โครงการหนึ่งอาจารย์หนึ่งผลงาน ประจำปี พ.ศ. 2552

เอกสารประกอบการสอนรายวิชา

108303 ภูมิคุ้มกันวิทยาทางการแพทย์

(MEDICAL IMMUNOLOGY)

จำนวน 5 บท

หลักสูตรแพทยศาสตร์

โดย

ผศ. ทนพญ. ดร. วิไลรัตน์ ลื้อนันต์ศักดิ์ศิริ

สำนักวิชาวิทยาศาสตร์

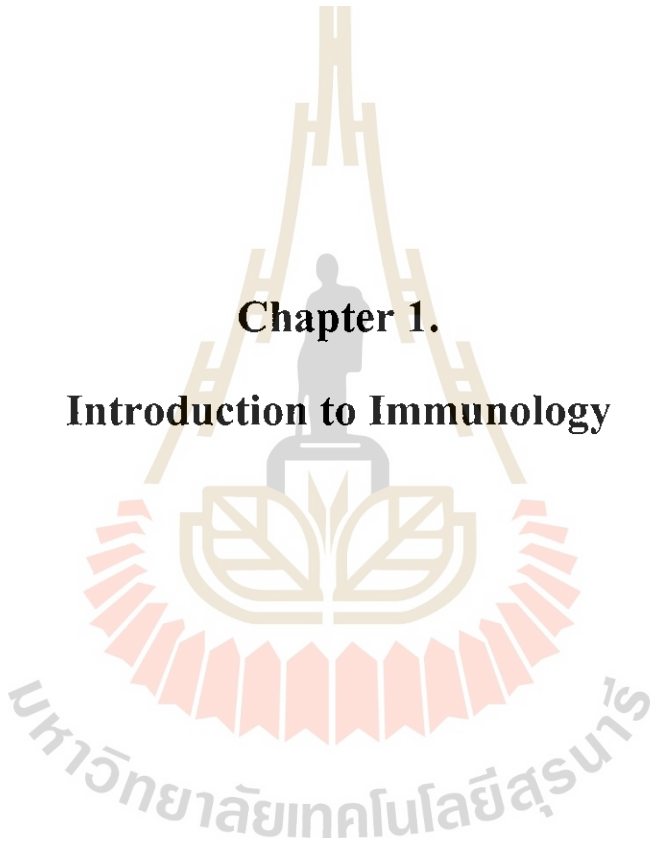
มหาวิทยาลัยเทคโนโลยีสุรนารี

Table of Contents

Topics

Chapter 1.

Introduction to Immunology



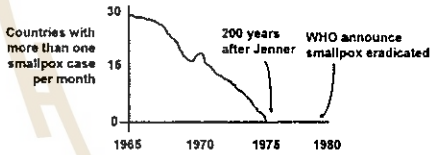
Introduction to Immunology

Asst. Prof. Dr. Wilairat Leeanansaksiri

Overview

Edward Jenner.

History & impact of immunology on human health



Why study immunology now?

Infectious diseases

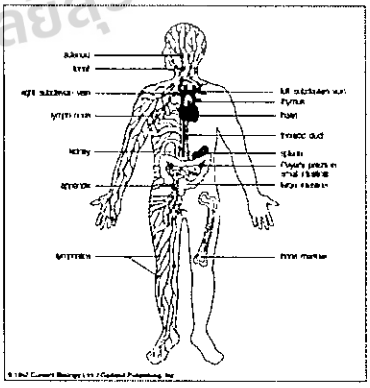
Mechanisms of pathogenicity
Vaccine development

Diseases caused by a disturbed immune system

ALLERGY: Immune responses to innocuous materials e.g. ASTHMA
AUTOIMMUNITY: Anti-self immunity e.g. MULTIPLE SCLEROSIS
GRAFT REJECTION: Immune responses to TRANSPLANTED TISSUE
IMMUNODEFICIENCY: Defects in immune responses e.g. SCID

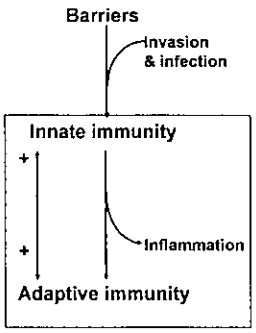
Manipulation of immunity to treat disease

IMMUNOSUPPRESSION: Treatment of immune diseases
IMMUNOREGULATION: Immunotherapeutic interventions



The distribution of lymphoid tissues in the body.

Immune responses

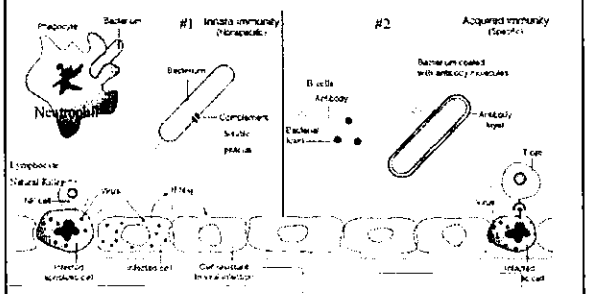


Skin & Mucous membranes
rapidly regenerating surfaces, peristaltic movement, mucociliary escalator, vomiting, flow of urine/tears, coughing

Cellular and humoral defences
lysosyme, sebaceous/mucous secretions, stomach acid, commensal organisms,complement proteins, phagocytosis, NK cells

Cellular and humoral defences
Antibodies, cytokines, T helper cells, cytotoxic T cells

An overview of some of the mechanisms by which the immune system rids the body of invading pathogens



1 Non Specific
2. inflammation

Highly Specific 2 types
1 Humoral (Abs) Extracellular
2. Cell-mediated (T-cells)

หน้าที่ของระบบภูมิคุ้มกันของร่างกาย แบ่งออกได้เป็น 3 ข้อใหญ่ๆ คือ

•**1. Defense** คือ หน้าที่หลักของระบบภูมิคุ้มกัน โดยมีหน้าที่ในการป้องกันการติดเชื้อของร่างกาย ทำลายสิ่งแปลกปลอมที่เข้าสู่ร่างกายทุกชนิด

•**2. Homeostasis** คือ หน้าที่ในการกำจัดเซลล์ปกติของร่างกายที่ใช้งานไม่ได้แล้ว โดยรักษาสภาวะสมดุลย์ของเซลล์ในร่างกายกำจัดเซลล์หรือเนื้อเยื่อที่หมดอายุ

•**3. Surveillance** ทำหน้าที่คอยสอดส่องดูแลการผิดปกติของเซลล์ต่างๆ ในร่างกาย ตลอดจนคอยกำจัดเซลล์ที่เปลี่ยนแปลงผิดไปจากปกติ เช่น เซลล์เนื้องอก

คุณสมบัติที่สำคัญของระบบภูมิคุ้มกัน

•**Diversity**

•**Specificity**

•**Memory**

•**Self regulation**

•**Self / non self discrimination**

Immune Syetem

•**Non specific Immune Response (Innate Immunity, Natural Immunity)**

•**Specific Immune System (Acquired Immunity)**

- •Humoral Immune Response (HIR)
- •Cell Mediated Immune Response (CMIR)

Innate immune response

Inbuilt immunity to resist infection

- Present from birth
- Not antigen-specific
- Not enhanced by second exposure
- Has no memory
- Is poorly effective without adaptive immunity

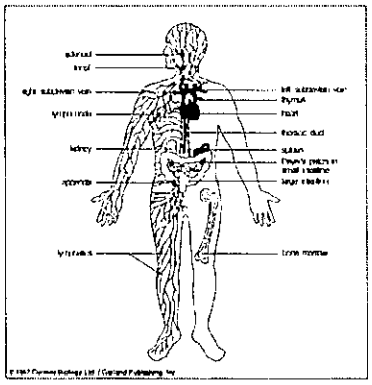
Also involved in the triggering and amplification of adaptive immune responses

Adaptive immunity

Immunity established to adapt to infection

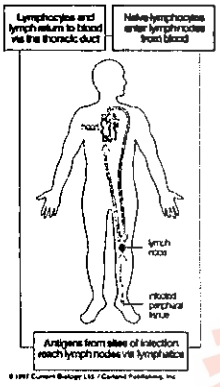
- Learnt by experience
- Confers pathogen-specific immunity
- Enhanced by second exposure
- Has memory
- Uses cellular and humoral components
- Is poorly effective without innate immunity

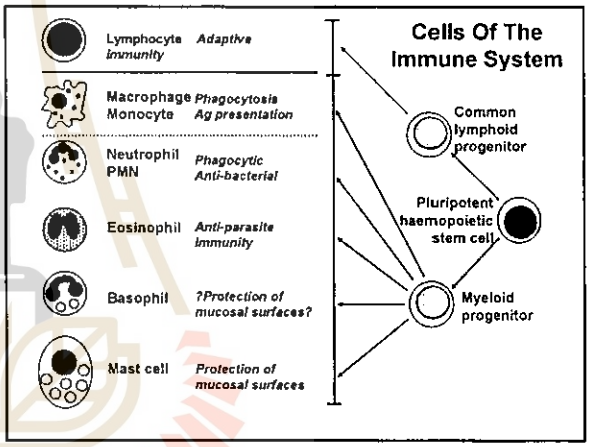
Antibodies reflect infections to which an individual has been exposed- diagnostic for infection



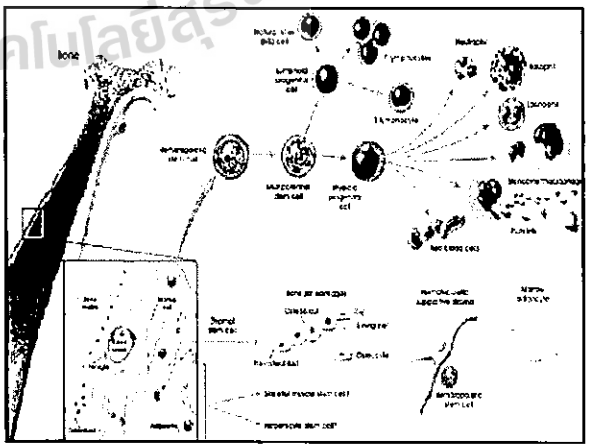
The distribution of lymphoid tissues in the body.

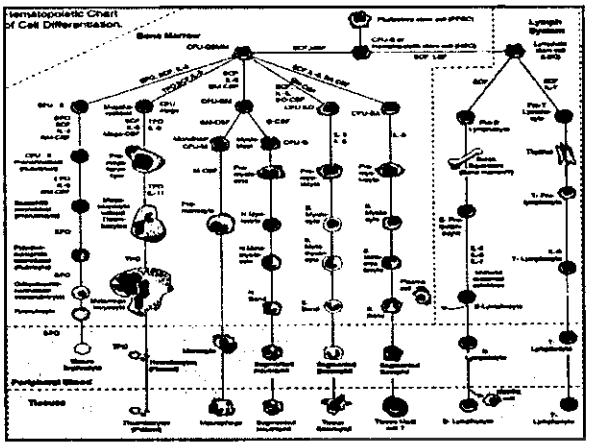
Circulating lymphocytes encounter antigen in peripheral lymphoid tissues.

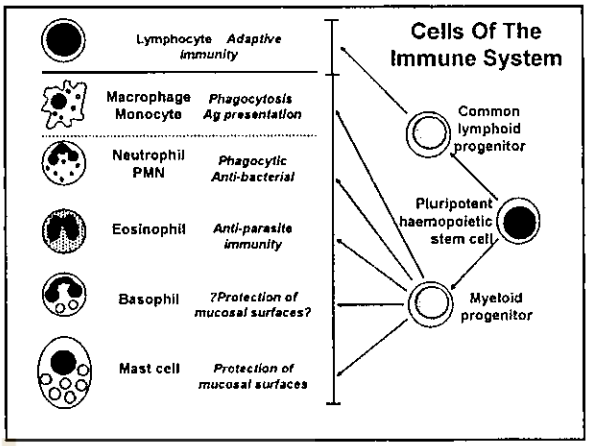


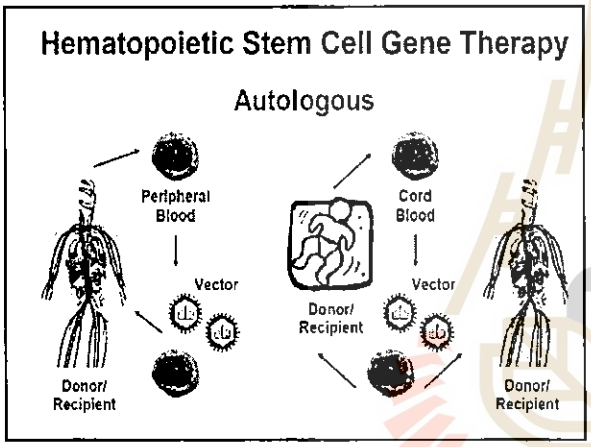


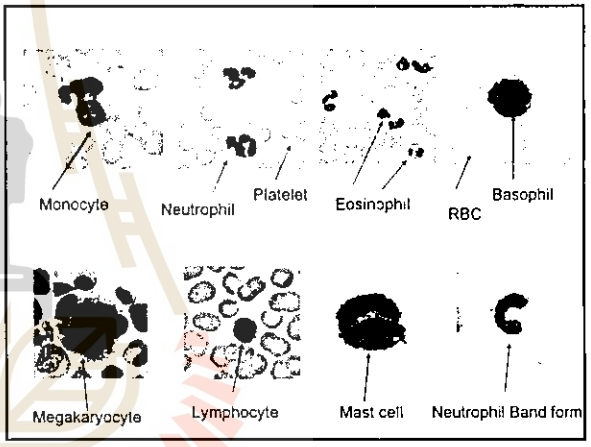
Hematopoiesis And Immune Cells

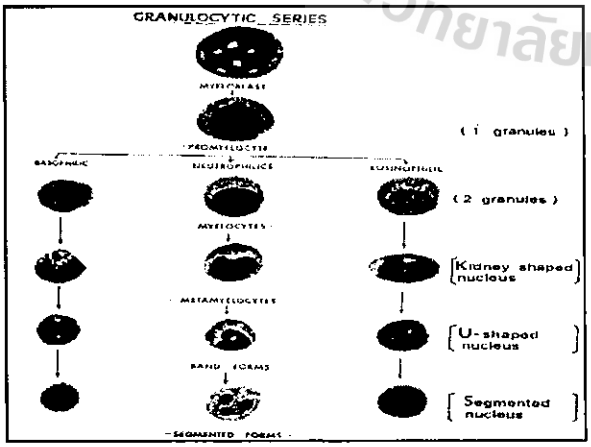


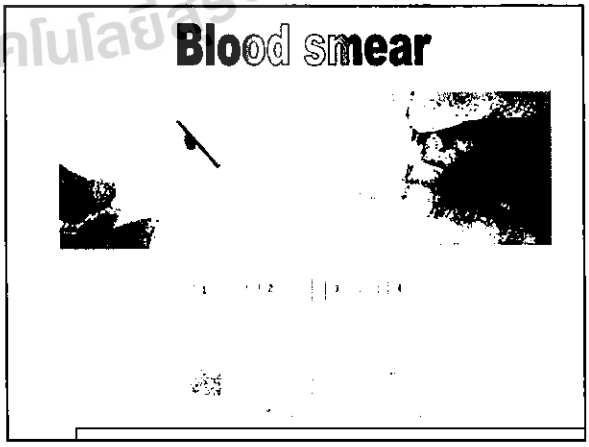


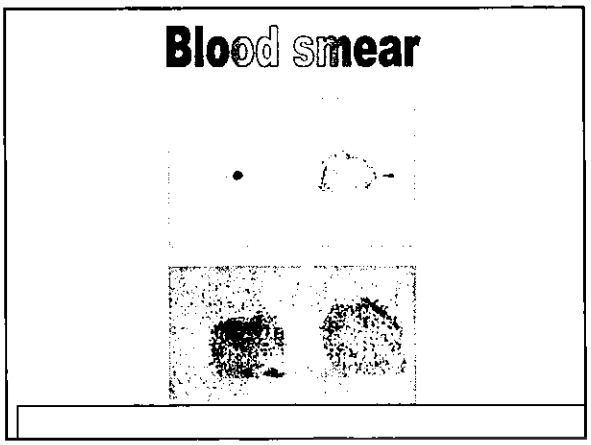


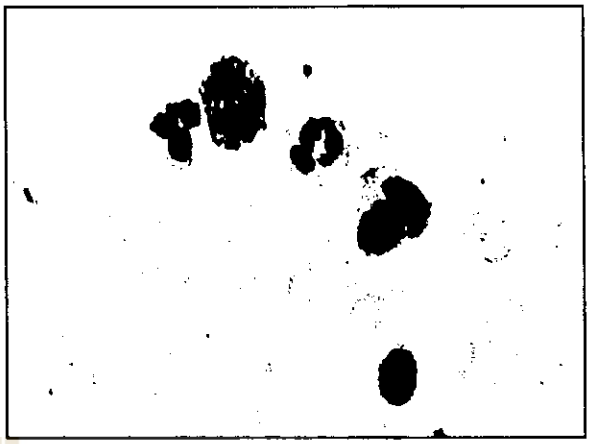


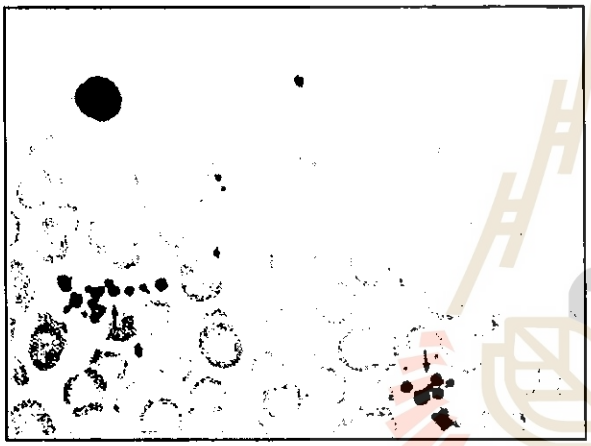


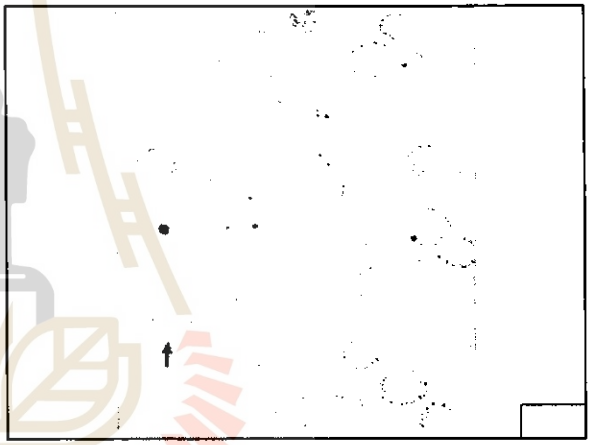












ABO System

Blood group	Antigen	Antibody	Agglutination with
A	A	Anti-B	Anti-A
B	B	Anti-A	Anti-B
O	-	Anti-A, Anti-B	-
AB	A and B	-	Anti-AB

