Republic of Iraq Ministry of Higher Education and Scientific Research

Training package
In
Clinical Immunology

For

Students of fourth class of
Medical Laboratory Techniques Department

Lecture No. 1

Rheumatoid Arthritis

Rheumatoid Arthritis is a chronic systemic inflammatory disease of undetermined etiology involving primarily the synovial membranes and articular structures of multiple joints. The disease is often progressive and re sults in pain, stiffness, and swelling of joints. In late stages, deformity and ankylosis develop. Women are affected more than men, with a female: male ratio of 3:1; the disease onset reaches its apex between 35 and 50 years.

Causes

The causes of RA is unknown.

- **Genetic**: Certain **HLA-DR4** molecules associated with RA (e.g. **HLA-DR beta *0401, 0404, or 0405**); in addition, **HLA-DR1** (**HLA-DR beta *0101**) carries this shared epitope and confer risk, particularly in certain southern European areas.
- **Environmental:** for many decades, numerous infection agents have been suggested to induce RA. Among these are Mycoplasma organisms, Epstein Barr virus, rubella virus, cytomegalovirus and herpes virus.
- **Hormonal:** Sex hormones may play a role, as evidenced by the disproportionate number of females with RA, its amelioration during pregnancy, its recurrence in the early postpartum period, and its reduced incidence in women using oral contraceptives. Hyperprolactinemia may be a risk factor for RA.

Immunopathogensis

Rheumatoid Arthritis is a disease result from immunological response to an antigen within the joint. This antigen could be self molecules expressed in the joint or could be foreign (e.g. bacterial or viral) antigen sequestered in joint tissue. The nature of this immunological response and the target antigen remain uncertain. Unknown antigen stimulates the activation of T lymphocytes that, in turn activate synovial macrophages. The macrophages secrete the proinflammatory cytokines, TNF-α and IL-1, which activate osteoclasts and chondrocytes. This "two-pronged attack" results in the destruction of cartilage and bone. The chondrocytes begin to produce high quantities of fibroblast growth factor and GM-CSF, which completes a harmful cycle that can result in reactivation of the macrophage. Also, B cells are activated by polyclonal stimulation and produce immunoglobulins, especially rheumatoid factors, that stimulate the activation of complement through immune complex. Moreover, proinflammatory cytokines, especially TNF-α and IL-1, lead to the increased of proliferation and activation of fibroblast. This result in synovitis with pannus formation and in consecutive bone and joint damage.

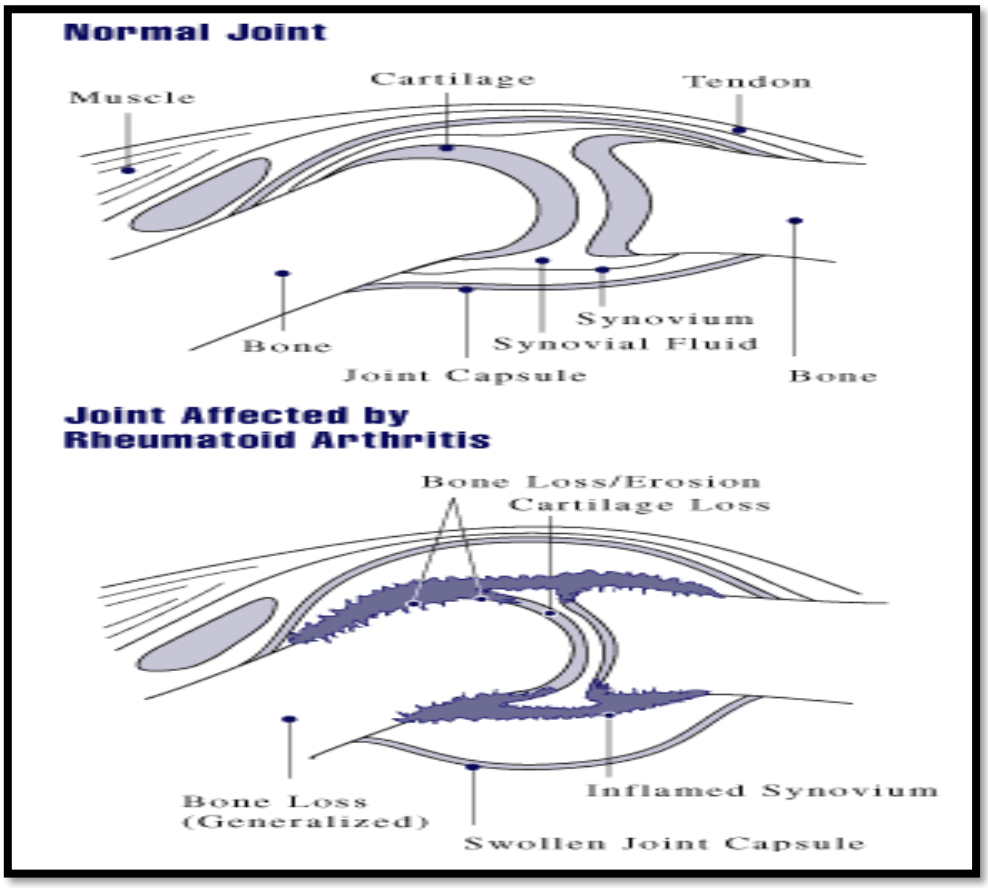
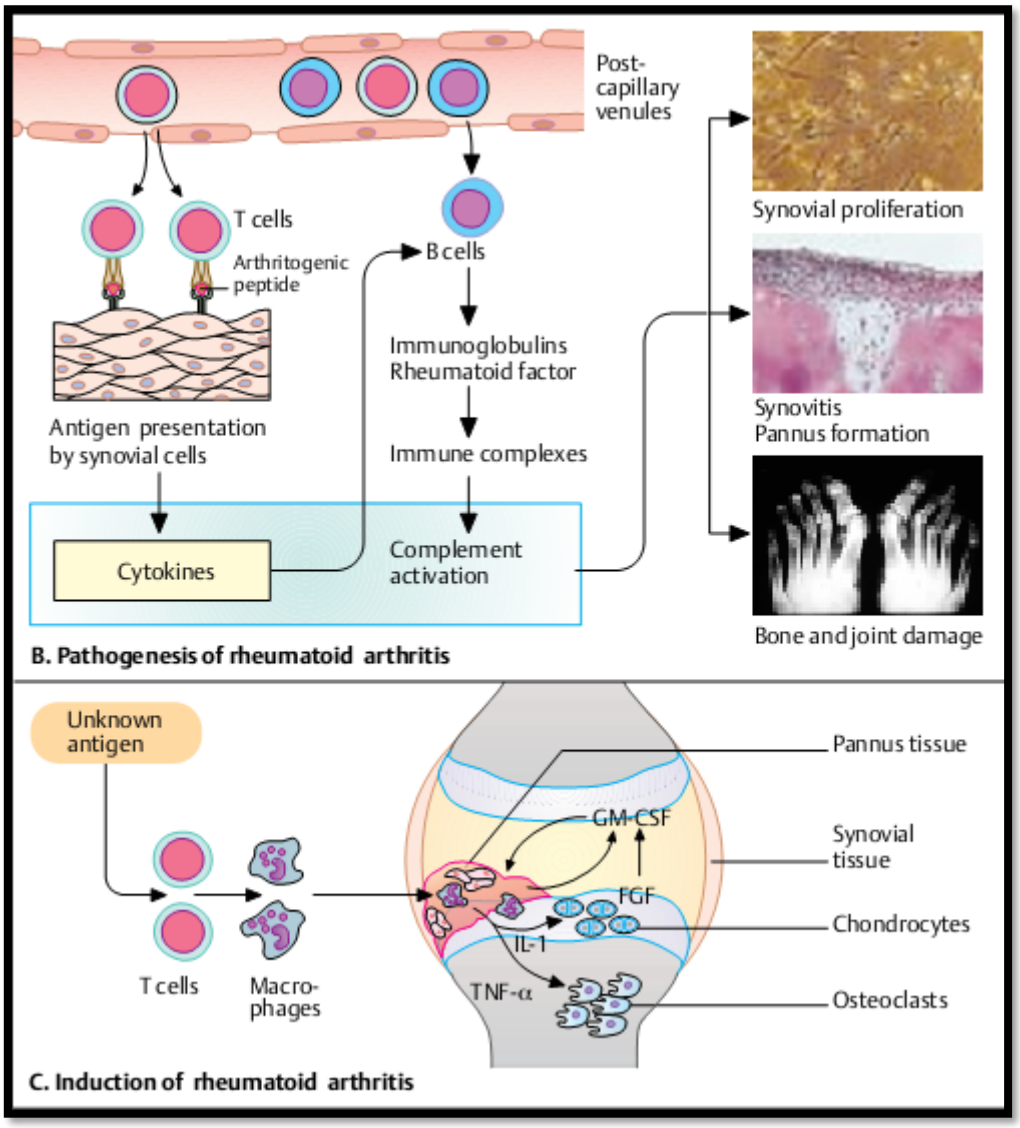


Figure: Comparison between normal and RA joint



Symptoms of RA Patients

RA usually affects joints on both sides of the body equally. Wrists, fingers, knees, feet, and ankles are the most commonly affected. The disease often begins slowly, usually with only minor joint pain, stiffness, and fatigue.

Other symptoms include:

- Chest pain when taking a breath (pleurisy).
- Pericarditis.
- Eye burning, itching, and discharge.
- Nodules under the skin (usually a sign of more severe disease).
- Burning in the hands and feet.
- Sleep difficulties.

Joint symptoms may include:

- Morning stiffness, which lasts more than 1 hour, is common. Joints may feel warm, tender, and stiff when not used for an hour.
- Joint pain is often felt on the same joint on both sides of the body.
- Over time, joints may lose their range of motion and may become deformed.

Laboratory diagnosis

A. **Synovial fluid analysis**

1. Inflammatory synovial fluid (WBC count > 2000/µL) is present with WBC counts generally from 5,000-50,000/µL.
2. Usually, neutrophil predominance (60-70%) is observed in the synovial fluid(in contrast with mononuclear cell predominance in the synovium). Because of a transport defect, the glucose levels of pleural, pericardial, and synovial fluids in patients with RA are often low compared to serum glucose levels.

B. **Immunological diagnosis** include autoantibodies (e.g., **RF, anti-RF33 (nuclear antigen), anti-CCP, ANA)**.

1. **Rheumatoid factor(RF)** refers only to the IgM antibody which binds aggregated IgG as antigen. During the first year of illness, rheumatoid factor is more likely to be negative with some individuals converting to seropositive status over time. RF is also seen in other illnesses, for example Sjögren's syndrome, Hepatitis C, chronic infections and in approximately 10% of the healthy population, therefore the test is not very specific.
2. **Anti-cyclic Citrullinated peptide** (**anti-CCP**) is the highly sensitivity (90-96%) for RA, can identify RA years before symptoms develop and is the most specific test for RA.
3. **Antinuclear antibodies (ANA)** are present in approximately 30% of patient with RA. This test is not routinely performed in the early disease.
4. **C-reactive protein (CRP)** for acute active arthritis.

Table: autoimmune response identified in patients with RA.

Autoantigen	**Antibodies in RA**	**T cell response in RA**	**Specificity for RA**
IgG	Yes: RF		No
Type II collagen and other cartilage antigens	Yes: in10-20%	Yes: in10-20%	No
Citrullinated proteins (CCP)	Yes	Probably	Yes

C. **Hematological tests**

1. **Complete blood count (CBC)** indicate the presence of anemia in normocytic and normochromic.
2. **Thrombocytosis** may be present.
3. **Erythrocyte sedimentation rate (ESR)** is elevated inapproximately 90% of patient with RA. This test is not routinely performed in the acute setting

Table 10.5 Some laboratory findings in active rheumatoid arthritis (RA) contrasted with those in active systemic lupus erythematosus (SLE).

	RA	SLE
Rheumatoid factor	Positive in 70%	Positive in 30%
• Titre	High	Low
Antinuclear antibody		
• Class	IgM	IgG
• Titre	Low	High
• Proportion of patients positive	40%	95%
DNA binding		
• Proportion of patients positive	<10%	70–85%
C3 + C4 levels	Normal or ↑	↓ or normal
C-reactive protein	↑	Usually normal
Immunofluorescent examination of a skin biopsy*	–ve	+ve
Serum IgG levels	Usually normal	Often ↑

Lecture No. 2

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of autoantibodies and a diversity of clinical manifestations. It most commonly presents in women during their child-bearing years, in which the immune system targets intracellular particles that contain both nucleic acids and nucleic acid binding proteins.

Etiology and Pathogenesis

Although the etiology of SLE is unknown, multiple factors are associated with the development of SLE, including genetic (HLA- DR2/DR3), racial, hormonal, immune abnormalities and environmental factors (ultraviolet light, viral infection involving molecular mimicry between organism and self for example anti-Sm autoantibody react with p24 gag protein of retroviruses and that anti- Ro recognizes a nucleocapsid protein on vesicular stomatitis virus). One proposed mechanism for the development of autoantibodies involves a defect in apoptosis or clearance of apoptotic cells, leading to a disturbance in immune tolerance. The redistribution of cellular antigens during apoptosis leads to a display of cytoplasmic and nuclear antigens on the cell surface, enhancing immune reactivity to antigens, which are normally protected intracellularly. Activation of antigen-presenting cells by IFN-α might promote presentation of autoantigens to self-reactive T cells. Immune complexes form in the microvasculature, leading to complement activation and inflammation. Antibody–antigen complexes deposit on the basement membranes of skin and kidneys. In active SLE, this process has been confirmed based on the presence of complexes of nuclear antigens such as DNA, immunoglobulins, and complement proteins at these sites.

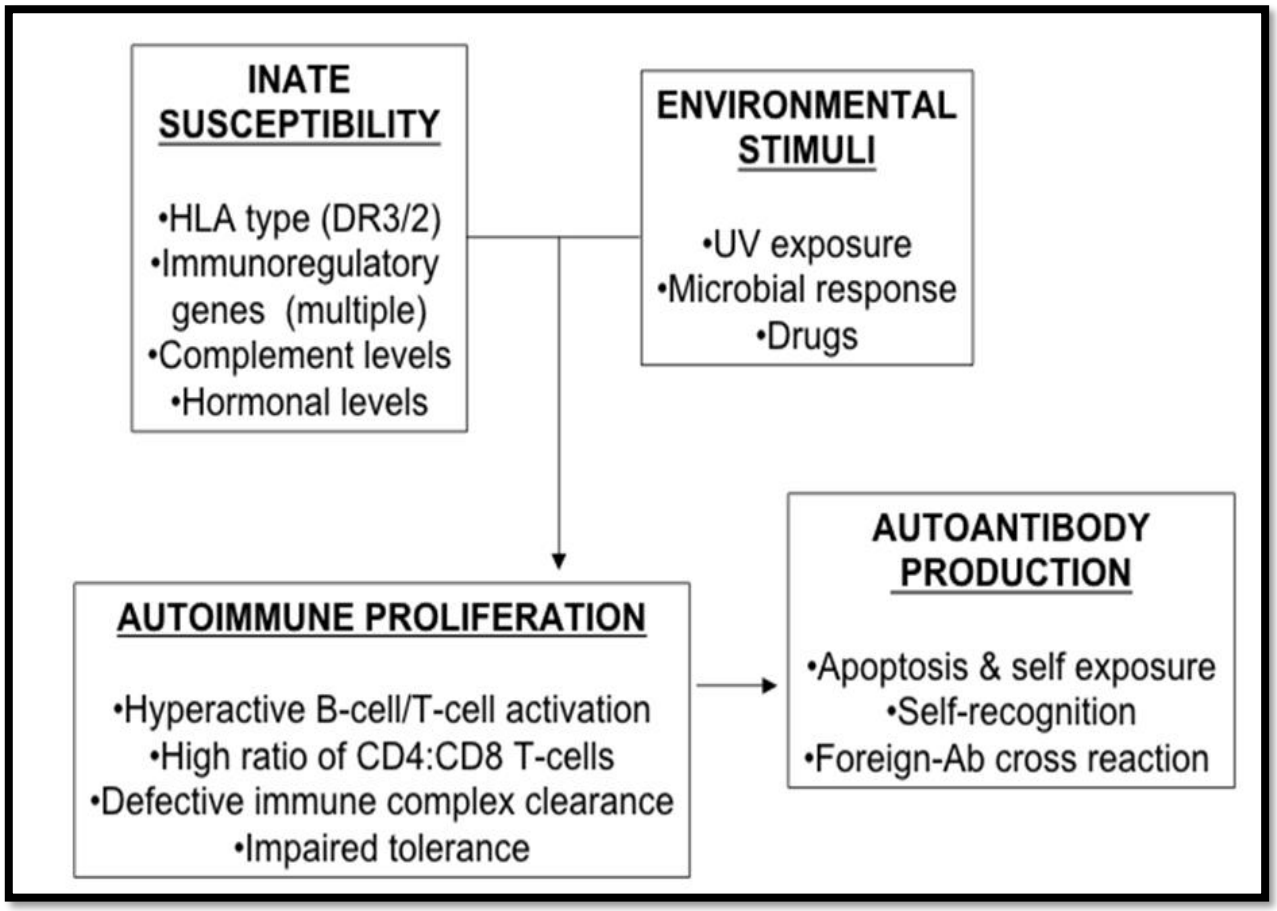
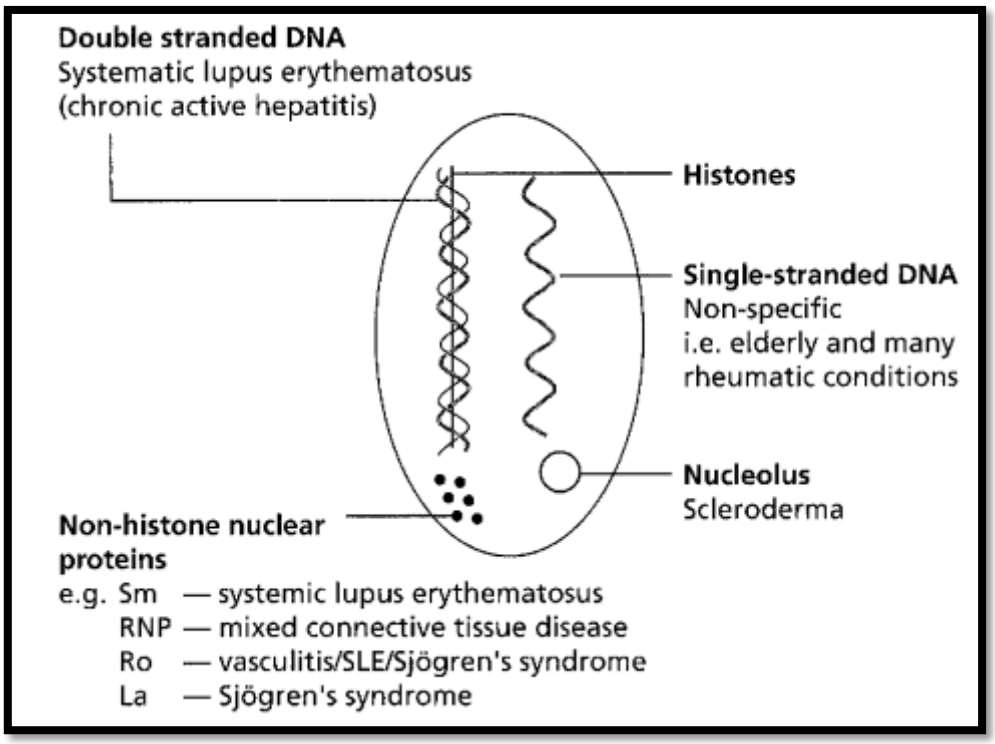


Figure: Etiology of SLE.



Nuclear antigens

Clinical Features

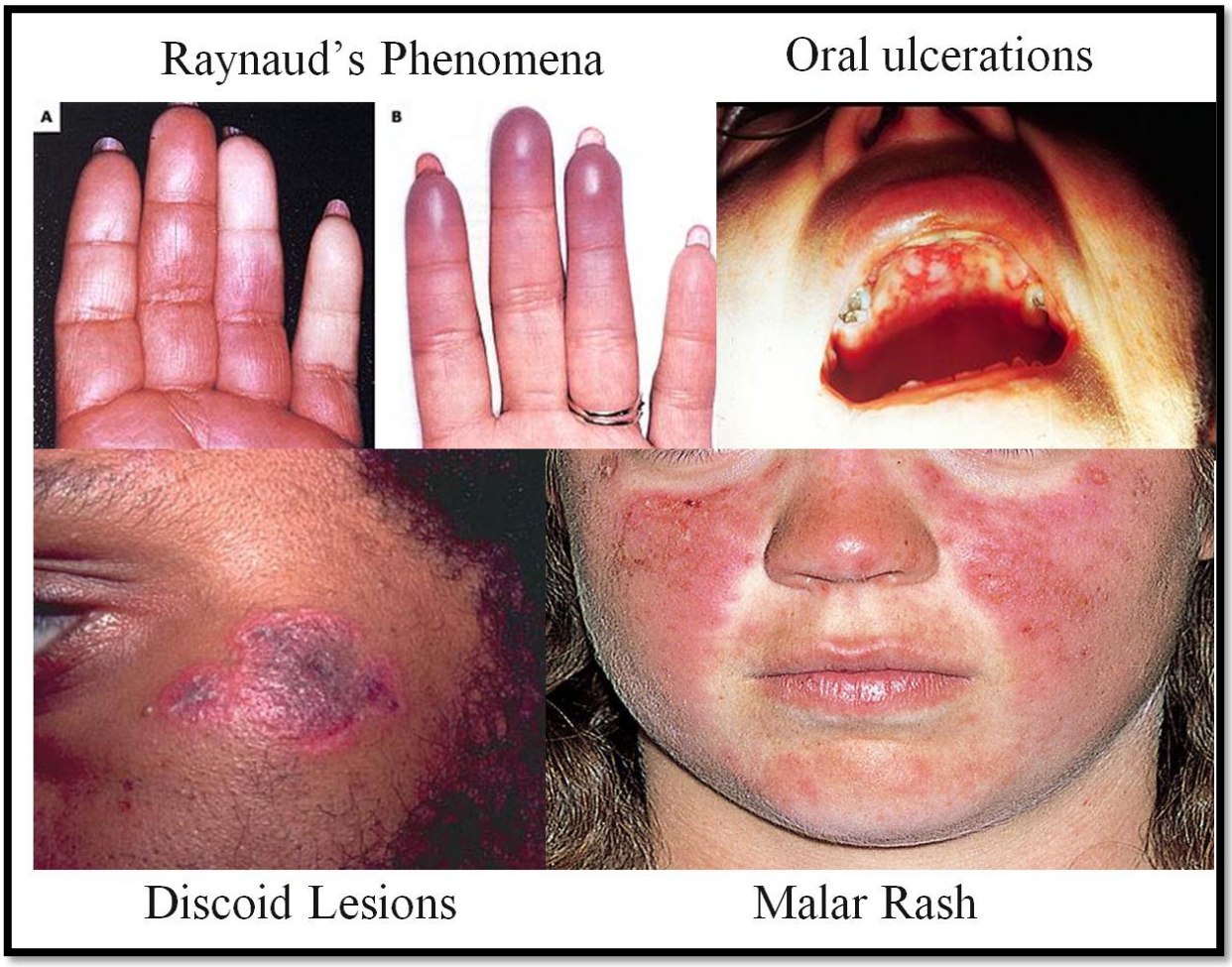
Systemic lupus erythematosus is a multisystem disease and can affect virtually all organs and system; whilst some manifestations are common, others are rare. Therefore, joint, skin and blood are affected in 80-100% of patients, kidneys, CNS and cardiopulmonary system in over 50%; while thrombosis, a typical lupus manifestation associated with possession of the anticardiolipin antibody, is present in 10% of patients. Systemic manifestations including fatigue, malaise, fever, anorexia, nausea and weight loss, are present in the great majority of patients.

The symptoms difference according to the infected organ and including arthritis, arthralgia, malar rash, an erythematous rash over the nasal bridge, photosensitivity, discoid lesions, headache, migraine, nephrotic syndrome, pleuritis and pericarditis.

Table 10.12 American College of Rheumatology criteria for diagnosis of systemic lupus erythematosus (SLE).*

Malar rash
Discoid rash
Photosensitivity
Oral ulcers
Non-erosive arthritis
Serositis (pleuritis/pericarditis)
Renal disease (persistent proteinuria/urinary casts)
Neurological disorder (seizures/psychosis)
Haemolytic anaemia/leucopenia/lymphopenia/thrombocytopenia
Antinuclear antibody
Antibodies to dsDNA/antibodies to extractable nuclear antigens/ antiphospholipid antibodies

* To establish a diagnosis of SLE a patient must have four or more of these criteria.



General laboratory findings

The most frequent laboratory alteration that is identified is normocromic normocytic anemia of chronic disorders. Occasinally a Coombs-positive haemolytic anemia is observed. Leukopenia (probably autoantibody mediated), especially lymphopenia, and thrombocytopenia are frequent. The erythrocyte sedimentation rate is typically elevated, while C-reactive protein tends to be normal. Urinalysis can show haematuria, proteinuria and renal casts in the presence of glomerulonephritis.

Immunological laboratory findings

All patients in whom SLE is suspected should be tested for antinuclear antibodies, including those to dsDNA and extractable nuclear antigens (ENA), and for antiphospholipid antibodies, as well as having their serum level of IgG and complement components, C3 and C4 measured, Antihistone antibodies are also present in patients with drug-induced SLE, most frequently associated with hydralazine and procainamide therapies.

The sero-immunological hallmark of SLE is **antinuclear antibodies (ANA)**, in the absence of ANA, the diagnosis of SLE is put into question, even though some 5% of patients may have an ANA-negative serology.ANA is currently detected by an indirect immunofluorescence technique, where diluted patients serum is applied to frozen tissue, especially liver, of rodent origin and cell lines of human origin, such as the HEp2 cell line derived from a laryngeal tumor, in which nuclei are prominent, are used as substrate to detect ANA. If the patient is ANA positive, the autoantibody will bind to nuclei. To reveal this binding, a second antibody tagged with fluorescent label is add. This second antibody will bind and ANA will then be seen by placing the preparation under a fluorescence microscope. Four patterns of fluorescence can be seen indicating different types of antinuclear antibodies.

Table. Immunofluorescence Patterns of Antinuclear Antibodies

Pattern	Antigen	Disease association(s)
Peripheral	Double-stranded DNA	SLE
Homogeneous	DNA-histone complexes	SLE and other connective tissue diseases
Speckled	Non-DNA nuclear antigens	
	Sm	SLE
	ribonucleoprotein	Mixed connective tissue disease, SLE, scleroderma, etc.
	SS-A, SS-B	Sjögren's disease
Nucleolar	Nucleolus-specific RNA	Scleroderma

ANA is a very sensitive test for SLE, being present in virtually all patients and frequently at high titers; its disease specificity is relatively low since it is frequently found in other rheumatic diseases, as well as in autoimmune liver disease, during viral infections and, occasionally, at low titers, in normal subjects.

DNA antibodies are the most important in SLE. They can react with **single-stranded DNA (ssDNA) or with double-stranded DNA (dsDNA)**.Although anti-ssDNA may be found in many diseases besides SLE, anti-dsDNA autoantibodies are found almost exclusively in

SLE (70%of the patients). While the disease specificity of dsDNA autoantibodies is high, that of ANA is low.

Anti-dsDNA autoantibodies are usually detected by very analytically sensitive technique, such as radioimmunoassay (RIA) or enzyme linked immunosorbent assay (ELISA).they can also be detected by immunofluorescence staining of an organelle called a kinotoplast in the flagellate *Crithdia luciliae,*which contains dsDNA.

In a patient with lupus nephritis, a kidney biopsy is frequently obtained for diagnostic reasons the glomeruli of such bioptic renal material contain antigen-antibody complexes. By applying a fluorescent antibody directed against human antibody (similar to that used in the second step of ANA detection) to frozen section of the kidney biopsy. This one step technique is known as direct immunofluorescence.

Extractable nuclear antigens (ENA) include **Sm** (Smith), **RNP** (ribonucleoprotein), **Ro** (Robert) also called SS-A (Sjogrens syndrome antigen A) and anti- **La** (Lane) or SS-B (Sjogrens syndrome antigen B).

