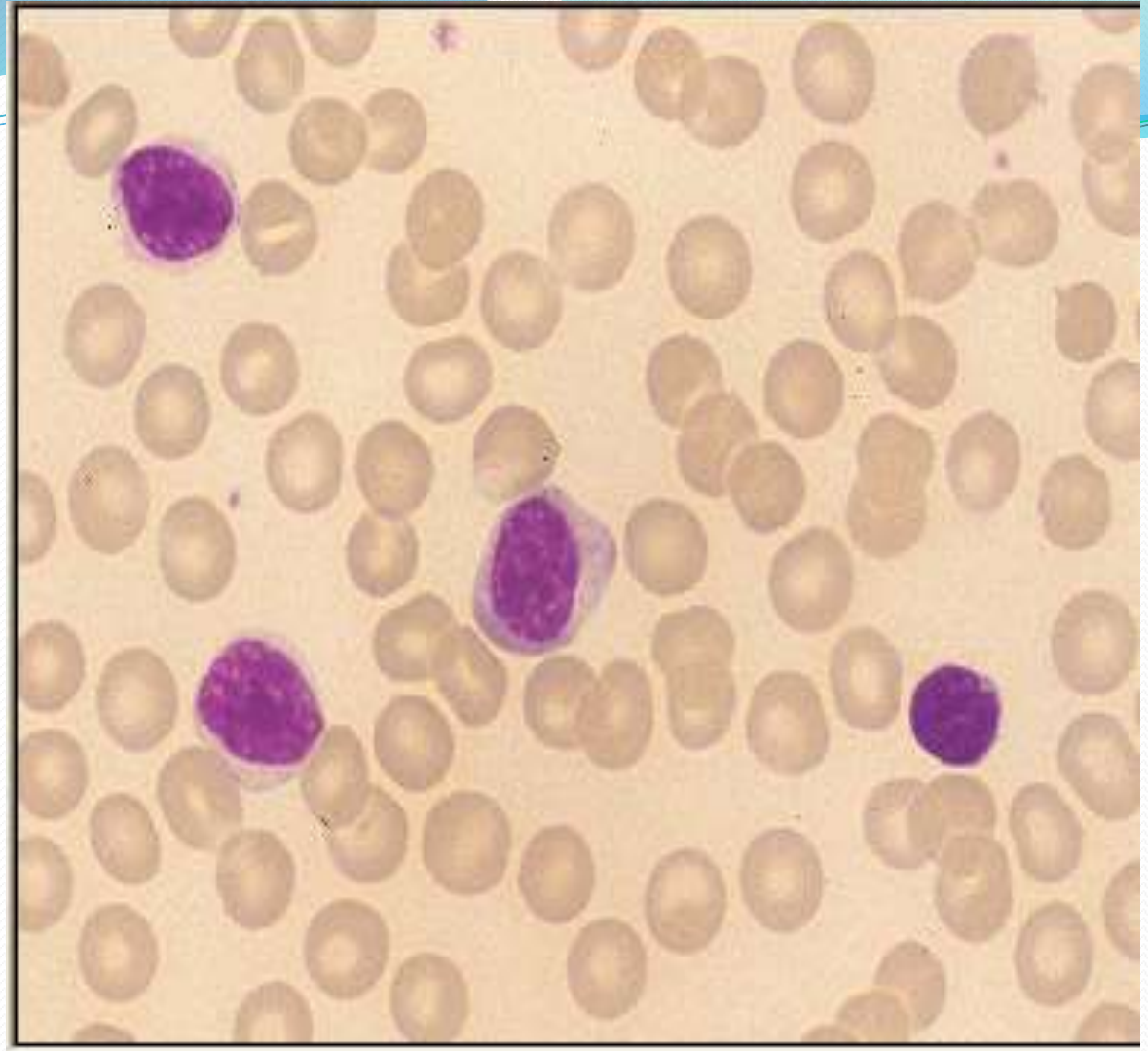
- The total leukocyte count, as a rule, returns to normal within 3 weeks.
- **The atypical lymphocytes** have **nuclear alterations** and an **increase in the amount and basophilia of cytoplasm**.
- Lymphocytes include **monocytoid lymphocytes,** which likely correspond to **immunoblasts** in lymph nodes
- Other atypical lymphocytes, which are more numerous, include **plasmacytoid lymphocytes** and those with **small nuclei but abundant cytoplasm**.