Rh System

Rh^+ Antigen D

Rh^- No Antigen D

White people : 85% Rh^+ 15% Rh^-

Thai people : 99.9% Rh^+ 0.1% Rh^-

Leukocytes

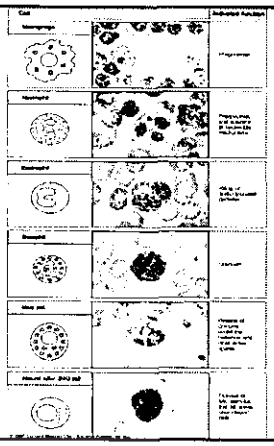
Adaptive and innate immunity depends upon LEUKOCYTES

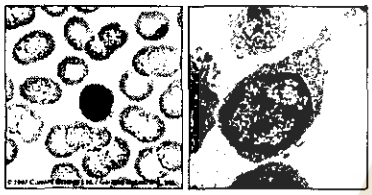
Innate immunity is mediated largely by GRANULOCYTES

Adaptive immunity mediated by LYMPHOCYTES

The growth, development and activities of granulocytes and lymphocytes are interconnected and often co-operative.

Myeloid cells in innate and adaptive immunity.

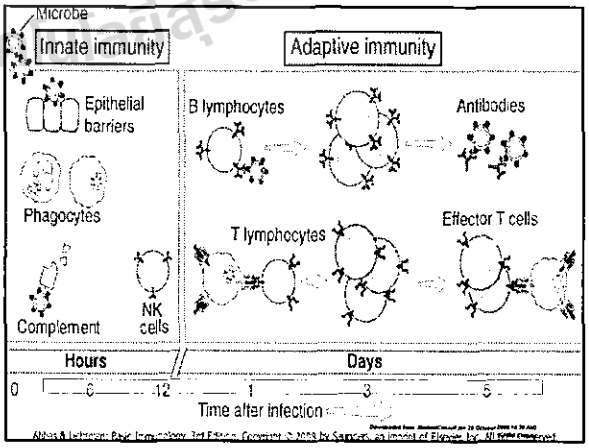




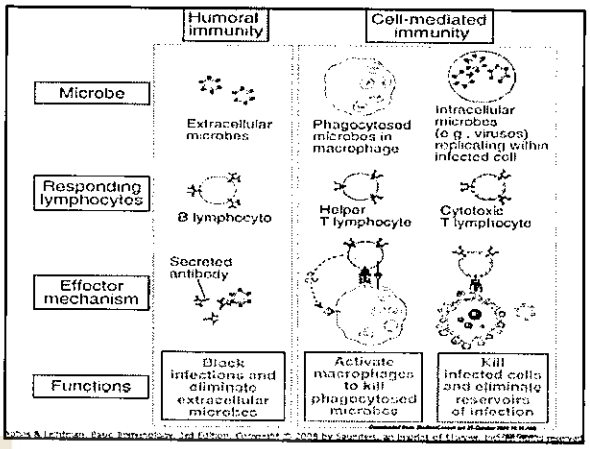
Small lymphocytes are cells whose main feature is inactivity.

Role of the immune system	Implications
Defense against infections	Deficient immunity results in increased susceptibility to infections; exemplified by AIDS
	Vaccination boosts immune defenses and protects against infections
The immune system recognizes and responds to tissue grafts and newly introduced proteins	Immune responses are barriers to transplantation and gene therapy
Defense against tumors	Potential for immunotherapy of cancer

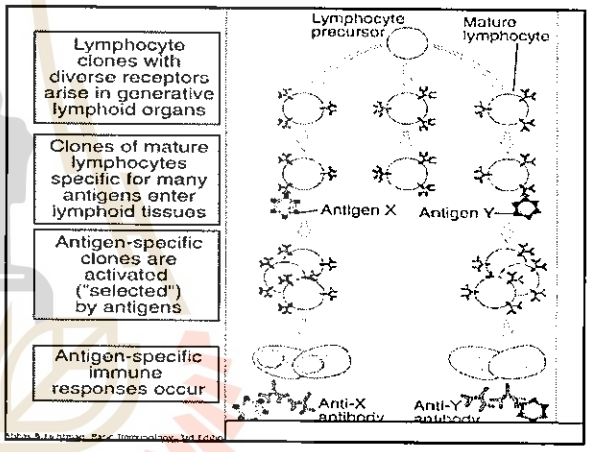
Disease	Maximum number of cases (year)	Number of cases in 2004	Percent change
Diphtheria	206,939 (1921)	0	-99.99
Measles	894,134 (1941)	37	-99.99
Mumps	152,209 (1968)	236	-99.90
Pertussis	265,269 (1934)	18,957	-96.84
Polio (paralytic)	21,269 (1952)	0	-100.0
Rubella	57,686 (1969)	12	-99.98
Tetanus	1,560 (1923)	26	-98.33
Haemophilus influenzae type b infection	~20,000 (1984)	16	-99.92
Hepatitis B	26,611 (1985)	6,632	-75.08

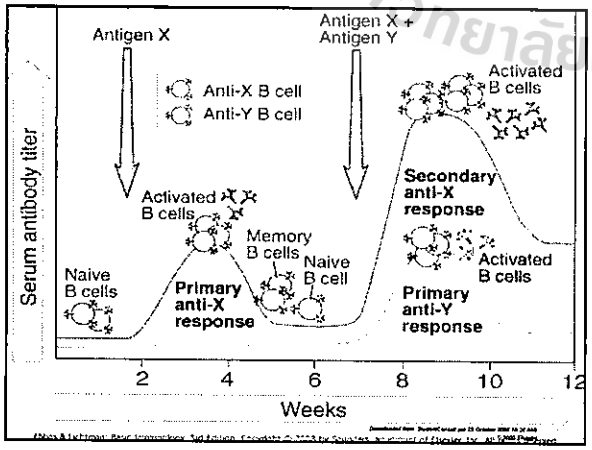


	Innate	Adaptive
Characteristics		
Specificity	For structures shared by groups of related microbes	For antigens of microbes and for nonmicrobial antigens
Diversity	Limited; germline-encoded	Very large; receptors are produced by somatic recombination of gene segments
Memory	None	Yes
Nonreactivity to self	Yes	Yes
Components		
Cellular and chemical barriers	Skin, mucosal epithelia; antimicrobial chemicals	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Blood proteins	Complement, others	Antibodies
Cells	Phagocytes (macrophages, neutrophils), natural killer cells	Lymphocytes

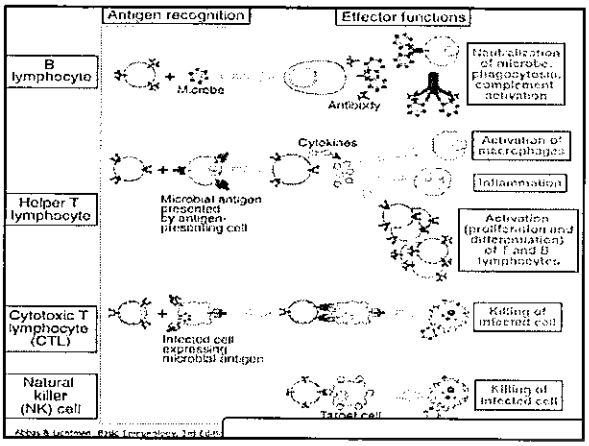


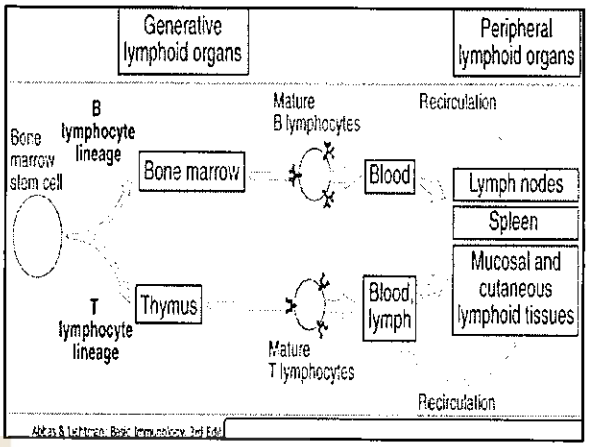
Feature	Functional significance
Specificity	Ensures that distinct antigens elicit specific responses
Diversity	Enables immune system to respond to a large variety of antigens
Memory	Leads to enhanced responses to repeated exposures to the same antigens
Clonal expansion	Increases number of antigen-specific lymphocytes to keep pace with microbes
Specialization	Generates responses that are optimal for defense against different types of microbes
Contraction and homeostasis	Allows immune system to respond to newly encountered antigens
Nonreactivity to self	Prevents injury to the host during responses to foreign antigens

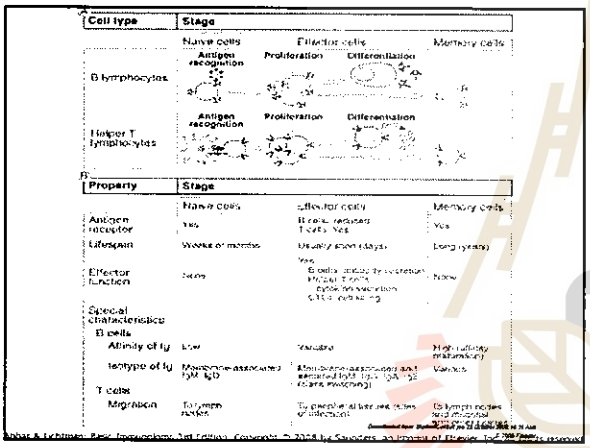


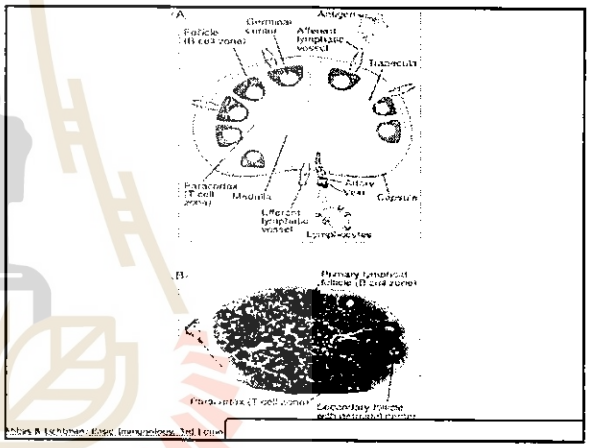


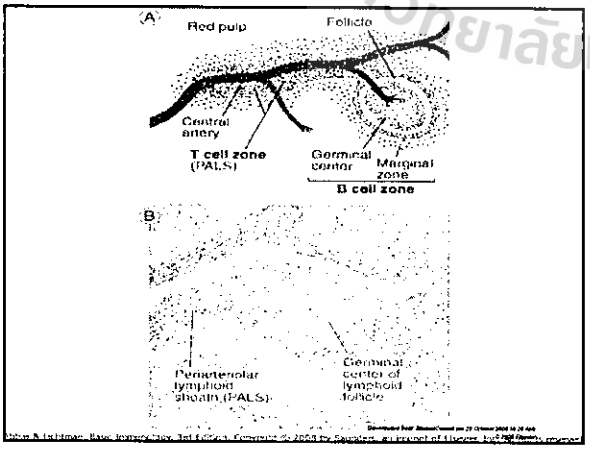
Cell type	Principal function(s)
Lymphocytes: B lymphocytes; T lymphocytes; natural killer cells	Specific recognition of antigens: B lymphocytes: mediators of humoral immunity T lymphocytes: mediators of cell-mediated immunity Natural killer cells: cells of innate immunity
Antigen-presenting cells: dendritic cells; macrophages; follicular dendritic cells	Capture of antigens for display to lymphocytes: Dendritic cells: initiation of T cell responses Macrophages: initiation and effector phase of cell-mediated immunity Follicular dendritic cells: display of antigens to B lymphocytes in humoral immune responses
Effector cells: T lymphocytes; macrophages; granulocytes	Elimination of antigens: T lymphocytes: helper T cells and cytotoxic T lymphocytes Macrophages and monocytes: cells of the mononuclear-phagocyte system Granulocytes: neutrophils, eosinophils