Anti-ENA antibodies are include anti-Sm found almost exclusively in SLE, and anti-RNP more typically associated with mixed connective tissue disease than with SLE, anti- Ro and anti-La are found in Sjogrens syndrome. Other Anti-ENA antibodies are **anti-Jo-1**, anti **Scl-70** and **anticentromere**, which are associated mainly with polmyositis, systemic sclerosis and CREST syndrome respectively. Anti-ENA antibodies are normally detected by immunodiffusion or ELISA technique.

The lupus anticoagulant causes a prolonged clotting time in vitro but thrombosis in vivo. It is often found in associated with other autoantibodies to phospholipids, such as anticardiolipin antibodies and false positive tests for syphilis.

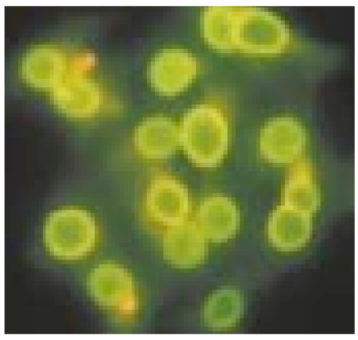
Assessment of the complement profile is of importance in management. Serial determinations of CH_{50}, a functional assay measuring complement hemolytic activity, and of the individual factors C3 and C4, inform on how much immune complexes are consuming complement.

Table 10.13 Laboratory findings in untreated systemic lupus erythematosus (SLE)*.

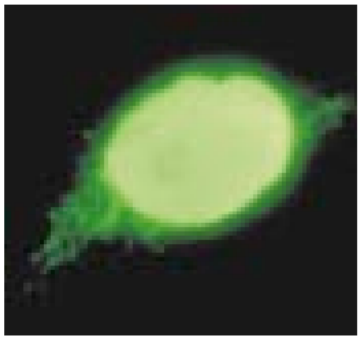
Immunological test	%	Haematological	%	Others	%
dsDNA binding	70–85	Raised ESR	60	C-reactive protein–normal unless infection present	
Antinuclear bodies (high titre; IgG class)	95	Leucopenia	45	Proteinuria	30
Raised serum IgG level	65	Direct Coombs' test positive	40		
Low serum complement C3/C4 levels	60	Lupus anticoagulant	10–20		
Platelet antibodies	60				
Cryoglobulinaemia	60				
Antibodies to ENA:					
Sm	30				
RNP	35				
Ro	30				
La	15				
Antibodies to phospholipids	30–40				
Rheumatoid factor (low titre)	30				
Skin biopsy IgG, C3 and C4 deposits in normal skin	75				

* Figures show percentage of patients with positive tests.

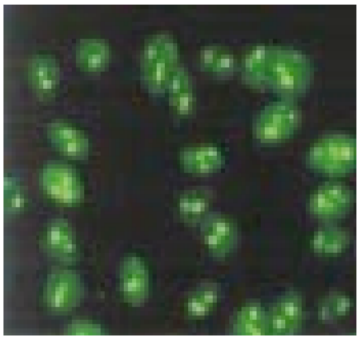
ESR, erythrocyte sedimentation rate; ENA, extractable nuclear antigens; RNP, ribonucleoprotein.



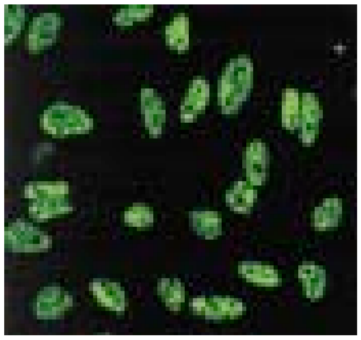
1. Rim pattern (150x magnif; anti-DNA)



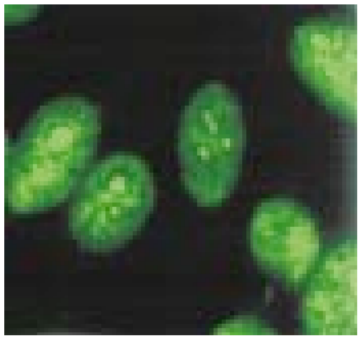
2. Homogenous pattern (435x enlarged; anti-DNA)



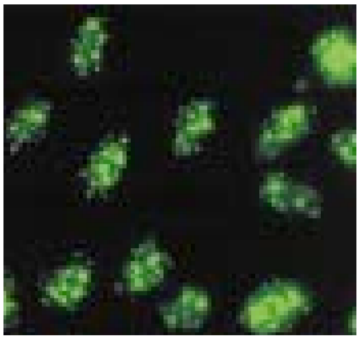
3. Nucleolar pattern (e.g. fibrillarin)



4. Coarse-speckled pattern (U1RNP/Sm)



5. Fine-speckled pattern (Ro/La)



6. Anti-centromere antibody pattern

Lecture No. 3

Ankylosing spondylitis

The term spondyloarthritis (SpA) (otherwise known as spondyloarthropathy) encompasses a heterogeneous group of inflammatory diseases characterized by spinal and peripheral joint oligoarthritis, inflammation of the attachments of ligaments and tendons to bones (enthesitis) and, at times, mucocutaneous, ocular, and/or cardiac manifestations. These disorders show familial aggregation and are typically associated with genes of the major histocompatibility complex (MHC), particularly human leukocyte antigen (HLA)-B27 The SpA include: (1) ankylosing spondylitis (AS); (2) reactive arthritis (ReA)— known previously as Reiter's syndrome; (3) psoriatic arthritis (PsA) and/or spondylitis; (4) enteropathic arthritis and/or spondylitis associated with the inflammatory bowel diseases (IBD), ulcerative colitis (UC), or Crohn's disease; and (5) undifferentiated SpA, which encompasses patients expressing elements of, but failing to fulfill, accepted criteria for one of the above diseases. In addition, isolated acute anterior uveitis (AAU)1 and spondylitic heart disease (complete heart block and/or lone aortic regurgitation) associated with HLA-B27 may also be classified within the spectrum of SpA.

Ankylosing spondylitis (AS) is a chronic inflammatory condition of the spine and sacroiliac joints. It is progressive disease in which restriction of movement is associated with intervertebral ossification of the ligaments. Men, usually below the age of 40, man develop the disease three times more frequently than women. Approximately 90% of the patients are HLA-B*27 positive, while the prevalence of this antigen in the general population is 7%. Of all the adult HLA-B*27- positive individuals, 1-2% have ankylosing spondylitis.

Etiology of AS

The etiology of the disease is unknown, but persistence of specific antigens of the infecting organisms has been demonstrated in these patients. This has led to suggestion that AS is also triggered by infection (possibly in the gastrointestinal tract) in susceptible HLA-B*27-positive individuals. Inflammation occurs and persists in different organs and joints in Ankylosing Spondylitis. Each individual tends to have their own unique pattern of presentation and activity of the illness. The initial inflammation may be a result of an activation of body's immune system, perhaps by a preceding bacterial infection or a combination of infectious microbes. Once activated, the body's immune system becomes unable to turn itself off, even though the initial bacterial infection may have long subsided. Chronic tissue inflammation resulting from the continued activation of the body's own immune system in the absence of active infection is the hallmark of an inflammatory autoimmune disease.

Clinical features

The onset of AS tends to be insidious with a dull lumbar pain; this persists over 3 months and is accompanied by morning stiffness relieved by exercise. Arthritis of the peripheral joints is seen in one third of the patients. Amongst extrarticular manifestation, iritis is the most troublesome: it tends to be unilateral and accompanied by photophobia pain. Inflammation of the colon and ileum is frequent but usually asymptomatic.

Criteria of AS Classification

- **Inflammatory Spinal Pain**: History or present symptoms of spinal pain in back, dorsal or cervical region with at least 4 of the following: **A.** Onset at age < 45 years. B. Insidious onset. **C.** Improved by exercise. **D.** Associated with morning stiffness **E.** at least for 3 months duration
- **Synovitis**: Asymmetric or Predominantly in the lower limbs**.** and one of the followings:
  - Positive family history
  - Psoriasis
  - Inflammatory bowel disease.
  - Alternating buttock pain.
  - Enthesopathy
  - Acute diarrhea
  - Urithritis
  - Sacroiliiatis

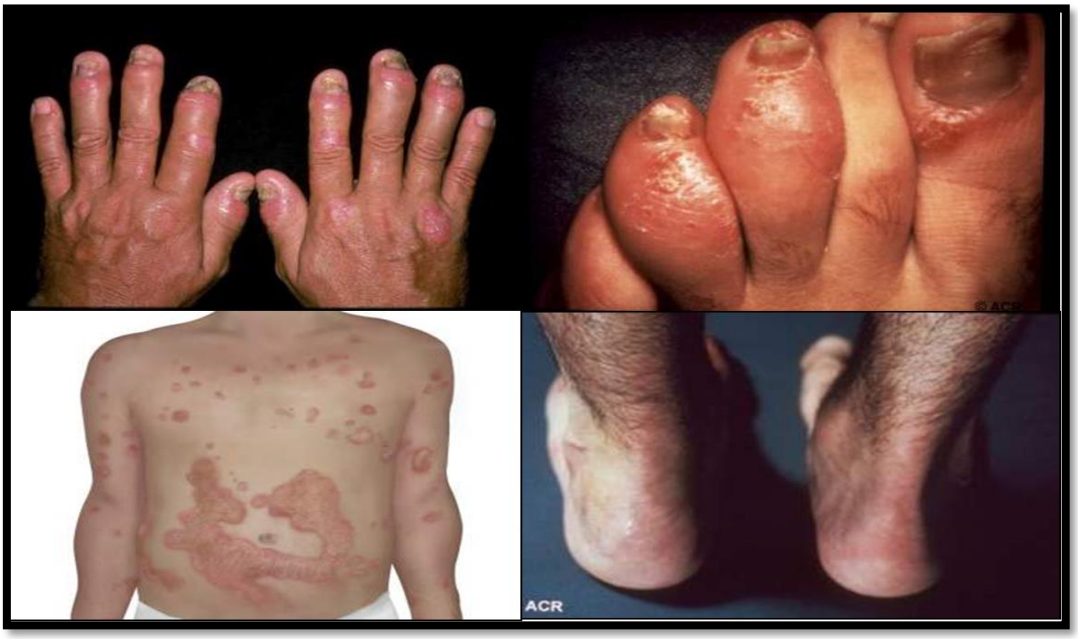
The diagnosis of Ankylosing Spondylitis is based on:

- Evaluating the patient's symptoms include pain and morning stiffness of the spine and sacral areas with or without accompanying inflammation in other joints, tendons, and organs.
- A physical examination: the Schober's test is a useful clinical measure of flexion of the lumbar spine performed during examination. Flexibility of the low back and/or neck can be decreased.
- X-ray findings
- Blood tests: Patients with AS tend to have elevated levels of IgA and, when the disease is active, elevated erythrocyte sedimentation rates and levels of C-reactive protein. Rheumatoid factor and antinuclear antibody are consistently negative. The clinical need to assess the HLA-B*27 status of a patient with symptoms and signs of AS is controversial.

Psoriatic Arthritis

Psoriatic Arthritis (PsA) is a chronic and inflammatory arthritis in association with skin psoriasis, characterized by osteolysis and bony proliferation. PsA is classified as one of the subtypes of spondyloarthropathies. Males and females are equally affected. PsA can range from mild nondestructive disease to a severely rapid and destructive arthropathy.

Clinical manifestations include skin and nail psoriasis, dactylitis, enthesitis, osteoperiostitis, large joint oligoarthritis, arthritis mutilans, sacroiliitis, spondylitis and distal interphalangeal arthritis.



Comorbidities in PsA Patients

- Ocular inflammation (Iritis/Uveitis/ Episcleritis).
- Irritable bowel disease (IBD).
- Metabolic Syndrome (Hyperlipidemia, Hypertension, Insulin resistent, Diabetes,Obesity) lead to Higher risk of Cardiovascular disease (CVD)
- Psychosocial burden, Reactive depression, Higher suicidal ideation and Alcoholism.

Two percent of patients with psoriasis develop psoriatic arthropathy; this may affect the peripheral joints or the spine. The psoriasis generally precedes the arthritis by many years; in rare cases where the arthritis comes first, diagnosis may be difficult. A family history of psoriasis is a helpful diagnostic clue and the characteristic nail changes of psoriasis are present in 80% of patients with psoriatic arthritis. Dactylitis – inflammation of an entire digit to look like a sausage – is a distinctive feature. Usually rheumatoid factor (RF) negative and ACPA negative. Radiographic damage can be noted in up to 47% of patients

at a median interval of two years despite clinical improvement with standard DMARD therapy. Treatment is similar to that for RA, including the use of anti- TNF drugs. The prognosis is usually good, although severe joint destruction can occur.

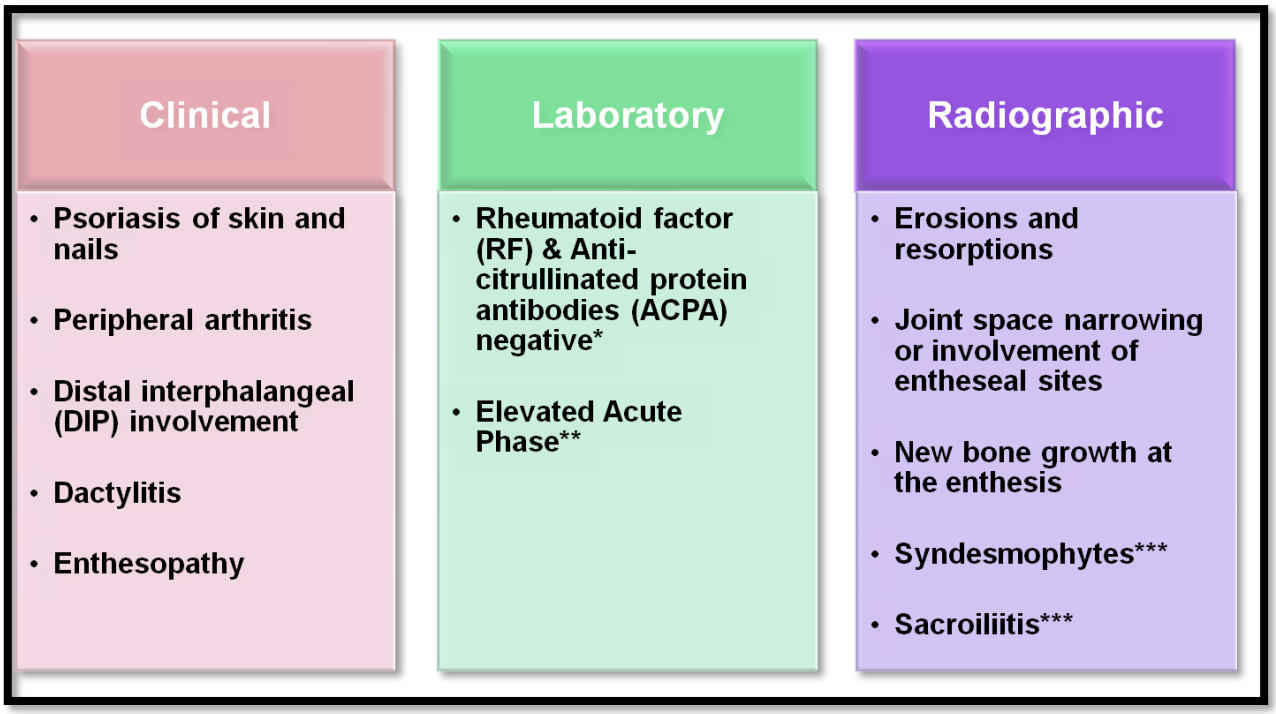


Figure: Main Features of Psoriatic Arthritis

Lecture No. 4

Sjögren's syndrome

Sjögren's syndrome (SS) is a systemic chronic inflammatory disorder characterized by lymphatic infiltrates in exocrine organs (lacrimal and salivary glands). Sjögren's syndrome predominantly effects females (female:male ratio 9:1) in their fourth and fifth decades of life. It was named after the Swedish ophthalmologist Henrik Sjögren's after he reported 19 cases of keratoconjunctivitis in 1933.1 The hallmark feature of SS is deficient tear and saliva production due to lymphocytic infiltration of the salivary and lachrymal glands leading to xerostomia (dry mouth) and xerophthalmia (dry eyes). In addition, SS can involve any organ system and present with a wide spectrum of clinical features.

There are two type of Sjögren's syndrome

- **Primary Sjögren's syndrome** (when the disease occurs alone) is a systemic autoimmune disease that targets the exocrine glands. It is characterized by xerostomia (dry mouth), xerophthalmia (dry eyes).
- **Secondary Sjögren's syndrome** is defined as the former definition of primary Sjögren's in the presence of another autoimmune diseases such as RA, SLE, Scleroderma, and polyarteritis nodosa.

Etiology and Pathogenesis

A number of factors play a role in the development of Sjögren's syndrome

1. Endogenous factors [**HLA-B*8-DRB1*03 ((B8 DR3) and hormones (estrogens)**].
2. Exogenous factors [**viruses (herpes and retrovirus)**].

The pathogenesis of SS is still largely unknown. In a genetically predisposed individual, various environmental factors, such as viral infections, may lead to epithelial cell activation and a protracted inflammatory response with features of autoimmunity. The actual trigger of glandular dysfunction is assumed to be a viral infection. Epithelial cells in the infected gland present viral antigens. This attracts T cells, which infiltrate the glandular tissue and cause a local inflammatory reaction, resulting in damage to glandular tissue. The T cells activate the glandular epithelium and, most importantly, B cells. This results in excessive, uncontrolled B-cell proliferation, which initially manifests in the peripheral blood as hypergammaglobulinemia in association with presence of immune complexes.

Clinical features

The principle feature of Sjögren's syndrome is autoimmune destruction of exocrine gland, most prominently the lacrimal and salivary glands, but also glands at other sites including respiratory mucosa and vagina. The disease commonly affects eyes, mouth, parotid gland, lung, kidney, skin and nerves system. Most individuals with Sjögren's syndrome present with sicca symptoms, such as xerophthalmia (dry eyes), xerostomia (dry mouth)**,** and parotid gland enlargement. Other xeroses such as dry skin and dry vaginal mucosa leading to irritation and dyspareunia may also occur. In addition, numerous extra glandular features may develop, such as arthralgia, arthritis, pulmonary disease, leucopenia, anemia, lymphoadenopathy, neuropathy, renal tubular acidosis and lymphoma.

Laboratory finding

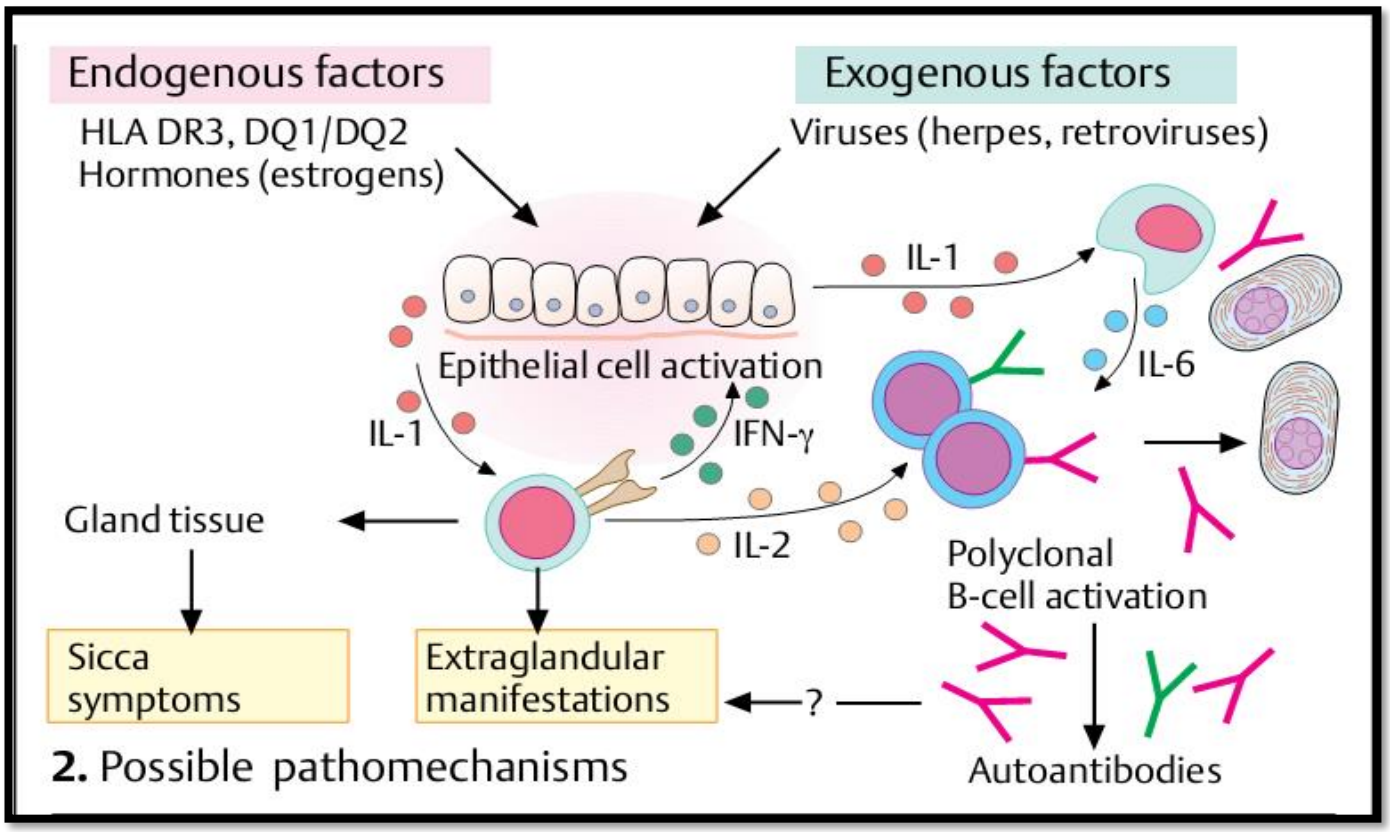
The most important diagnostic tests for Sjögren's syndrome are

1. **Schirmer's test:** Lachrymal function can be assessed by the Schirmer's test to quantify the amount of tear production, a slip of sterile filter paper is placed over the lower eyelid, failure to produce sufficient tears within 5 min wet 10 mm of the paper suggest defective tear production, then this is positive for Sjögren's syndrome.
2. **Erythrocyte sedimentation rate (ESR)** is elevated in 80% of patients.
3. **C reactive protein (CRP)** is also elevated in patients.
4. **Rheumatoid factor (RF)** is present in 52% of cases of primary-type Sjögren's syndrome and in 98% of secondary-type cases.
5. **Complete blood count (CBC)** shows anemia and leucopenia.
6. **Autoantibodies**
    - **Anti-SS-A (anti-Ro) and anti-SS-B (anti-La)** are present in most cases of primary-type Sjögren's syndrome. While anti salivary duct antibodies are present in most cases of the secondary type. Anti-Ro/SS-A antibodies are found in over 70% of patients with SS, but are also frequently found in SLE and other autoimmune diseases even in the absence of oral or ocular dryness. Anti-La/SS-B is more specific; it is present in 50% of patients with primary SS or SS/SLE but is rarely seen in other diseases. The pathogenic role of these antibodies is not yet defined except in newborns born to women with anti-

Ro/SS-A and/or anti-La/SS-B antibodies. These antibodies can cross the placenta and bind to Ro and La antigens located on the cell surface of fetal myocardial tissue, leading to fetal heart block.

- **Antinuclear antibodies (ANA)** of the speckled type are present in most cases of primary Sjögren's syndrome.
- In recent years, research has focused on identifying antibodies more specific for SS, such as anti-a-fodrin and anti-muscarinic acetylcholine receptor antibodies, but the results have been controversial. The major stimulus for saliva production is the binding of acetylcholine to muscarinic acetylcholine receptors. The hypothesis that oral and ocular dryness could result from antibodies antagonizing the muscarinic acetylcholine receptor-3 is intriguing.

7. **Creatinine clearance** may be diminished in up to 50% of patients.
8. **Rose Bengal, fluorescein and lissamine green staining** are performed on an outpatient basis to the detection corneal and conjunctival damage due to dryness.
9. **Salivary gland evaluation** is done by collection of unstimulated saliva or salivary scintigraphy.



2. Possible pathomechanisms

Lecture No. 5

Behcet's Disease

Behcet's disease is a rare, chronic, lifelong disorder that involves **inflammation of blood vessels** throughout the body. It is a form of vasculitis that can lead to ulceration and other lesions, slightly affecting more men than woman characterized by a triad of symptoms, including aphthous ulceration of the oral mucous membranes and genitalia and uveitis. **Rare manifestations** include oligoarthritis of the lower extremities, vasculitis of the pulmonary vessels, cerebrovascular symptoms. **The etiology** of the Behcet's disease is unkown. It is believed to be partly genetic, an association with HLA-B51 has been found.

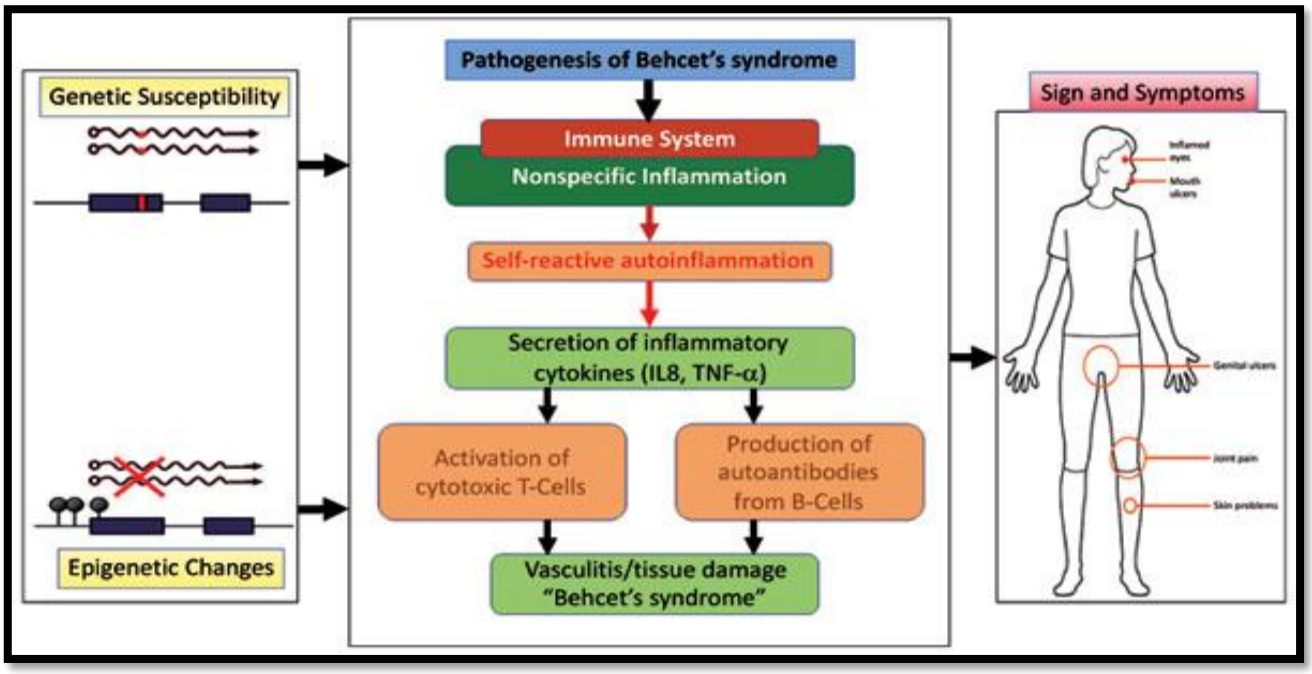


Figure: Pathogencity of Behcet's disease

Risk factors

- **Age.** Behcet's disease most commonly affects men and women in their 20s, 30s and 40s, though children and older adults also can develop the condition. When the condition occurs at an earlier age, it tends to be more severe.
- **Geography.** Although the disease occurs worldwide, people from countries in the Middle East and Asia, including Turkey, Iran, Iraq, Japan and China, are more likely to develop Behcet's.
- **Sex.** While Behcet's disease occurs in both men and women, the disease is usually more severe in men.
- **Genes.** Having certain genes HLA-B51 is associated with a higher risk of developing Behcet's.