Infectious mononucleosis, showing a large activated lymphoid cell

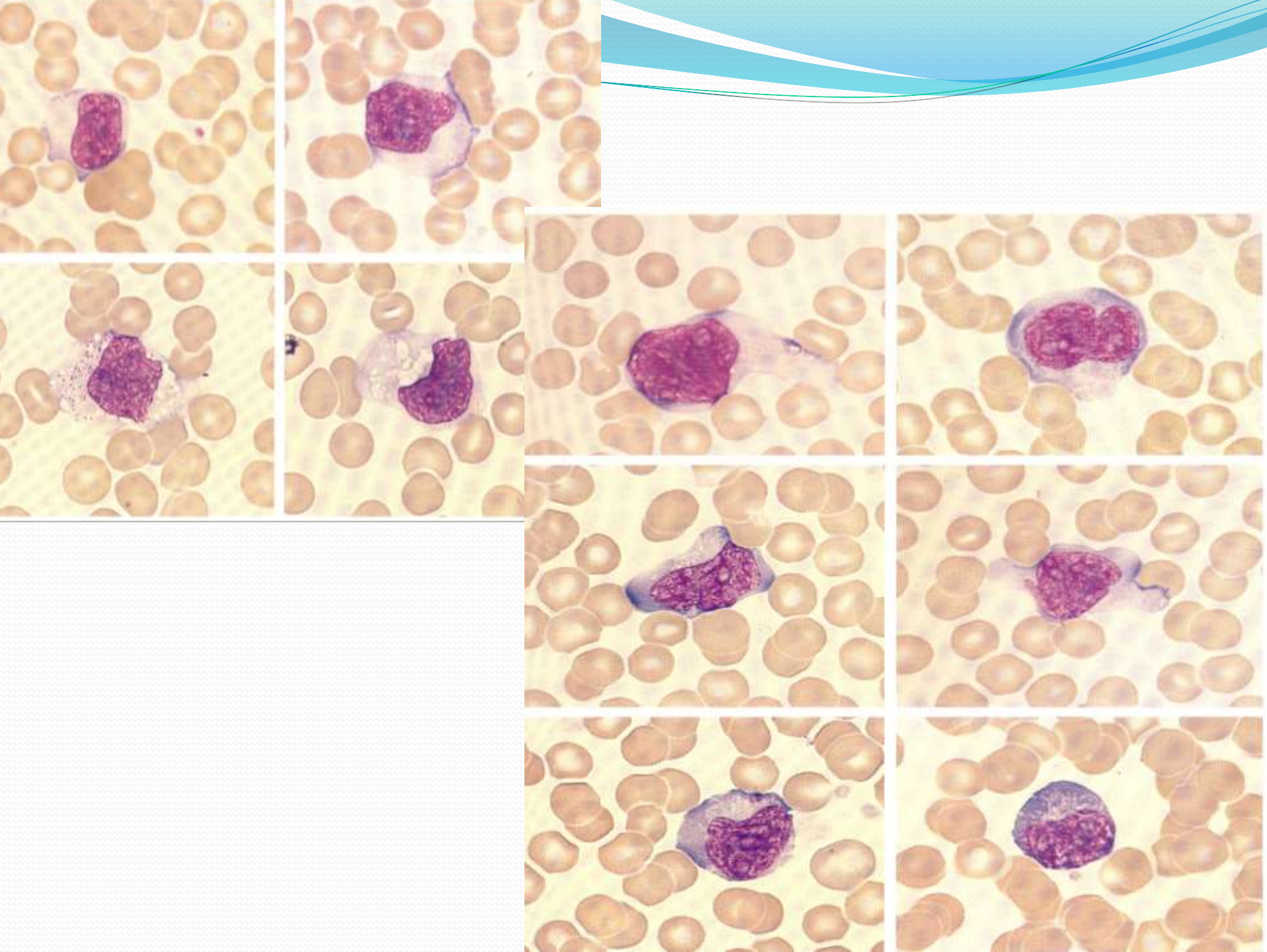


A plasmacytoid lymphocyte with a small nucleolus and a neutrophil.



Small lymphocytes and a plasmacytoid lymphocyte (center). Notice a slight degree of hypochromia.

The hemogram and peripheral blood film appearance may be mistaken for a chronic lymphoproliferative disorder if the patient's age is not taken into account.



The morphologic features of blasts

- Large cells size
- High N/C ratio
- Scant to moderate pale blue cytoplasm which may contain rare small granules and/or vacuoles
- Round to irregular nuclei
- Conspicuous nucleoli
- Fine chromatin

Laboratory Features

Cytologic alterations are **not pathognomonic of IM**.
Similar cells are found in:

cytomegalovirus mononucleosis,

toxoplasmosis,

infectious **hepatitis**,

viral pneumonia,

varicella,

mumps,

viral exanthemas of children.