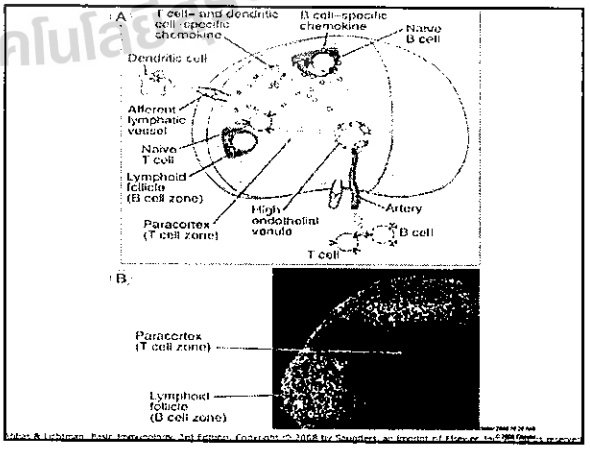


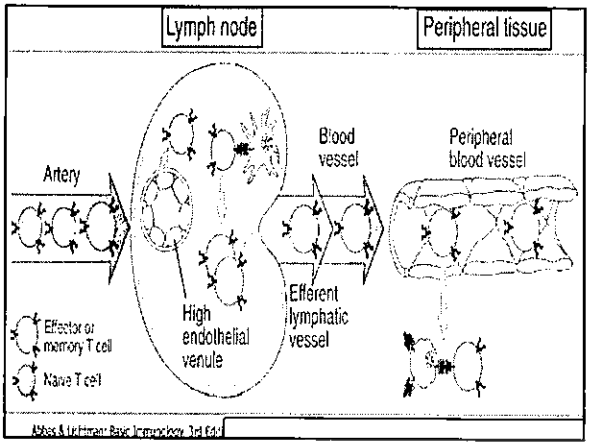


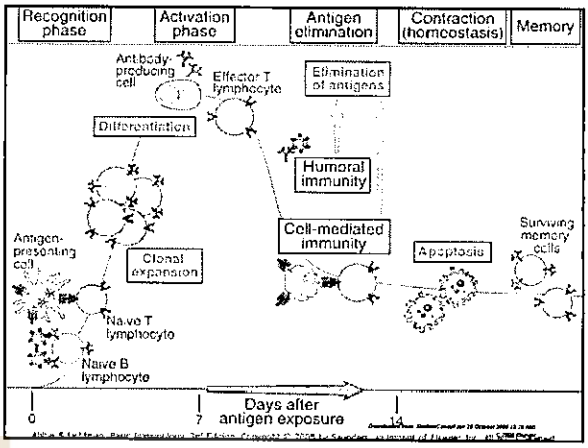




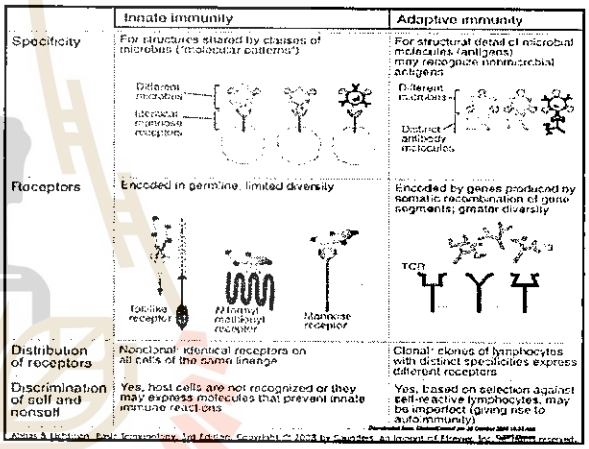


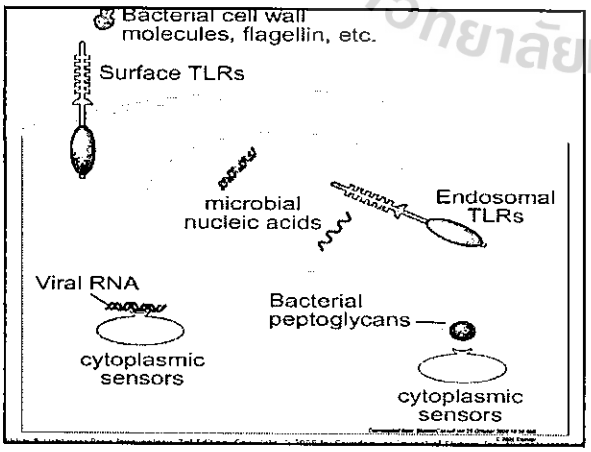


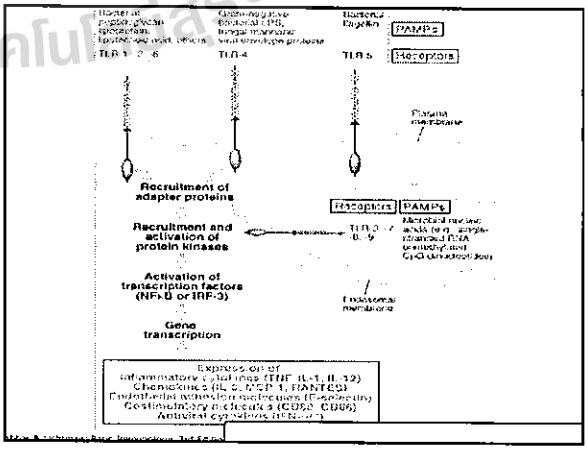


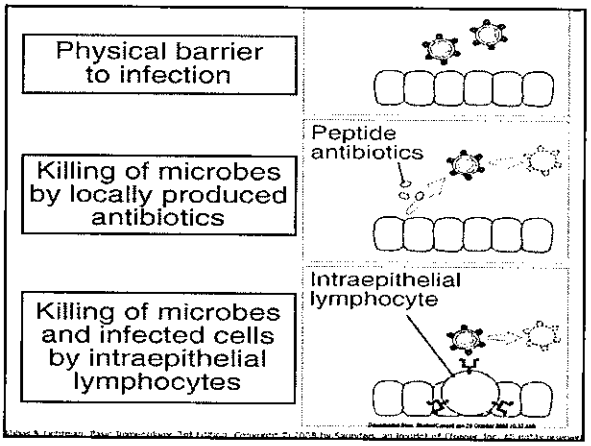


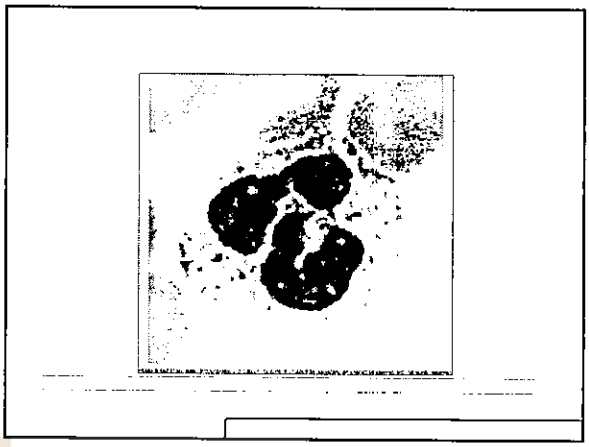
Adaptive Immunity

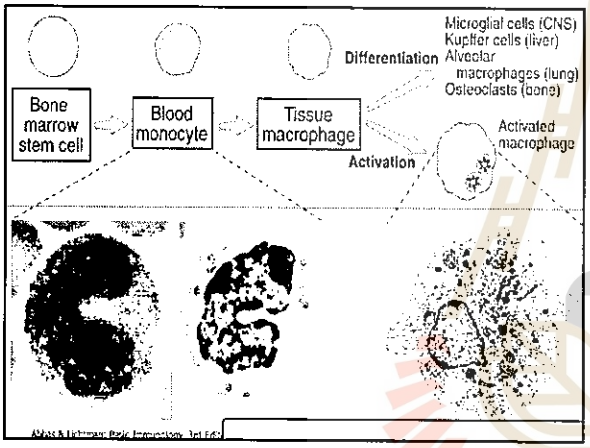


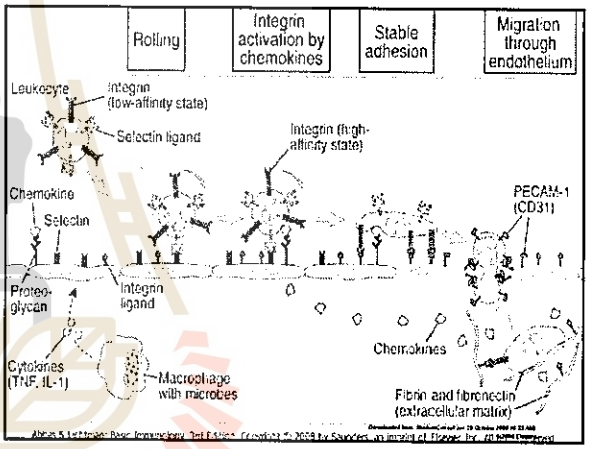


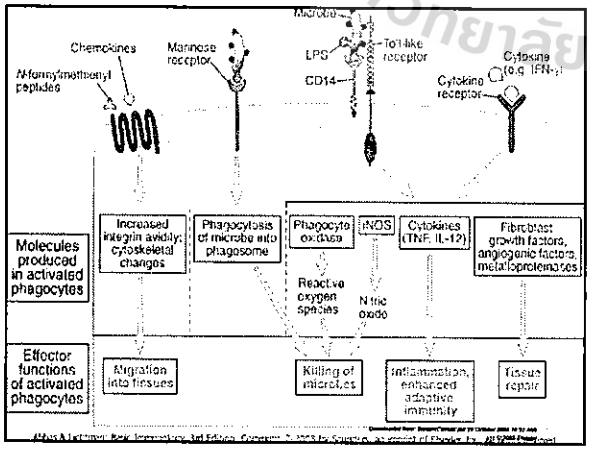


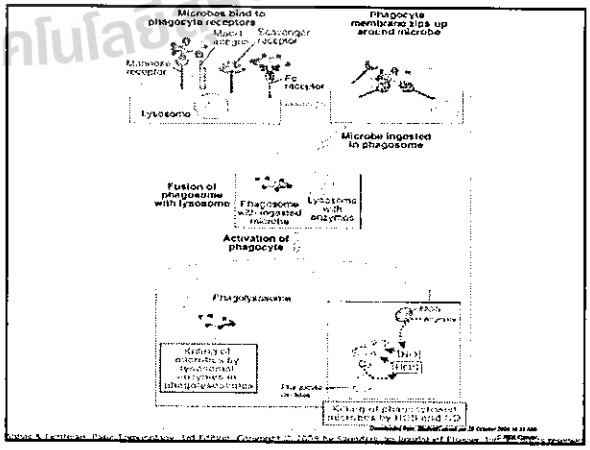


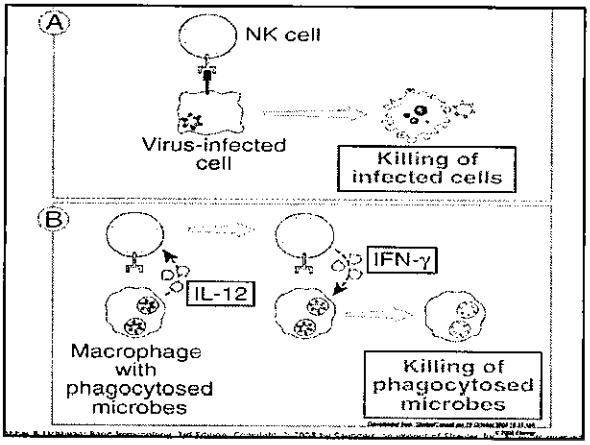


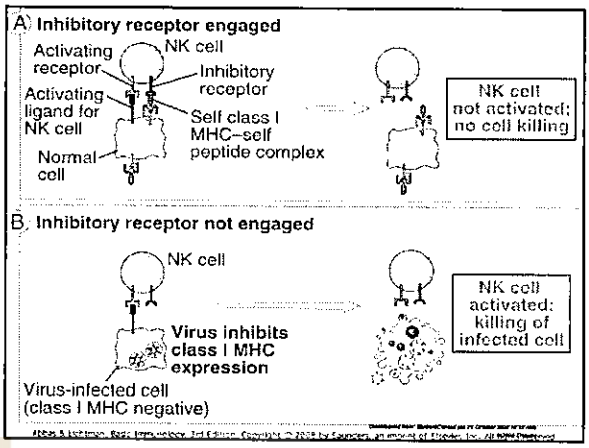


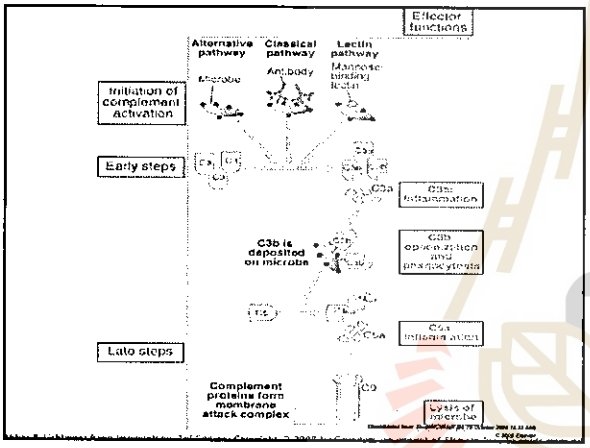


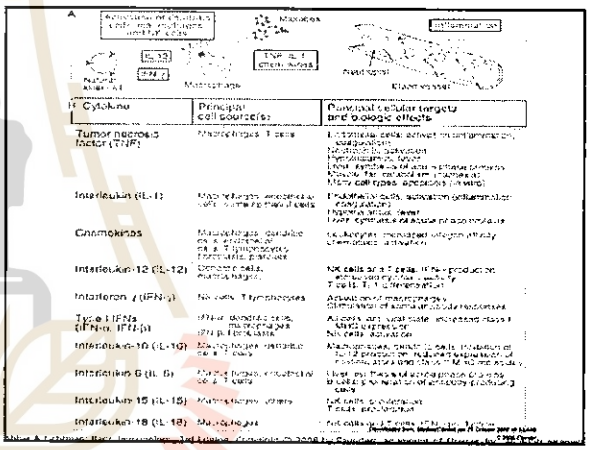




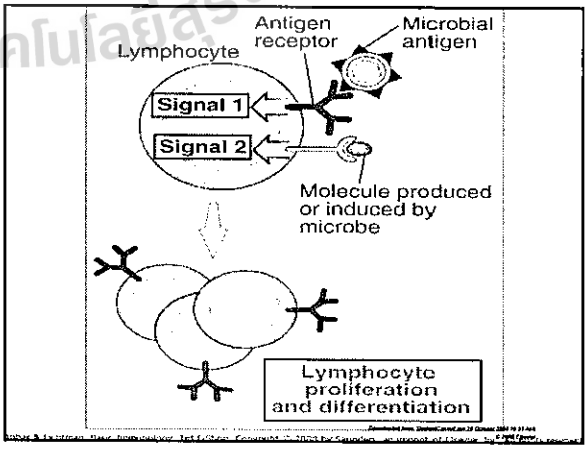


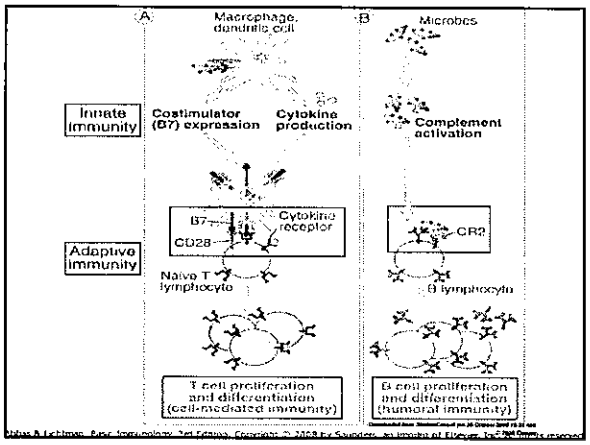




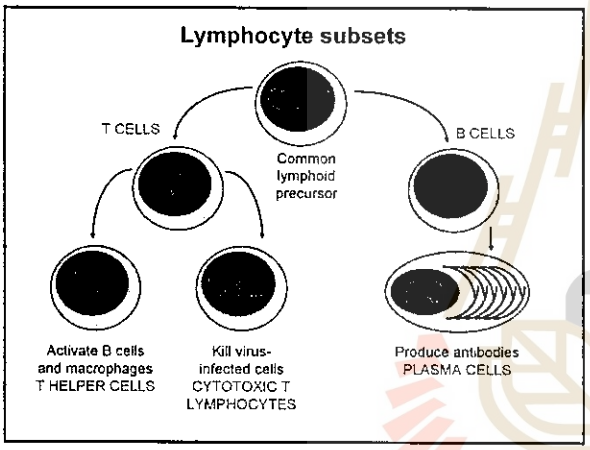


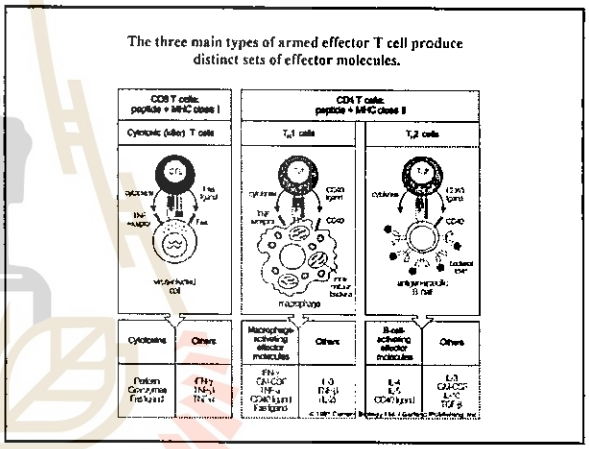
Mechanism of immune evasion	Organism (example)	Mechanism
Resistance to phagocytosis	Pneumococci	Capsular polysaccharide inhibits phagocytosis
Resistance to reactive oxygen species in phagocytes	Staphylococci	Production of catalase, which breaks down reactive oxygen intermediates
Resistance to complement activation (alternative pathway)	*Neisseria meningitidis*	Sialic acid expression inhibits C3 and C5 convertases
	Streptococci	M protein blocks C3 binding to organism, and C3b binding to complement receptors
Resistance to antimicrobial peptide antibiotics	*Pseudomonas*	Synthesis of modified LPS that resists action of peptide antibiotics