Clinical features

- **Mouth:** Painful mouth sores, identical to canker sores, are the most common sign of Behcet's disease. Sores begin as raised, round lesions in the mouth that quickly turn into painful ulcers. The sores heal usually in seven to 21 days, though they do recur.
- **Skin:** Skin lesions may occur in people with Behcet's disease. Skin problems can vary. Some people may develop acne-like sores on their bodies. Others may develop nodules on the lower legs.
- **Genitals:** People with Behcet's disease may develop sores on their genitals. The sores most commonly occur on the scrotum or the vulva.
- **Eyes:** Behcet's disease may cause inflammation in the eye — a condition called uveitis In people with Behcet's disease, uveitis causes redness, pain and blurred vision in one or both eyes
- **Joints:** Joint swelling and pain most commonly affect the knee in people with Behcet's disease.
- **Vascular system:** Inflammation in veins and large arteries may occur in Behcet's disease, causing redness, pain and swelling in the arms or legs when a blood clot results.
- **Digestive system:** Behcet's disease may cause abdominal pain, diarrhea or bleeding.
- Brain: Inflammation in the brain and nervous system that leads to headache, fever, disorientation, poor balance or stroke.

Diagnosis

There is no diagnostic test and the diagnosis is entirely clinical. Laboratory findings are nonspecific and reflect the inflammatory state, C-reactive protein levels, erythrocyte sedimentation rate (ESR), leukocyte count, complement components, and acute-phase reactants may all be elevated during an acute attack. Internationally accepted diagnosis criteria have been published recently.

International Clinical Criteria for Behcet's Disease

An international group of physicians has established a set of guidelines to aid in the classification of Behcet's patients. The International Clinical Criteria for Behcet's Disease classification states patients must present with:

- Recurrent oral ulcerations (apthous or herpetiform) at least three times in one year.
- Additionally, patients must present any two of the following:
    - Recurrent genital ulcerations.
    - Eye lesions (uveitis or retinal vasculitis) observed by an ophthalmologist.
    - Skin lesions (a variety of rashes or acne-like sores) may be caused by Behcet's disease. Positive
    - Positive pathergy test, your doctor inserts a sterile needle into your skin and then examines the area two days later. If the pathergy test is positive, a small

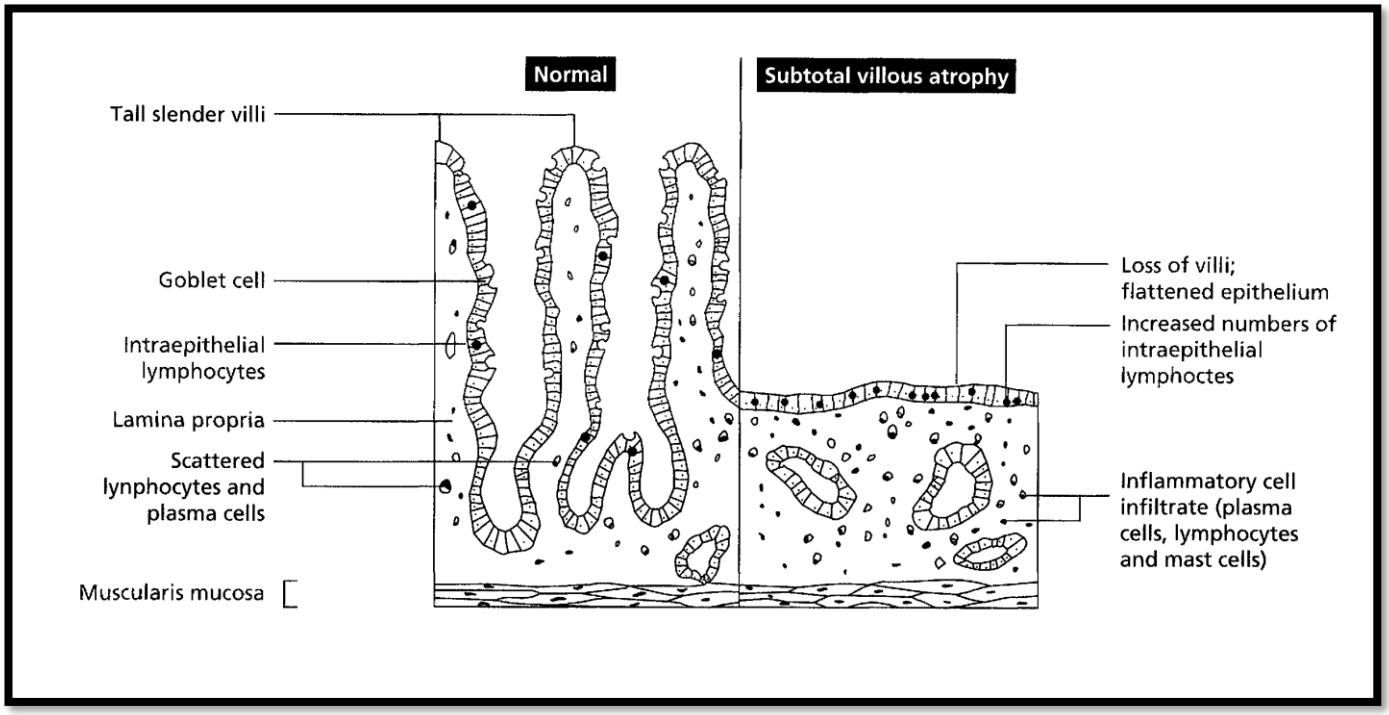
red bump forms under your skin where the needle was inserted. This indicates your immune system is overreacting to the minor injury.

Lecture No. 6

Celiac disease

Celiac disease (CD), an immune-mediated mucosal disorder primarily affecting the small intestine in genetically susceptible individuals, is triggered by the ingestion of dietary gluten. Gluten is the alcohol-soluble protein component of the cereals wheat, rye and barley. It is composed of 2 major protein fractions: glutenin and gliadin; most of the toxic activity exerted by gluten in CD is due to gliadin.

It is, also known as celiac sprue, gluten-sensitive enteropathy, non-tropical sprue, characterized by inflammation leading to injury to the mucosal lining of the small intestine, including villous atrophy with crypt hyperplasia, intraepithelial lymphocytosis, and subsequent nutrient malabsorption.



The disorder is a multifactorial condition, originating from the interplay of genetic and environmental factors. The necessary environmental trigger is gluten, timing of gluten introduction into the diet could play a role in pathogenesis, since initial exposure to wheat, barley, or rye in the first 3 months of life or after the 7th months proved to be related to an increased risk of CD. Breast-feeding could have a protective effect, since introduction of gluten to the infant's diet when infant is still at the age of being breastfed has markedly reduced the risk of celiac disease. While, the genetic predisposition has been identified in the major histocompatibility complex region on chromosome 6p21, with over 90% of CD patients expressing human leukocyte antigens HLA DQ2 and the remaining celiac patients

express DQ8.Some infectious agents could increase the risk of celiac disease, like repeated infection with rotavirus, the most common cause of childhood gastroenteritis, represent an independent risk factor for celiac disease in genetically susceptible individuals. Some drugs can have a role in enhancing a person's susceptibility to gluten, a course of interferon alfa could activate celiac disease in predisposed people.

Clinical features

The clinical manifestations of CD vary markedly with the age of the patient, the duration and extent of disease, and the presence of extraintestinal pathology. Depending on the features at the time of presentation, together with the histologic and immunologic abnormalities at the time of diagnosis, CD can be subdivided into the following clinical forms.

1. **Classical (typical) form**

The so-called typical form of CD is present characteristically between 6 and 24 months of age. Symptoms begin at various times after the introduction of weaning foods containing gluten. Infants and young children typically present with chronic diarrhea, anorexia, abdominal distension, abdominal pain, poor weight gain or weight loss and vomiting. Malnutrition can be severe if the diagnosis is delayed. Behavioral changes are common and include irritability.

2. **Atypical forms**

An increasing number of patients, especially at an older age, are being diagnosed with CD without having typical gastrointestinal manifestations but there are various extraintestinal manifestations present such as dermatitis herpetiformis, anemia, osteoporosis, autoimmune hepatitis, dental enamel defects, recurrent aphthous stomatitis, epilepsy, and neuropathy. Serology for CD is positive and bioptic findings confirm the diagnosis.

3. **Silent form**

Silent celiac disease patients are those who are asymptomatic but small intestinal biopsy show villous atrophy. Silent cases are detected by population screening and screening of first degree relatives of celiac disease, 10% of whom are found to have CD. Serological tests are positive in them.

4. **Latent form**

Latent (or "potential") form is asymptomatic patients, with a normal or minimally abnormal mucosa. These individuals have a genetic susceptibility to CD and may also have positive autoimmune serology.

Refractory celiac disease (RCD) is defined by persistent or recurrent malabsorptive symptoms and villous atrophy despite strict adherence to a gluten-free diet (GFD) for at least 6–12 months in the absence of other causes of non-responsive treated celiac disease and overt malignancy

Celiac disease prevalence is increased in at-risk conditions such as family history of celiac disease, autoimmune diseases, especially type1diabetes (T1D) and thyroiditis, IgA deficiency, and some genetic syndromes.

Immunopathogensis

Celiac patients present with a complex immunological reaction to ingested gluten encompassing both innate and adaptive immunity and leading to progressive inflammation and severe destruction of the mucosal lining of the small bowel.

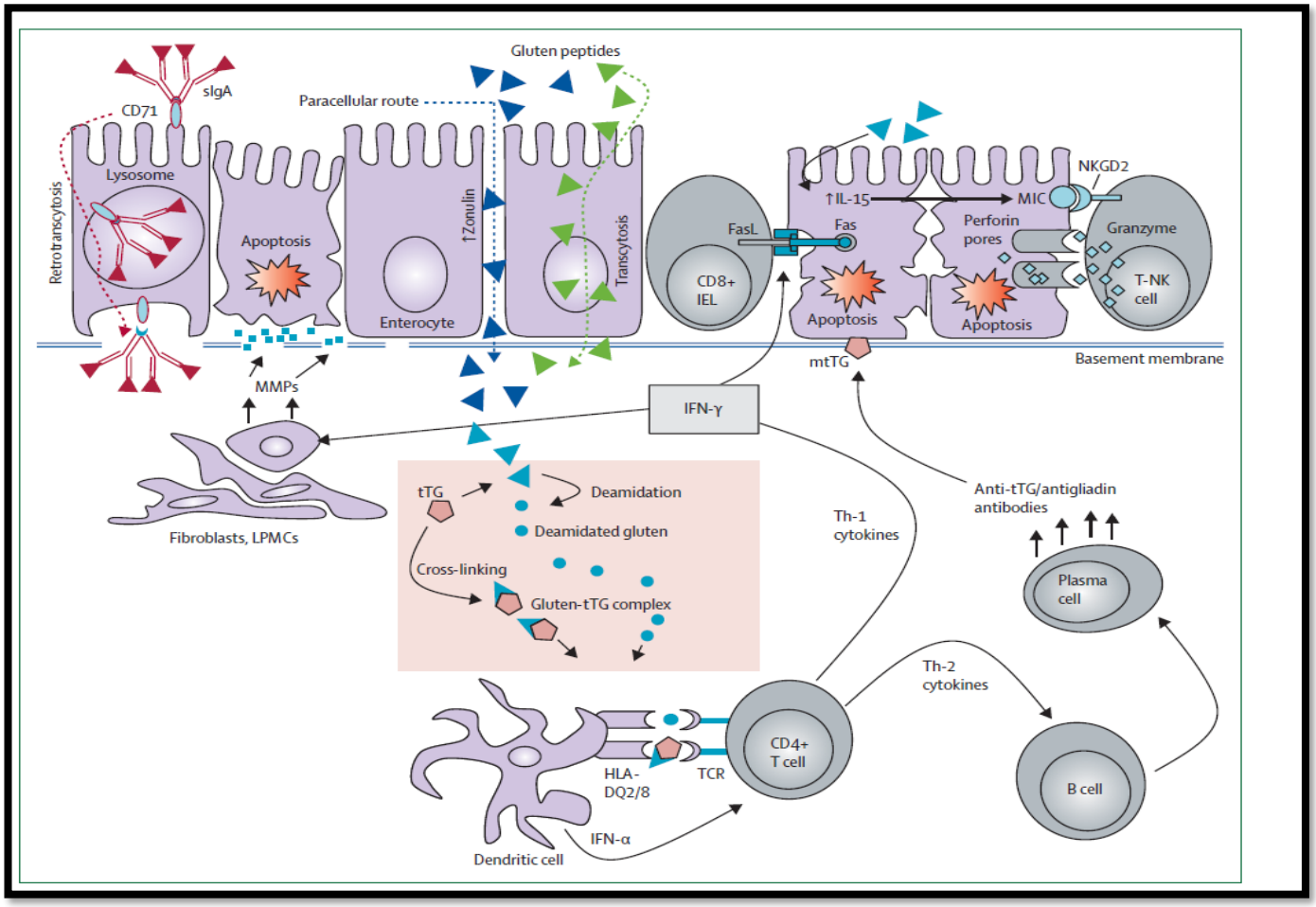


Figure: Mechanisms of mucosal damage in celiac disease.

Diagnosis

1. **Small intestine biopsy**

The most relevant feature of the disease was histological change, and histology became the gold standard for diagnosis. The diagnosis required three small bowel biopsies-the first during the gluten containing diet, which had to show “flat” mucosa; the second, during gluten free diet which showed improvement in villous structure, and the third, at gluten challenge 2 years later which had to show histological relapse.

The degree of the intestinal lesion is defined on the basis of the widely used Marsh-Oberhuber classification, it ranges from type 0 (Marsh 0) to Marsh type 4:

- **Type 0** concerns the normal stage of the small bowel mucosa.
- **Type 1 or infiltrative lesion** comprises normal mucosal architecture in which the villous epithelium is infiltrated by small, non mitotic intraepithelial lymphocytes and it is characteristically present in first-degree relatives of children with celiac disease.
- **Type 2, or hyperplastic lesion,** consists of a type 1 lesion with enlarged crypts.
- **Type 3or destructive lesion** is synonymous with the typical flat mucosa of CD and it is subclassified according to the different degrees of villous atrophy present: Marsh type 3a, with partial villous atrophy; Marsh type 3b, in the presence of subtotal villous atrophy; and Marsh type 3c, when total villous atrophy is present.
- **Marsh type 4 or hypoplastic lesion** (total villous atrophy with crypt hypoplasia) represents the extreme end of the gluten-sensitivity spectrum and an irreversible lesion is present in some adult CD patients whose small bowel mucosa is unresponsive to gluten withdrawal: the so-called refractory CD.

2. Serology tests

Serologic testing is primarily used to identify symptomatic or at-risk individuals who need to undergo biopsy. Because of their high sensitivity and specificity, serologic tests are excellent for screening asymptomatic at-risk individuals; they also can be used for monitoring dietary compliance.

- Anti-gliadin antibodies (AGA) are not specific for CD as they are also found in healthy individuals and patients with other gastrointestinal diseases such as gastritis, gastroenteritis and irritable bowel syndrome, except in children younger than 2 years of age, in whom anti-gliadin antibodies measure is more sensitive test**.** IgG-AGA is very sensitive but less specific, and IgA-AGA is less sensitive but more specific. Their use in combination can give results of a high detection rate. Several methods have been used to analyze AGA, but currently ELISA is the most used method.
- Anti-endomysial antibodies (EMAs) are used as the “gold standard” for CD screening because of their high sensitivity and specificity. The test was developed in the early 1980s and rapidly gained use as part of "a celiac panel" by commercial labs in combination with AGA IgG and IgA. IgA-EMA and IgG-EMA are measured by indirect immunofluorescence, using tissue sections from either monkey esophagus or human umbilical cord [140]. Its major drawbacks are false negatives in young children, and in the hands of an inexperienced laboratory because of the subjective nature of the test. Also IgA-EMA give false negative in patients with IgA deficiency.

- Anti-tissue transglutaminase (tTG) antibodies are more specific have shown to be correlated with mucosal damage and are used widely in CD screening**.** IgG-tTG and IgA-tTG were used in combination as a screening test for celiac disease to assess IgA deficiency. ELISA is the most used method to analyze tTG**.** However, it represents an improvement over the antiendomysial antibody assay because it is inexpensive, rapid and easy to perform**.**
- Anti-reticuline antibody is best detected by an indirect immunofluorescent method using unfixed cryostat sections of rat liver and kidney as antigens. IgA class reticulin antibodies react with connective tissue fibers and are found in 60% of celiac disease patients. IgG class reticulin antibodies are occasionally found in other disease states, especially bullous dermatoses and in some normal subjects.

3. Genetic testing

Up to 95% of patients with celiac disease are positive for HLA-DQ2, and most of the remaining patients are positive for HLA-DQ8. . However, these alleles are also found in 40% of the general population. Although HLA-DQ2 and HLA-DQ8 are necessary in the disease process, they alone are not sufficient for celiac disease to develop. HLA testing has a high negative predictive value and can be useful in certain situations, such as when a diagnosis is unclear, when serologic testing or biopsy is performed in patients on a gluten-free diet, or in determining which family members to screen for celiac disease.

Pernicious Anemia

Pernicious anemia (PA) is a megaloblastic anemia caused by a deficiency of vitamin B_{12} resulting from malabsorption. Impaired absorption is the result of defective intrinsic factor (IF) secretion. This is due to atrophy of the gastric mucosa caused by autoimmune reactions to gastric parietal cells and their products.

Virtually all patients will have gastric parietal cells antibody targeting antigens in the secretory canaliculi, which are the intracellular channels carrying hydrochloric acid into the gastric lumen and its major target is the α subunit of the proton pump (H^+, K^+, ATPase), an enzyme composed of two transmembrane components, the α and β subunits. In addition, there are at least two types of antibody against intrinsic factor: blocking and binding antibodies; the blocking type reacts with the combining site for vitamin B_{12} on IF and is found in most patients (over 70%), while the binding antibody reacts with other epitopes on IF (whether this is free or complexed to vitamin B_{12}) and is present in some 60% of pateints.

During the course of the digestion of foods containing B_{12}, the B_{12} becomes attached to a substance called intrinsic factor. Intrinsic factor is produced by parietal cells that line the stomach. The B_{12}-intrinsic factor complex then enters the intestine, where the vitamin is

absorbed into the bloodstream. In fact, B_{12} can only be absorbed when it is attached to intrinsic factor.

In pernicious anemia, the parietal cells stop producing intrinsic factor. The intestine is then completely unable to absorb B_{12}. So, the vitamin passes out of the body as waste. Although the body has significant amounts of stored B_{12}, this will eventually be used up. At this point, the symptoms of pernicious anemia will develop.
Pernicious anemia occurs in equal numbers in both men and women. Most patients with pernicious anemia are older, usually over 60 years. Occasionally, a child will have an inherited condition that results in defective intrinsic factor.

Causes
Intrinsic factor is produced by specialized cells within the stomach called parietal cells. When these parietal cells shrink in size (atrophy), they produce less intrinsic factor. Eventually, the parietal cells stop functioning altogether. Other important products of parietal cells are also lessened, including stomach acid, and an enzyme involved in the digestion of proteins. Other conditions that interfere with either the production of intrinsic factor, or the body's use of B_{12}, include conditions that require surgical removal of the stomach, or poisonings with corrosive substances which destroy the lining of the stomach. Certain structural defects of the intestinal system can result in an overgrowth of normal bacteria. These bacteria then absorb B_{12} themselves, for use in their own growth. Intestinal worms (especially one called fish tapeworm) may also use B_{12}, resulting in anemia. Various conditions that affect the part of the intestine (the ileum), from which B_{12} is absorbed, can also cause anemia due to B_{12} deficiency. These ileum-related disorders include tropical sprue, Crohn's disease, tuberculosis.

Symptoms
Symptoms of pernicious anemia and decreased B_{12} affect three systems of the body

- **The hematopoietic system** is harmed because B_{12} is required for the proper formation of red blood cells. Without B_{12}, red blood cell production is greatly reduced. Those red blood cells that are produced are abnormally large and abnormal in shape. Because red blood cells are responsible for carrying oxygen around the body, decreased numbers (termed anemia) result in a number of symptoms, including fatigue, dizziness, ringing in the ears, pale or yellowish skin, fast heart rate, enlarged heart with an abnormal heart sound (murmur) evident on examination, and chest pain.
- **The gastrointestinal system** include a sore and brightly red tongue, loss of appetite, weight loss, diarrhea, and abdominal cramping.

- **The nervous system** is severely affected when pernicious anemia goes untreated. Symptoms include numbness, tingling, or burning in the arms, legs, hands, and feet; muscle weakness; difficulty and loss of balance while walking; changes in reflexes; irritability, confusion, and depression.

Diagnosis

Tests that may be used to diagnosis pernicious anemia include

- Blood smear reveals abnormally large red blood cells.
- White blood cells and platelet counts may also be decreased in number.
- Reticulocyte count will be low in number.
- Serum vitamin B_{12} level will be low.
- Schilling test, in this test, a patient is given radioactive B_{12} under two different sets of conditions: once alone, and once attached to intrinsic factor. Normally, large amounts of B_{12} are absorbed through the intestine, then circulate through the blood, and enter the kidneys, where a certain amount of B_{12} is then passed out in the urine. When a patient has pernicious anemia, the dose of B_{12} given by itself will not be absorbed by the intestine, so it will not pass into the urine. Therefore, levels of B_{12} in the urine will be low. When the B_{12} is given along with intrinsic factor, the intestine is able to absorb the vitamin. Urine levels of B_{12} will therefore be higher.
- Immunology, specifically anti-parietal cell antibody (APCA) and intrinsic factor antibody (IFA). APCAs bind to the alpha- and beta-subunits of the membrane-bound H(+)/K(+)-ATPase. In contrast, IFAs bind directly to intrinsic factor, blocking its ability to bind vitamin B12 and can be detected by means of immunofluorescence, enzyme-linked immunosorbent assay - currently the most commonly used method, and radioimmunoprecipitation assay (RIA). APCA can be found in 85-90% of patients with PA. Their presence is not sufficient for diagnosis, because they are not specific for PA as they are also found in the circulation of individuals with other diseases. APCA are more prevalent in the serum of patients with T1D, autoimmune thyroid diseases, vitiligo, celiac disease. So that a combination of PCA and IFA testing was the optimal strategy for the evaluation of patients with suspected PA.

Diabetes mellitus

Diabetes is a state of high blood sugar (hyperglycemia) in which different mechanisms lead to deficiency of insulin and/ or impaired insulin action and persistent hyperglycemia and is classified into:

1. **Insulin-dependent diabetes mellitus** (IDDM) or type1.
2. **Non-insulin-dependent diabetes mellitus** (NIDDM) or type 2.
3. **Gestational diabetes mellitus.**

Type 1 diabetes mellitus

Type 1 diabetes mellitus (type 1 DM or T1DM) is a major clinical problem in both children and adults. It is an organ-specific autoimmune disease represents 10-15% of all diabetes. Healthy human islets of Langerhans are composed of a core of some 80% β cells (making the glucose-regulating hormone insulin), with a mantle of other endocrine cells types, producing glucogan (α cells), somatostatin (δ cells) and pancreatic polypeptide (PP cells) making up the remainder.In type 1 DM, the hyperglycemia results from insufficient insulin secretion by β cells in the islets of Langerhans of the pancreas.

Causes

1. Genetic: DR3/DQ2 or DR4/DQ8 haplotypes have strong link for the incidence of the disease, but other genetic associations (non HLA) are CTLA-4 (cytotoxic lymphocyte associated protein 4) also found in many family that play a role in the onset of type 1 DM.
2. Environmental factors:
   - Seasonal variation in the incidence rate (peaks in autumn and winter).
   - Infection with pathogens that have specific tropism toward the pancreatic tissue, mumps and coxsakie viruses. Similarities in the protein sequence of these viruses and certain islet cell cytoplasmic (ICA), glutamic acid decarboxylase (GAD) would initiate molecular mimicry mechanism in tolerance breakdown.

Immunopathogenesis

A virus infection in the pancreatic β islets cells leads to inflammation, damaged and releasing β cells antigens, their recruit antigen presenting cells (dendritic cells) which capture the virus protein and auto antigens released from the damaged β islets cells to local lymph node and present them to T cells. T cells are activated to eradicate the virus. Inadvertently, T cells are activated against β cells and the slow process of β cells damage starts. In type 1 DM insulin production is failed due to destruction of β cells in the islets of Langerhans in pancreatic tissue without any destruction in the other cells as (α or δ cells) which is mediated by specific immune response).

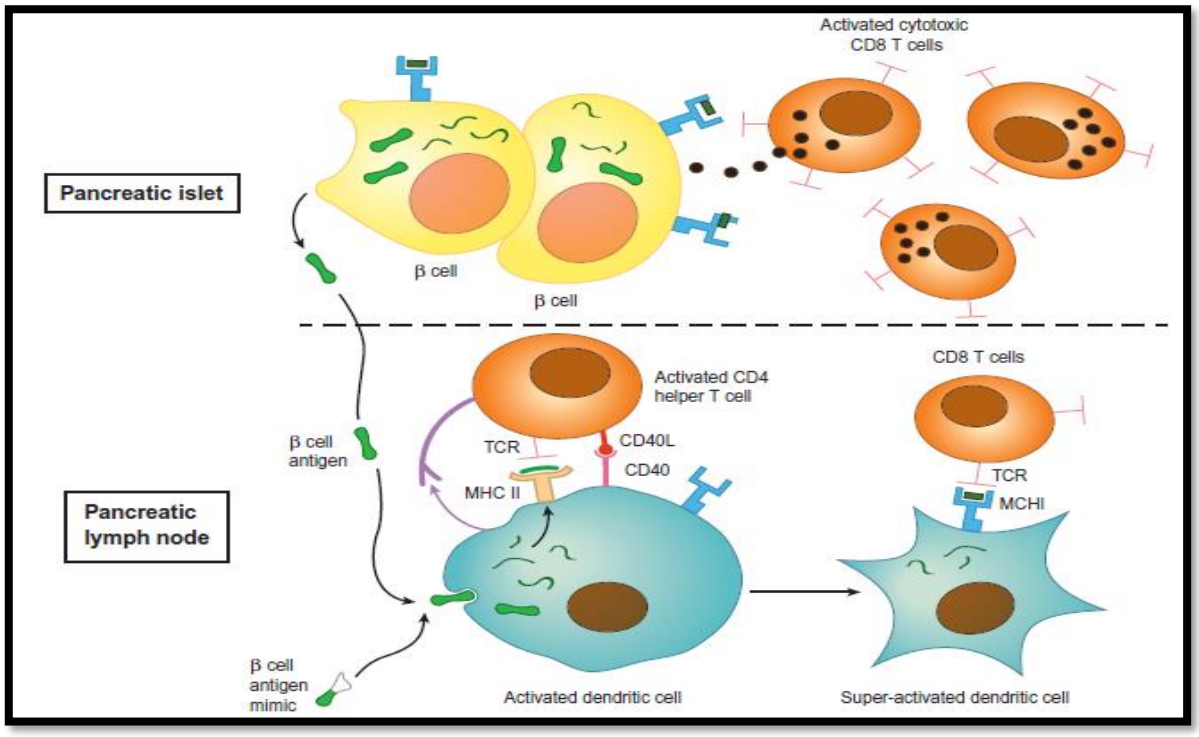


Figure: Immune mechanisms of β-cell destruction in type 1diabetes.