Laboratory Features

- Approximately **one third** of patients with IM carry β-hemolytic streptococci in the pharynx.
- Transient **monocytosis(**The term **mononucleosis** refers to an increase in lymphocytes, not in monocytes)
- Relative and absolute **neutropenia** during the first week (shift to the left may occur-an increase in band cells and metamyelocytes)
- **Mild thrombocytopenia** in half of cases
- **Hemolytic anemia** in 1%–3% of cases, often with anti-I specificity
- Elevated **transaminases in 85%–100%** of cases, but clinical **jaundice rare**

- **The BM** from patients with IM usually shows **increased cellularity**. Numbers of **lymphocytes, macrophages, plasma cells, megakaryocytes**, and **erythroid cells** are increased. The **neutrophilic series appears decreased**.

  About half of cases may have **collections of mononuclear cells** forming loose granulomas.

Laboratory Features

- **Spot test** is simple, rapid, specific, based on agglutination of horse red blood cells (RBCs)
- **Heterophil antibody** (HA) test is based on differential absorption of IM-specific HA by beef RBC stroma and guinea pig kidney

Heterophil Antibody

- Paul : **Sheep cell agglutinins** in the sera of patients with IM
- Sheep cell agglutinins are **not specific for IM** and can be present in **other disorders**
- Davidsohn : heterophil antibodies in patients with IM are absorbed by **Beef erythrocytes**, in contrast to heterophil antibodies present in other disorders **(specific for IM)**
- heterophil antibodies present in other disorders absorbed by **Forssman's antigen**, such as that found in **Guinea pig kidney (specific for non IM)**
- **Differential absorption test** (Paul-Bunnell-Davidsohn test) is **highly specific for IM**

Heterophil Antibody

- **Spot test** for IM simple, rapid, **highly specific, and sensitive** test for the heterophil antibodies of IM

  (**Horse erythrocytes** are **more sensitive than sheep** erythrocytes in testing for IM)

- **A positive Spot test:**

***<u>Agglutination</u> of horse erythrocytes*-------- by serum absorbed with guinea pig kidney stroma**(specific for non IM heterophil antibodies)

***<u>Not agglutination</u> of horse erythrocytes*--- by serum absorbed with beef erythrocyte stroma**(specific for IM heterophil antibody)

- **False-negative** tests occur particularly in **young children** who produce heterophil antibodies (IgM) in limited amounts. In heterophil-negative IM, the diagnosis may be substantiated by:

  assay for antibody to EBV.

- In addition to heterophil and EBV antibodies, patients with IM frequently produce antibodies to a wide variety of antigens. Antibodies **against human erythrocytes, leukocytes, and platelets** have been described. Patients with IM have an increased frequency of **cold agglutinins**. Positive tests for **rheumatoid factor** and **antinuclear factor** have been reported.

- **In immunocompromised patients**, serologic tests are of limited value,

Direct detection methods (**PCR**)

Cytomegalovirus Infection

- Cytomegalovirus infection, is the most common cause of **heterophil-negative mononucleosis**.
- Posttransfusion mononucleosis
- Patient has fever, chills, profound malaise, and myalgia.
- Sore throat (but **not exudative** pharyngitis) and lymphadenopathy may be noted
- **Leukocytosis** is characteristic with **absolute lymphocytosis**. Usually **20%** or more of the leukocytes are **atypical lymphocytes**.
- BM aspirates have shown increased numbers of normal lymphocytes and atypical lymphocytes.
- **Abnormal liver function test** results are the most frequent abnormal laboratory finding

Toxoplasmosis

- **Toxoplasma gondii**, a **protozoan** parasite, can produce in both young and old a disease **similar to infectious mononucleosis**.
- Only a small proportion (≈10%) of immunocompetent individuals are symptomatic.
- **fever**, headache, **sore throat**, **hepatosplenomegaly**, **chorioretinitis**, **lymphadenopathy** commonly cervical and an increased number of **atypical lymphocytes** in the peripheral blood
- congenitally infected **fetuses and newborns**, women infected **during pregnancy**, **immunocompromised** patients, and patients with **chorioretinitis**

Lymphocytopenia

- Absolute lymphocyte count is

  below **1000 /µL in adults**

  below **2000 /µL in children**.