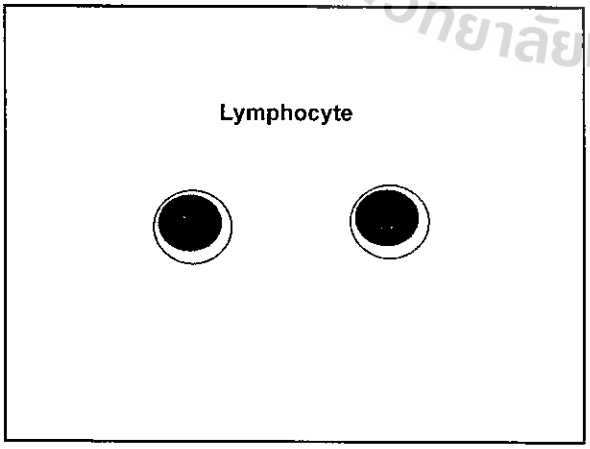


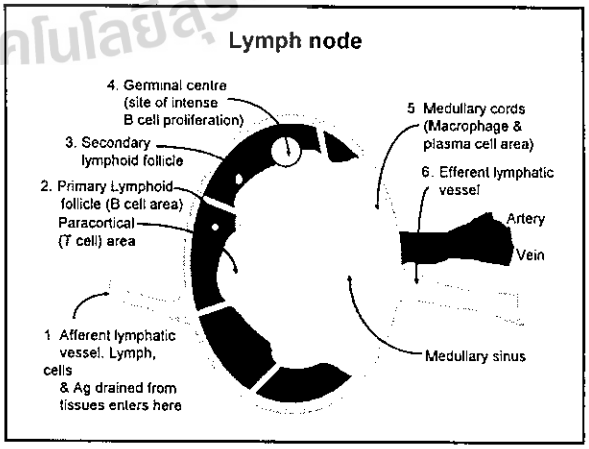


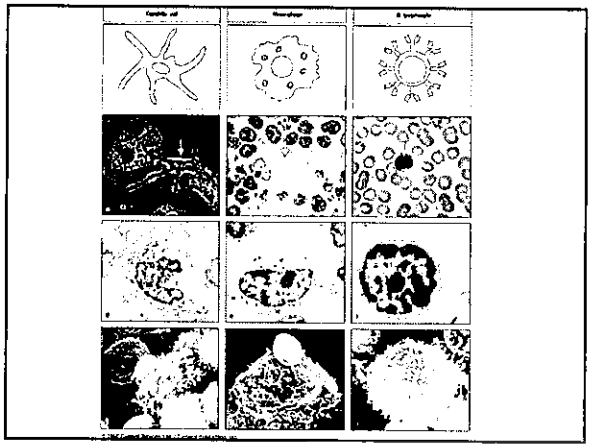
Adaptive Immunity

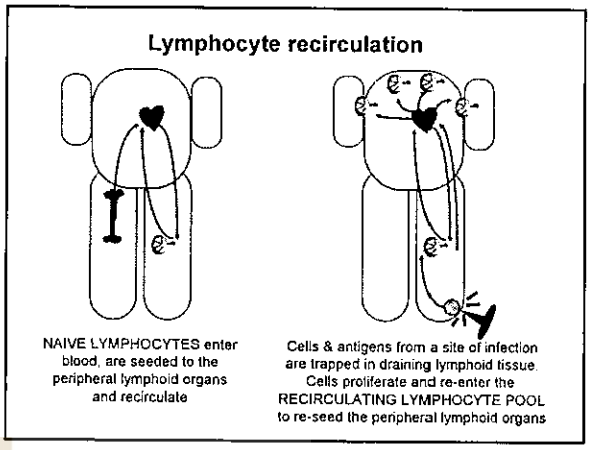


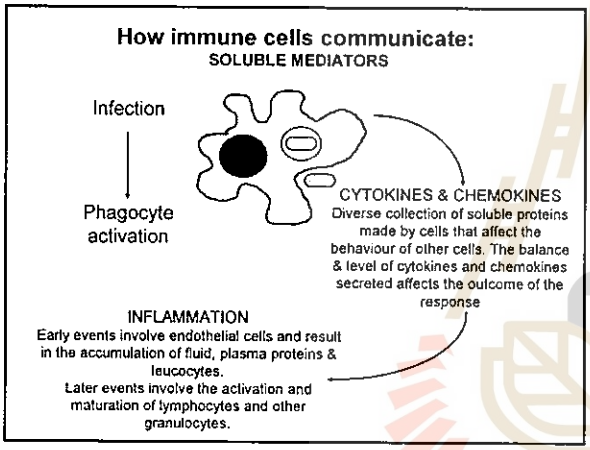


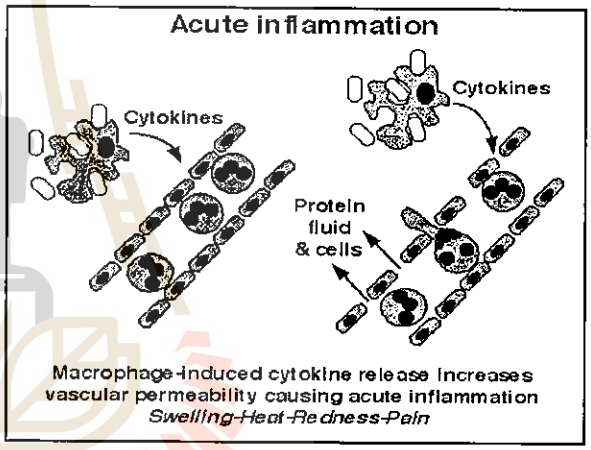


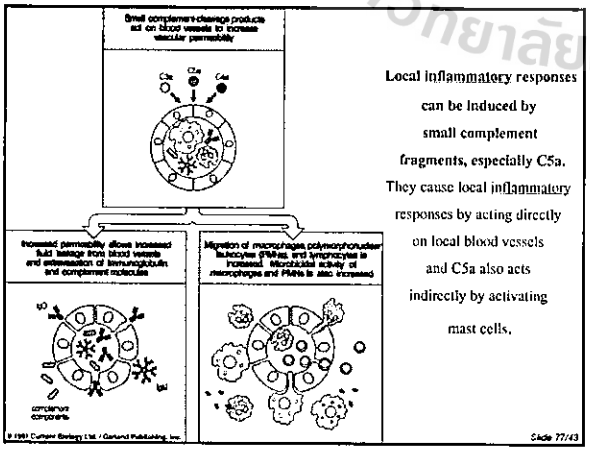


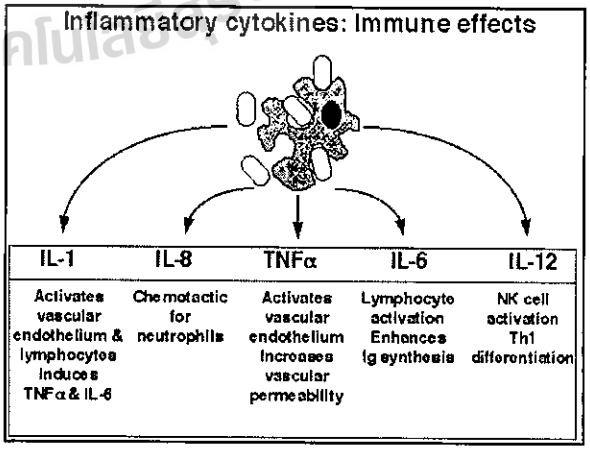










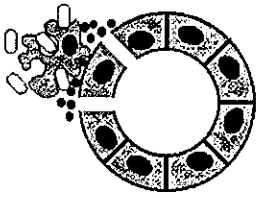


Inflammatory cytokines: Systemic effects and co-ordination of response

IL-1 IL-6 TNFα

Liver	Bone marrow & endothelium	Hypothalamus, fat & muscle	Lymphocytes
Acute phase proteins (CRP, MBP)	Neutrophil mobilisation	Increased metabolism & temperature	Increased activation
Activation of complement & phagocytosis	Phagocytosis	Decreased viral/bacterial replication	Increased Ag processing

Local and systemic inflammation



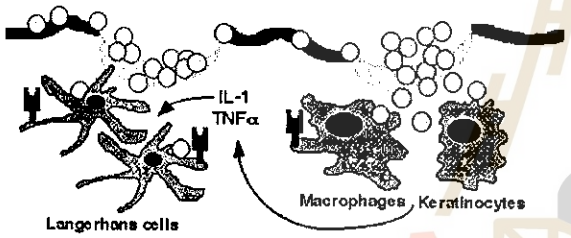
Localised infection
Local release of TNFα

Local oedema & inflammation
Removal of infection and adaptive immunity

Systemic infection (sepsis)
Systemic release of TNFα

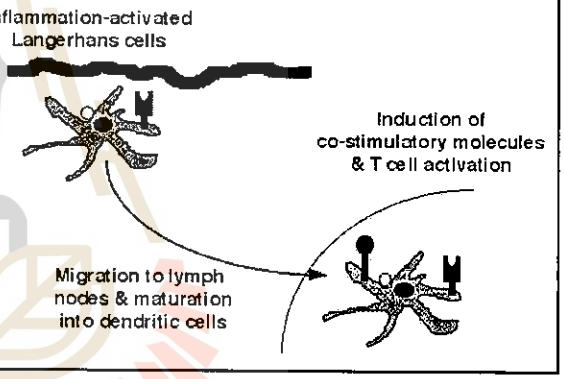
Systemic oedema
DIC, organ failure, death

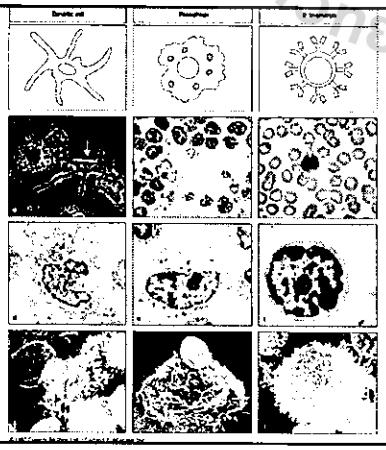
Inflammation and the induction of adaptive immunity



Inflammation activates and recruits the adaptive immune system by activating potent antigen-presenting cells

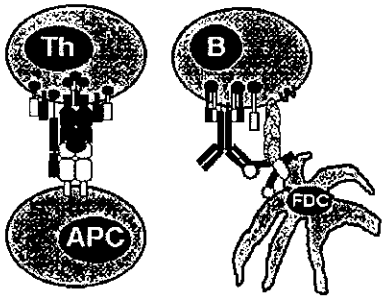
Induction of adaptive immunity





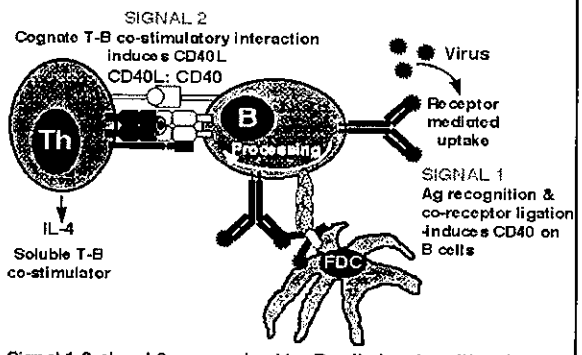
Costimulation of antigen and co-receptor activated T and B cells

SIGNAL 1:
Antigen Recognition
(± co-receptors)



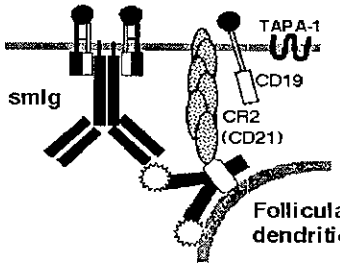
Antigen-receptor and co-receptor ligation are often insufficient for proliferation and the expression of effector function

Co-stimulation of B cells by T helper cells



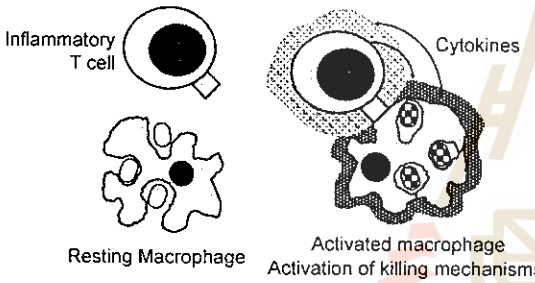
Signal 1 & signal 2 are required for B cell clonal proliferation and differentiation to plasma cells

Amplification of B cell activation: The B cell co-receptor complex

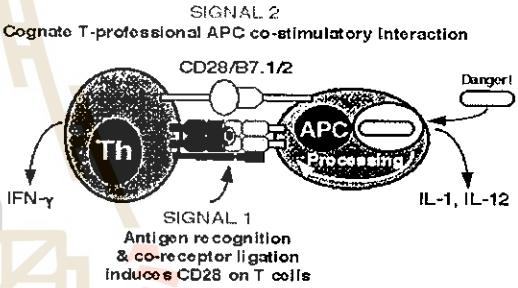


Signals through the co-receptor complex induce proliferation (clonal expansion) and the expression of molecules on B cells that allow them to communicate with T helper cells.

Effector mechanisms against intracellular bacteria MACROPHAGE ACTIVATION



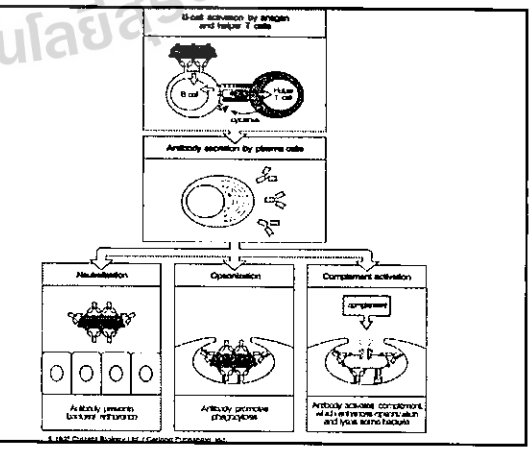
Co-stimulation of T helper cells by professional APC

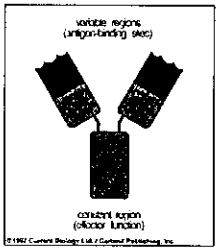


Signal 1 & signal 2 are required for T cell clonal proliferation and differentiation to effector cells

Immune Syetem

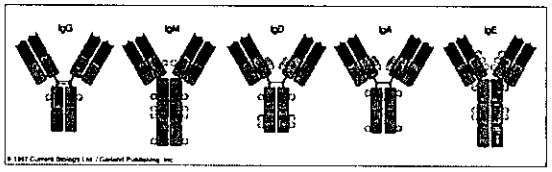
- **Non specific Immune Response (Innate Immunity, Natural Immunity)**
- **Specific Immune System (Acquired Immunity)**
  - Humoral Immune Response (HIR)
  - Cell Mediated Immune Response (CMIR)

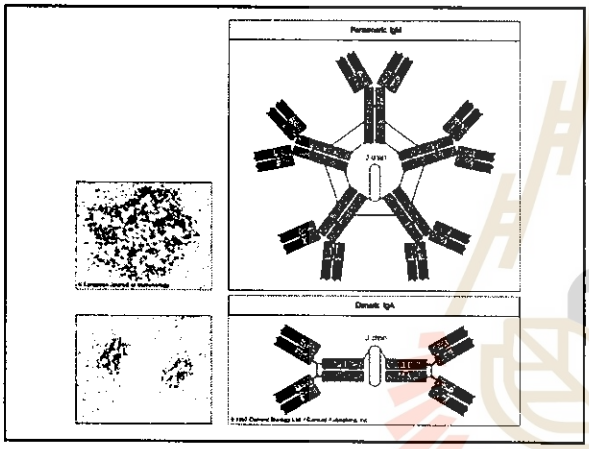




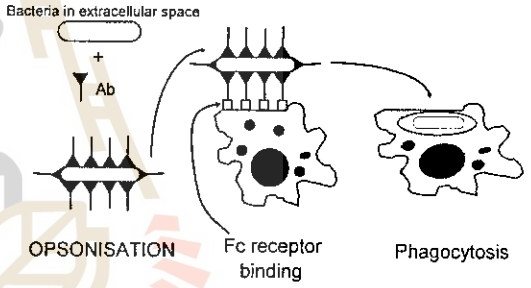
Structure of the antibody molecule.