Features of islet autoantigens in type 1 DM

Autoantigens	Islet specific	Function	Autoantibody
Insulin	Yes, and β cells specific	Regulates glucose	Insulin autoantibody (IAA)
glutamic acid decarboxylase	No, present in other islet cells and CNS	Catalyses synthesis of γ-amino butyric acid (GABA), a negative neurotransmitter probably regulates insulin release.	glutamic acid decarboxylase autoantibody (GADA)
Islet tyrosine phosphatase	No, present in other islet cells and CNS	Unknown	insulinoma-2 associated autoantibody (IA-2A)
Zinc transport 8	Yes, and β cells specific	Zinc transport	Zinc transport 8 autoantibody (ZNT8A)

Clinical features

Many pre- or subclinical stages occur in the DM patient before clinical diagnosis can be done:

1. Stage 1: the cell mass and function of β cells is normal but individuals who carry genetic susceptibility alleles to type 1 suffer exposure to an environmental stimulus triggering islets inflammation (insulitis). The release of sequestered or altered self antigens explains in part the later development of islet Autoantibodies that mark the recognition of stage 2.
2. Stage 2: serological evidence of humoral and cell-mediated autoimmunity indicated by the appearance of different types of autoantibody as islet cell cytoplasmic autoantibody (ICA), glutamic acid decarboxylase autoantibody (GADA), insulinoma-2 associated autoantibody (IA-2A) or insulin autoantibody (IAA). This occurs without any clinical metabolic signs. However, during this stage, there can be a 50% decline in β cells mass without detectable abnormalities by any form of glucose tolerance testing.
3. Stage 3: The earliest functional β cells abnormalities which manifestation by the intravenous glucose tolerance test (IVGTT) which decrease.
4. Stage 4: intolerance to oral glucose challenges appears as indicated by oral glucose tolerance test (OGTT).
5. Stage 5: after 1-2 years of glucose intolerance upon oral testing, atypical history of polyuria, polydipsia, polyphagia with weight lose impaired visual acuity, tingling or numbness in the hands or feet resulting from sensory nerve changes are identified. Finally by a true hyperglycemia a full diagnosis can be done. If diabetes is undiagnosed or untreated, failure to metabolize glucose will result in the breakdown of fat, leading to ketonemia and ketoacidosis, which may be accompanied by nausea and hyperventilation before life-threatening ketoacidotic coma

Lecture No. 7

Inflammatory bowel disease

It is a chronic inflammatory disease of gastrointestinal tract due to immune response to the commensal microflora in the lumen of basal consistent and may be divided into two major groups:

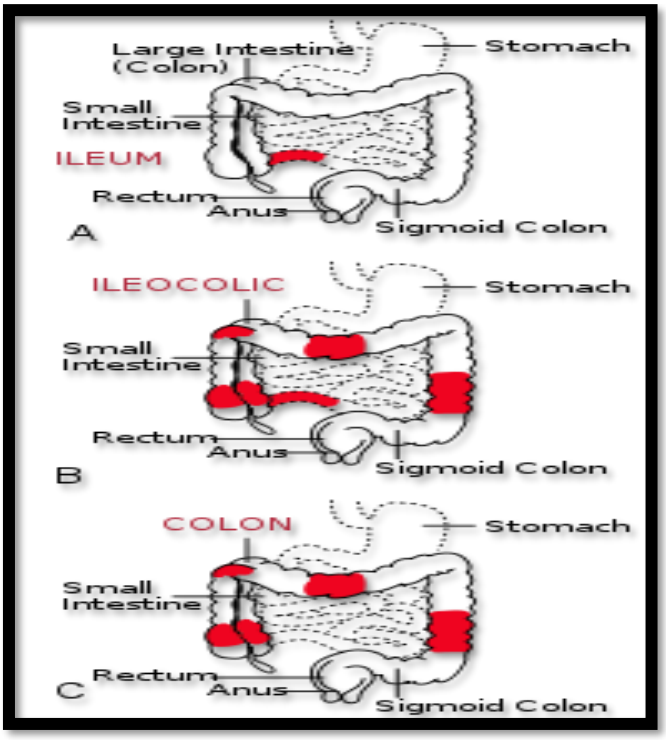
- Crohn's disease
- Ulcerative colitis

Crohn's disease also known as granulomatous colitis and regional enteritis, it is classified as a type of inflammatory bowel disease, in which the body immune system attacks the gastrointestinal tract, causing a transmural inflammation, that may affect any part of the

gastrointestinal tract from mouth to anus. It is onset patient between 15-30 year, males and females are equally affected.

Most gastroenterologists categorize the presenting disease by the affected areas:

- ***Ileocolic Crohn's diseases,*** which affect both the ileum (the last part of the small intestine that connect to the large intestine) and the large intestine, accounts for 50% of cases.
- ***Crohn's ileitis,*** affecting the ileum only, accounts for 30% of cases.
- ***Crohn's colitis,*** affecting the large intestine, accounts for the remaining 20% of cases and may be difficult to distinguish from ulcerative colitis.



However, individual affected by the disease rarely fall outside these three classification, being affected in other parts of the gastrointestinal tract such as the stomach and esophagus. Crohns disease may also be categorized by the behavior of disease as it progresses. There are three categories of disease presentation in Crohns disease:

- ***Stricturing disease:*** narrowing of the bowel which may lead to bowel obstruction or changes in the caliber of the feces.
- ***Penetrating disease:*** creates abnormal passageways (fistulae) between the bowel and other structures such as the skin.
- ***Inflammatory disease:*** cause inflammation without causing stricture or fistulae.

Causes

- **Genetic factor:** many studies that is suggested relationship between genetic and Crohns disease such as mutation in gene nucleotide-binding oligomerisation domain 2 (NOD2 gene) on chromosome 16.
- **Environmental factor:** by diet, smoking, drugs, hormonal contraception.
- **Immune system:** abnormalities in immune system causes Crohns disease and the inflammation that is occur in this disease causes activation of T_H1 by an overproduction of IL-12 by macrophages and of IFN-γ by T lymphocytes.
- **Microbes:** there are many bacteria causes of Crohns disease such as Mycobacterium ovum, Yersinia spp and Listeria spp.

Clinical Features

A. Gastrointestinal Features

- Abdominal pain.
- Diarrhea may be bloody or may not be bloody, is different according to the part of the small intestine or large intestine, in ileitis large-volume watery feces, while, in colitis small volume semisolid or watery feces.
- Vomiting &nausea.
- Perianal discomfort (itching around the anus).
- Aphthousof mouth (ulceration of mouth).

B. Systemic Features

- In children causes growth failure, acute myelogenous leukemia in blood (myeloid) and lymphoma (cancer of lymph).
- In adult causes weight loss.

Ulcerative colitis

Ulcerative colitis is confined to the colon and affects the mucosal layer only and causing a continuous inflammation. It is result of immune response to commensal microflora with T_H2 profile, through there is an increase of the T_H2 cytokine IL- 5. Favouring a T_H2 pattern is the fact that ulcerative colitis is associated with the production of various autoantibodies, such as perinuclear anti-neutrophil cytoplasmic antibody (PANCA) and anti- tropomyosin.

Clinical Features

- The clinical presentation of ulcerative colitis depends on the extent of the disease process. Patients usually present with diarrhea mixed with blood [Relapsing rectal bleeding] and mucus, of gradual onset.
- They also may have signs of weight loss, and blood on rectal examination.
- The disease is usually accompanied with different degrees of abdominal pain, from mild discomfort to severely painful cramp [**Tenesmus**].

Diagnosis

1. In both inflammatory bowel diseases, the key diagnostic procedures are radiologic, endoscopic and histologic.
2. In Crohn's disease, typical laboratory findings include anemia (chronic disease, iron deficiency, vitamin B12 deficiency, folate deficiency), leukocytosis, thrombocytosis, elevation of the sedimentation rate, hypoalbuminaemia and electrolyte imbalance in the presence of severe diarrhea. The measurement of C-reactive protein appears to be of use in monitoring the progress of the disease.
   In the setting of supportive findings through imaging or endoscopy, the measurement of certain serum antibodies can further strengthen the diagnosis of Crohn disease and even help differentiate it from UC, but they should not be used by themselves as diagnostic tests. It has been shown that up to 68% of patients with Crohn disease are seropositive for antibodies targeting microbial antigens, such as anti–Saccharomyces cerevisiae antibody (up to 16% of patients with UC are seropositive).
3. While, in ulcerative colitis, the laboratory findings are mostly non-specific, reflecting blood loss and inflammation, and include anemia, leukocytosis, elevated sedimentation rate and C-reactive protein levels. Seventy percent of patients with ulcerative colitis, but not with Crohn's disease, have been reported to have in their sera an anti-neutrophil cytoplasmic antibody (ANCA) that give a characteristic perinuclear staining (PANCA) that can also be seen in primary sclerosing cholangitis.
4. General stool examination for occult blood.

Table 14.6 Some differences between ulcerative colitis and Crohn's disease.

	Ulcerative colitis	Crohn's disease
Disease site	Colon	Any part of gastrointestinal tract
Inflammation	Mucosal	Transmural, granulomatous
Cytokine profile	T_{H2}	T_{H1}
ANCA positivity	50–80%	5–20%

Helicobacter pylori associated Chronic Gastritis & Mucosa-Associated Lymphoid Tissue Lymphoma (MALT)

Gastritis is a histological term that describes stomach inflammation resulting from toxic exposures, infection, idiopathic inflammation, and autoimmunity. The most common cause of gastritis is **H pylori infection**. Other causes include acid reflux, prolonged use of

nonsteroidal anti-inflammatory drugs (NSAIDS), alcohol use, and tobacco use, all of which can irritate the lining of the stomach. Severe illness and radiation therapy can also cause gastritis

Erosive gastritis is most commonly caused by alcohol use, tobacco use, and prolonged use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDS). Severe illness and consumption of caustic substances have also been associated with the development of erosive gastritis. The most common cause of chronic, **nonerosive gastritis** is a stomach infection caused by Helicobacter pylori (H pylori), a type of bacteria found in up to half of all people in industrialized nations.

Symptoms

The signs and symptoms of gastritis vary among individuals. If infection with H pylori bacteria is the cause, symptoms will remain as long as the infection is untreated. H. pylori is uniquely adapted to the acidic environment of the stomach through its ability to metabolize urea to ammonia, which provides a buffered microenvironment that allows prolonged asymptomatic colonization.

Some people with gastritis have no symptoms at all, while others may have burning abdominal pain, Loss of appetite, nausea with or without vomiting.

In some cases, gastritis can be life threatening, with symptoms including**:**

- Bloody stool (blood may be red, black, or tarry in texture)
- Severe abdominal pain
- Vomiting blood or black material (resembling coffee grounds)

Although acute infection can cause abdominal pain and dyspepsia, there is typically no clinical recognition of acute infection. Rather, the burden of H. pylori results from chronic infection of the stomach.The development of peptic ulcer disease and adenocarcinoma caused by chronic H. pylori infection correlates with the anatomical distribution of inflammation. When H. pylori chronic gastritis affects the antrum predominantly, there is an association with duodenal ulcers, increased serum gastrin levels and excess acid production, and no gastric mucosal atrophy. However, when H. pylori affects the body and the antrum in a confluent or patchy manner, intestinal metaplasia develops, oxyntic mucosa atrophies, and acid production decreases. This latter type of H. pylori chronic gastritis is associated with gastric ulcerations and increased risk for adenocarcinoma and mucosa-associated lymphoreticular tissue (MALT) B-cell lymphoma. Although eradication of H. pylori can reverse the mucosal atrophy and restore acid production in this setting, mucosal restoration occurs only in a minority of patients and does not necessarily reverse the intestinal metaplasia.

Immune pathophysiology

Although there are many pieces of evidence to support immune mechanisms for the persistence of HP infection in the stomach, data suggest that pro-regulatory effects of H.

pylori infection, including local IL-10 production, increases in regulatory T cells (Tregs) in the gastric mucosa and increased antigen-presenting cell (APC) phagocytosis of apoptotic cells all contribute to persistence of chronic H. pylori gastritis.

Diagnosis

Active disease can be diagnosed with endoscopic biopsy, which has high sensitivity and specificity, while simultaneously assessing peptic and malignant complications. Noninvasive testing for *H. pylori* infection includes serum antibody detection (best used in highly endemic areas to predict active infection), urea breath testing (limited by expense and possible false-positive results), and fecal antigen testing (which has potential advantages in the setting of intestinal metaplasia and after antibiotic treatment).

Lecture No. 8

Autoimmune Hepatitis

Autoimmune hepatitis(AIH) is a progressive inflammation of the liver that has been identified by a number of different names, including **autoimmune chronic active hepatitis (CAH), idiopathic chronic active hepatitis** and **lupoid hepatitis**. The reason for this inflammation is not certain, but it is associated with an abnormality of the body immune system. It is a rare condition characterized by active inflammation, liver cell necrosis and fibrosis, which may lead to hepatic failure, cirrhosis and ultimately death.The disease affects young to middle-aged women, many of whom (60%) are associated with other autoimmune diseases, such as diabetes mellitus, thyroiditis, rheumatoid arthritis, ulcerative colitis crohns disease and glomerulonephritis.

Pathophysiology

Evidence suggests that liver injury in a patient with autoimmune hepatitis is the result of a cell-mediated immunological attack. This attack is directed against genetically predisposed hepatocytes. Aberrant display of human leukocyte antigen (HLA) class II on the surface of hepatocytes (normally not expressed on liver cells), facilitates the presentation of normal liver cell membrane constituents as autoantigenic peptides to $CD4^{+}$T cells.

Causes

It is not clear why autoimmune hepatitis develops. Researchers suspect that some people inherit a genetic disposition that could make them more likely to develop it. Sometimes drugs (e.g., interferon) or viral infections (e.g., acute hepatitis A or B, Epstein-Barr virus infection) have been suggested to play a role in triggering AIH, possibly through molecular mimicry and cross-reactivity between their epitopes and liver antigens, trigger the development of the disease.

Symptoms

The clinical features of AIH can be quite variable. About 25% of individuals are asymptomatic and are diagnosed only after abnormal liver function tests are found

coincidentally when blood work is performed. Adults usually present with an unexpected onset of vague symptoms, including: fatigue, nausea, weight loss, abdominal pain, itching, and maculopapular rashes. Less often, patients have symptoms of portal hypertension such as gastrointestinal bleeding or hypersplenism. Jaundice may also be present. Rarely, the initial presentation is fulminant liver failure requiring liver transplantation.

Types of Autoimmune Hepatitis

Based on autoantibody marker, **Autoimmune hepatitis** is recognized as a heterogeneous disorder and has been subclassified into 3 types:

- Type 1 autoimmune hepatitis.
- Type 2 autoimmune hepatitis.
- Type 3 autoimmune hepatitis.

Clinical feature	**Type 1**	**Type 2**	**Type 3**
Diagnostic autoantibodies	ASMA, ANA, Anti-actin	Anti-LKM	Anti-SLA
Age	10 y elderly	Pediatric (2-14y) Rare in adults	Adults (30-50 y)
Women (%)	78	89	90
Concurrent immune disease (%)	41	34	58
Gamma globulin elevation	+++	+	+++
Low IgA	No	Occasional	No
HLA association	B8, DR3, DR4	B14, DR7, C4AQO	Uncertain
Steroid response	+++	++	+++
Progression to cirrhosis (%)	45	82	75

ASMA: anti-smooth muscle antibody.

ANA: antinuclear antibody, primarily in homogenous pattern.

Anti-actin: antibodies to actin a cytoskeletal protein.
Anti-LKM: anti-liver-kidney microsomal antibodyreact with epitopes on the 2D6 isoform of cytochrome P450 as autoantigen.
Anti-SLA: anti- soluble liver antigen.

	Autoimmune hepatitis	Hepatitis B or C associated
Proportion of all cases of CAH in the UK*	50–80%	20–50%
Sex	Female > male (6 : 1)	Male > female (9 : 1)
Age at onset	10–30 years 40–60 years	Elderly
Associated autoimmune disease	Common	Rare
Smooth-muscle antibodies	Positive 70% High titre	Low titre or absent
Antinuclear antibodies	Positive in 80%	Negative
Anti-DNA antibodies	May be positive	Negative
Antimitochondrial antibodies	Positive 25%	Negative
Antibodies to liver and kidney microsomes	Positive 4% (especially children)	Negative
Serum immunoglobulins	IgG ↑↑	Normal or IgG ↑
HLA type	HLA-B8, -DR3	?
Response to steroids	Good	?
Risk of hepatoma	Low	High

Lecture No. 9

Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver that results in chronic injury to the intrahepatic bile duct epithelium. The gradual inflammatory destruction of the bile ducts causes cholestasis with the subsequent retention of toxins, inciting further hepatic injury and resulting in fibrosis, cirrhosis, and eventual liver failure. It is most common in women over the age of 50. The ratio of affected women to men has been reported to be as high as 9:1.

Causes

The cause of the disease is unknown, but research indicates that there is immunological basis for the disease, making it an autoimmune disorder. Most of the patients (>90%)seem to have anti-mitochondria antibodies (AMAs) against pyruvate dehydrogenase complex (PDC-E2), an enzyme complex that is found in the inner mitochondria membrane. Molecular mimicry is the most widely proposed explanation as to the induction of autoimmunity in PBC. Briefly, a host is infected with a microorganism that contains antigens similar to antigens present in the host. These microbial antigens induce an immunologic response when presented to the immune system of the host. As a result, what began as a pathogen-specific response then cross-reacts with the host antigens and results in tissue injury and disease.

The predisposing role of the HLA system to the disease has not been fully clarified, although a weak but significant association with HLA-DR8 has been reported.

The pathogensis of the bile duct damage in PBS is unclear. Bile ducts in PBS patients express increased densities of adhesion molecules, MHC classII antigens, IL-2 and pyruvate dehydrogenase compared with normal ducts, and so represent potential targets for the infiltrating activated T cells ($CD4^+$ and $CD8^+$).

Symptoms

The most common presenting symptoms include pruritis and fatigue. Jaundice, darkening of the skin in exposed areas and manifestations resulting from impaired bile excretion follow.latter range from steatorhoea to impaired absorption of lipid soluble vitamins , leading to osteomalacia (from vitamin D malabsorption), bruising (vitamin K) and occasionally night blindness (vitamin A).

Diagnosis

The diagnostic criteria for PBC include an elevation in liver enzymes (most notably alkaline phosphatase) for a duration of six or more months, histologic findings, and the presence of antimitochondrial antibodies in the serum. The presence of two criteria is highly suggestive of the disease while a definite diagnosis requires all three.

Involvement of the liver is heterogeneous, so a biopsy may demonstrate different stages of disease

- Stage I is characterized by portal inflammation comprised of predominantly lymphoplasmacytic infiltrates. The pathognomonic lesion of PBC, the florid duct lesion, represents focal duct obliteration by granuloma formation.
- Stage II there is extension of inflammation to theperiportal areas.
- Stage III. There is formation of fibrous septa that link adjacent portal triads and bile duct loss (ductopenia).
- Stage IV is defined by frank cirrhosis.

Primary Sclerosing Cholangitis

Primary Sclerosing Cholangitis(PSC) is a chronic liver disease that is characterized by chronic inflammation and fibrosis leading to narrowing and dilatation of the intrahepatic or extrahepatic bile duct, or both.

PSC typically presents in the fourth to fifth decades of life. Men are affected more often than women.

Although the exact cause of PSC is unknown, it is considered autoimmune due to the presence of autoantibodies.The condition is associated in the majority of cases with chronic inflammatory bowel disease, particularly ulcerative colitis.

Patient may present with clinical, biochemical, immunological and histological features indistinguishable from those of type I autoimmune hepatitis, though they have more frequently an atypical peri-nuclear anti-neutrophil cytoplasmic antibody. This pANCA is atypical in that its target antigen appears to be nuclear and not cytoplasmic.

The correct diagnosis can be made only by demonstrating the characteristic bile duct abnormalities by specialized imaging such as endoscopic retrograde cholangio-pancreatography or magnetic resonance cholangiography.

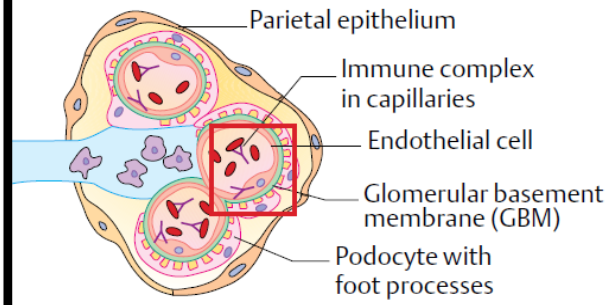
Lecture No. 10-11

Renal diseases

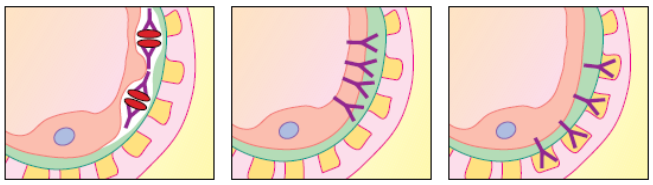
Many renal diseases have underlying immunological mechanisms. Antibody-mediated effects are primarily involved, whereas cellular mechanisms are less important. Immunological diseases of the kidney mainly affect the glomerulus, which is most likely due to its filter function. Circulating antibody-mediated renal diseases are induced in three mechanisms, circulating performed immune complexes accumulate subendothelially on the capillary aspect of the basement membrane, alternatively antibodies may react in situ with the glomerular basement membrane or with antigens of the visceral epithelial cells.

Antibody deposits can cause direct damage to epithelial or endothelial cells of glomerulus due to complement activation and pore formation. On the other hand, the antibodies can also bind to the FC receptors of monocytes, macrophages, granulocytes and platelets. This leads to the activation, or in the case of platelets aggregation of the cells. The glomerular damage can cause two distinct symptom complexes: the nephrotic syndrome and the nephritis syndrome.

Differentiation Between Nephrotic Syndrome and Nephritic Syndrome		
Typical Features	**Nephrotic**	**Nephritic**
Onset	Insidious	Abrupt
Edema	++++	++
Blood pressure	Normal	Raised
Jugular venous pressure	Normal/low	Raised
Proteinuria	++++	++
Hematuria	May/may not occur	+++
Red blood cell casts	Absent	Present
Serum albumin	Low	Normal/slightly reduced



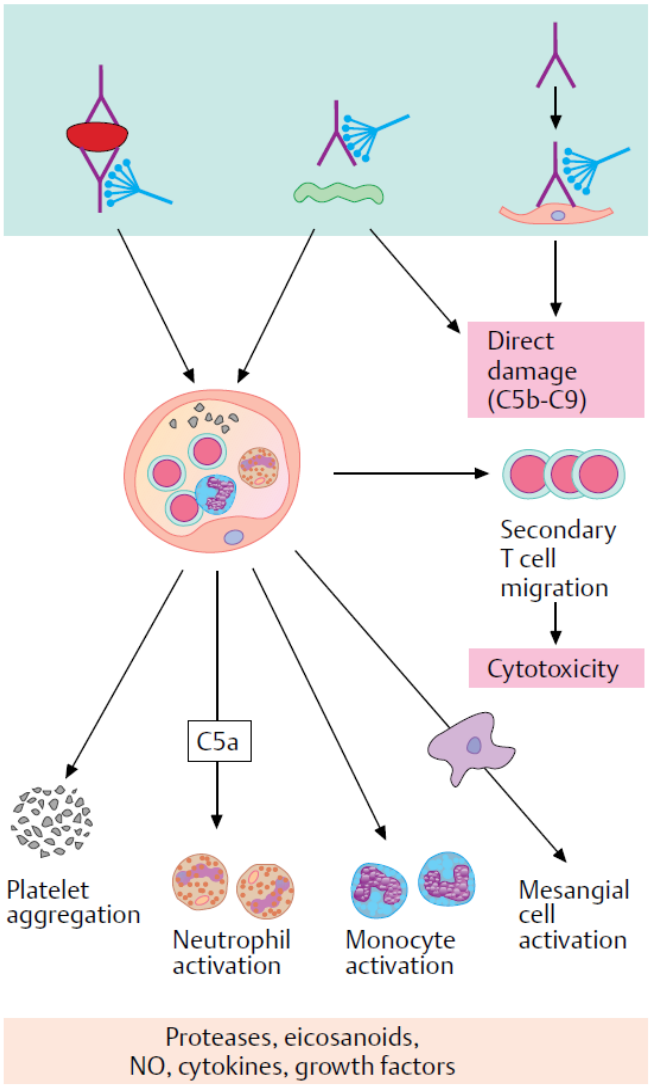
1. Anatomy



2a. Immune complex deposition

2b. Anti-GBM Ab

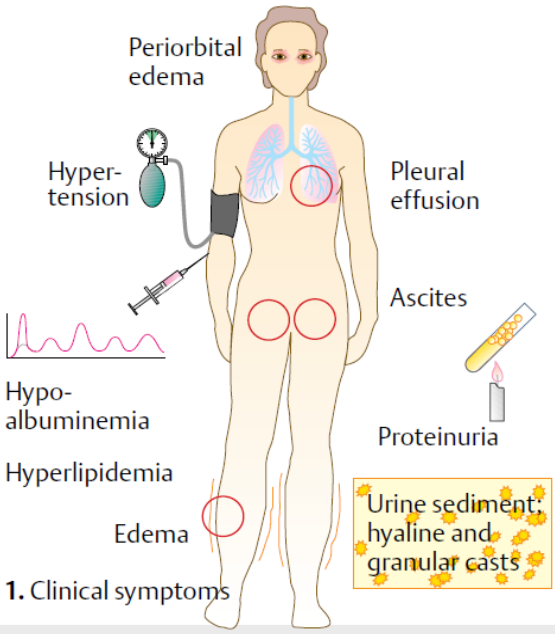
2c. Anti-epithelial cell Ab



Proteases, eicosanoids, NO, cytokines, growth factors

3. Mediators of glomerular damage

A. Mechanisms

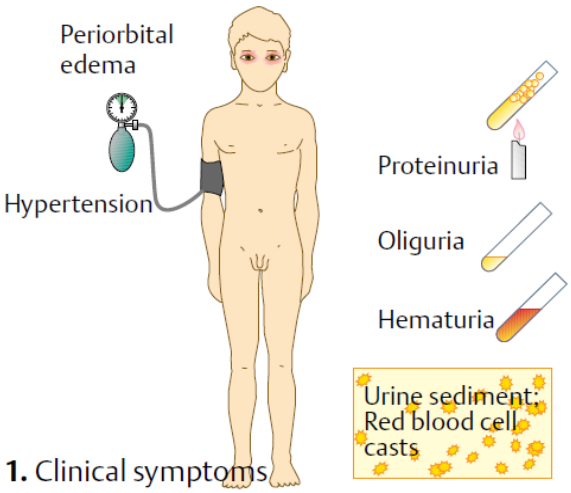


1. Clinical symptoms

	Children	Adults
Membranous glomerulonephritis	5%	20%
Lipoid nephrosis minimal change GN	60%	10%
Focal segmental glomerulosclerosis	10%	10%
Membranoproliferative glomerulonephritis	10%	5%
Proliferative GN (focal, IgA....)	10%	15%
Systemic diseases: diabetes, SLE, amyloidosis...	5%	40%

2. Causes of nephrotic syndrome

B. Nephrotic syndrome



1. Clinical symptoms

Postinfectious GN
Rapidly progressive GN
IgA nephropathy

2. Causes of the nephritic syndrome

C. Nephritic syndrome

Antineutrophil cytoplasmic antibody associated glomerulonephritis

Patients with ANCA-associated glomerulonephritis are usually aged from 40 to70 years and most have had a flu-like illness with arthralgia and myalgia a few days or weeks prior to the onset of renal disease or vasculitis. A spectrum of vasculitis is seen, ranging from disease limited to the kidneys in about a quarter of cases to a systemic vasculitic process with pulmonary involvement in about half the patients. ANCA-associated glomerulonephritis is now the commonest form of crescentic or rapidly progressive glomerulonephritis.

The renal lesion is characterized by few or no deposits of immunoglobulin or complement in the kidney **(so-called pauci-immune glomerulonephritis)** and by necrosis and crescent formation

Two pattern of antineutrophil cytoplasmic antibody (ANCA) reactivity are important clinically: generalized cytoplasmic staining **(cANCA)** and a perinuclear pattern **(pANCA)**. Most cANCA sera react with a serine proteinase called proteinase 3 (PR3), while most pANCA sera react with myeloperoxidase (MPO). A further pattern is associated with inflammatory bowel disease, particularly ulcerative colitis, some cANCA/ Panca positive sera react with neutrophil antigens other than PR3/MPO.

Raised ANCA titres are generally detectable during active granulomatosis with polyangiitis, and rising titres may herald a relapse. There has been debate whether ANCAs are pathogenic in vasculitis or simply a marker, but there is mounting evidence that they are pathogenic. The exact pathogenesis of granulomatosis with polyangiitis is not completely understood, but T cells, B cells, neutrophils and endothelial cells have all been implicated in the process.

Lecture No. 12-13

Membranous glomerulonephritis

Membranous glomerulonephritis can occur at any age, with the peak incidence in adults aged between 30-50 years and is characterized by the formation of immune complexes on the subepithelial surface of the basement membrane. The antibodies react in situ with endogenous podocyte antigens. The majority (70-80%) of patients with primary MN have circulating autoantibodies to **M-type phospholipase A2 receptor (PLA2R),** a transmembrane receptor that is expressed in glomerular podocytes. Antibodies to thrombospondin type-1 domain containing 7A protein (THSDA7A) and neutral endopeptidase have been identified in smaller subsets of patients.