- About **80% of circulating peripheral blood** lymphocytes are **CD3+ T cells**, and a majority (≈65%) of these cells are CD4+ helper T cells

Causes of Lymphopenia

- **Destructive**—radiation, chemotherapy, corticosteroids
- **Debilitative**—starvation, aplastic anemia, terminal cancer, collagen vascular disease, renal failure
- **Infectious**—viral hepatitis, influenza, typhoid fever, TB
- **AIDS associated**—HIV cytopathic effect, nutritional imbalance, drug effect
- **Congenital immunodeficiency**—Wiskott-Aldrich syndrome
- **Abnormal lymphatic circulation**—intestinal lymphangiectasia, obstruction, thoracic duct drainage/rupture, CHF

Acquired Immunodeficiency Syndrome

- Infection with **HIV-1** and **HIV- 2—RNA retroviruses** that are cytotropic for **CD4+ T cells** and for other cells, including **macrophages, monocytes**, megakaryocytes, and CNS microglial cells.
- There is a marked **decrease in the number of T-helper** cells and an **imbalance in T-suppressor/cytotoxic** cells in the blood and lymphoid tissues
- **Cellular immune depression** occurs, characterized by infection with a variety of **opportunistic organisms**
- AIDS-associated cancers include **Kaposi's sarcoma**, **non-HL**, and **cervical cancer**, **HL** and **anogenital cancers**

Acquired Immunodeficiency Syndrome

- **Anemia** of chronic disease
- **Lymphopenia (80%–85%** of cases), particularly of the T-helper/inducer (CD4) subset.
- **Thrombocytopenia** in 30%
- **Neutropenia** in 40%, oftn with a left shift
- Peripheral blood film usually displays **atypical lymphocytes** that have a **plasmacytoid appearance**.
- **Monocytes are often large** with a fine nuclear chromatin and cytoplasmic vacuoles.

Functional Disorders of Lymphocytes

Acquired disorders :

- **Malnutrition,**
- **Infection,**
- **Malignancy**

Decreased B cell function is observed in **chronic lymphocytic leukemia**, in which two thirds of patients have **hypogammaglobulinemia**.

In **multiple myeloma**, synthesis of **normal immunoglobulin** in the presence of high levels of paraprotein is **diminished**.

Diminished **T cell activity** has been described in patients with **HL**, **sarcoidosis**, and **leprosy.**

Diagnosis of functional disorders of lymphocytes

- **Skin tests**,
- Enumeration of **B and T cells** and their subsets,
- Measurement of serum **immunoglobulin** and **antibodies**,

Plasmacytosis

- Plasma cells are **not normally** present in circulating blood

Causes of Plasmacytosis

- **Viral**—infectious mononucleosis, measles, rubella, HIV
- **Bacterial**—tuberculosis, syphilis, streptococcus, staphylococcus
- **Parasitic**—malaria, trichinosis
- **Inflammatory**—SLE, RA, inflammatory bowel disease, alcoholic liver disease
- **Neoplastic**—plasma cell leukemia, myeloma
- **Immune stimulation**—immune complex disease (serum sickness), drug sensitivity, transfusion
- **Trauma**

Plasmacytosis in marrow

- An **average of 1%–2%** of plasma cells are present in **adults**.
- An **increase beyond 4% is significant**;
- lower values are found in children
- **Increases of up to 20%** of plasma cells may be found in a variety of conditions **other than multiple myeloma**, including

  Metastatic carcinoma,

  Chronic **granulomatous infection**,

  Conditions linked with **hypersensitivity**,

  Following administration of **cytotoxic drugs**

  Aplastic anemia(this is probably just a relative increase).

  Inflammatory—SLE
- On the other hand, they are decreased or absent in **agammaglobulinemia**.