The structural organization of the main human immunoglobulin isotype monomers

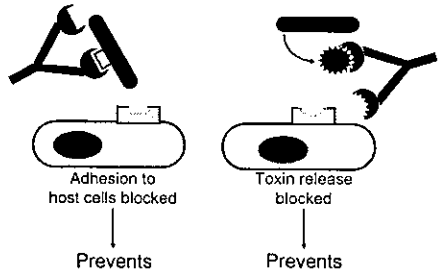




Effector mechanisms against extracellular pathogens
OPSONISATION

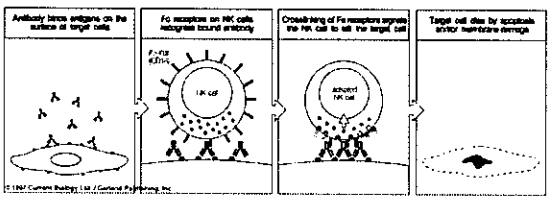


Immune effector mechanisms against extracellular pathogens & toxins
NEUTRALISATION



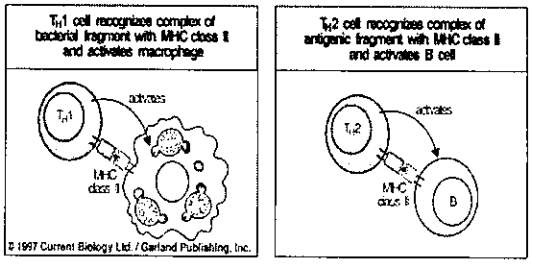
NEUTRALISING ANTIBODIES

Antibody-coated target cells can be killed by natural killer cells (NK cells) in antibody-dependent cell-mediated cytotoxicity (ADCC).

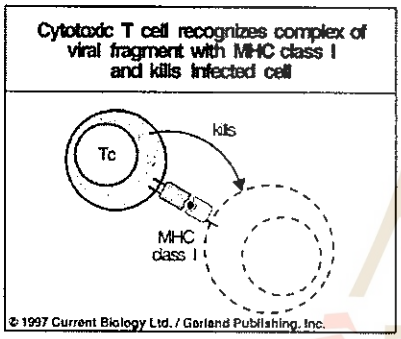


Immune System

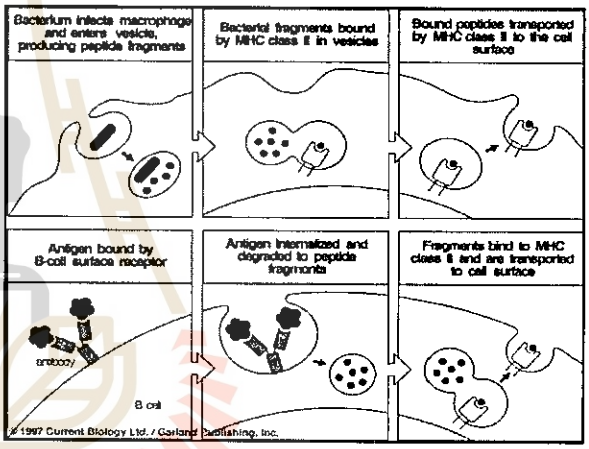
- **Non specific Immune Response (Innate Immunity, Natural Immunity)**
- **Specific Immune System (Acquired Immunity)**
  - Humoral Immune Response (HIR)
  - Cell Mediated Immune Response (CMIR)

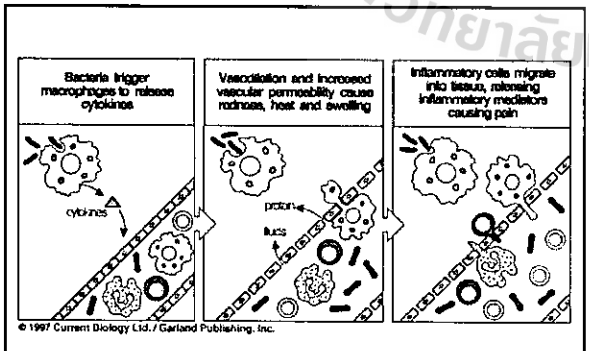


T_{H1} and TH2 cells recognize antigen presented by MHC class II molecules

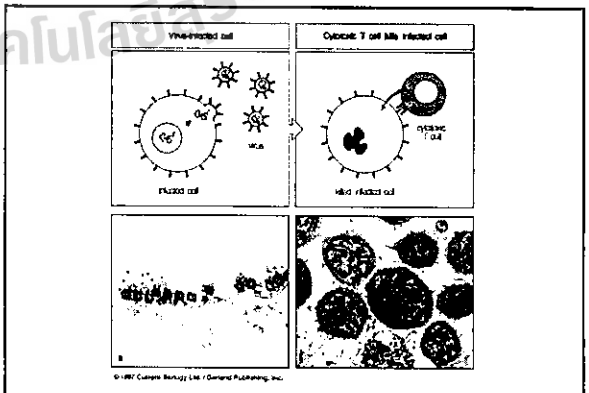


Cytotoxic T cells recognize antigen presented by MHC class I molecules and kill the cell

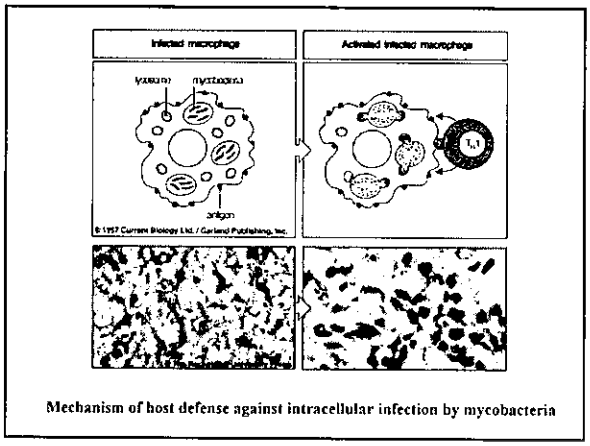




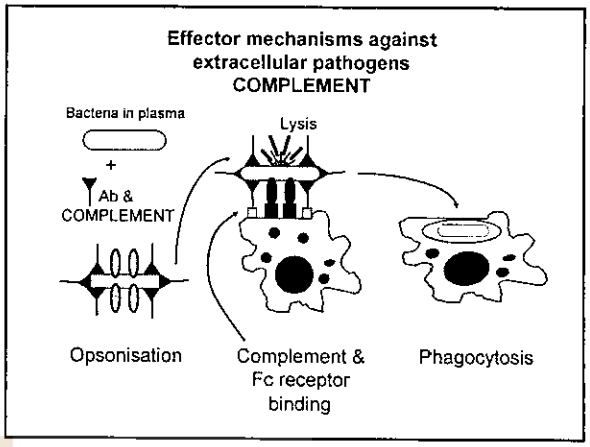
Bacterial infection triggers an inflammatory response.

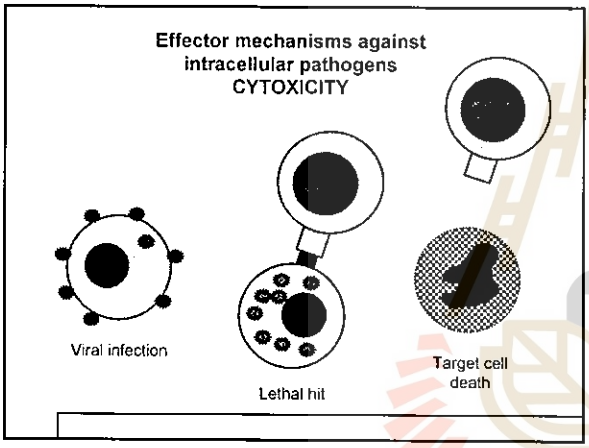


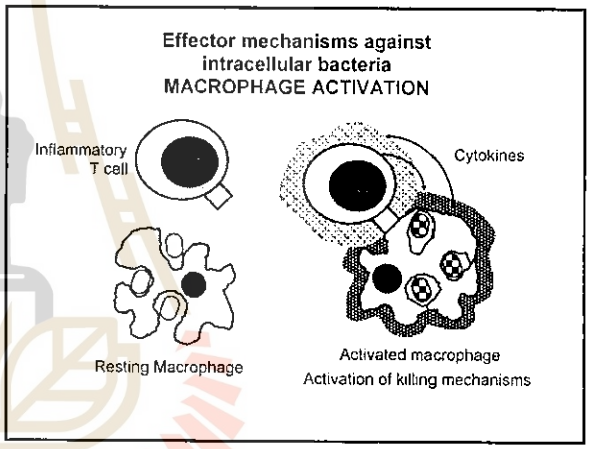
Mechanism of host defense against intracellular infection by viruses



Mechanism of host defense against intracellular infection by mycobacteria



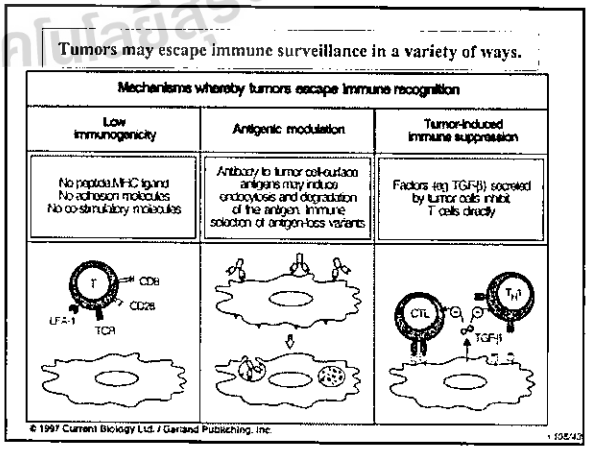




There are several proteins that are selectively expressed in human tumors and are therefore candidate tumor-rejection antigens.

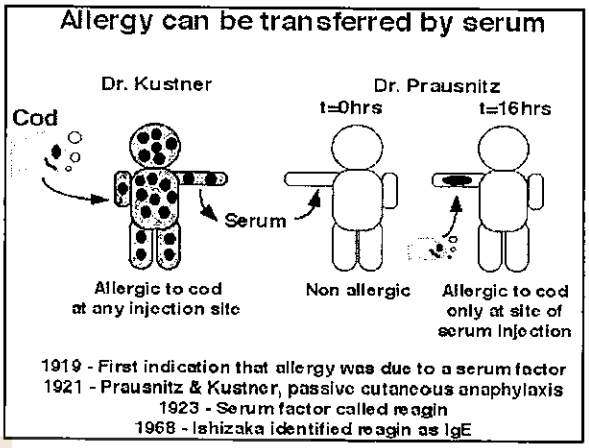
Potential tumor-rejection antigens have a variety of origins

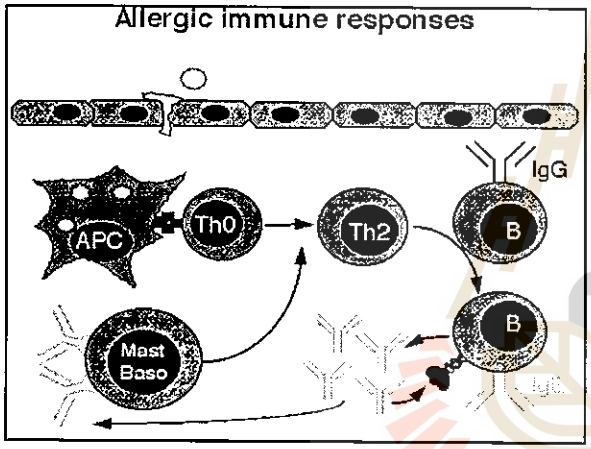
Class of antigen	Antigen	Nature of antigen	Tumor type
Embryonic	MAGE-1 MAGE-3	Normal testicular proteins	Melanoma Breast Glioma
Abnormal post-translational modification	MUC-1	Underglycosylated mucin	Breast Pancreas
Differentiation	Tyrosinase	Enzyme in pathway of melanin synthesis	Melanoma
	Idiotype	Clonally restricted, following gene rearrangements in B-cell clone	Lymphoma
Mutated oncogene or tumor-suppressor	Ras	GTP-binding protein, key in signal transduction pathway	Many tumors
	p53	Cell-cycle regulator; tumor-suppressor gene	Lung Breast Colorectal Brain Hematologic
Fusion protein	bcr-abl	Fusion protein with tyrosine kinase activity resulting from chromosome translocation t(9;22) (Philadelphia chromosome)	Chronic myeloid leukemia
Oncoviral protein	HPV types 16, 18 E6 and E7 proteins	Viral transforming gene products	Cervical carcinoma

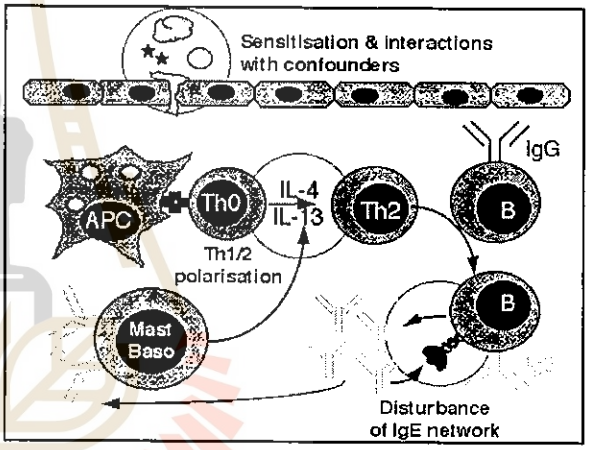


Immune responses can be beneficial or harmful depending on the nature of the antigen.

Antigen	Effect of response to antigen	
	Normal response	Deficient response
Infectious agent	Protective immunity	Recurrent infection
Innocuous substance	Allergy	No response
Grafted organ	Rejection	Acceptance
Self organ	Autoimmunity	Self tolerance
Tumor	Tumor immunity	Cancer







ปัจจัยที่มีผลต่อการทำงาน

•**1. Genetic factor** ระบบ ภูมิคุ้มกันของร่างกายก็เหมือนระบบอื่นๆ ที่การทำงานถูกควบคุมทางพันธุกรรมจีน **(gene)**

•**2. Age factor** เป็นอีกปัจจัยหนึ่งที่เกี่ยวข้องกับการตอบ สนองต่อสิ่งแปลกจะเห็นได้ชัดในเด็กเล็กๆ หรือ ผู้ใหญ่ที่มีอายุมากจะมีโอกาสติดเชื้อและเกิดโรคได้ง่ายกว่า
•คนในระยะหนุ่มสาว

•**3. Metabolic factor** เป็นปัจจัยซึ่งมากจากกลไกการเมตาบอลิสม **(metabolism)** ของร่างกาย ซึ่งกลไกเกี่ยวข้องกับฮอร์โมนบางชนิดที่อาจมีฤทธิ์ต่อการทำงานของระบบภูมิคุ้มกัน

4. Environmental factor เป็นปัจจัยที่เกี่ยวข้องกับความเป็นอยู่การดำรงชีวิตตลอดจนคุณภาพชีวิต

5. Anatomic factor เป็นจัยซึ่งเกี่ยวข้องกับความเป็นอยู่การดำรงชีวิตตลอดจนคุณภาพชีวิต

6. Microbial factor เป็นปัจจัยที่เกี่ยวกับจุลชีพประจำถิ่น **(normal flora)** ที่อาศัยอยู่ในร่างกายของมนุษย์ โดยไม่ทำให้เกิดโรค

7. Physiological Factor เป็นปัจจัยที่เกิดจากสรีรวิทยา และหน้าที่ต่างๆ ที่มีอยู่ในร่างกาย โดยสามารถป้องกันได้ เช่น น้ำย่อยในกระเพาะอาหาร

Chapter 2.

Introduction to Leukemia

Introduction to Leukemia

Asst. Prof. Dr. Wilairat Leeanansaksiri

Scope of Introduction to Leukemia

1. Definition
2. Classification
3. Diagnosis
4. Treatment
5. Follow up after treatment

Scope of Introduction to Leukemia

1. Acute leukemias
-Acute lymphocytic leukemia (ALL)
-Acute myelogenous (granulocytic) leukemia (AML or AGL, rarely ANLL)

2. Chronic leukemias
-Chronic myelogenous (granulocytic) leukemia (CML or CGL)
-Chronic lymphocytic leukemia (CLL)

3. Lymphomas

3.1 Hodgkin's Disease (HD)
3.2 Non-Hodgkin's Lymphomas (NHL)
- Multiple myeloma (plasma cell)
- Burkitt's lymphoma

Introduction to Leukemia

- Definition
- Historic Perspective
- Etiology and Risk Factors
- Incidence
- Classification
- Comparison of Acute and Chronic Leukemia

Leukemia

Definition

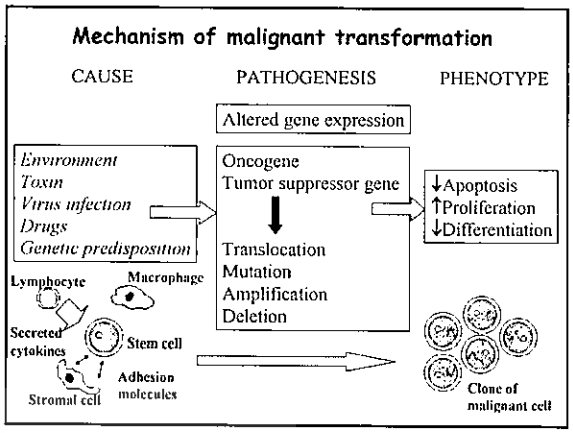
Leukemia is a malignant disease of hematopoietic tissue characterized by the accumulation abnormal white cells (neoplastic or leukemic) in the bone marrow leading to bone marrow failure, a raised circulating white cell count (leukocytosis) and infiltrate organs (e.g liver, spleen, lymph nodes, brain)

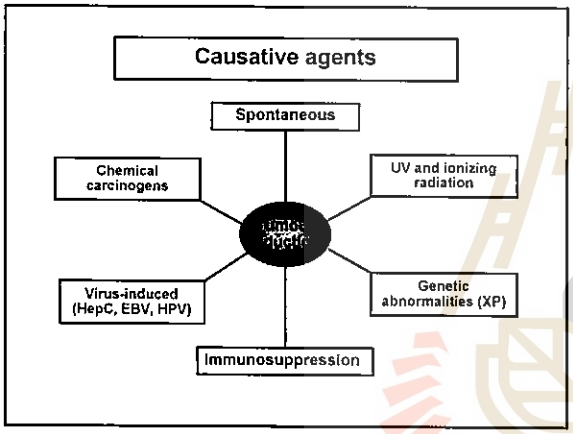
Leukemia

Historic Perspective

•1945

•The initial description of leukemia as a clinical entity was made by Bennett in Scotland and in Germany.