Typical features include diffuse thickening of the basement membrane with fusion of the foot processes. The inhomogeneous distribution of the IgG and C3 deposits results in

granular pattern upon immunofluorescence. Glomerular sclerosis may occur in the latter stage of the disease. Clinically, membranous glomerulonephritis appears as a relatively mild nephrotic syndrome. Around 40% of the patients gradually develop progressive renal failure. The response to corticosteroid is poor.

Etiology

The disease is idiopathic or primary in 80% of cases; the causal antigen is never found. The remaining 20%, however, are secondary to another disease or to drugs. The most important causes are drugs (gold, penicillamine, captopril), infections (hepatitis B or C), systemic lupus erthymatosus, or carcinoma of bronchus, breast, colon or kidney.

Diagnosis

1. Renal biopsy is usually required to establish the diagnosis,
2. Serological testing for relevant autoantibodies (*i.e.*,anti–PLA2R) may be informative if renal biopsy is contraindicated.
3. Identification of PLA2R in glomerular immune deposits (by immunofluorescence or immunohistochemistry) favors the diagnosis of primary MN; mesangial deposits are often present in secondary MN.

Lecture No. 14

Postinfectious glomerulonephritis

Acute poststreptococcal glomerulonephritis is the prototype postinfectious glomerulonephritis, it is a disease of children and adolescents, but adults may be affected. Over 90% of cases are preceded by streptococcal infection of the throat or skin. Patients typically present with acute nephritis 7-12 days sfter a throat infection or about 3 weeks after a skin infection.

Etiology and pathogenesis

Glomerular injury results from passive glomerular trapping of circulating immune complexes composed of nephritogenic bacterial antigens and IgG antibody or by the *in situ* formation of immune complexes. This is followed by immune cell recruitment, production of chemical mediators and cytokines, and local activation of the complement and coagulation cascades that drive an inflammatory response

Diagnosis

1. Increasing titres of streptococcal antibodies and a low serum C3 level. Laboratories can often test for a range of antistreptococcal antibodies including antistreptolysin (ASO), antihyaluronidase (AHase), antistreptokinase (ASKase),antinicotinamide-adenine dinucleotidase (anti-NAD) and anti-DNAse B antibodies. These antibodies

are useful in approx.95% of cases following pharyngitis and 80% in those following pyoderma.

2. Poststreptococcal glomerulonephritis is characterized by a nephritic syndrome consisting of smoky or rust-colored urine, generalized edema, hypertension, and nephritic urine sediment. Proteinuria is typically mild.
3. Patients have rising titers of anti-streptolysin and depressed C3 levels early in nephritis but normal or minimally depressed C4 levels, indicating activation of the alternative complement pathway.
4. Proliferative glomerulonephritis with polymorphonuclear leukocyte and monocyte infiltration, granular immune deposits of IgG and C3, and dome-shaped electron-dense subepithelial deposits (humps) are characteristic.
5. Kidney biopsy is rarely needed in the child but may be warranted if there is an atypical presentation or evolution.

IgA nephropathy

IgA nephropathy (mesangial IgA deposition or Bergers disease) is the most common form of primary glomerulonephritis in the world. It affects mainly older children or young adults, and present typically as recurrent episodes of macroscopic haematuria occuring after an upper respiratory tract infection or, less frequently, a gastrointestinal or urinary tract infection, or strenuous exercise. Presentation with acute nephritis, hypertension, the nephrotic syndrome or as a chance finding of microscopic haematuria is less frequent. In contrast to poststreptococcal glomeruonephritis, the period between infection and haematuria is short, ranging from hours to a few days.

Etiology and Pathogenesis

IgA nephropathy can be considered a type of renal limited vasculitis caused by an innate defect in IgA mucosal immunity in the gut or lung: repeated exposure to a variety of environmental antigens results in an abnormal IgA response, namely the generation of nephritogenic polymeric IgA antibodies with defective galactosylation of the IgA hinge region resulting in deposition in the mesangium and the induction of inflammation in genetically susceptible individual.

Diagnosis

1. On light microscopy, the glomeruli show focal and segmental mesangial proliferation and, prominent deposite of IgA are found in the mesangium of every glomerulus, together with complement components of the alternative pathway.
2. Serum IgA levels are variable but may be significantly elevated. However, this is not a specific test, as liver disease and infections may also lead to persistent elevation of IgA.

Lecture No. 15

Lupus nephritis

Lupus nephritis is glomerulonephritis caused by (SLE) Although only 25% of patients with SLE present with renal disease as the first manifestation of lupus . clinical glomerulonephritis occurs in about 50% of cases of SLE at some time, and evidence of renal involvement can be detected in most patients, even in the absence of proteinuria.

Clinical Features

Asymptomatic hematuria or proteinuria may be the presenting features, but they often progress to nephritic and/or nephrotic syndrome. Hypertension, azotemia, nephritic urine sediment (with hematuria and cellular casts), hypocomplementemia and high anti–double-stranded DNA (dsDNA) titers are more commonly found in patients with proliferative lupus nephritis.

Pathophysiology of lupus nephritis

Pathophysiology involves immune complex deposition with development of glomerulonephritis. The immune complexes consist of

- Nuclear antigens (especially DNA)
- High-affinity complement-fixing IgG antinuclear antibodies
- Antibodies to DNA

Deposition of immune complexes from the circulation into the kidney appears to be the initiating event in proliferative lupus nephritis; however, only a subset of immune complexes appears to be nephritogenic.

DNA and anti-DNA antibodies are known to be concentrated in glomerular deposits in the subendothelial location and are likely to play a central role in the pathogenesis of proliferative lupus nephritis.

Classification of lupus nephritis is based on histologic finding

Class I. Minimal mesangial lupus nephritis: normal glomeruli under light microscope, but with minimal mesangial deposits in immunofluorescence. (**Normal serum creatinine and urine laboratory results. Incidental finding**)

Class II. Proliferative mesangial lupus nephritis: hypercellularity and mild mesangial expansion under light microscope, with mesangial deposits evident in immunofluorescence; there may be subepithelial or subendothelial deposits visible in an electron microscope or with immunofluorescence.

Class III. Focal lupus nephritis :lesions present in less than 50% of glomeruli with diffuse subendothelial deposits, with or without mesangial alterations. (Proteinuria and haematuria)

Class IV. Diffuse lupus nephritis : Damage that amounts to more than 50% (Haematuria, proteinuria, nephrotic syndrome, renal failure, arterial hypertension. Associated with elevated anti-nDNA titre and hypocomplementaemia May evolve towards renal failure)

Class V. **Membranous lupus nephritis**: thickening of the basal glomerular membrane with global or segmental immune deposits on the subepithelial wall of the basal membrane; may be associated with mesangial expansion.

Class VI. Sclerosing lupus nephritis, with involvement of over 90% of glomeruli, with no residual activity.

Diagnosis

1. Urinalysis and serum creatinine (all patients with SLE)
2. Renal biopsy
3. Diagnosis is suspected in all patients with SLE, particularly in patients who have proteinuria, microscopic hematuria, red blood cell (RBC) casts, or hypertension. Diagnosis is also suspected in patients with unexplained hypertension, elevated serum creatinine levels, or abnormalities on urinalysis who have clinical features suggesting SLE.
4. Elevated anti–double-stranded-DNA (anti-dsDNA) antibody titers and low complement (C3 and C4) levels often indicate active lupus nephritis and support the diagnosis.
5. If the aforementioned studies are abnormal, **renal biopsy** is usually done to confirm the diagnosis and classify the disorder histologically.
6. Histologic classification helps determine prognosis and direct treatment.

Henoch-Schonlein nephritis

Henoch–Schonlein nephritis (Henoch–Schonlein purpura or anaphylactoid purpura) is a common form of systemic vasculitis in which small blood vessels in a number of organs are involved.

It is usually a disease of children, with a peak age of onset between 4 and 10 years. The syndrome is characterized by nonthrombocytopenic purpura of the skin (particularly around joints) arthralgia, gastrointestinal pain and glomerulonephritis. Kidney disease is the most important manifestation of HSP as renal failure is the main cause of death.

The **prevalence of renal disease** varies from 40% to 100% but in most patients this is mild; progression to renal failure occurs in fewer than 10%. Those with the most severe clinical presentation have the worst outcome: about 40% of those with nephritic or nephrotic syndromes at onset show long-term impairment of renal function.

Immunohistology of the renal biopsy shows irregular,granular deposits of IgA, C3 and fibrin in the glomeruli. Deposits of IgA and C3 are also found in the skin, even in non-affected areas, and are diagnostic of the condition.
As in IgA nephropathy, the available evidence suggests an IgA dominant immune-complex pathogenesis with complement activation occurring via the alternative pathway. A variety of bacterial or viral antigens could be involved, as there is an association with preceding upper respiratory tract infection. In addition, HSN is a seasonal disease: most patients present during the winter. The clinical and immunological similarity between HSN and IgA nephropathy suggests that IgA nephropathy is a renal limited form of HSN.

Lecture No. 16

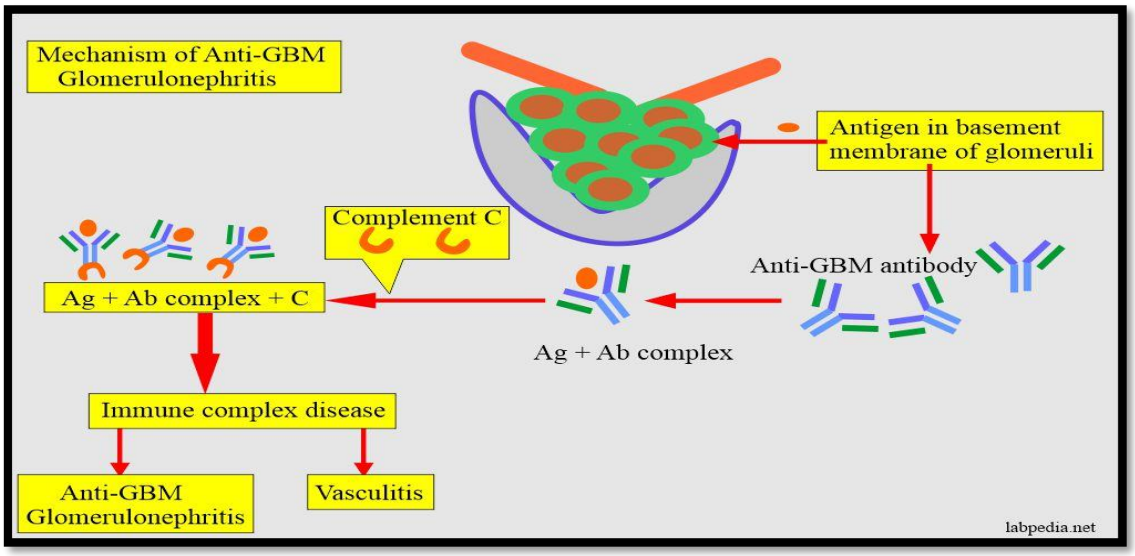
Anti-glomerular basement membrane disease

Acute glomerulonephritis mediated by anti-glomerular basement membrane (anti-GBM) antibody account for about 1-2% of all cases of glomerulonephritis. Anti-CBM nephritis is more common in men and in those who possess HLA-DR2. Patients present with nephritis alone or, more commonly, with glomerulonephritis and lung haemorrhage, a combination termed Goodpasture's syndrome. However, rapidly progressive nephritis and pulmonary haemorrhage can occur in other multisystem disorders such as SLE or Wegener's granulomatosis so the combination of renal and lung involvement is not synonymous with anti-GBM disease.

The target antigen is the α3 chain of type IV collagen, a major constituent of the GBM. Lung damage results from antibodies to antigens common to both alveolar and glomerular basement membranes. In Goodpasture's syndrome, respiratory symptoms often precede renal disease by 1 year or longer. Haemoptysis, usually leading to anemia, is a prominent feature and the sputum typically contains haemosiderin-laden macrophages. Lung biopsies show intra-alveolar haemorrhage and necrotizing alveolitis.

Etiology

Although the cause is unknown, anti-GBM disease follows upper respiratory tract infections in 20-60% 0f patients, or exposure to certain hydrocarbons. These agents may damage alveolar basement membrane, generating new and potent antigens able to stimulate autoantibody production. Alternatively, the agent responsible (e.g. a virus may cross-react with basement membrane antigens. Pulmonary haemorrhage in anti-GBM disease is strongly associated with cigarette smoking.



Figure(1):Mechanism of Anti-GBM glomerulonephritis

Diagnosis

1. **Renal involvement includes**
   - **a)** Gross or microscopic hematuria, proteinuria, a decreased 24-hour creatinine clearance, and elevated blood urea and serum creatinine levels.
   - **b)** Abnormally shaped RBCs and casts can be found in the urine sediment.
2. **In those patients with pulmonary involvement,** decreased total lung capacity and increased uptake of carbon monoxide is evident. An iron deficiency anemia with decreased hemoglobin and hematocrit can develop if pulmonary hemorrhage is severe.
3. The ESR and CRP level may be normal or increased.
4. Circulating antibodies to the GBM (anti - GBM) glomerular basement membrane can be detected in about 87% of patients. These antibodies can be identified by IIF, ELISA, or Western blot.

Lecture No. 17

Respiratory disease

Drug-Induced Pulmonary Disease

Drug-induced pulmonary disease is lung disease brought on by a bad reaction to a medicine. Pulmonary means related to the lungs.

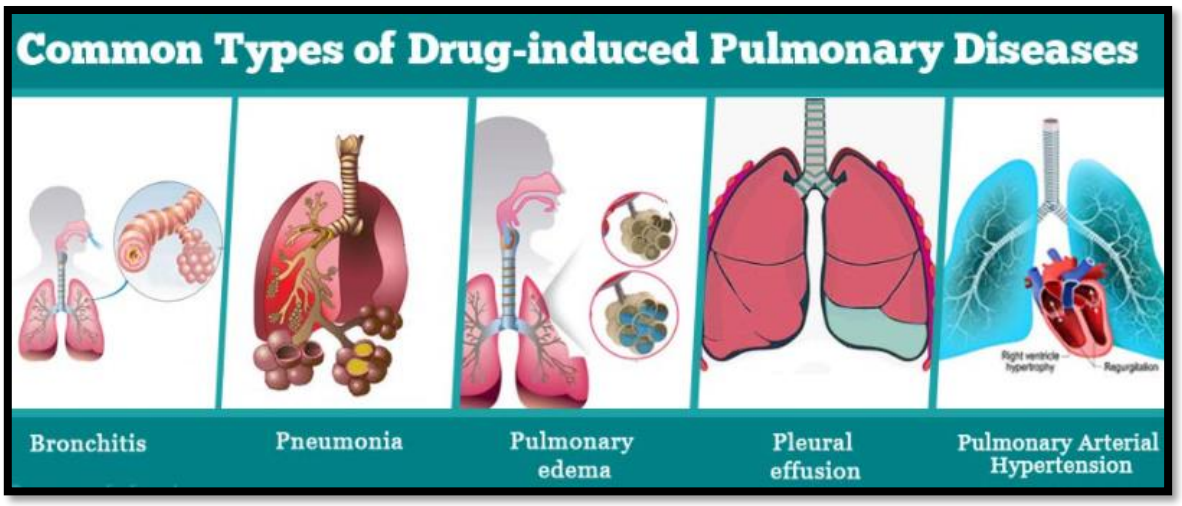
What is the most common drug-induced respiratory problem?

Interstitial pneumonitis (ie, inflammation of the lung interstitium, such as the alveolar septa) is the most common manifestation of drug-induced lung disease.

The Common Types of Drug-induced Pulmonary Diseases

There are different types of lung or pulmonary diseases caused by drugs are:

1. **Allergic reactions** like asthma, hypersensitivity pneumonitis, or eosinophilic pneumoni
2. **-Lymph node swelling**
3. **Alveolar haemorrhage**, i.e. bleeding into lung sacs .
4. **Bronchitis,** i.e., inflammation of the airways .
5. **Pneumonia**
6. **Pulmonary edema**, i.e., fluid accumulation in the lungs .
7. **Pleural effusion** i.e., fluid accumulation around the lungs .
8. **Pulmonary fibrosis i**.e., formation of scar tissue in the lungs .
9. **Pulmonary arterial hypertension**i.e defined as a mean pulmonary arterial pressure greater than 25 mm Hg at rest or greater than 30 mm Hg during exercise .
10. **Lung failure.**



Many medicines and substances are known to cause lung disease in some people. These include:

- Antibiotics, such as nitrofurantoin and sulfa drugs
- Heart medicines, such as amiodarone
- Chemotherapy drugs such as bleomycin, cyclophosphamide, and methotrexate
- Street drugs

Symptoms

Symptoms may include any of the following:

- Bloody sputum
- Chest pain
- Cough
- Fever
- Shortness of breath

- Wheezing

Diagnosis of Drug-induced Pulmonary Diseases

It has always been a challenge for pulmonologists to diagnose drug-induced pulmonary disease. The medications can cause reactions in varied forms, which makes it difficult for pulmonologists to identify the drug or its reaction.

Tests that could detect changes in the lungs include the following:

1. **Imaging tests like chest x-ray and chest CT scan .**
2. **Lung function tests :** The primary purpose of pulmonary function testing is to identify the severity of pulmonary impairment**.** The tests measure lung volume, capacity, rates of flow, and gas exchange.
3. **Bronchoscopy** : is a procedure to look directly at the airways in the lungs using a thin, lighted tube (bronchoscope). The bronchoscope is put in the nose or mouth. It is moved down the throat and windpipe (trachea), and into the airways **.**
4. **Blood tests to rule out SLE-like reactions as a cause of the lung disease**
5. Lung Biopsy, in rare cases

Lecture No. 18

Eosinophilic Pneumonia

Chronic eosinophilic pneumonia (CEP) is an idiopathic disorder characterized by an abnormal and marked accumulation of eosinophils in the interstitium and alveolar spaces of the lung causing inflammation and damage. Causes include smoking, allergic reactions and parasitic infections. EP may occur suddenly or worsen slowly.

What are the types of eosinophilic pneumonia?

There are three main types of eosinophilic pneumonia. They include:

- **Acute eosinophilic pneumonia:** This type worsens quickly as your blood oxygen level falls. Most patients with AEP completely recover with treatment.
- **Chronic eosinophilic pneumonia:** This type worsens slowly, over days or weeks. If untreated, it may persist over weeks or months and result in severe symptoms.
- **Löffler syndrome (simple pulmonary eosinophilic, or SPE):** This form of eosinophilic pneumonia may cause no symptoms or only mild symptoms such as a dry cough. Löffler syndrome occurs due to a parasitic infection (roundworms). With treatment, the condition typically resolves within one month.

Causes of Eosinophilic pneumonia

Eosinophilic pneumonia has many causes, both infectious and noninfectious. But healthcare providers don't always know the exact cause.

Common noninfectious triggers include:

- Allergic reactions.
- Fungus (usually aspergillosis).
- Inhaled toxins, such as chemical fumes or particulate metals (found in the air) or dust.
- Medication, including commonly used antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs) or selective serotonin reuptake inhibitors (SSRIs).
- Smoking, especially if you've had a change in cigarette smoking habits (starting smoking for the first time or smoking more often).
- Underlying conditions, including cancer, autoimmune disease or inflammatory disease.

The symptoms of eosinophilic pneumonia

Signs of eosinophilic pneumonia vary, depending on the type and cause. Common symptoms include:

- Cough.
- Fever.
- Shortness of breath (dyspnea).

Acute eosinophilic pneumonia can worsen quickly, often within two weeks. Symptoms are usually more severe in people who smoke and may include:

- Chest pain.
- Chills.
- Fatigue.
- Muscle aches or muscle pain (myalgia).

Without prompt diagnosis and treatment, the oxygen in your blood may fall to dangerously low levels. This can lead to acute respiratory failure in a few hours, requiring emergency treatment.

Common symptoms include:

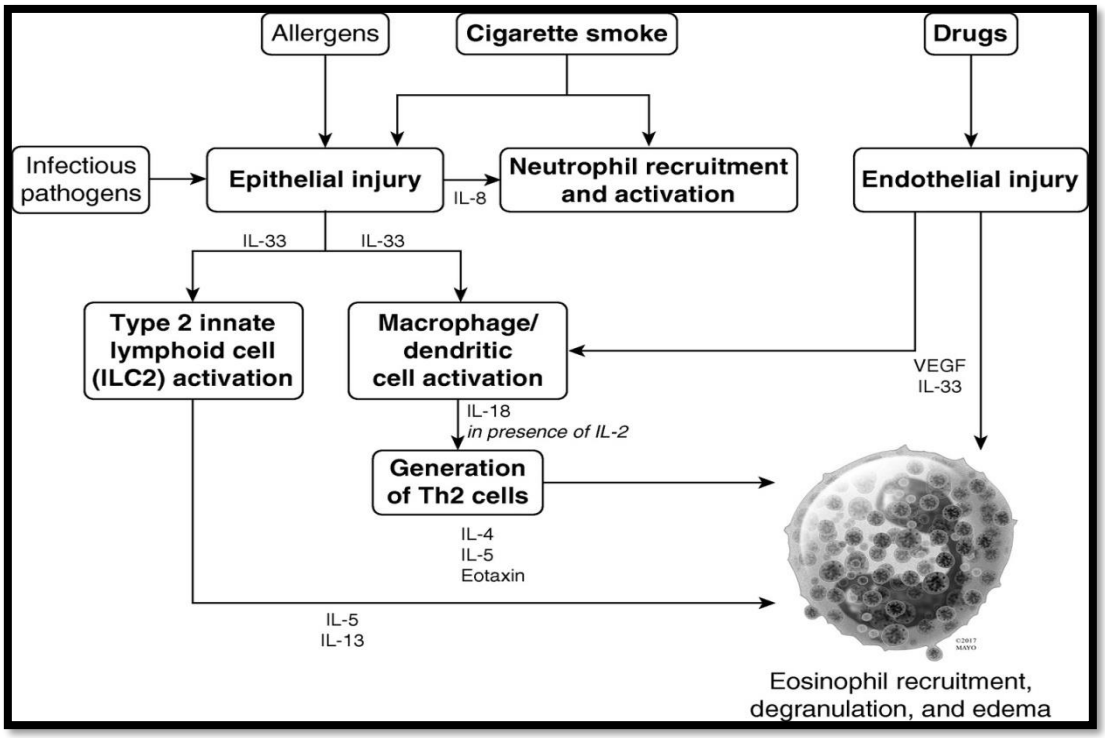
- Shortness of breath that worsens.
- Night sweats.
- Unexplained weight loss.
- Wheezing.

Pathogenicity of Chronic Eosinophilic Pneumonia

The pathophysiological role of eosinophils in autoimmune diseases is not well defined; however, it has been shown that the production of pro-inflammatory cytokines stimulates and activates different cell groups, and can simultaneously induce autoantibodies and/or increased infiltration of eosinophils in various tissues, without an underlying autoimmune disease.

A proposed model for the pathogenesis of acute eosinophilic pneumonia. IL-33 may be released by damaged epithelial cells responding to noxious stimulants such as allergens,

infectious pathogens, and other inhalational toxins, including cigarette smoke. IL-33 and vascular endothelial grow factor (VEGF) may also be released by endothelial cells after drug-induced injury. In addition to IL-33, acute exposure to cigarette smoking induces epithelial cell release of IL-8, which mediates recruitment and activation of neutrophils. An additional source of IL-33 in the lung may be the activation of innate type 2 lymphoid cells, which have the capacity to rapidly generate IL-33 in response to certain stimuli. Subsequent generation and binding of IL-33 to cells expressing its receptor (ST2), including macrophages and dendritic cells, may lead to recruitment and activation of T-helper cell type 2 (Th2)-polarized T lymphocytes and production of cytokines like IL-5, which further promote recruitment and activation of eosinophils in the lung tissue. Eosinophils may also migrate into the lung because of chemokine gradients and increased permeability in the context of endothelial injury.



Diagnosis of Eosinophilic pneumonia

- medical history and travel.
- physical exam.
- Blood tests, including a complete blood count, to detect abnormalities.
- Broncho alveolar lavage (BAL) is the most important test to diagnose EP. Uses a flexible tube (bronchoscope) to collect fluid from your lungs to look for signs of disease.
- Chest x-ray and CT scan.
- Peripheral blood eosinophilia count , peripheral eosinophilia is often present in chronic eosinophilic pneumonia.
- Sedimentation rate (ESR)

Occupational lung diseases

Occupational or work-related lung diseases are lung conditions that have been caused or made worse by long-term exposure to certain irritants in the workplace. Dust particles, chemicals, fungal spores, and certain animal droppings are examples of exposures that may increase your risk of developing occupational lung disease.

There is no cure for occupational lung diseases. Controlling your exposure to lung irritants and treatment can help slow the disease progression, lessen symptoms, and improve your quality of life. If you smoke, quit. Smoking can cause or worsen lung disease.

The symptoms of an occupational lung disease

- Coughing
- Shortness of breath
- Chest pain
- Chest tightness
- Abnormal breathing pattern .

Types of occupational lung diseases

- Asthma.
- Bronchiolitis obliterans.
- COPD.(Chronic obstructive pulmonary disease)
- Hypersensitivity pneumonitis.
- Lung cancer.
- Mesothelioma.
- Pneumoconiosis.

The difference between inorganic and organic dust

Inorganic refers to any substances that do not contain carbon, excluding certain simple carbon oxides, such as carbon monoxide and carbon dioxide.

Organic refers to any substances that do contain carbon, excluding simple carbon oxides, sulfides, and metal carbonates .

Exposure to environmental and occupational lung irritants may put you at risk of developing chronic lung disease, including:

1. **Silicosis** is caused by breathing in tiny bits of silica, a mineral found in sand, quartz, and many other types of rock. Silicosis mainly affects workers exposed to silica dust in jobs such as construction and mining.
2. **Coccidioidomycosis or Valley fever** is an infection caused by breathing in the spores of the fungus Coccidioides found in the soil. Valley fever mainly affects workers exposed to dust storms or areas where contaminated soil is being disturbed, in jobs like construction or farming.
3. **Hypersensitive pneumonitis** is caused when you breathe in a specific substance (allergen) that triggers an allergic reaction in the body.

4. **Histoplasmosis** is caused by breathing fungal spores from soil that has been contaminated by bird or bat droppings. Some occupations that may expose workers to spores are farmers, pest control workers, poultry keepers, construction workers and landscapers.
5. **Asbestosis** is a naturally occurring mineral used as an insulation material and as a fire retardant. The main group at risk for asbestosis is people who worked in mining, milling, manufacturing, installation, or removal of asbestos products .
6. **Coal workers pneumoconiosis**, commonly known as black lung disease, occurs when coal dust is inhaled. Continued exposure to coal dust causes scarring in the lungs.
7. **Mesothelioma** is a rare type of cancer that occurs in the lining of the lungs and less commonly the lining of the abdomen. Asbestos exposure is the primary risk factor for mesothelioma. Occupations such as mining or milling, electricians, plumbers, pipe-fitters, insulators, or even remodelers of older homes still have a high risk of exposure.
8. **Work-related asthma:-** Men working in forestry and minerals and women working in service industries (waitresses, cleaners, and dental workers) are most likely to develop occupational asthma.

Diagnose of an occupational lung disease

- **Pulmonary function tests**: diagnostic tests that help to measure the lungs' ability to move air into and out of the lungs effectively. The tests are usually performed with special machines into which the person must breathe.
- **Microscopic examination** from biopsy or autopsy of tissue, cells, and fluids from the lungs
- **Measurement of respiratory or gas exchange functions**
- **Examination of airway or bronchial activity**

How can occupational lung diseases be prevented?

The best prevention for occupational lung diseases is avoidance of the inhaled substances that cause lung diseases and Do not smoke. Smoking can actually increase the risk for occupational lung disease.

Lecture No. 19

Asthma

A chronic disease in which the bronchial airways in the lungs become narrowed and swollen, making it difficult to breathe. Symptoms include wheezing, coughing, tightness in the chest, shortness of breath, and rapid breathing. An asthma attack may be brought on by pet hair, dust, smoke, pollen, mold, exercise, cold air, or stress.