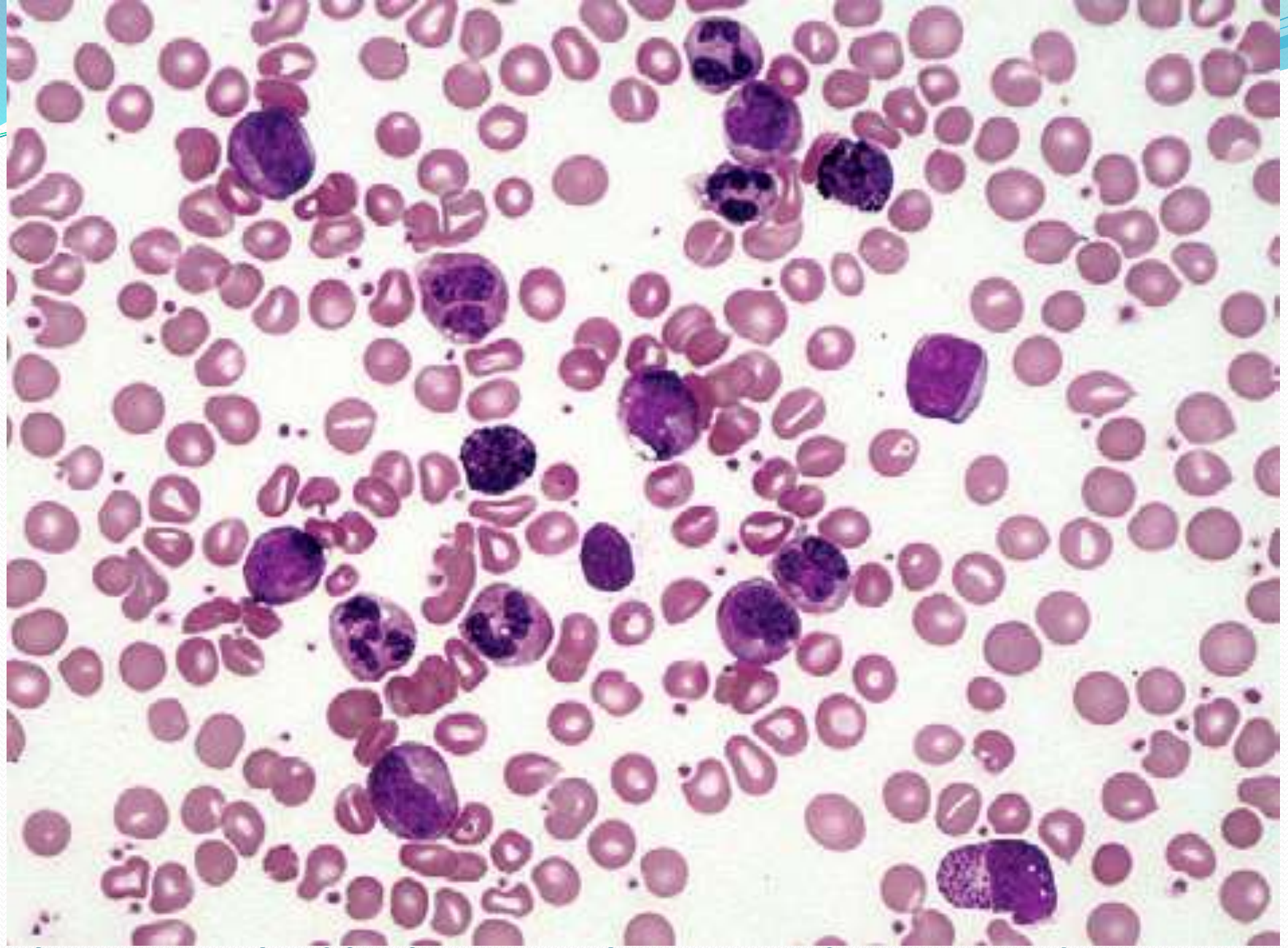
LEUKEMOID REACTIONS

- Excessive leukocytic response in the peripheral Blood ,includes:
- **leukocytosis of 50000** or higher **with a shift to the left**
- **lower counts**, even below normal, **with considerable numbers of immature granulocytes**
- Similar quantitative or qualitative changes in **lymphocytes** or **monocytes**

Neutrophilic Leukemoid Reactions

- hemolysis,
- hemorrhage,
- malignancy with bone involvement,
- HL,
- myelofibrosis,
- infection (especially tuberculosis),
- severe burns,
- Eclampsia
- Certain intoxications

Leukemoid reactions lack the characteristic differential count that is seen in CML, including the myelocyte "peak," eosinophilia, and basophilia



Chronic myeloid leukemia with increased segmented neutrophils, myelocytes, and basophils and an occasional blast

Eosinophilic Leukemoid Reactions

- usually occur in children and usually are caused by parasitic infection
- some eosinophilic leukemoid reactions have been associated with tumor

Erythroblastosis and Leukoerythroblastosis

- Metastatic carcinoma involving BM
- Marrow infection and/ or fibrosis,
- GI bleeding
- Hemolytic anemia

Lymphocytic Leukemoid Reactions

- Infectious lymphocytosis and in Pertussis
- Infectious Mononucleosis

When atypical lymphocytes are strikingly increased or immature the **distinction from leukemia** may be difficult.

Examination of the marrow may be useful because **lymphocytes are minimally increased**, if at all, in most leukemoid reactions in contrast to leukemia.

Flow cytometric studies of peripheral blood and/or BM