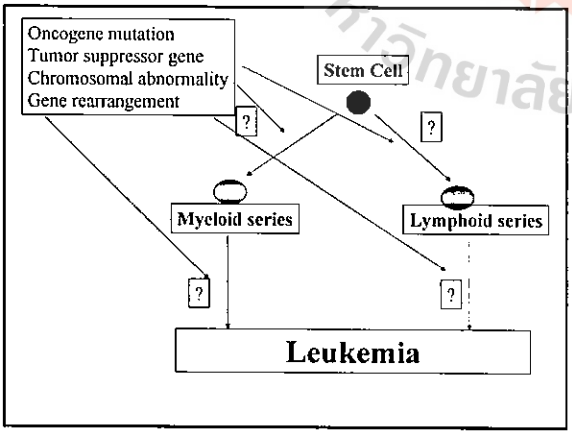


Leukemia

Etiology and Risk Factors

The etiology of leukemia is unknown.

- Oncogenes mutation and tumor suppressor gene alteration.
- Host factors.
- Environmental factors



Host Factors

- **Congenital chromosomal abnormalities**
  - Increased frequency in patients with congenital disorders that have tendency for chromosomal abnormality.
  - Such as : Bloom's syndrome, Fanconi anemia, Down's and Klinefelter's syndromes.
  - 18-20 fold increase incidence of AL is seen in children win DS.

- **Immunodeficiency**
  - An unusually high incidence of lymphoid leukemia and lymphoma has been described in patients with hereditary immunodeficiency states (ataxia-telangiectasia and sex-linked agamaglobulinemia).
  - Usually related to T and B-lymphocyte gene rearrangement.

Chronic bone marrow dysfunction

- **Patients with CBMD syndromes have an increased risk of acute leukemic transformation.**
- **Examples include the myelodypalstic syndromes, myeloproliferative disorders, aplastic anemia and PNH**

Environmental factors

Ionizing radiation

- Leukemia is associated with exposure to ionizing radiation such as nuclear weapons in Hiroshima and Nagasaki.
- Both acute and chronic forms of leukemia including AML, ALL and CML were associated.

Chemical drugs

- A variety of chemicals and drugs have been associated with the development of leukemic transformation
- Examples: Benzene, Chloramphenecol, Phenylbutazone and Cytotoxic alkylating chemotherapeutic agents.

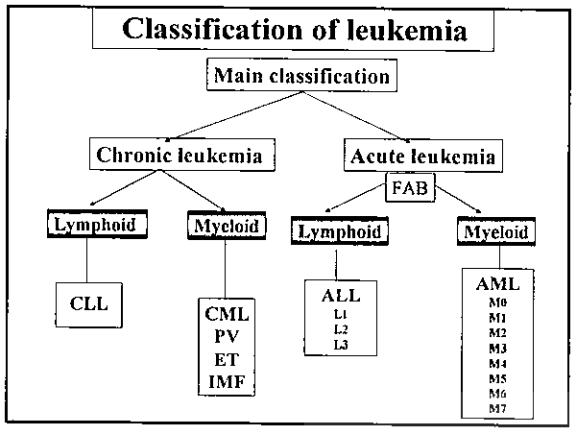
Viruses

- The human T-cell leukemia-lymphoma virus-I (HTLV-I) has been implicated as a causative agent of adult T-Cell leukemia-lymphoma.
- Another related virus HTLV-II has been isolated from patients with atypical hairy cell leukemia (CLL)
- The Epstein's Barr virus has been linked to Burkitt's lymphoma.

Incidence

- Incident increase every year
- Leukemia strike more in adult than children (10:1) and has slightly increase incidence in males than females (1-2:1)

Classification of leukemia

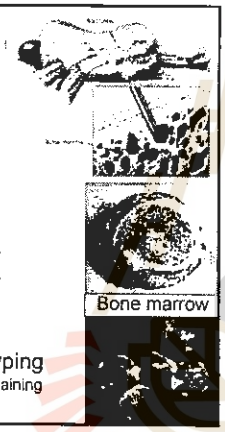


Comparison of acute and chronic leukemia

	Acute	Chronic
Age	All ages	Adults
Clinical onset	Sudden	Insidious
Leukemic cells	More Immature Blast cell ≥30%	More Mature BLAST CELL<30%
Anemia	Mild to severe	Mild
Thrombocytopenia	Mild to severe	Mild
WBC	Variable	Increased
Organomegaly	Mild	prominent

Diagnostic Studies

- Peripheral blood
- Bone marrow
- Cytogenetics
- Flow cytometry
- Cytochemistry (special stains)
- Molecular markers
- For myeloma - SPEP, UPEP, Quantitative immunoglobulins, immunoelectrophoresis



Immunophenotyping
Cell surface antigen staining

Staging

- Most leukemias are not staged
- Chronic lymphocytic leukemia (CLL)
  - 5 Stages (Rai-Sawitsky) classification, 0 - IV
  - Based upon lymphocyte numbers, adenopathy, anemia, thrombocytopenia
- Multiple myeloma
  - 3 stages, based upon multiple factors, hemoglobin, M protein, calcium, bone lesions

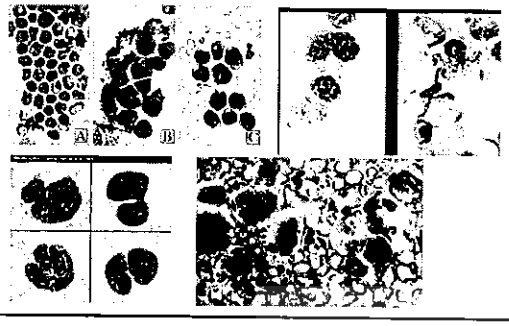
Acute Myelogenous Leukemia (AML)

- All acute leukemias arising from the myeloid cell lineage
- May affect neutrophil, monocyte, erythrocyte, and megakaryocyte cell lines
- Eight subtypes of AML from M0 to M7.

AML Subtypes

- M0- Myeloid leukemia with minimal differentiation.
- M1- Myeloblastic leukemia
- M2- Myeloblastic leukemia (undifferentiated)
- M3- Promyelocytic leukemia
- M4- Myelomonocytic leukemia
- M5- Monocytic leukemia
- M6- Erythroblastic leukemia
- M7- Megakaryoblastic leukemia

Leukemia



Leukemia

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in young children. This disease also affects adults, especially those age 65 and older.

- Acute myeloid leukemia (AML) occurs in both adults and children. This type of leukemia is sometimes called acute nonlymphocytic leukemia (ANLL).

Chronic lymphocytic leukemia (CLL) most often affects adults over the age of 55. It sometimes occurs in younger adults, but it almost never effects children.

Chronic myeloid leukemia (CML) occurs mainly in adults. A very small number of children also develop this disease.

Leukemic patients


Austin


Ryan Tamayoshi


Michael Wu


Sara Ruehling

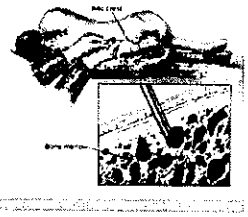
www.aadp.org/pages/page.php?pageid=52

Leukemia

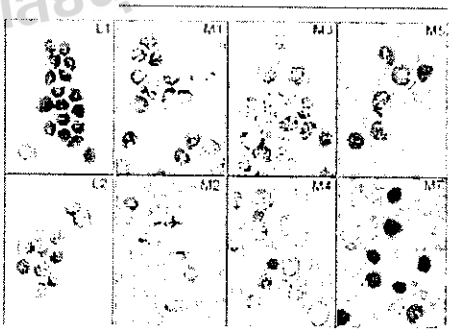
Symptom
more frequent infections and fevers
* anemia and its symptoms: pale skin, fatigue, weakness
* bleeding
* bruising
* fever, chills
* loss of appetite
* loss of weight)
* swollen or tender lymph nodes, liver, or spleen
* petechiae (tiny red spots under the skin)

www.susheewa.com/blog/?m=2009

Leukemia



•swollen or bleeding gums
* sweating
* bone or joint pain
In addition, acute leukemia may cause:
• headaches
* vomiting
* confusion
* loss of muscle control
* seizures
* swollen testicles
* sores in the eyes or on the skin



AML- Epidemiology

- 17% of all childhood leukemias
- Rates highest in the first two years of life
- Between 1995 and 1999 AML accounted for 4.8% of all cancers in children under 15 years of age in Canada.
- Occurs in 1 in 130,000 people under 20 years of age each year.

AML- Prognosis

- Overall survival is 60%
- Acute promyelocytic leukemia (APL-M3) has a favourable prognosis.
- Acute megakaryoblastic leukemia (M7) has a much poorer prognosis than other types of AML.
- Down's syndrome children with AML have better outcome than other patients with AML
- Cytogenetics impact outcome

AML- Clinical Presentation

- Children with AML usually have an elevated WBC at diagnosis (median 24,000/mm^3).
- Peripheral blasts are seen in more than 90% of cases and most children are neutropenic.
- About half have a hemoglobin < 80 and a platelet count <50,000/mm^3.

AML- Clinical Presentation

- Onset of symptoms is a median of 6 weeks prior to diagnosis.
- As with ALL, pallor, fatigue, petechiae, fever, and infection may be seen at presentation due to abnormal blood counts.
- Anorexia and sore throat may also be seen
- Skin, gums, and the head and neck area may be sites of extramedullary disease

AML- Clinical Presentation

- Chloromas are extramedullary accumulations of leukemic cells.
  - occur in the spinal cord, brain, soft tissues, bones, and eyes.
  - Orbital chloroma may cause ptosis.
- Leukemia cutis defined as the accumulation of leukemic cells in the skin.
  - Papular rash with salmon or bluish to slate gray lesions
  - Palpable rubbery subcutaneous nodules

AML

AML- Diagnosis

- Work-up includes bone marrow aspirate, biopsy and lumbar puncture
- Greater than 20% blasts need to be seen on the aspirate for the patient to be diagnosed with AML.
- CNS disease less common in AML than ALL
  - Factors associated with CNS disease include hyperleukocytosis (↑WBC), monocytic leukemia (M4 or M5), and young age.

AML- Treatment

- Involves systemic chemotherapy with a multiagent protocol. Most treatment plans are short and intense.
- Treatment is done as an inpatient.
- High dose cytarabine plays a role in most treatment plans.
- Intrathecal chemotherapy is given to all children with AML
- Patients with AML are at high risk for infection and sepsis.

AML- Treatment

- Risk stratification in AML is relatively new.
- Patients with low risk disease based on cytogenetics are treated with chemotherapy alone.
- Patients with intermediate risk disease (neither favourable nor unfavourable features) are treated with chemotherapy. Stem cell transplant is done if there is a matched family donor.
- High risk patients undergo chemotherapy followed by stem cell transplant (may be either family donor or matched unrelated donor).

Myelodysplastic syndromes (MDS)

- Myelodysplastic syndromes (MDS) –rare set of disorders characterized by ineffective hematopoiesis.
- 32% of children with MDS go on to develop AML
- Present with signs and symptoms of hematopoietic failure including bleeding, pallor and petechiae.
- Supportive care includes transfusions and IV antibiotics
- Only known curative therapy is allogeneic stem cell transplant

Juvenile Myelomonocytic Leukemia (JMML)

- A myelodysplastic syndrome
- Hypercellular marrow with <20% blasts
- Peripheral monocyte count > 1000/mm³
- Most have WBC >10,000/mm³ and elevated hemoglobin F
- Do not have Philadelphia chromosome
- Typically under 4 years of age
- Clinical presentation may include hepatosplenomegaly (>90%), lymphadenopathy (75%), pallour (69%), fever (61%), skin rash (39%).
- Poor prognostic features include older age, elevated hemoglobin F, and thrombocytopenia.
- Only curative therapy is allogeneic stem cell transplant (~ 50% cure rate)

Myeloproliferative Disorders

- Clonal proliferation of the myeloid cell line due to an intrinsic abnormality of the hematopoietic stem cell.
- Includes Chronic myeloid leukemia (CML)
  - Ninety-five percent of pediatric CML cases have the Philadelphia chromosome.
  - Rare in children.
  - Typically presents in chronic phase (mean duration 4-6 years). Clinical symptoms include weight loss, fatigue, malaise, bone and joint pain, fever, night sweats, abdominal fullness/pain. Splenomegaly in 80-95% of cases.
  - 40% of children with CML are asymptomatic.
  - WBC >100,000 in 80% of patients.

Myeloproliferative Disorders

- CML
  - Disease eventually enters an accelerated phase (mean duration 3-9 months) and then terminal or blastic phase (mean duration 3-6 months).
  - Chronic phase managed with imatinib (Gleevec®) which selectively targets CML cells and has few toxic effects.
  - Patients on imatinib had progression free survival of 84% after 5 years.
  - Patients with progressive disease or intolerance to imatinib may undergo allogeneic stem cell transplant

Relapse

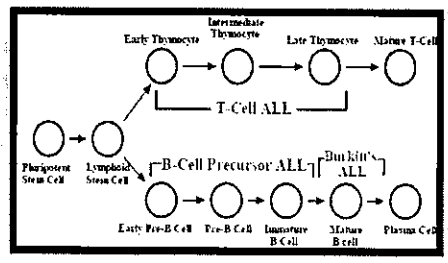
- Relapse defined as recurrence of leukemia after remission has occurred.
- Most relapse occur during treatment or within the first 2 years after completion of treatment.
- ALL known to relapses as late as 10 years after diagnosis.
- In both ALL and AML bone marrow is most common site of relapse.

Relapse

- In ALL relapse to testes occurs in 2% of cases and CNS to 5% of cases.
- Bone marrow relapse is predictive for a poor outcome in most patients.
- Time to relapse in ALL associated with outcome
- Allogeneic stem cell transplant is the treatment of choice for patients with hematologic relapse during or shortly after the completion of therapy and for patients with T-cell ALL.
- In AML relapse less than 18 months after therapy confers a dismal prognosis. Allogeneic cell transplant is the treatment of choice for all relapsed AML s.