Asthma signs and symptoms include:

1. Shortness of breath
2. Chest tightness or pain
3. Wheezing when exhaling, which is a common sign of asthma in children
4. Trouble sleeping caused by shortness of breath, coughing or wheezing
5. Coughing or wheezing attacks that are worsened by a respiratory virus, such as a cold or the flu

Types of asthma

1. Allergic asthma
2. Seasonal asthma
3. Non allergic asthma
4. Exercise induced asthma
5. Difficult asthma
6. Childhood asthma

Causes

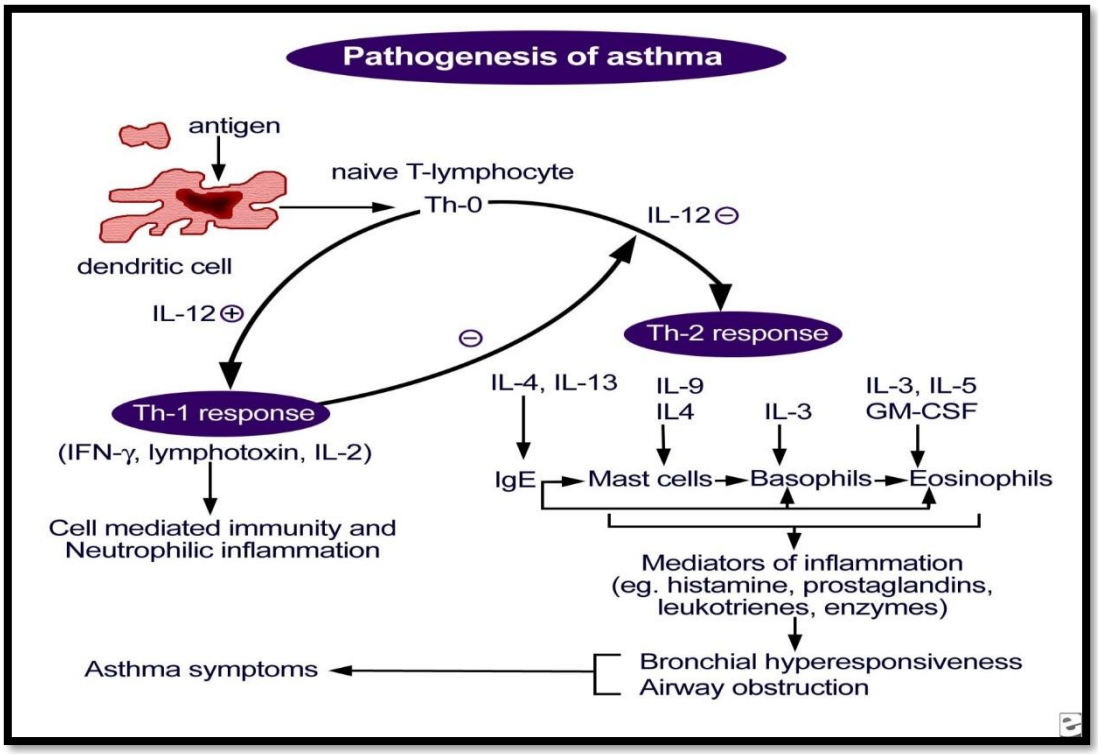
It isn't clear why some people get asthma and others don't, but it's probably due to a combination of environmental and inherited (genetic) factors. Exposure to various irritants and substances that trigger allergies (allergens) can trigger signs and symptoms of asthma. Asthma triggers are different from person to person and can include:

1. Airborne allergens, such as pollen, dust mites, mold spores, pet dander or particles of cockroach waste
2. Respiratory infections, such as the common cold
3. Physical activity
4. Cold air
5. Air pollutants and irritants, such as smoke
6. Certain medications, including beta blockers, aspirin, and nonsteroidal anti-inflammatory drugs, such as ibuprofen (Advil, Motrin IB, others) and naproxen sodium (Aleve)
7. Strong emotions and stress
8. Sulfites and preservatives added to some types of foods and beverages, including shrimp, dried fruit, processed potatoes .
9. Gastroesophageal reflux disease (GERD), a condition in which stomach acids back up into your throat

Pathophysiology

There are two phases of an asthma exacerbation, which include the early phase and late phase. The early phase is initiated by IgE antibodies that are sensitized and released by plasma cells. These antibodies respond to certain triggers in the environment, such as the risk factors listed above. IgE antibodies then bind to high-affinity mast cells and basophils. When a pollutant or risk factor gets inhaled, the mast cells release cytokines and eventually de-granulate. Released from mast cells are histamine, prostaglandins, and leukotrienes. Simultaneously, cytokines derived from the mast cell will signal other inflammatory cells and their mediators to the lung. The result is airway inflammation, increased vascular permeability, mucus secretion, bronchospasm, and wheezing. These events are referred to as the *early asthmatic response* because they occur within minutes. A major component of the early response is bronchospasm.

The *late asthmatic response* is delayed by hours. It is caused by a multitude of inflammatory cells continuing the inflammatory process. Of the inflammatory cells, the T cells play an important role. Antigen presenting cells may present a variety of allergenic antigens to chronically activated T helper cells. These cells then secrete multiple cytokines that maintain and intensify the local inflammatory response. Many other inflammatory cells, including mast cells and eosinophils, will respond to the T cells' cytokines. These inflammatory cells will produce cytokines, which amplify the cellular response and the inflammatory reaction. There is a migration of inflammatory cells from the circulation into the pulmonary vasculature and the airway submucosa. A central component to the inflammatory process as well as treatment is the arachidonic acid pathway, which leads to the generation of leukotrienes.



Diagnosis

1- Physical exam

2-**Lung function tests :-** These are also called (pulmonary function tests.) Lung function tests detect how well you inhale (breathe in) and exhale (breathe out) air from your lungs. These tests measure breathing.

Lung function tests are often done before and after inhaling a medication known as a bronchodilator. This medicine opens the airways. If lung function improves a lot with a bronchodilator, the patient likely has asthma.

Common Lung function tests used to assess airways include:

a. **Spirometry:** A type of lung function test that measures how much you breathe in and out and how fast you breathe out.

b. **FeNO test (exhaled nitric oxide):** A test that helps assess inflammation in the airways.

c. **Bronchial provocation or "trigger" tests:** Tests that measure if lungs are sensitive to certain irritants or triggers such as methacholine or histamine.

d. **Diffusion Capacity:** Diffusion capacity measures how well oxygen flows from the lungs into your blood. Poor diffusion indicates damage to the lung where the oxygen and blood meet in the lungs. Diffusion capacity is usually normal in asthmatics.

3- Allergy tests

4- Blood tests: measured the levels of immunoglobulin E (IgE) and Eosinophil . If the levels are high, this could be a sign of severe asthma.

5-**Chest X-Ray:-**in asthma, the chest X-ray is likely to show air trapping or hyperexpansion.

Lecture No. 20

Non-allergic bronchitis

It is a form of lower respiratory tract infection occurs due to a viral or bacterial infection. Some people develop non-allergic bronchitis after a cold, for instance. Bronchitis can be acute or chronic. Acute form leads to cough, which may contain mucus, while in case of chronic bronchitis, cough last for more than a few months. Air pollution and smoking are some major causes of bronchitis.

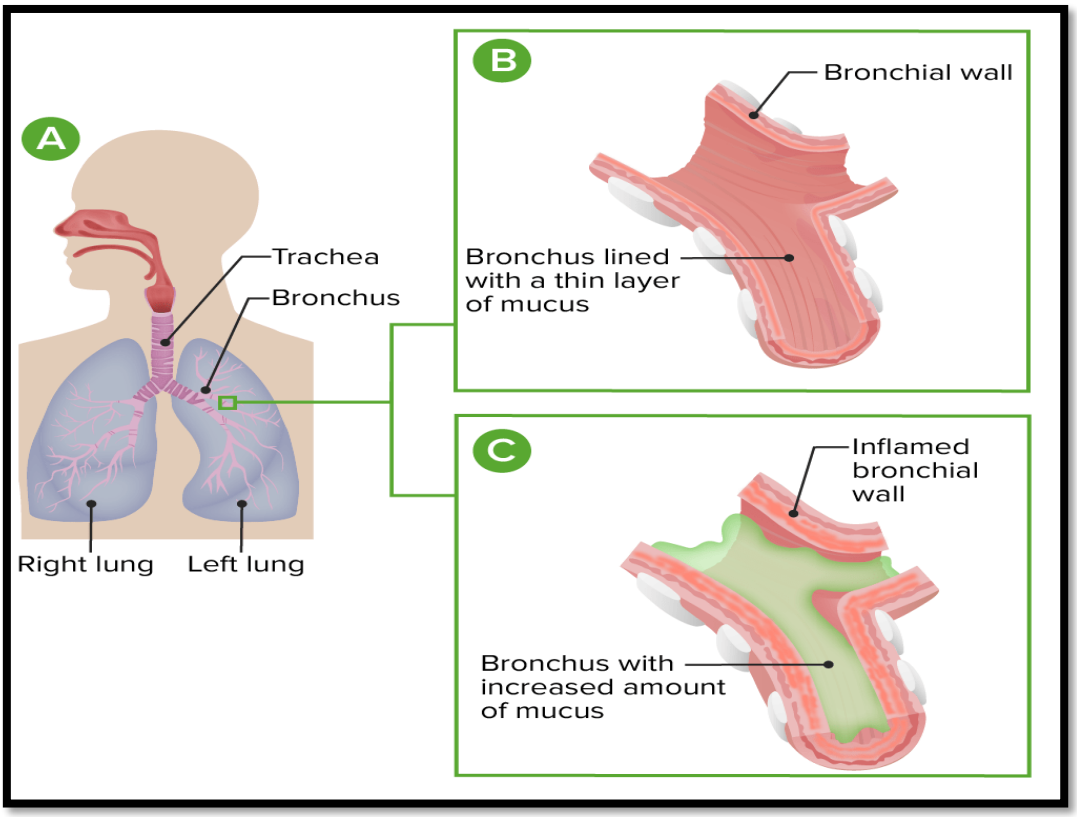
Symptoms of Acute Bronchitis

Each person is different, and symptoms will vary depending on the cause of inflammation. The symptoms associated with acute bronchitis are similar to those of the cold and flu and last less than 3 weeks .

- Coughing with or without mucus
  - A runny nose
  - A sore throat

- Sneezing
- Fever & chills
- Breathing difficulties
- Extreme fatigue

- Mild headache
- Mild body ache



Causes

A virus usually causes acute bronchitis. Bacteria can sometimes cause acute bronchitis. But, even in these cases, taking antibiotics is NOT advised and will not help you get better.

Diagnosis

1. **Spirometry :-** A test that measures lung function as breathe in and out of a mouthpiece that is attached to a device called a spirometer.
2. **Peak expiratory flow :-** A test that measures the force of air breathe out (exhale) into the mouthpiece of a device called a peak expiratory flow meter
3. **Chest X-ray:-** A radiology test that produces images of the chest to look for evidence of other conditions that could be causing your coughand breathing problems.
4. **Complete blood count (CBC) with differential**
5. **Procalcitonin levels** (to distinguish bacterial from nonbacterial infections)
6. **Sputum cytology** (if the cough is persistent)
7. **Blood culture** (if bacterial superinfection is suspected)

8. **Chest radiography** (if the patient is elderly or physical findings suggest pneumonia)
9. **Bronchoscopy** (to exclude foreign body aspiration, tuberculosis, tumors, and other chronic diseases)
10. **Influenza tests**
11. **Laryngoscopy** (to exclude epiglottitis)

Lecture No. 21-22

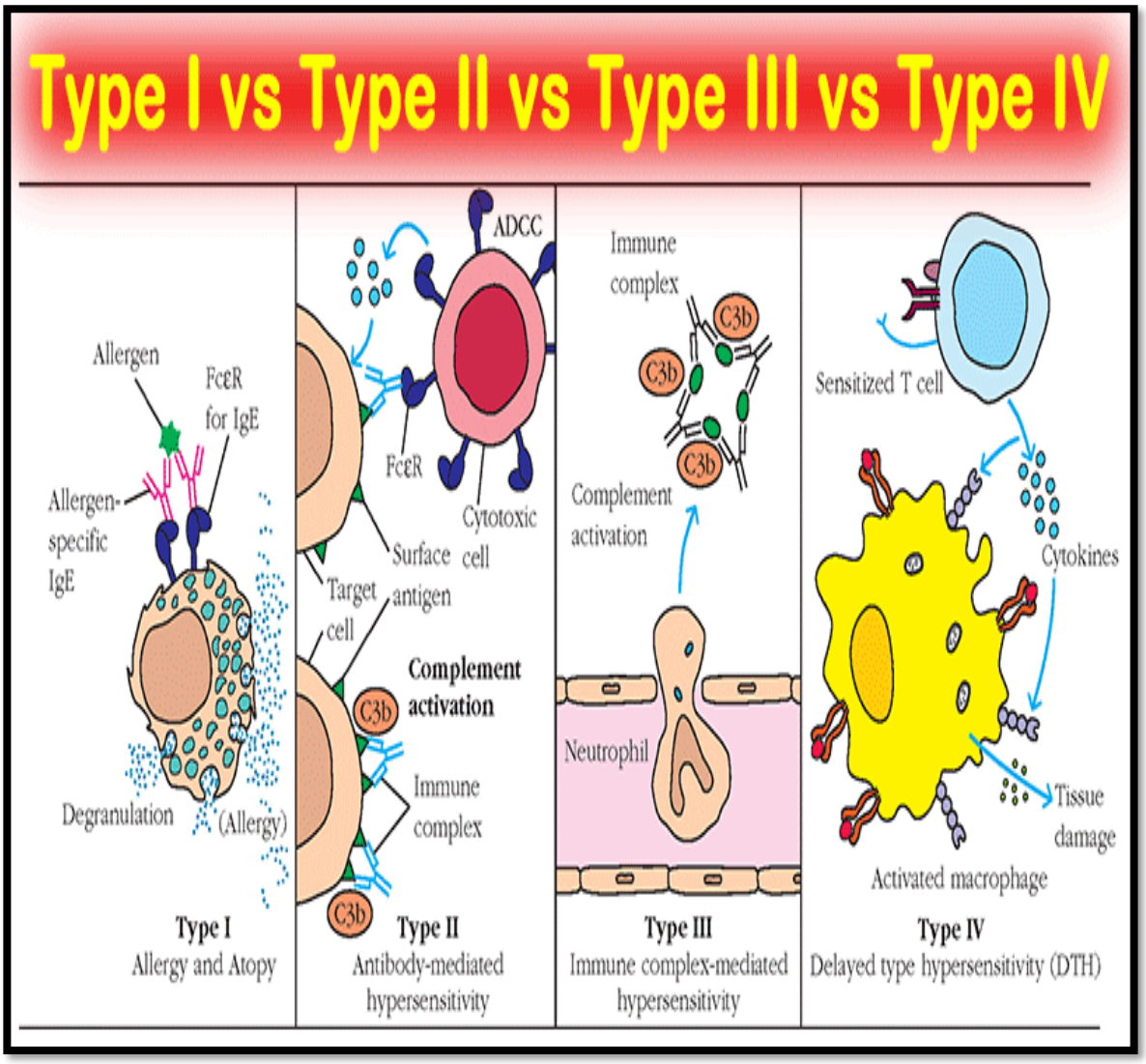
Hypersensitivity

Hypersensitivity, which is defined as an exaggerated response to atypically harmless antigen that results in injury to the tissue, disease, or even death. Antigens that trigger allergic reactions are called **allergens.**

Classification of hypersensitivity reactions:

Table 26-1 Classification of Hypersensitivity Reactions

	Type of Reaction			
Parameter	**I**	**II**	**III**	**IV**
Reaction	Anaphylactic	Cytotoxic	Immune complex	T cell–dependent
Antibody	IgE*	IgG, possibly other immunoglobulins	Antigen-antibody complexes (IgG, IgM)*	None
Complement involved	No	Yes*	Yes*	No
Cells involved	Mast cells, basophils, granules (histamine)*	Effector cells (macrophages, polymorphonuclear leukocytes)*	Macrophages, mast cells	Antigen-specific T cells
Cytokines involved	Yes*	No	Yes*	Yes (T cell cytokines)*
Comparative description	Antibody mediated, immediate	Antibody dependent; complement or cell mediated	Immune complex mediated (immune complex disease)	T cell-mediated, delayed type
Mechanism of tissue injury	Allergic and anaphylactic reactions	Target cell lysis; cell-mediated cytotoxicity	Immune complex deposition, inflammation	Inflammation, cellular infiltration
Examples	Anaphylaxis Hay fever Asthma Food allergy	Transfusion reactions Hemolytic disease of newborn Thrombocytopenia	Arthus reaction Serum sickness Systemic lupus erythematosus	Allergy or infection Contact dermatitis



Testing of Hypersensitivity

1. In Vitro Tests: Total IgE

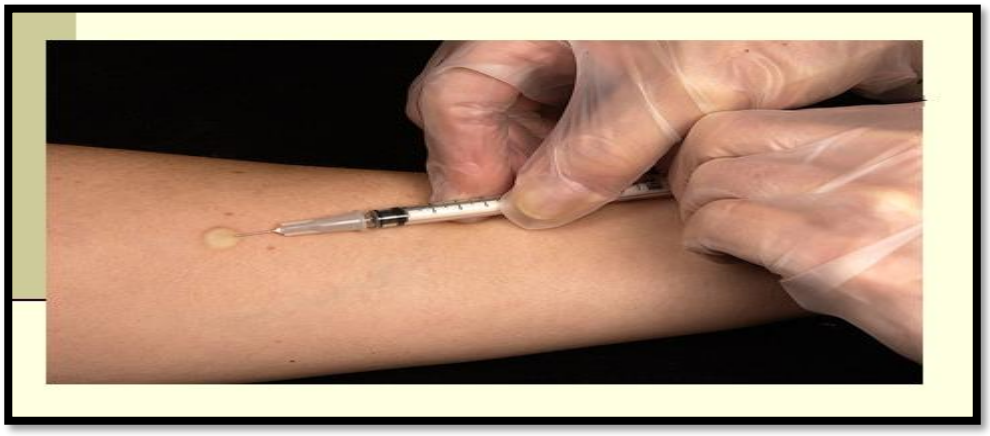
Testing Principles In vitro tests involve measurement of either total IgE or antigen-specific IgE. These are less sensitive than skin testing but usually are less traumatic to the patient. Total IgE testing has become more important as a screening test before a patient is referred to an allergy specialist**.** Total serum IgE testing is used clinically to aid in diagnosis of allergic rhinitis, asthma, or other allergic conditions that may be indicated by patient symptoms

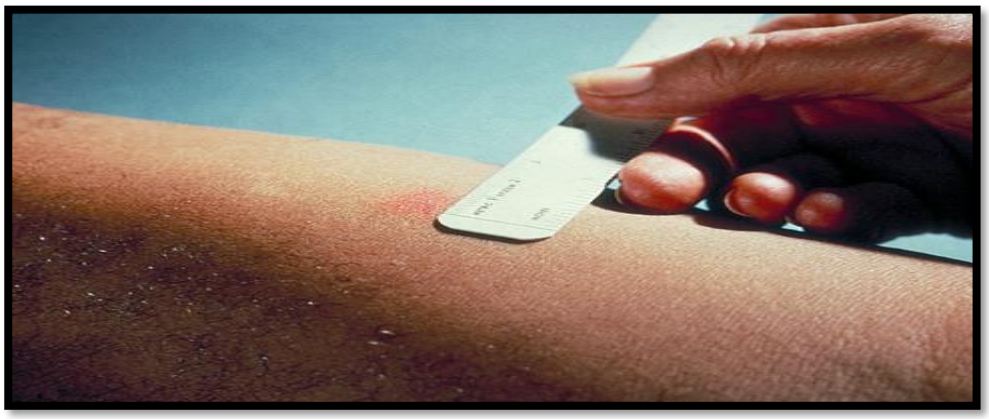
2. Antigen-Specific IgE Testing

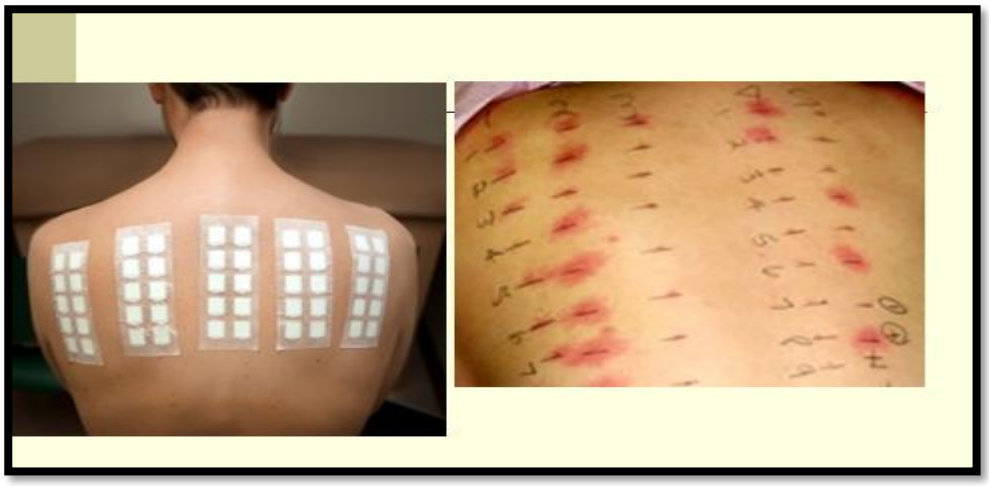
The original commercial testing method for determining specific IgE was known as the **radioallergosorbent test (RAST),** introduced in 1966. Principles of the test remain the same, but newer testing methods involve the use of enzyme or fluorescent labels rather than radioactivity. Allergen-specific IgE testing is safer to perform than skin testing and is easier on some patients, especially children or apprehensive adults, and the sensitivity now approaches that of skin testing. It is especially useful in detecting allergies to

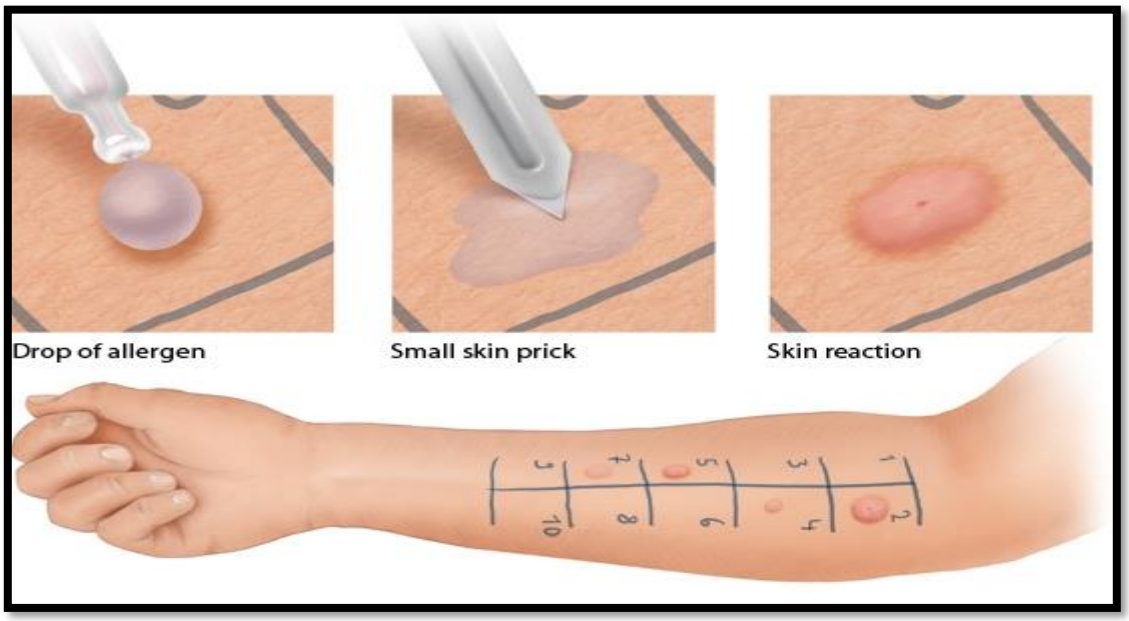
common triggers such as ragweed, trees, grasses, molds, animal dander, milk, and egg albumin.

3. **The patch test** is considered the gold standard in testing for contact dermatitis. This must be done when the patient is free of symptoms or when he or she at least has a clear test site. A nonabsorbent adhesive patch containing the suspected allergen is applied on the patient's back, and the skin is checked for a reaction over the next 48 hours. Redness with papules or tiny blisters is considered a positive test. Final evaluation is conducted at 96 to 120 hours. All readings should be done by a skilled evaluator. False negatives can result from inadequate contact with the skin.
4. Skin testing can be performed by a skin puncture test (SPT) to assist in the identification of foods that may provoke IgE-mediated, food induced allergic reactions
5. **MELISA** (**Memory Lymphocyte Immunostimulation Assay**) is a blood test that detects type IV hypersensitivity to metals, chemicals, environmental toxins and molds. Type IV hypersensitivity reactions, particularly to nickel, are well established and may affect 20% of the population
6. **The oral food challenge (OFC**) remains the gold standard for the diagnosis of food allergy. During the OFC, a standard serving size of the allergen is divided into 4–7 servings and administered over 60–90 minutes, with each dose being given 15–20 minutes apart. The initial amount fed to the patient is typically a very small proportion of the total serving, and each successive dose administers a larger amount of protein. At the first sign of an objective reaction, the OFC is stopped and appropriate treatment administere








Drop of allergen
Small skin prick
Skin reaction

Lecture No. 23

Autoimmune hemolytic anemia (AIHA)

In this disease, red blood cells (RBC) survive for a shorter time than in normal (less than 120 days) due to immune mechanism destructions of these RBC. In all conditions, the immune destruction is mediated by autoantibodies against certain components of the RBCs. AIHA are classified according to the thermic activity of the autoantibodies:

1. **Warm antibody hemolytic anemias** bind more efficiently to RBC at 37 C.
2. **Cold antibody hemolytic anemias** bind more efficiently to RBC at 4 C.

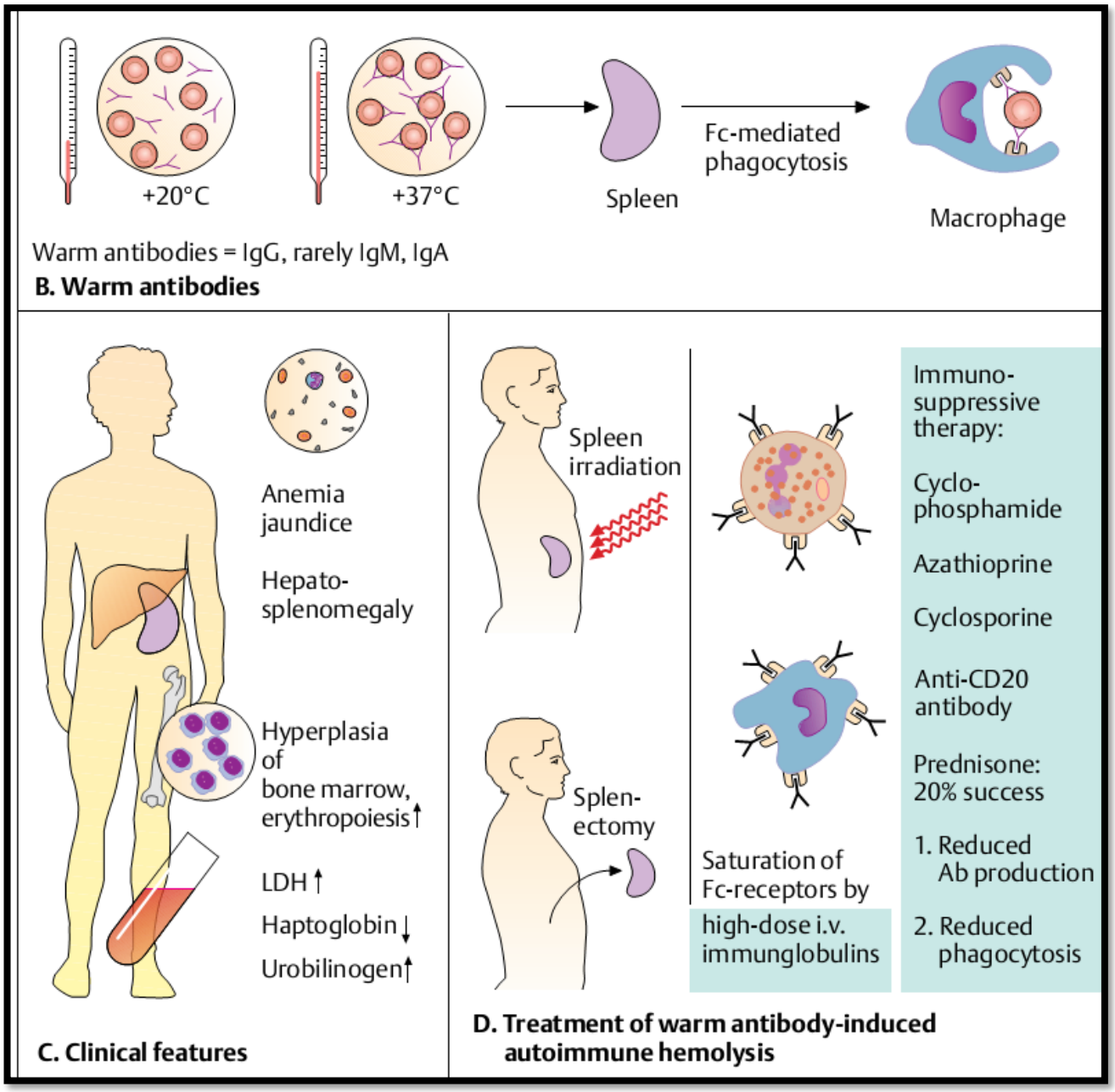
In all types of AIHA, there are autoantibody against certain components of the RBC surface which are attached to the patients RBC or free in the serum. In many occasion, complement may participate in the reaction to produce an Ab-Ag-complement complex. The screening test that used for the diagnosis of AIHA is called (Coombs test) which can demonstrate the presence of these autoantibody either attached to the patients RBC or free in serum. Coombs test could be direct or indirect.

Warm antibody hemolytic anemias

Affects all ages and mostly over 30 years of age and could be transient or persistent. About the half of the cases are idiopathic and the other half are due to secondary causes (lymphoproliferation, autoimmune disease as SLE, drugs and infections).the commonest pathogenesis of hemolytic anemia is the destruction of the opsonized RBC (with IgG and/or C3) by splenic macrophages and liver kupffers cells.

With warm antibodies, erythrocyte lysis takes place mainly in the RE system; the serum concentration of the hemoglobin-binding protein haptoglobin decreases only if there is severe hemolysis. Hepatosplenomegaly occurs due to the increased rate of hemolysis in the spleen and liver. Intracellular enzymes, such as lactate dehydrogenase (LDH), are released. Erythropoiesis is stimulated in the bone marrow, and reticulocytes are increased. The freed hemoglobin is reduced to bilirubin, which binds to glucuronate in the liver and is excreted in the bile. Hyperbilirubinemia, which leads to yellowish discoloration of the sclera and skin (jaundice), is frequently seen. Urobilinogen, another degradation product, cause dark discoloration of the urine.

Most cases are positive for direct Coombs test (50% are positive for both IgGand C3, 40% are positive for only IgG, 10% are positive for only C3). About 35% are positive for free autoantibody (indirect Coombs test).



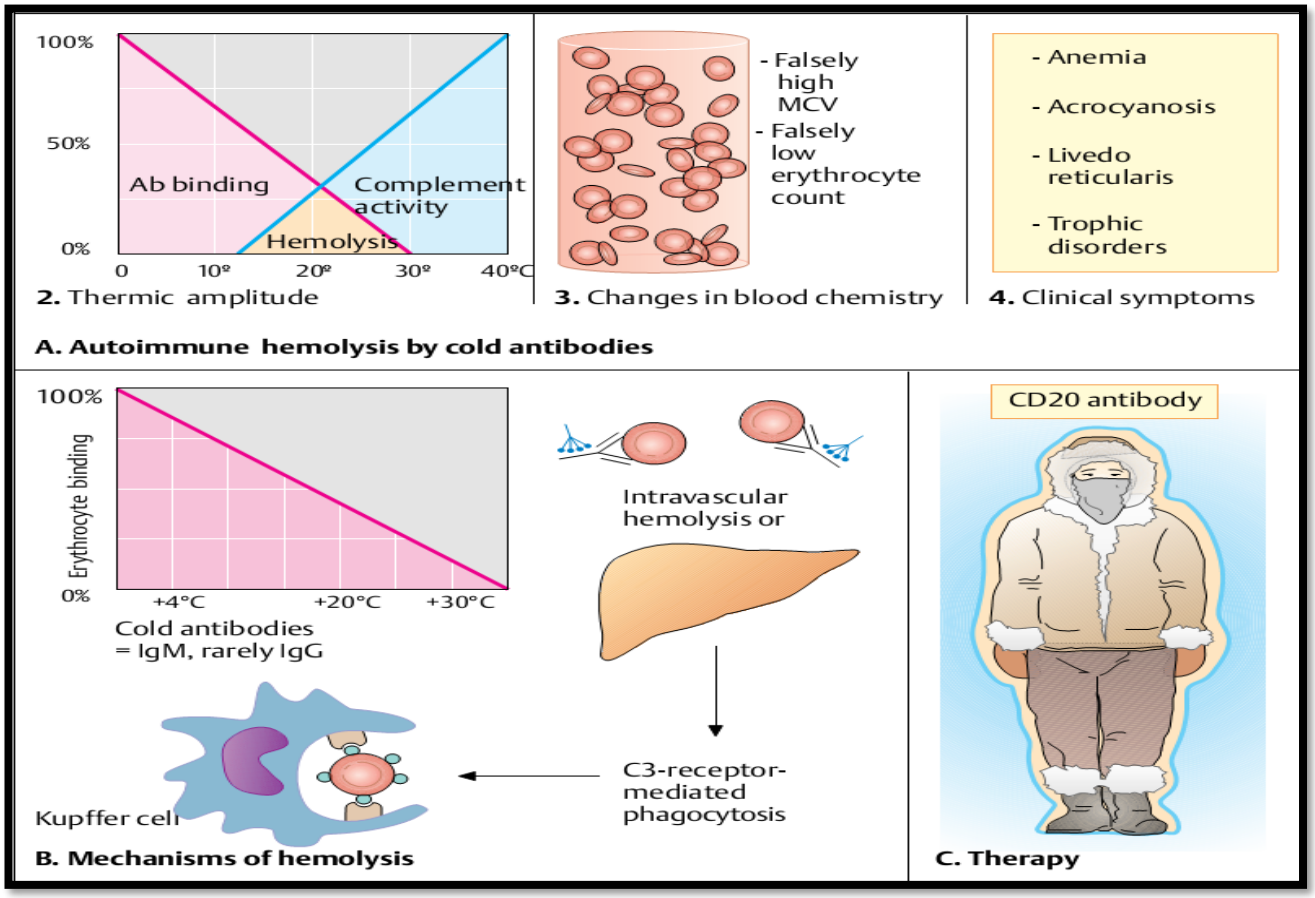
Cold antibody hemolytic anemias

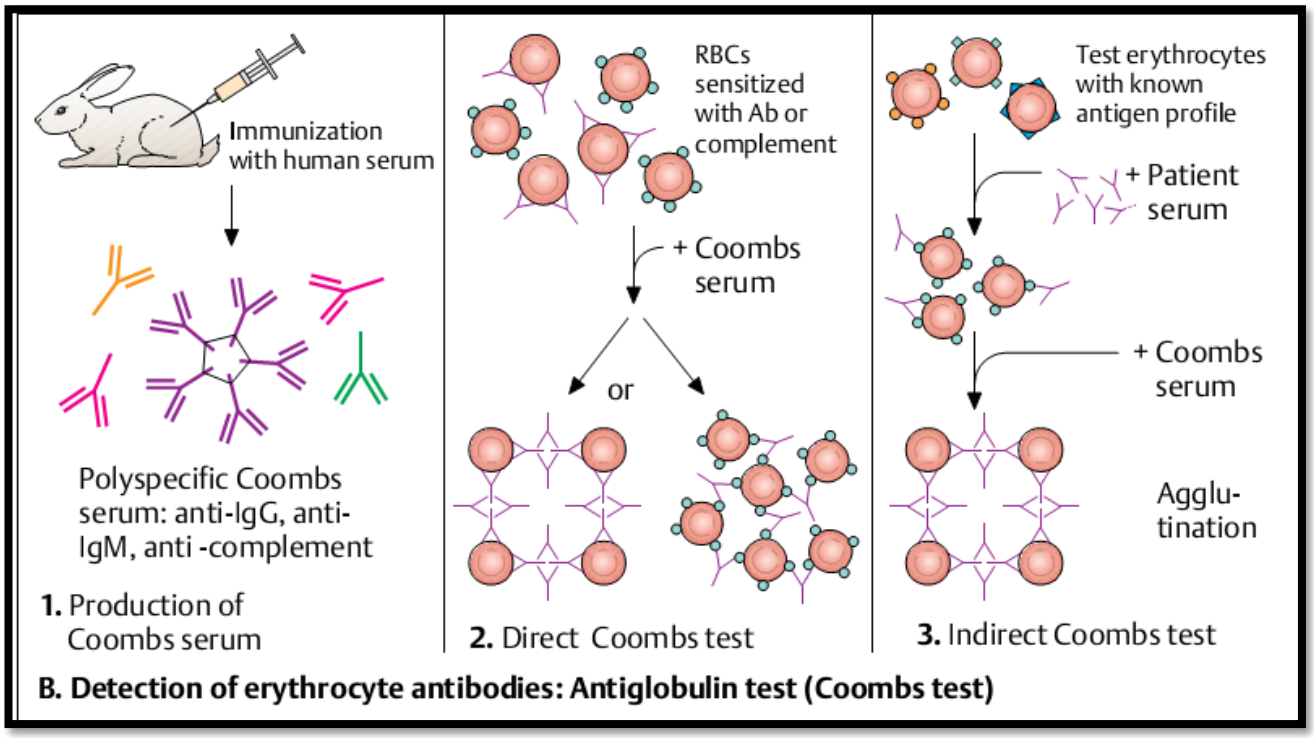
Cold antibodies are usually IgM and only occasionally IgG. Accordingly, they can cause agglutination of erythrocytes and are therefore called agglutinins.It is a disease of elderly people (over60years) mainly due to primary (idiopathic causes) and rarely due to secondary cause (infections, lymphoproliferation). They are most commonly observed following infections, especially by Mycoplasma, Epstein-Barr virus, or cytomegalovirus, and rarely after bacterial diseases. These infections usually lead to the formation of polyclonal cold reactive antibodies that bind to erythrocytes most efficiently at low temperature. In most cases, cold antibodies are directed against I antigen, which is mainly expressed on mature RBCs, but also on some pathogens. Some malignant lymphatic diseases may lead to

secretion of monoclonal agglutinins. Monoclonal agglutinins may be directed against both I and i antigen (immature fetal erythrocytes).

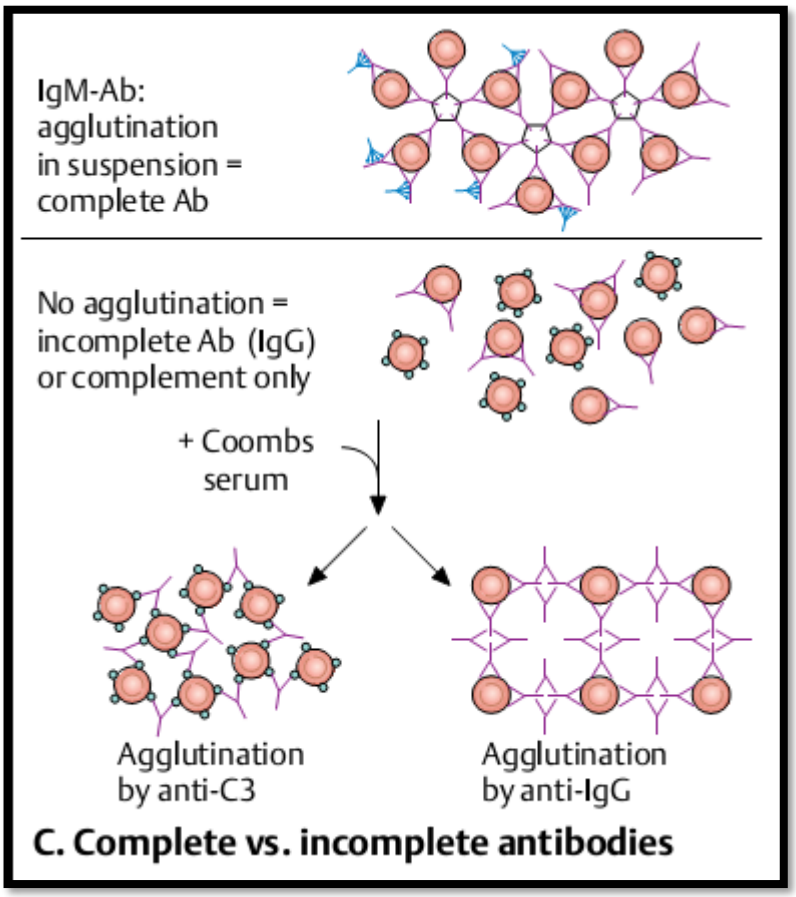
All patients have IgM coated RBC at 4c, but on warming the blood these Abs detached from RBC, however, fixed C3d can still be detected by direct Coombs test. The commonest pathogenesis of hemolysis is complement mediated mechanism. Free cold auto-Ab (cold agglutinins) are also found in the patients serum.

Since the temperature in the capillaries of the skin can drop below 30 C, the cold agglutinins cause the erythrocytes to clump together. This intravascular agglutination process leads to capillary obstruction, which manifests as acrocyanosis (bluish discoloration of the fingers, ears, and tip of the nose) or livedo reticularis (reddish/bluish reticular pattern of the skin). Trophic lesions (ulcer, necrosis) may occur in sever forms.





B. Detection of erythrocyte antibodies: Antiglobulin test (Coombs test)



C. Complete vs. incomplete antibodies

Lecture No. 24

Eczema

Eczema defines an inflammatory skin disorder with many possible causes, the hallmark being a histological process called spongiosis: the accumulation of oedema fluid within and between keratinocytes in the epidermis, giving a "spongy" appearance. It is common, affecting between 5 and 10% of the population, and 10-20% of children.

The main symptoms are **itching or, in the infant the appearance of dry, red patches with occasional vesicles overtaken by crusting**. In infancy, the cheeks, abdomen and limb surface are involved, whilst in older children the classical distribution is on the elbow, knee and wrist flexor surfaces. Prolonged scratching leads to the development of discoloured plaque with a leathery texture (lichenification). In approximately 75% of cases the disorder is self-limiting and clears in the first few years of life. The pathogenesis of allergic eczema is less clear.

The relationship between diet and eczema is perhaps the most intriguing and controversial aspect of the disease. Well designed studies in which potential triggers in food (**cow's milk, hen's egg proteins, peanuts**) are avoided appear to bring about an improvement in eczema.

The diagnosis is made on **history, examination and skin testing** (skin prick test evokes a wheal and flare response but not eczematous lesions, it is possible to replicate eczema like plaques by patch testing with allergens such as house dust mite extracts in sensitive individual), with **the total serum IgE level** also usually raised and IgE directed against airborne and food allergens is a prominent feature.

Contact dermatitis

Contact dermatitis is an inflammatory skin disease caused **T_H1-cell mediated (type IV) hypersensitivity** to external agents (see figure below) which come into contact with the skin. These agents (known as **haptens**) are usually molecules of relatively of low molecular weight (<1 KDa) and are not immunogenic, but they can penetrate the epidermis and bind to certain proteins in the skin (carrier proteins) and become highly reactive molecules.

Classification

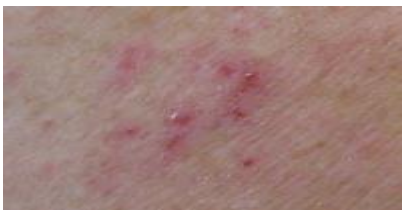
1. Acute toxic dermatitis.
2. Cumulative dermatitis.
3. Allergic Contact dermatitis.

Pathogenesis

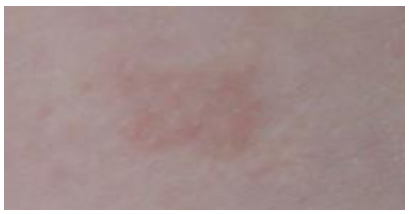
Two phase of pathogenesis are recognized: an **induction phase**, from time of initial antigen contact to sensitization of T lymphocytes, and an **elicitation phase**, from antigen re-exposure to the appearance of dermatitis. In **the induction phase**, Langerhans cells bind the hapten-carrier protein complex and present it in association with MHC class II antigens to T lymphocytes ($CD4^+$). Induction of cellular immunity to a contact skin sensitizer can occur within 7-10 days of first contact but it usually happens after many months or years of exposure to small amounts of antigens. Individual sensitivity varies according to the nature of the agent, its concentration and the genetic susceptibility of the person exposed. Re-exposure to the relevant antigen triggers **the elicitation phase** which produce dermatitis. In this phase, effector T lymphocytes carried via the circulation to the skin meet the antigen (composed of hapten complexed to carrier protein) presented by Langerhans cells and other antigen-presenting cells in the epidermis. Activation of T lymphocytes releases cytokines which cause induce skin inflammation, with keratinocytes proliferation, hyperplasia of the epidermis and consequent protective thickening.

Diagnosis

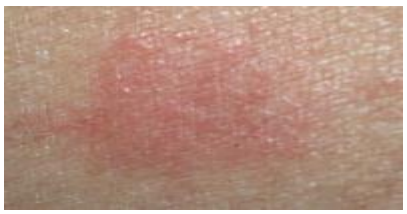
The diagnosis of contact dermatitis depends on **a careful medical history, the distribution of the lesions, and patch testing**. In the patch test, a suspected contact sensitizer is applied to normal skin (usually on the upper back) and covered for 48h. The reaction is read after 2 and 4 days. In a positive response, there is inflammation and induration at the test site.



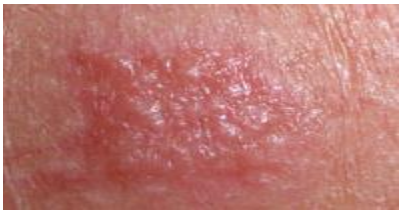
Irritant reaction



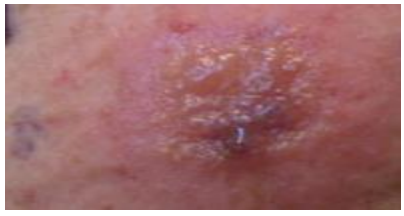
+/- reaction



+ reaction



++ reaction



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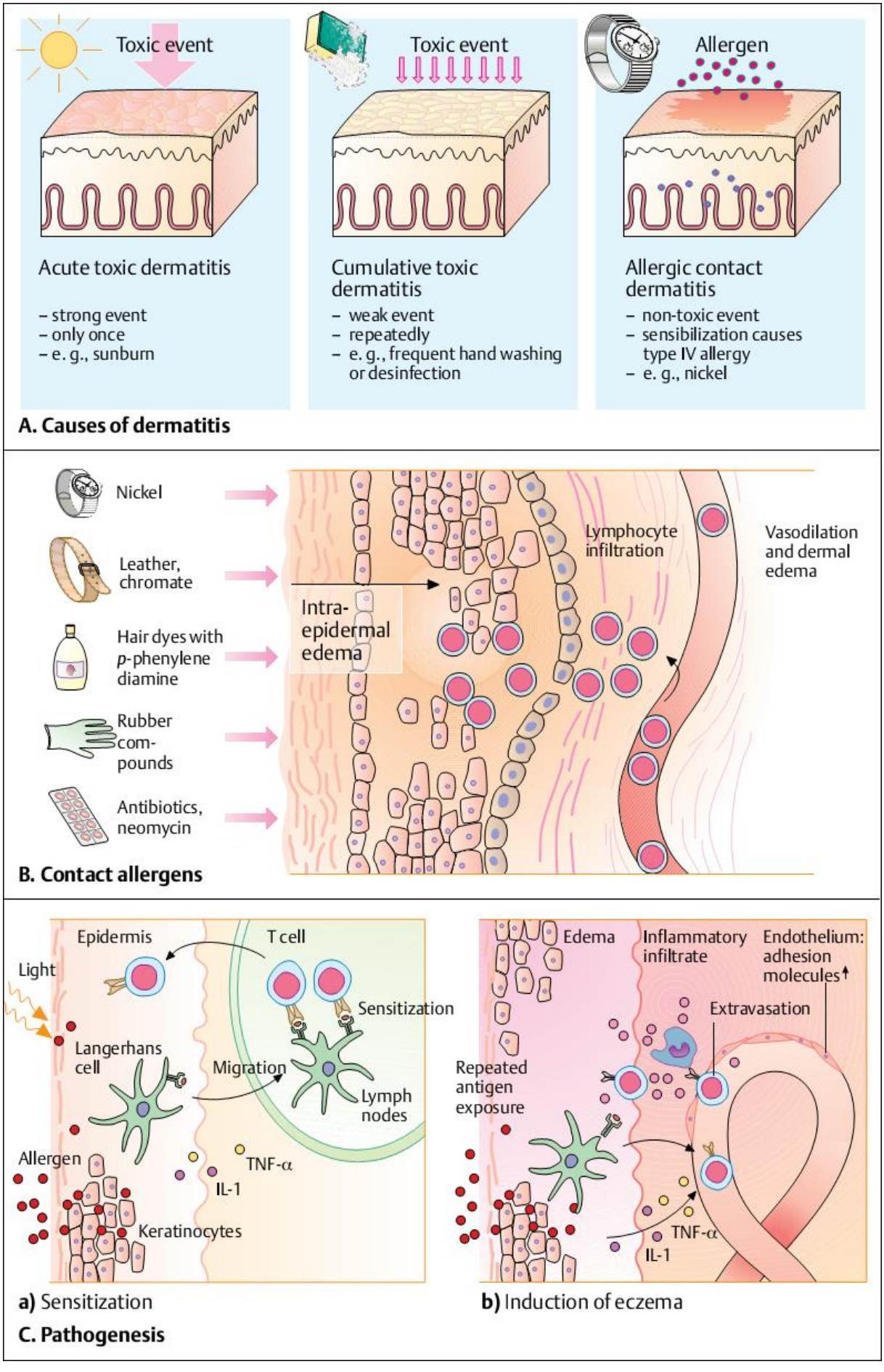
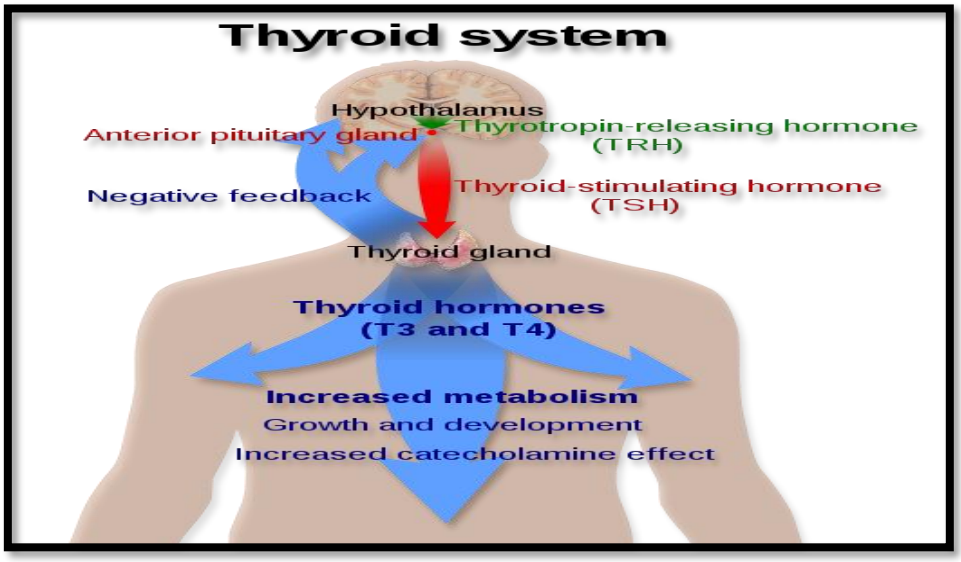

Toxic event
Acute toxic dermatitis
– strong event
– only once
– e. g., sunburn
Toxic event
Cumulative toxic dermatitis
– weak event
– repeatedly
– e. g., frequent hand washing or desinfection
Allergen
Allergic contact dermatitis
– non-toxic event
– sensibilization causes type IV allergy
– e. g., nickel
A. Causes of dermatitis
Nickel
Leather, chromate
Hair dyes with p-phenylene diamine
Rubber com-pounds
Antibiotics, neomycin
Intra-epidermal edema
Lymphocyte infiltration
Vasodilation and dermal edema
B. Contact allergens
Epidermis
T cell
Light
Sensitization
Langerhans cell
Migration
Lymph nodes
Allergen
TNF-α
IL-1
Keratinocytes
a) Sensitization
Edema
Inflammatory infiltrate
Endothelium: adhesion molecules
Extravasation
Repeated antigen exposure
TNF-α
IL-1
b) Induction of eczema
C. Pathogenesis

Lecture No. 25-26

Thyroid gland

The human thyroid gland is a major component of the endocrine system. Thyroid hormones perform many important functions. They exert powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance of the body, as well as on the maintenance of metabolic activity and the development of the skeletal and organ system.

The hormones Thyroxine (T4) and Triiodothyronine (T3) are secreted from the thyroid gland and regulated by a sensitive feedback system involving the hypothalamus and pituitary gland. The hypothalamus is an endocrine gland in the brain releases the thyrotropin releasing hormone (TRH), which stimulates the pituitary to release the thyroid stimulating hormone (TSH). This causes the thyroid to release T3 and T4 and these in turn regulate the release of TRH and TSH via a feedback control mechanism.



Synthesis of T3 and T4

Thyroglobulin (Tg) is a large tyrosine-rich protein bind to iodine after oxidation of iodide, a reaction catalysed by thyroid peroxidase (TPO) and release monoiodotyrosin (MIT) and di-iodotyrosine (DIT). Then peroxidase links 2 DIT to form T4 or one DIT and one MIT to form T3 released by thyroid gland cells in the circulation. In liver deiodinases enzymes covert about 1/3 of the T4 into T3.

Oxidation

Iodide $\xrightarrow[\text{TPO}]{}$ **Iodine +** **Thyroglobulin (Tg)** $\longrightarrow$ **Monoiodotyrosin + Di-iodotyrosine**

Monoiodotyrosine + Diiodotyrosine $\longrightarrow$ **Triiodothyronine (T3)**

Diiodotyrosine + Diiodotyrosine **Thyroxine (T4)**

T4 and T3 may stay free in plasma or bind to several serum proteins mainly; thyroid binding globulin (TBG), transthyretin and albumin. Only about 0.02% of T4 and 0.3% of T3 is free in plasma.

Thyrotoxicosis is a characteristic feature of overproduction of the thyroid hormone; Thyroxine (T4) and Triiodothyronine (T3). This is known as hyperthyroidism in which the level of these hormones increased above normal level of blood. Thyrotoxicosis can occur for other reasons as ingestion of exogenous thyroid hormone as tablets, toxic thyroid adenoma, thyroiditis and anti-arrhythmic drug.

Autoimmune thyroid disease is broadly classified into categories on the basis of the effect on gland function: **autoimmune hyperthyroidism** is seen in **Graves's disease** and **hypothyroidism** is seen in **Hashimotos thyroiditis**.

Patient's serum also containing different types of auto-antibody that directed against different thyroid self antigens mainly Tg, Tpo, thyroid growth stimulating Ig (TGI) and thyroid stimulating hormone receptor (TSH-R).

Two types of anti-TSH-R antibodies may exist in the patient serum:

1. Stimulatory (Thyroid Stimulating Ig or TSI).
2. Blocking or inhibitory (Thyroid Binding Inhibitory Ig or TBII).

Graves's disease

It is the most common cause of hyperthyroidism which driven by an autoimmune mechanism. Graves's disease has a peak incidence in the 3rd and 4th decades and is found in approximately 0.1-0.5 % of the general population. It is more common in women than man (7:1). Predispositions to Graves's disease include living in an area of high iodine intake, female sex, stress and possession of HLA-DR3, which confer a relative risk of disease.