Types of ALL

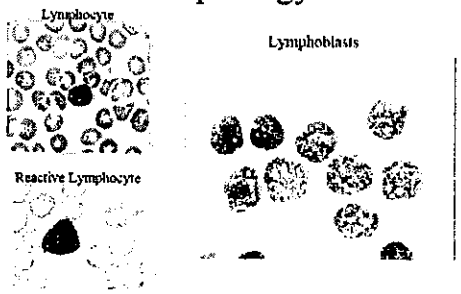
Pre-B cell (84%) T-cell (15%) B-cell (1%)



Subtypes of ALL

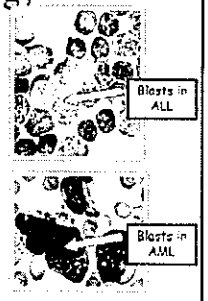
- **Precursor-B Cell**
  - Most common type
  - Commonly seen in preschoolers
- **T-Cell**
  - Associated with high WBC
  - Often associated with mediastinal mass
  - Commonly seen in adolescent males
- **Mature B-Cell (Burkitt's)**
  - Responds poorly to standard ALL therapy
  - Treatment same as for Burkitt's lymphoma

Peripheral blood blast morphology



Bone Marrow Analysis: Morphology

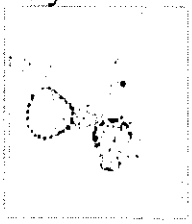
- Description of leukemic cells
  - Size, shape, amount of cytoplasm
  - Other characteristics (e.g., vacuoles, Auer rods)
- Cells classified according to the FAB (French-American-British) system
  - ALL: FAB L1 – L3
  - AML: FAB M0 – M7



Morphology in ALL: FAB Classification

Bone Marrow Analysis: Cytochemistry

- Special stains applied to slides
- Helps to differentiate cell lineage (AML: Sudan black, myeloperoxidase versus ALL: periodic acid-Schiff/PAS)
- Helps to differentiate AML subtypes



Bone Marrow Analysis: Immunophenotyping

- Identifies markers (antigens) on blast cells
- Helps to differentiate:
  - ALL vs. AML
  - T vs. B lineage ALL
  - Certain subtypes of AML

Bone Marrow Analysis: Cytogenetics

- Analysis of leukemic cell chromosomes
- Chromosome number (ploidy)
- Chromosome structure
  - Deletions
  - Translocations

Cytogenetics in ALL: Ploidy

- **Ploidy** = number of chromosomes
  - **Normal** – diploid (46)
  - **Hyperdiploid** – extra copies of chromosomes (usually favorable)
  - **Hypodiploid** – missing copies of chromosomes (always unfavorable)

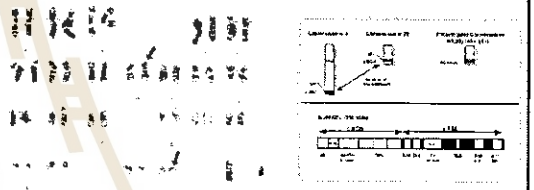
Cytogenetics in ALL: Structural Changes

Type	Associated Gene Product	Prognostic Implication
t(12;21)	TEL-AML1	Favorable
t(4;11)	MLL	Unfavorable
t(9;22)	BCR/ABL (Philadelphia Chromosome)	Unfavorable
Trisomy 4, 10, 17	None	Favorable

NCI classification system of ALL

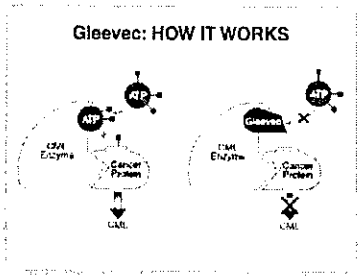
	Standard Risk	High Risk
Age	1-9.999 yrs	≥10 yrs
WBC	<50,000	≥50,000
Steroid pre-treatment	No	Yes

Philadelphia Chromosome



Translocation seen in CML (>95%) and ALL (4%)

Gleevec



T-cell ALL

- Make up 15% of childhood ALL
- Slightly worse prognosis (EFS 85%) than B-cell ALL
- Current study stratifies patients according to response to therapy and tests high dose methotrexate & Nelarabine (nucleoside analogue) in the treatment of T-cell ALL
- All but low risk get cranial XRT

Infant ALL

- Patients diagnosed less than one year of age are eligible
- EFS 35-40%
- Commonly associated w/ 11q23 (MLL gene) rearrangement
  - Same genetic defect in leukemia post- etoposide chemotherapy
  - Signs of leukemia retrospectively found in NBS samples
  - ? In utero exposure to carcinogen
- Treated aggressively, often including bone marrow transplant

Off-therapy ALL care

- Patients need to come to clinic monthly in the first year off therapy for follow-up CBCs & physical exams
- PCP prophylaxis may be discontinued 3 months after stopping therapy
- Subsequent years visits will become less frequent
- "Cure" is defined as remission 5 yrs off therapy

Long term complications from therapy for ALL

- Literature is fairly depressing
  - Cohort of longest term survivors treated in an era when cranial XRT given prophylactically to all kids with ALL
  - Craniospinal XRT affects pituitary function, growth, pubertal development, neurocognitive function, dentition

Long term toxicity of ALL treatment

- Fertility not affected (unless receive BMT)
- Cardiotoxicity from anthracyclines
  - Rare w/ ALL cumulative doses
- Osteoporosis, AVN, & "metabolic syndrome" from steroids
- Secondary malignancies rare (esp. w/o CNS XRT) but dreaded complication

Long Term Outlook for ALL

- Quality of life generally good for most survivors
- Probably a lot fewer complications in patients treated on newer protocols
- We're nearing (or at) the end of our ability to improve survival using conventional chemo
- The future is in molecularly targeted therapies

Treatment - Acute Leukemias

- Chemotherapy
  - Induction
  - Consolidation/re-induction
  - Maintenance
- Central nervous system prophylaxis-intrathecal chemotherapy
- Supportive therapy
  - Growth factors

Treatment - Acute Leukemias

- High dose chemotherapy with stem cell rescue
- Monoclonal antibody
  - Mylotarg (anti CD 33 Antibody)
  - Campath-1H (anti CD 52 Antibody)
- For acute programulocytic leukemia (M3)
  - All-trans retinoic acid (ATRA)

Treatment - Chronic Leukemias

- CLL - chlorambucil, cyclophosphamide, prednisone, fludarabine, rituximab, Campath 1-H
- CML - Hydroxyurea, α-interferon, Gleevec
- MM - alkeran/prednisone, thalidomide, dexamethasone, VAD (vincristine, adriamycin, dexamethasone), bortezomib, stem cell transplant

Response Assessment

- Complete (hematologic) response
  - ANC >1500, Plt >100, Marrow < 5% Blasts
  - Maintain > 4 weeks
- Partial response
- New categories of response: CR-plt
  - CR without complete ANC and or Platelet
- Relapse

Follow-up

- Blood counts
- Bone marrows
- Cytogenetics
- Other genetic markers
- Myeloma - SPEP, UPEP, bone x-rays,
  - β2 microglobulin

3. Lymphomas

3.1 Hodgkin's Disease (HD)
3.2 Non-Hodgkin's Lymphomas (NHL)
- Multiple myeloma (plasma cell)
- Burkitt's lymphoma

Diagnostic Studies

- Lymph node biopsy
- Bone marrow aspiration and biopsy
- Flow cytometry
- Genetic studies
- Cytogenetics

Staging Studies

- Bone marrow aspiration and biopsy
- CTs
- Radionuclide scans: bone, Gallium, PET
- GI x-rays
- Spinal fluid analysis
- Others

Staging

- Same system for HD and NHL
- 4 Stages
  - I One lymph node group
  - II Two lymph node groups
  - III Nodes above and below diaphragm
  - IV Organ involvement
- Add "A" for no systemic symptoms, "B" for systemic symptoms, "E" for extranodal disease, "X" for bulky adenopathy

Prognostic Factors

- Stage - which factors in systemic symptoms, extranodal disease, and tumor bulk
- Histologic subtype
  - Hodgkin's Disease
    - Lymphocyte dominant
    - Nodular sclerosing
    - Mixed cellularity
    - Lymphocyte depletion

Prognostic Factors

- Stage - which factors in systemic symptoms, extranodal disease, and tumor bulk
- Histologic subtype
  - Non Hodgkin's Disease (up to 17 subtypes)
    - Follicular/diffuse
    - Cell type
    - Patterns

Prognostic Factors

- Histologic subtype
  - Non Hodgkin's Disease
    - Low grade (indolent)
    - Intermediate grade
    - High grade (aggressive)

Treatment

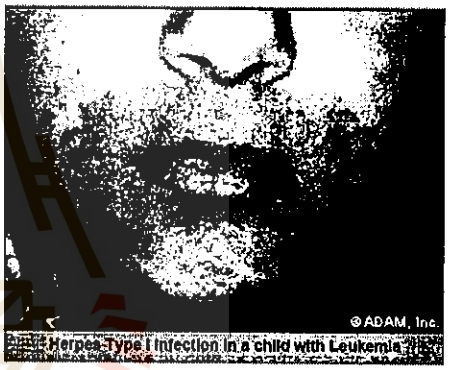
- Chemotherapy
- Radiation therapy
- Monoclonal antibodies - with or without radiolabel or toxin (Rituximab, Zevalin)
- High dose chemotherapy with stem cell rescue

Follow-up

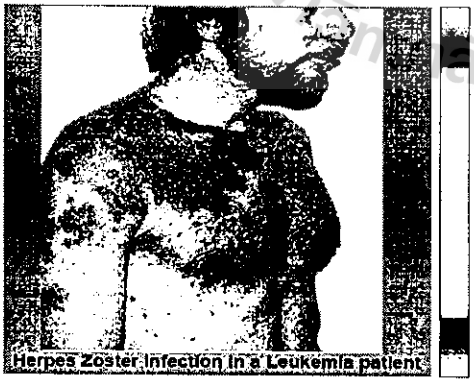
- Relapse
- Survival
- Toxicity (including second malignancies)
- Quality of life

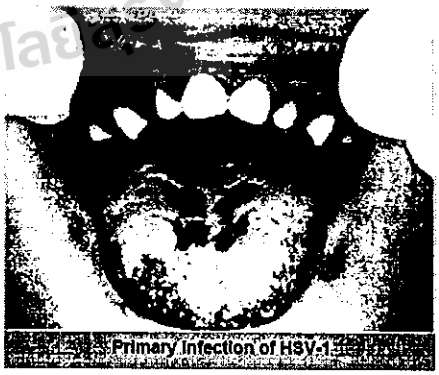
Complications of Leukemias

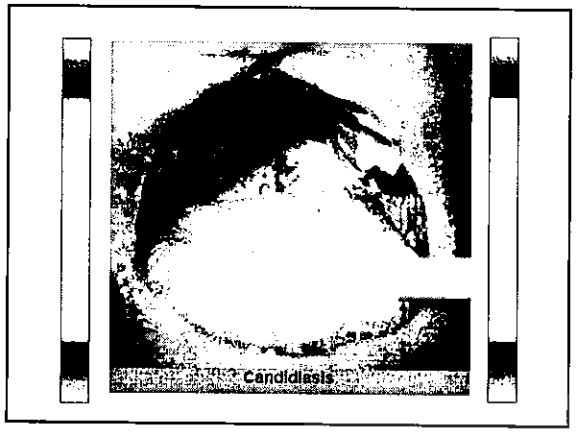
1) **Complications of low white blood count**
   - Lasting low grade fever
   - Night sweats
   - Frequent infections
2) **Complications of Anemia**
   - unexplained fatigue, tiredness, lack of energy
   - pale skin
   - shortness of breath upon physical activities
3) **Complications of low platelets**
4) **Excessive bleeding**
   - Bruises for no clear reason (black or blue marks)
   - Pin head sized red spots under the skin
5) **Complications of other organs involved**
   - Enlarged lymph node
   - Enlarged spleen (abdominal discomfort)
   - Enlarged liver
   - Involvements of skin, CNS, testes, and meninges

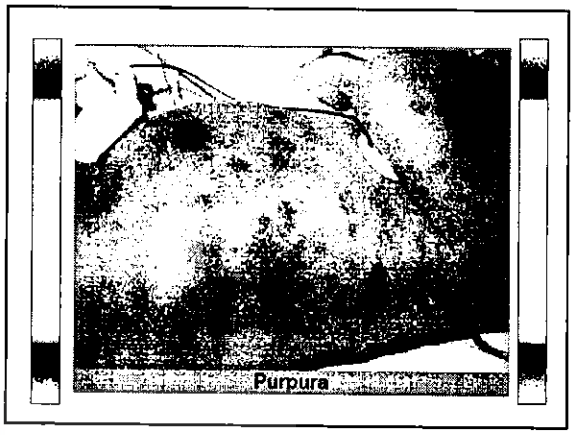


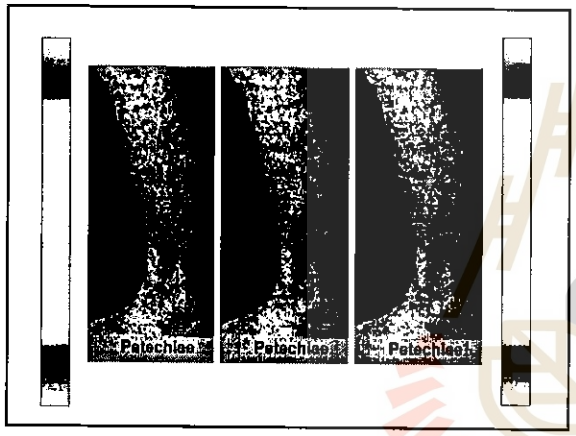
Herpes Type I infection in a child with Leukemia