In Graves's disease the dominant type of anti-TSH-R Abs is the TSI, however, the presence of both types of Ab (TSI &TBII) in some patients may explain the fluctuation from over activity to under activity of the gland. The TSI mimic the TSH in its action, even more, it has a more prolonged action on the activation of thyroid gland cells than TSH do.

Pathogenesis of Graves's disease depends on the humoral and cell-mediated immunity participation. However, T cells (T_H2, $CD8^+$) are responsible for the glandular thyroid T cells infiltration, whereas the antibodies are acting as a disturbing factor for the normal physiological function of the gland. A clear application for that is the autoimmune

syndrome in neonates which is caused by the transplacental transfer of IgG which cause a transient disturbance in the endocrine physiology that disappears with time after birth in proportion with the half-life time of IgG without any significant damage of the target organs.

The level of thyroid Abs in pregnant women with **Graves's disease** and **Hashimotos thyroiditis** decreased during pregnancy, but increased again after word.

Symptoms

Patients typically present with the symptoms or signs of hyperthyroidism (palpitations, tachycardia, arrhythmias, heat intolerance, increased appetite with weight loss, diarrhea, weakness and proximal myopathy, nervousness and tremor). One characteristic feature of Graves's disease is eye disease characterized by protrusion of the eyeball and lid retraction resulting from tissue inflammation in the retro-orbital space. Goiter which is an enlargement or hypertrophy of the thyroid tissue is of diffuse pattern in Graves's disease.

Diagnosis

Laboratory findings are of

1. Elevated thyroid hormones- thyroxine (T4) and triiodothyronine (T3)- with suppressed levels of thyroid stimulating hormone(TSH).
2. Measuring of auto-Abs

- Anti-TSH-R in most case (TSI, TGI).
- Anti-TPO in 50% of cases (more common in **Hashimotos thyroiditis)** and anti-Tg in lesscases.
- Thyroid growth stimulating Ig (TGI) is seen in the serum of Graves's disease patient with goiter and in some patients with toxic multinodula and non-toxic goiter. The titer of these Ab is correlated with the size of goiter, but not associated with the level of T4 and T3 as the case with TSI in which there is strong association between the high level of T4 and T3 and the level of TSI.

3. The radioactive iodine uptake test and thyroid scan test. The uptake test uses radioactive iodine (I-123) injected or taken orally on an empty stomach to measure the amount of iodine absorbed by the thyroid gland. Person with hyperthyroidism absorb too much iodine. The thyroid scan producing images is typically conducted in connection with the uptake test to allow visual examination of the over-functioning gland.

Hashimotos thyroiditis

Hashimotos thyroiditis (autoimmune thyroiditis) is a chronic disease typically characterized by enlargement (goiter) and dense lymphatic infiltration of the thyroid gland. It is four times more common in women and has incidence of approximately 0.5% in the general population; the incidence peaks in middle age.

Causes

- HLA-DR5 gene most strongly implicated conferring a relative risk. In addition, HT may be associated with polymorphism of CTLA-4 gene.
- Environmental factors (high iodine intake, infection as chronic HCV, certain drugs, exposure to radioactive isotypes, presence of other autoimmune diseases as celiac disease and type 1 diabetes.

Pathogenesis of **Hashimotos thyroiditis** depends on the humoral and cell-mediated immunity participation. However, T cells ($CD4^+$ (T_H1), $CD8^+$) are responsible for the destruction of thyroid tissue that targeting the auto antigens Tg and Tpo. Microbial mimicry by viral or bacterial antigens may drive this destructive mechanism.

Symptoms

Patients usually complain of goiter as the main symptom, with an enlarged, firm, sometimes nodular thyroid gland on examination. At presentation, patients may still be euthyroid, but with time the pathological processes result in loss of thyroid tissue and hypothyroidism. Symptoms and signs of hypothyroidism may be seen at the first consultation (fatigue, cold intolerance, dryness of skin, anorexia, weight gain, menstrual disturbance, huskiness of voice, mental slowing, abnormal reflexes).

Diagnosis

1. Low level of free T3 and free T4.
2. Low level of total T3 and free T4.
3. High level of TSH.
4. Measuring of auto-Abs
   - Anti-Tg Ab is found in 90% of cases.
   - Anti-TPO Ab correlate with the severity of the disease.

   Both Abs contribute in decreasing the uptake of iodine leading to hypothyroidism.

- Other auto-Ab are detected (anti- TSH-R blocking Abs(low), anti-thyrotropin-R Ab, anti-second colloid Ag) these antibodies have an inhibitory effect on the production of the thyroid hormones.

Graves's disease	Hashimotos thyroiditis
1. Hyperactivity	1. Fatigue, lethergy
2. Weight loss with increase of Appetite	2. Weight again
3. Heat intolerance	3. Cold intolerance
4. Thirst/polyuria	4. Dry coarse skin
5. Diffuse goiter	5. Rubbery, nodular goiter
6. Ophthalamopathy, eyelid Retraction, exophthalmous, peri-orbital odema	6. Facial edema (myxodema)
7. Tachycardia	7. Mostly bradycardia
8. Free T3 ↑ Free T4 ↑ Total T3 ↑ Total T4 ↑ Anti-Tg N(rarely ↑ in few cases) Anti-TPO N (slightly in 50% of cases) Anti-TSH-R ↑ TSH ↓	8. Free T3 N- ↓ Free T4 ↓ Total T3 N- ↓ Total T4 ↓ Anti-Tg ↑ Anti-TPO ↑ Anti-TSH-R N TSH ↑
9. Treatment Anti-thyroid drugs Radioactive iodine Thyroidectomy	9. thyroxine
10. TH2	10. TH1

Lecture No. 27-28

Tumor

Tumor is an overgrowth (uncontrolled growth) of tissues and cells in certain organs in the body which result in a mass of tissue that has result in destruction of normal architecture of the tissue and lost the normal function of the healthy original tissue.

Tumor can be generally classified into

1. **Benign tumor:** cluster of tumor cells that are localized in a restricted area in the body without the ability to move to other areas in the body.
2. **Malignant tumor:** cluster of tumor cells that can move and invade (**metastasis**) other adjacent and far away tissues.

Metastasis means spreading of the invasion tumor cells from the primary focus of tumor to other parts of the body via blood or lymph circulation to form a secondary focus of tumor.

Tumor classifications

A. According to the involved tissue:
   1. **Carcinoma:** Tumor of epithelial cells.
   2. **Sarcoma:** Tumor of muscle and connective tissues.
   3. **Adenoma:** Tumor of the glandular tissue which is benign.
   4. **Adenocarcinoma:** Tumor of the glandular tissue which is malignant.

B. According to the system or organ:
   1. **Lymphoma:** Tumor of lymph nodes.
   2. **Leukemia:** Tumor of the blood, bone marrow and immune system.
   3. **Hepatocarcinoma:** Tumor of the liver.
   4. **Astrocytoma, gilioma, retinoblastoma, neuroblastoma:** Tumor of the central nervous system.

Causes

1. **Environmental:** there are so many environmental carcinogenic factor including:
   A. Chemical carcinogens: including wide range of food preservatives, dyes, smokes and many others.
   B. Physiological carcinogens: including UV light, X ray, nuclear radiation and many others.
   C. Biological carcinogens: including mostly viruses and some bacteria:
      1. Hepatitis B virus (HBV) and (HCV) can cause hepatocellular carcinoma in chronically infected persons.
      2. Epstein Barr virus (EBV) can cause Burkitt's lymphoma.
      3. Human T lymphocyte virus-1 (HTLV-1) can cause T cell leukemia.
      4. Human papilloma virus (HPV) can cause cervical carcinoma in women.
      5. Helicobacter pylori can cause gastric carcinoma.
      6. Human herpes virus-8 (HHV-8) can cause Kaposi sarcoma in AIDS patients.
2. **Genetic:** many of the people with tumors have certain genetic constitutions which indicate a familial association of tumors like breast cancer and ovarian cancer.
3. **Hormonal:** hormonal changes (mainly in women) may trigger certain types of tumors. The best example of that is the breast cancer which occurs more commonly in women with
   1. Pregnancy at old ages (>35 years).
   2. Early age of menstruation.
   3. Late onset of menopause.
   4. Post menopausal hormone replacement therapy.

Tumor antigens

In tumor, there are many antigens (which they are not found normally or found in different forms or quantities) are expressed on the tumor cells surface or generalized inside the tumor cell. The earliest classification of tumor antigens was based on their patterns of expression.

1. **Tumor-specific antigens:** Antigens that are expressed on tumor cells but not on normal cells were called tumor-specific antigens; some of these antigens are **unique** to individual tumors **(chemical carcinogens)**, whereas others are **common**, shared among tumors of the same type **(viral carcinogens)**.
2. **Tumor-associated antigens:** Many of tumor antigens that expressed in case of tumor could also expressed in normal cells but either with low level or at different development stage. For examples
   A. **Alpha-feto protein (αFP):** is a protein that secreted by the liver of embryo, but it should be disappear after birth. When it appears again in adult serum it may indicate liver and gonadal (testes) cancer.
   B. **Carcino-embryonic antigen(CEA):** this types of antigens is expressed in certain tumors of the gastrointestinal tract (as colonic cancer),however it can also expressed in the patient serum with inflammation of pancreas (pancreatitis) or inflammation of colon (colitis). The presence of CEA is not specific to the tumor only and not diagnostic. However it can used to monitor the response to therapy against colonic cancer by measuring its level in the patient serum.
   C. **Cancer testes antigens:** these antigens are expressed normally in the tissue of the testes, but when expressed in other tissue as lung or breast it indicates tumors of these organs.
   D. **Mutated antigen:** certain antigens are found normally in the body, but when they mutated they change into tumor antigens example: protein 53 (P53) this protein normally inhibit cellular proliferation.
   E. **Melanoma-melanocyte differentiation antigens:** certain enzymes like tyrosinase enzyme is found normally in skin cells (melanocytes) in small amount, but it also found in skin tumor cells (melanoma) in large amount (quantitative difference). **Example (tyrosinase enzyme, melanoma antigen recognized by T cells)**

Immune responses to tumors

Innate immunity

The role of **macrophages** in anti-tumor immunity is largely inferred from the demonstration that invitro, activated macrophages can kill many tumor cells more efficiently than they can kill normal cells.

NK cells kill many types of tumor cells, especially cells that have reduced class I MHC expression and can escape killing by CTLs. Invitro, NK cells can kill virally infected cells and certain tumor cell lines, especially hematopoietic tumors.

Adaptive immunity

The effector mechanisms of both cell-mediatedimmunity and humoral immunity have been shown to kill tumor cells in vitro.

Cell-mediated immunity

The principal mechanism of tumor immunity is killing of tumor cells by CD8 CTLs.

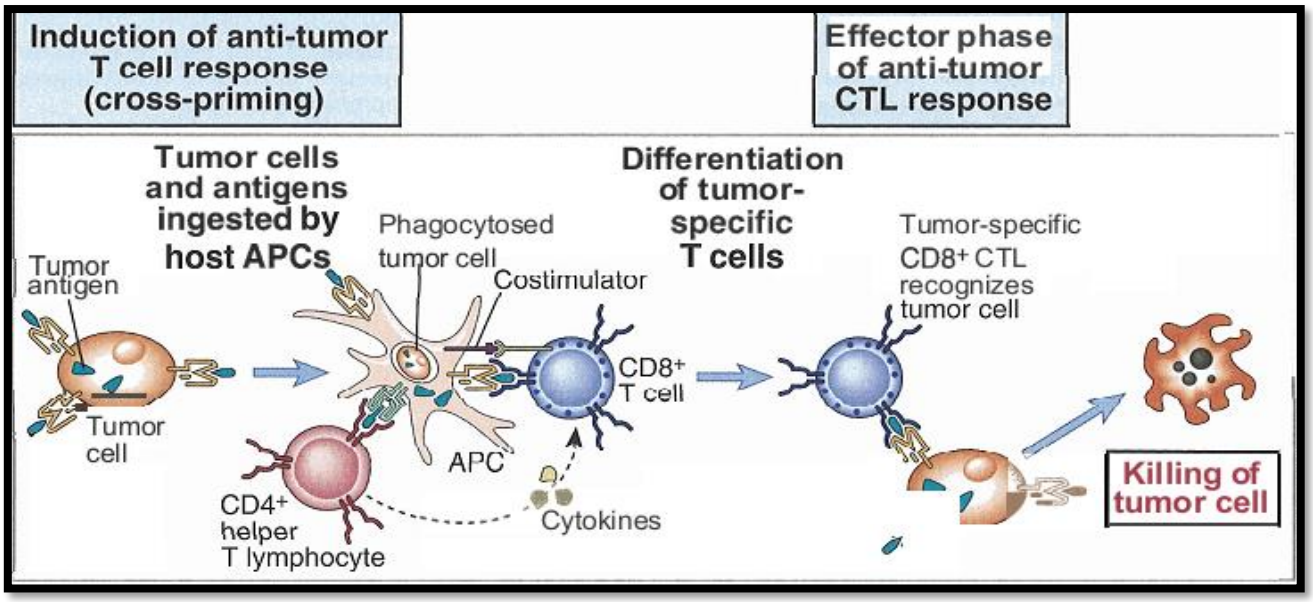


Figure: lnduction of T cell responses to tumors. antigens.

Humoral immunity

Tumor-bearing hosts may produce antibodies against various tumor antigens. For example, patients with serum antibodies against EBV encoded antigens expressed on the surface of the lymphoma cells. Antibodies may kill tumor cells by activating complement or by antibody-dependent cell-mediated cytotoxicity, in which Fc receptor-bearing macrophages or NK cells mediate the killing. However, the ability of antibodies to eliminate tumor cells has been demonstrated largely invitro, and there is little evidence for effective humoral immunity against tumors.

Evasion of immune responses by tumors

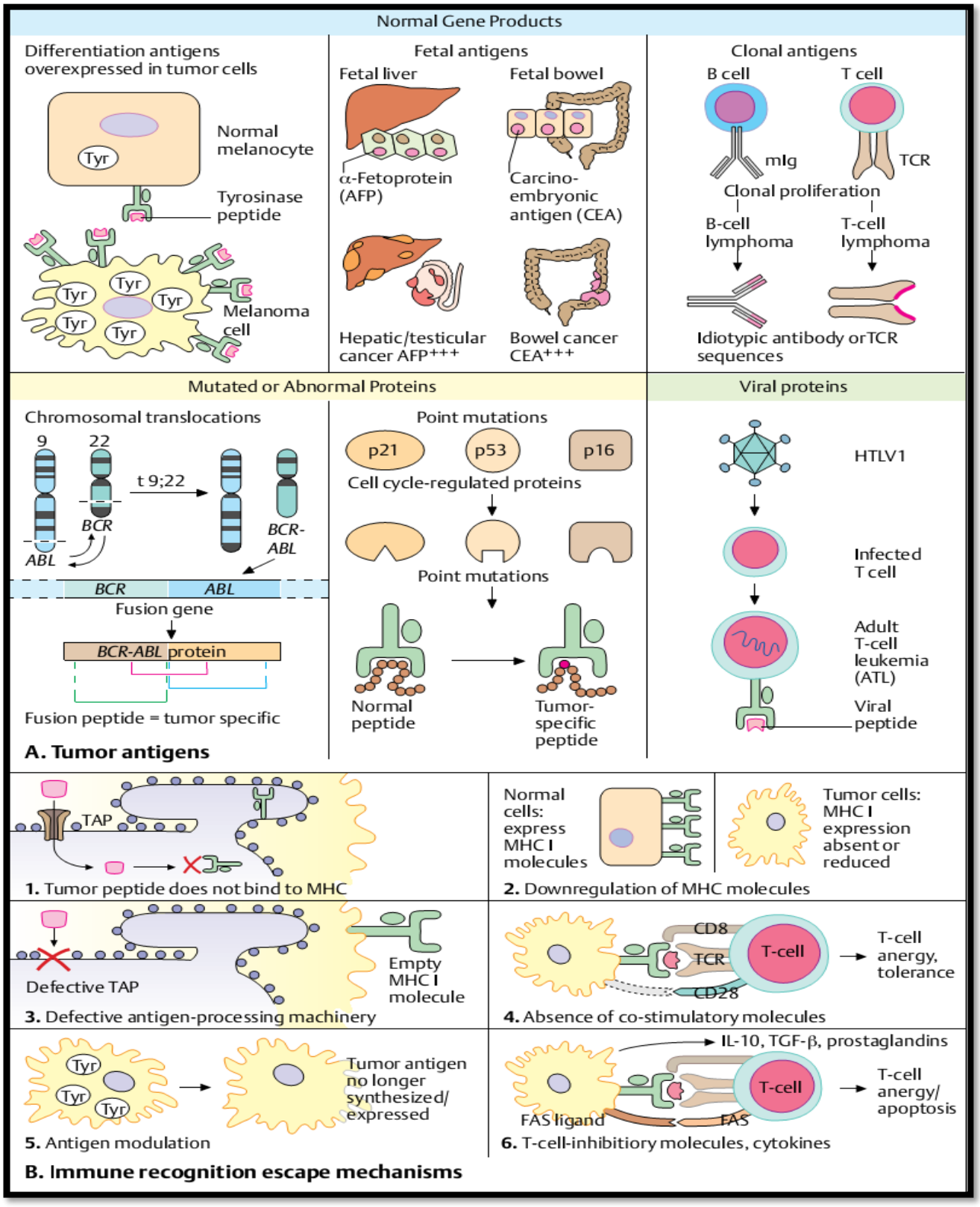
Many malignant tumors possess mechanisms that enable them to evade or resist host immune responses:

1. Class I MHC expression may be down-regulated on tumor cells so that they cannot be recognized by CTLs.
2. Tumors lose expression of antigens that elicit immune responses.
3. Tumors may fail to induce CTLs because most tumor cells do not express costimulators or class II MHC molecules.
4. The products of tumor cells may suppress anti-tumor immune responses.
5. Tumor antigens may induce specific immunologic tolerance.

Diagnosis

1. **Histopathology:** by taking a biopsy of the tumor mass and recognize the transforming cells.
2. **Enzymatic:** measuring certain enzymes that may change during the course of the disease.
3. **Clinical presentation.**
4. **Immunodiagnosis:** in many occasions immunodiagnosis is more sensitive and can diagnosis tumor more early than other diagnostic methods. This method depends basically on the detection of tumor antigens (markers) in the patient serum or tissue biopsy. Generally, there are two method for that:
    1. **In vivo method** which means injecting of radiolabelled mAb against suspected tumor marker in the body then to follow any binding reaction between the Ab and the Ag inside the body by a device called immune-scintigraphy. This method is used to monitor the level of CEA in people with colonic cancer.
    2. **In vitro method** which means taking sample (blood or tissue) from the patient and reacting it with the specific Ab for the expected tumor marker. This method is used to detect α-feto protein in the serum of patients with suspected liver tumors.

Other application for this method is to use an anti-MHC-class I Ab with breast biopsy to detect the presence or absence of MHC-class I molecules on breast cells. Breast cells with tumor are usually lack the presence of these molecules on their surfaces (IFAT method).


Normal Gene Products
Differentiation antigens overexpressed in tumor cells
Tyr
Normal melanocyte
Tyrosinase peptide
Melanoma cell
Fetal antigens
Fetal liver
Fetal bowel
α-Fetoprotein (AFP)
Carcino-embryonic antigen (CEA)
Hepatic/testicular cancer AFP+++
Bowel cancer CEA+++
Clonal antigens
B cell
T cell
mIg
TCR
Clonal proliferation
B-cell lymphoma
T-cell lymphoma
Idiotypic antibody or TCR sequences
Mutated or Abnormal Proteins
Chromosomal translocations
9
22
t 9;22
BCR
ABL
BCR-ABL
Fusion gene
BCR-ABL protein
Fusion peptide = tumor specific
Point mutations
p21
p53
p16
Cell cycle-regulated proteins
Point mutations
Normal peptide
Tumor-specific peptide
Viral proteins
HTLV1
Infected T cell
Adult T-cell leukemia (ATL)
Viral peptide
A. Tumor antigens
TAP
1. Tumor peptide does not bind to MHC
Normal cells: express MHC I molecules
Tumor cells: MHC I expression absent or reduced
2. Downregulation of MHC molecules
Defective TAP
Empty MHC I molecule
3. Defective antigen-processing machinery
CD8
TCR
T-cell
CD28
T-cell anergy, tolerance
4. Absence of co-stimulatory molecules
Tumor antigen no longer synthesized/ expressed
5. Antigen modulation
IL-10, TGF-β, prostaglandins
T-cell
FAS ligand
FAS
T-cell anergy/ apoptosis
6. T-cell-inhibitory molecules, cytokines
B. Immune recognition escape mechanisms

Lecture No. 29-30

Transplantation

Transplantation is the process of taking cells, tissues, or organs, called **a graft**, from one individual and placing them into a different individual.A graft transplanted between two genetically different individuals of the same species is called an **allogeneic graft (or allograft)**.

Transplantation of tissues from one individual to a genetically nonidentical recipient leads to a specific immune response called **rejection** that can destroy the graft. The antigens recognised during rejection are referred to as **alloantigens**. The key alloantigens are those encoded by the MHC. In humans these are known as **HLA** molecules is to present peptide antigen to a complementary T cell receptor.

Allogeneic MHC molecules may be presented on donor APCs to recipient T cells (**direct allorecognition**), or the alloantigens may be picked up by host APCs that enter the graft or reside in draining lymphoid organs and be processed and presented to T cells as peptides associated with self MHC molecules (**indirect allorecognition**).

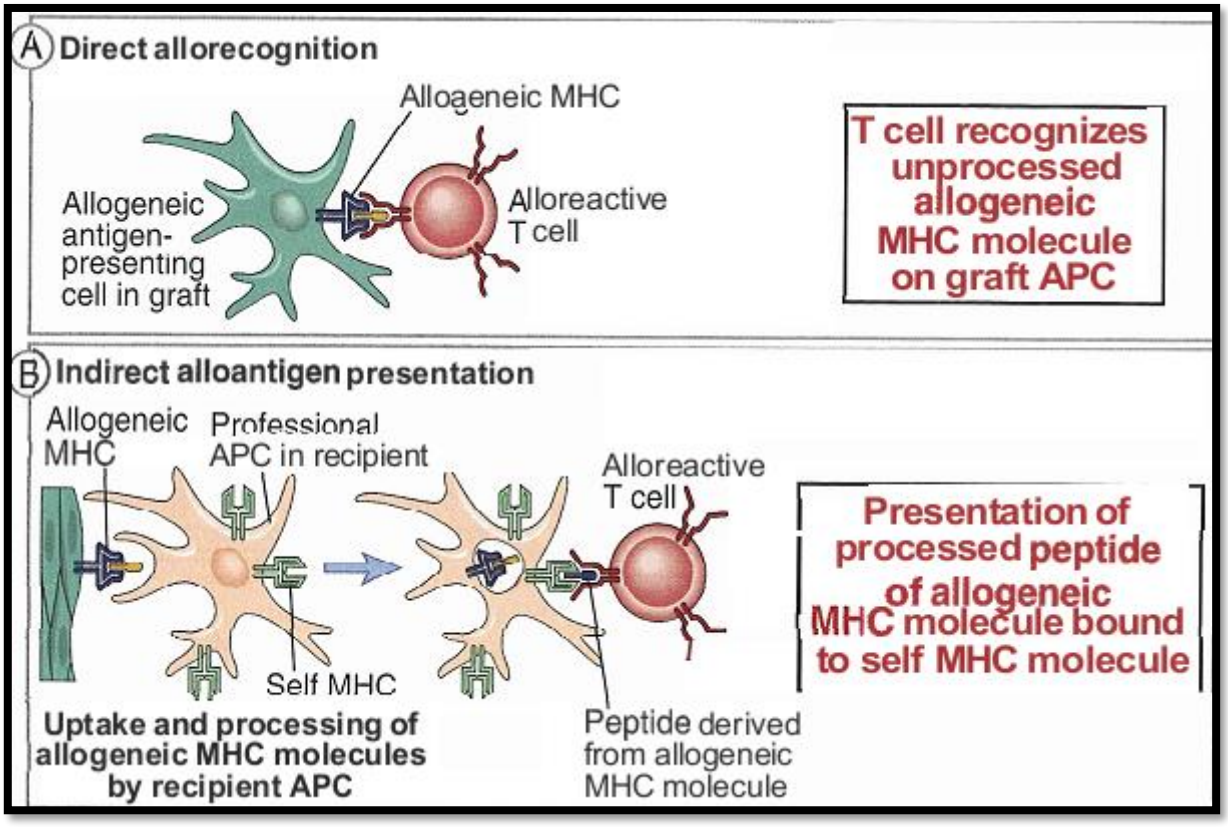


Figure: Direct and indirect alloantigen recognition.

Types of transplant

- **Autograft or autologous transplant:** the organ/tissue is transplanted within the same individual. It does not undergo rejection.
- **Syngraft or syngeneic transplant:** the organ/tissue is transplanted between genetically identical subjects, such as monozygotic twins or inbred laboratory animals. It does not undergo rejection.
- **Allograft or allogeneic transplant:** the organ/tissue is transplanted between genetically non-identical members of the same species. It is rejectes unless immunosuppression is instituted.
- **Xenograft or xenogeneic transplant:** the organ/tissue is transplanted between members of the different species. It is rejected hyperacutely.

Classification of rejection

Rejection can be classified according to the timescale of its appearance and to the immune mechanism involved (see Table.1)**.**

Table.1 Classification of rejection

Type	Time after transplantation	Probable mechanism
Hyperacute	Minutes	Preformed antibodies
Accelerated acute	1-5 days	T lympocytes
Acute	From 2nd	T lympocytes
Chronic	Months or years	Antibodies, complement, adhesion molecules

In classical immune responses, it is the balance between the different components of the immune system that decrees the magnitude and manifestation of the rejection process. Once a naïve helper CD4 cell designated to T_H0 has recognized as alloantigen, presented by a professional antigen-presenting cell such as a dendritic cell, which is singularly competent in providing the co-stimulation signals needed to arouse naïve cells, it can become either a T_H1 or a T_H2 cell according to the microenvironment it encounters and the nature of the alloantigenic stimulus. If the surrounding medium is rich in IL-12 a macrophage-derived cytokines, the naïve T_H0 CD4 cell will commit itself to the T_H1 phenotype and function and orchestrate the activation of CD8 cytotoxic T cells and of macrophage through the release of IL-2 and IFN-γ. If ,however, the prevailing cytokine is IL-4, the naïve CD4 cell will differentiate into the T_H2 phenotype, and though the secretion of IL-4 and IL-10 will direct the activation of B lympocytes and antibody production.

Graft-versus-host reaction

Immunocomponent cells from thegraft recognise alloantigens of the recipient and the recipient develops a disorder known as **the graft-versus-host (GVH) reaction**. This reaction is common after transplantation of **bone marrow**, even when the matching between donor and recipient has been stringent. When GVH becomes symptomatic the term **graft-versus-host disease (GVHD)** is more appropriate. GVHD has been described not only following bone marrow transplantation but also, occasionally, after **liver transplantation** and even after **blood transfusions**. GVHD can be divided into two distinct entities: **acute disease**, occuring in the first 1 or 2 months after transplantation, and **chronic disease**, developing at least 2 or 3 months after transplantation.

In humans, GVHD typically affects **the skin, liver, intestinal tract and immune system** and appears within days or weeks after bone marrow transplantation. In **mild GVH** reactions, patients manifest erythema of the palms, soles and ears. Hepatic signs of mild reactions are limited to asymptomatic hyperbilirubinaemia, and gastrointestinal involvement is indicated by mild diarrhoea, in the case of **sever GVHD**, the skin lesions can include a necrolytic disorder, characterized by blister formation and desquamation. Severe liver abnormalities include jaundice, elevation of alkaline phosphatase, which denotes cholestasis, and of transaminase, a sign of liver cell damage. Sever gastrointestinal GVHD includes abdominal pain and diarrhea, with life-threating electrolyte abnormalities. These manifestations are the result of injury to the epithelial cells of the target organs. Mild GVH may resolve spontaneously or with mild immunosuppressive treatments. Sever GVH is usually unresponsive to treatments and has a fatal outcomes.