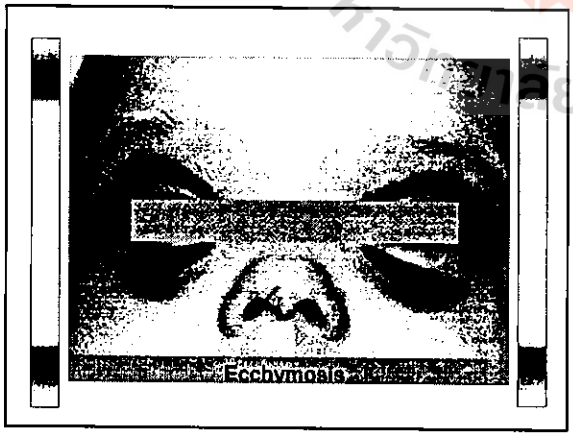

Herpes Zoster infection in a Leukemia patient

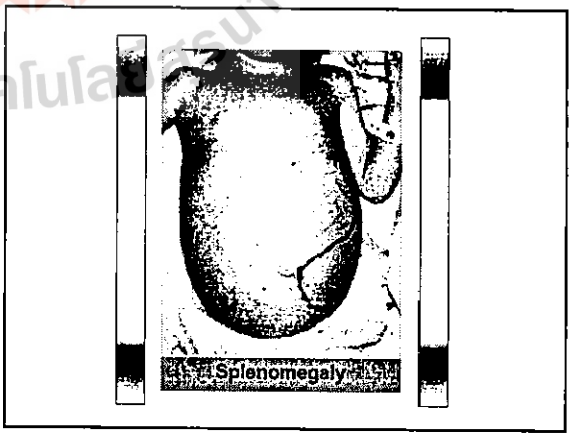

Primary infection of HSV-1

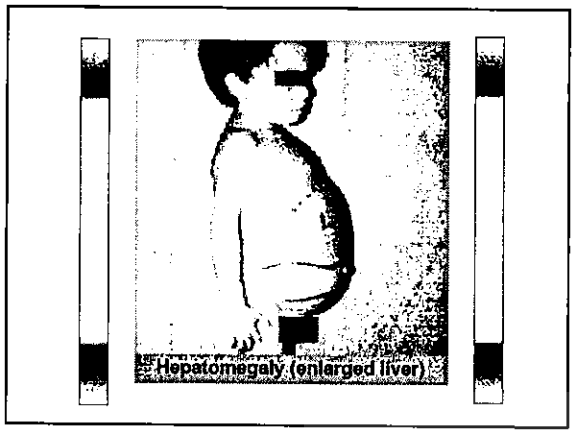


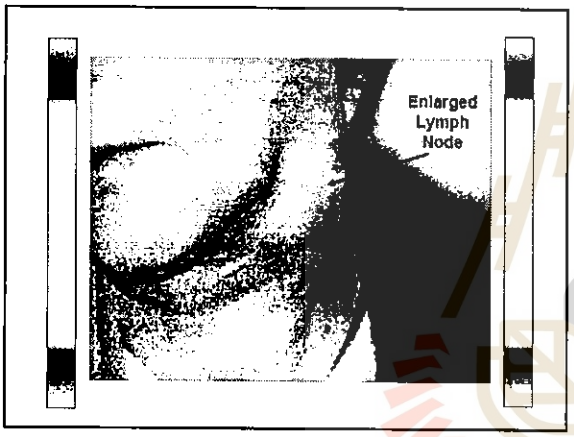


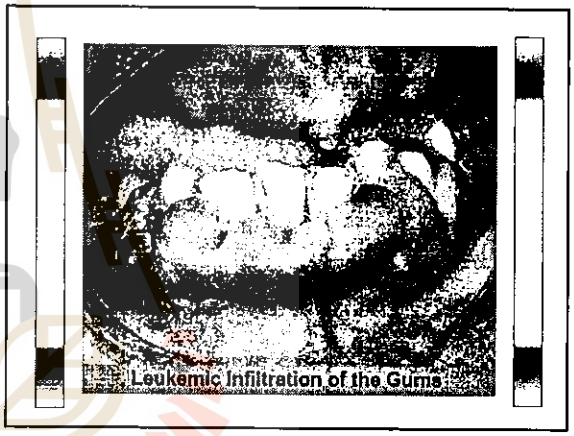


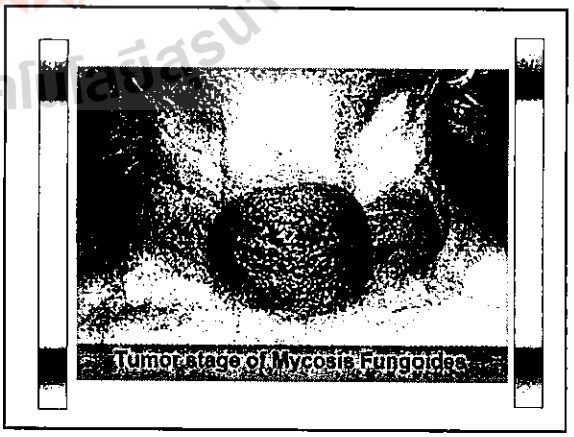




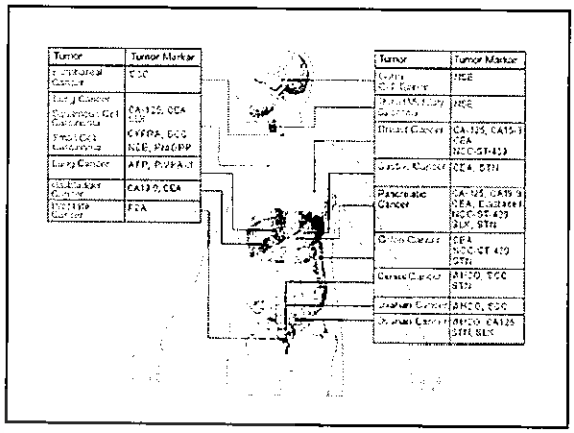






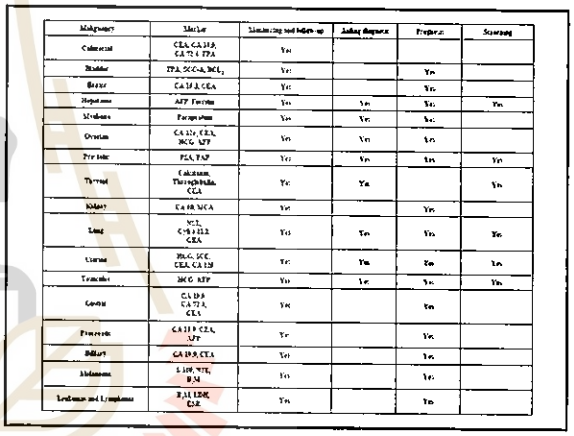


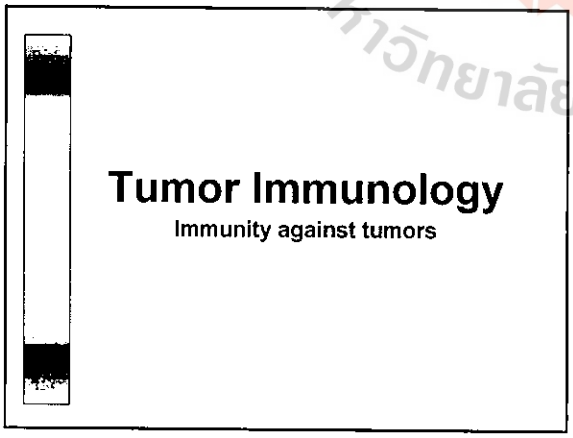
Other Tumors and Tumor Markers



Tumor Markers commonly used in clinical practice

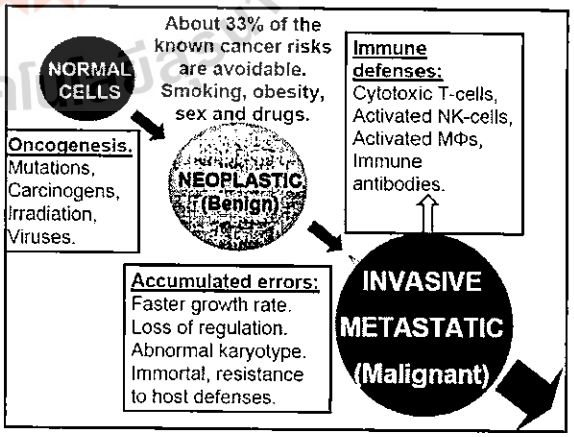
Malignancy	Marker	Monitoring and follow-up	Aiding diagnosis	Prognosis	Screening
Chorio-carcinoma	HCG	Yes	Yes	Yes	Yes
Colorectal	CEA	Yes			
Bladder	TPA	Yes		Yes	
Breast	CA 15-3	Yes		Yes	
Hepatoma	AFP	Yes	Yes	Yes	Yes
Myeloma	Paraproteins	Yes	Yes	Yes	
Ovarian	CA-125	Yes	Yes	Yes	
Prostate	PSA	Yes	Yes	Yes	Yes
Thyroid medullary	Calcitonin	Yes	Yes		Yes
Thyroid follicular papillary	Thyroglobulin	Yes			
Kidney	CA 50	Yes		Yes	
Lung	NSE	Yes	Yes	Yes	Yes
Uterine	HCG	Yes	Yes	Yes	Yes
Testicular	HCG	Yes	Yes	Yes	Yes
Gastric	CA 72-4	Yes		Yes	
Pancreatic	CA 19-9	Yes		Yes	
Biliary	CA 19-9	Yes		Yes	
Melanoma	S-100	Yes		Yes	

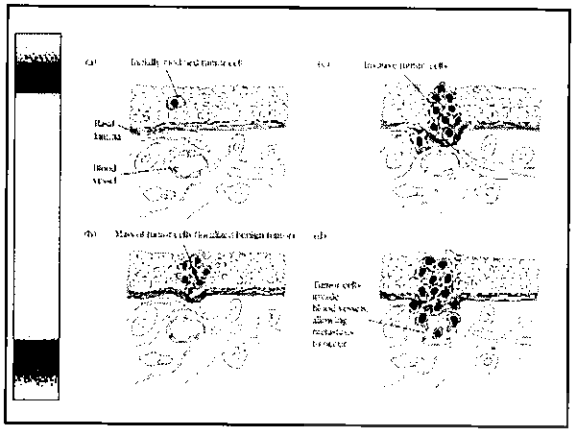




Tumor Immunology

Immunity against tumors





How do cancer cells differ from normal?

- Clonal in origin
- Deregulated growth and lifespan
- Altered tissue affinity
- Resistance to control via apoptotic signals
- Change in surface phenotype and markers
- Structural and biochemical changes
- Presence of tumour-specific antigens

Cancer stages

- Stage-1: **Cancer in situ.**
  - Primary focus, confined to original site, displaces normal tissue during slow growth, no invasion.
- Stage-2: **Local growth** within original organ.
  - Invasion through basement membranes in organ.
- Stage-3: **Invasion** of other adjacent organs.
  - Invasion through organ 'capsule' to adjacent tissues.
- Stage-4: **Metastasis**, to secondary sites.
  - Vascular/lymphatic spread to lung, liver, brain.

AVOIDABLE CANCER RISKS.

- Poor fetal nutrition, maternal low protein diet.
  - Children should be breast fed for 6 months
- Foods high in sugar and/or fat. Energy dense foods and salt.
- Red meats and processed meats.
  - Eat mostly foods of plant origins
- Obesity, particularly abdominal obesity.
  - Be as lean as possible. BMI between 21 - 25
- Carcinogen exposure.
  - Minimize exposure to metals in cereals and legumes
- Sedentary lifestyle.
  - Be physically active every day
- Alcoholic drinks in excess of 30 gm Ethanol/day.
- Tobacco products.

American Institute for Cancer Research, 2007.

IS IMMUNE SURVEILLANCE IMPORTANT?

Patient group	Annual Incidence	Adjusted Risk	Rate of growth
Normal	1/300	1	None
Allograft	1/100	25	Fast
No T-cells	1/100	25	Very fast
No B-cells	1/10	5,000	Fast
S.C.I.D.	1/10	10,000	Very fast
No NK/MΦ	1/10	5,000	Fast

SCID

The GAZETTE, Montreal, Tuesday, November 11, 1980

Cancer kills 'bubble boy'

DURHAM, N.C. (UPI) — Ricky the "bubble boy" has died — without ever having felt the winter wind on his face or summer's warm grass under his feet.

For little Ricky had to spend the whole three years of his life inside a germ-free plexiglass chamber in a hospital.

Yesterday the nurses at that hospital mourned him. "He had become more like our child than a patient," one said.

Ricky — Richard Joel Bradley Jr. — was born with an hereditary condition that prevented his body generating normal organisms that combat bacteria.

His sterilized plexiglass bubble at Duke University Medical Centre could only be entered by people who put on surgical gowns, masks and gloves.

Nurses dressed him and performed other chores for him through long rubber gloves built into one wall of the "bubble".

The shadow of tragedy fell over Ricky last July. He contracted cancer of the white blood cells.

Ricky Bradley: Three years in a germ-free chamber

Three years in a germ free chamber and then dies of leukemia.

MALIGNANT CELLS

- No factor dependent growth regulation.
- Loss of contact inhibition of growth.
- Surface independent growth in suspension.
- High mitotic index or 'S' phase fraction.
- Production of angiogenic factors.
- Immortal (apoptosis inactivated/inhibited).
- Loss of "tumor suppressor genes".
- Abnormal DNA content (ploidy).
- Invasive phenotype, produce invasion factors.
- Spread by metastasis, seed to other sites.

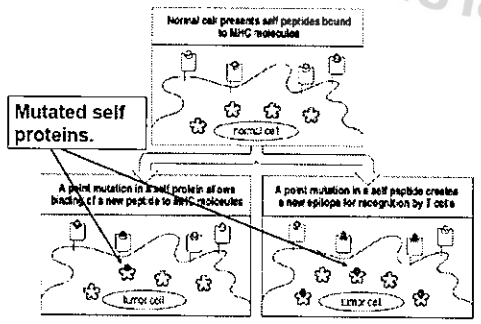
TUMOR SPECIFIC ANTIGENS

- Tumor specific transplantation antigens (TSTA). Cell antigens that are specific targets of tumor rejection mechanisms. MHC-I dependent peptide presentation.
- Tumor associated antigens (TAA). Normal tissue proteins and glycoproteins that are produced in abnormal quantities by tumor cells. Not usually involved in tumor rejection but may be useful in detection.

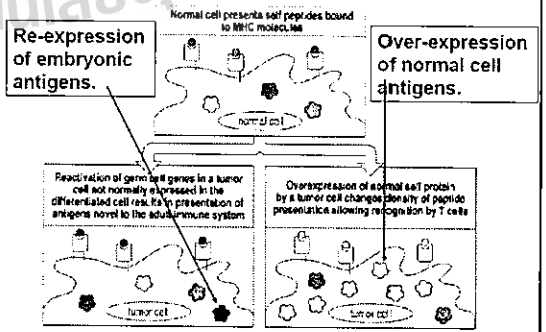
Tumor antigens

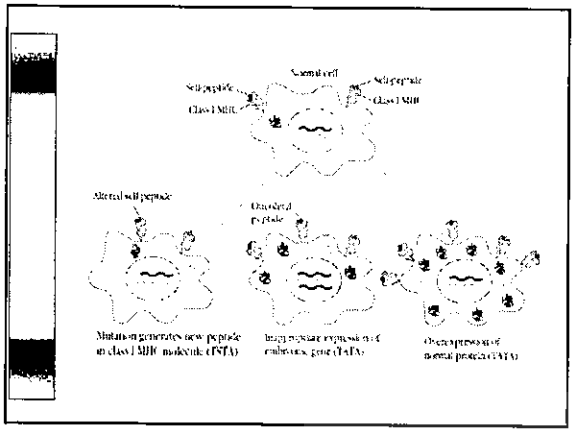
- **tumor specific antigens (TSA)** (no general tumor specific antigen)
- **tumor specific transplantation antigens (TSTA)**
  (mutated self antigens expressed on MHC-I)
- **tumor associated antigens (TAA)**
  (AFP, CEA, hCGβ, PSA, CA, LDH, β2 microglobulin)
- **tumor associated transplantation antigens (TATA)** (tumor associated antigens expressed on MHC-I)

TUMOR SPECIFIC ANTIGENS (TSA)



TUMOR SPECIFIC ANTIGENS (TSA)





TUMOR-ASSOCIATED AND TUMOR-SPECIFIC ANTIGEN PEPTIDES RECOGNIZED BY HUMAN T CELLS

Malignant melanoma, esophageal carcinomas, non-small-cell lung carcinomas and hepatocellular carcinomas	MAGE 1	EADPTGHSY and ASSCETKL
Melanoma	Tyrosinase	MLLAVLYCL YVNLTMGIC, YMNGTMSQV, and others
Colon cancer	Carcinoembryonic antigen (CEA)	YLSGANLNL
Breast and ovarian cancer	HER2/neu	KIFGSLAFL
Head and neck squamous-cell carcinoma	Caspase 8	LPSDSWDGF
Chronic myeloid leukemia	b3a2 fusion protein (product of fusion of bcr gene with abl gene)	ATGFKQSSKALQRPVAS
Prostate cancer	PSA	FLTPKKLQCV and FLSNKVVCV

Fig. 20.3 EBV is associated with Burkitt's lymphoma in Africa and nasopharyngeal cancer in China, suggesting that co-factors, either genetic or environmental, are required to cause the tumours. Adult T-cell leukaemia is found mainly in Japan and the Caribbean. Roitt

Viruses and human tumours

tumour	virus
liver cancer **(Hepatocellular carcinoma)**	hepatitis B
cervical cancer	human papillomaviruses (HPV 16, 18 and others)
Burkitt's lymphoma and other lymphomas in immunosuppression	EBV (Epstein-Barr Virus)
nasopharyngeal cancer	EBV **(Herpesvirus)**
adult T-cell leukaemia	human T leukaemia virus I (HTLV-I) **(Retrovirus)**

Tumor associated antigens.

- Igs produced by myeloma cells (B-cell).
- Alpha-fetoprotein produced by liver cell tumors (hepatocellular carcinoma).
- Chorionic gonadotrophin produced by trophoblast cell tumors (choriocarcinoma).
- Carcinoembryonic antigen produced by colon cancer cells (colon carcinoma).

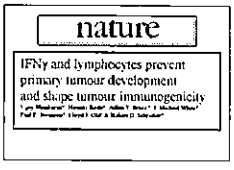
Immune Surveillance of Cancer

- Proposed originally in 1909 by Paul Ehrlich
- Refined in late 1950s by Burnet and Thomas

> "In animals…genetic changes must be common and a proportion…will represent a step towards malignancy
>
> …there should be some mechanism for eliminating such potentially dangerous mutant cells and it is postulated that this mechanism is of immunological character "
>
> FM Burnet "The concept of immunological surveillance" (1970

Immune Surveillance of Cancer

- Subsequent evidence against immune surveillance, particularly from nude mice studies.
- More recent studies identify effector populations and KO models utilised.
- Definitive evidence of immune surveillance published by Schreiber *et al* in 2001



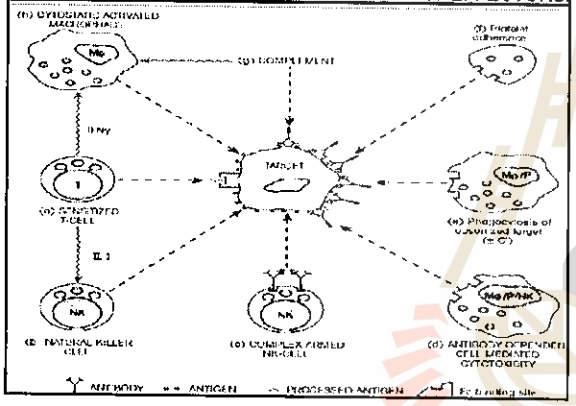
Evidence of Immune Surveillance in Humans

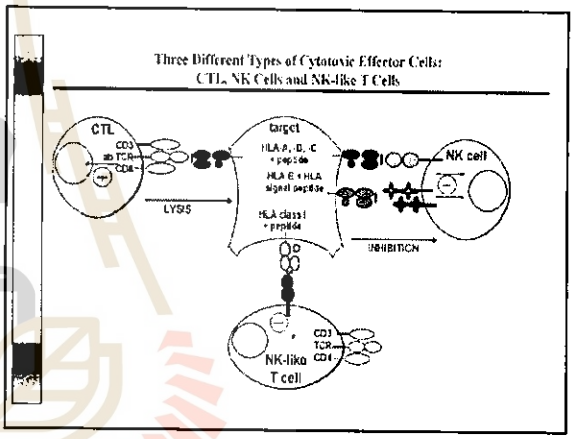
- Immunosuppression leads to increased development of viral-derived tumours (Kaposi / NHL / HPV).
- Organ transplant increases malignant melanoma risk. (0.3% general paediatric popn., 4% paediatric transplants)
- 3-fold higher risk of sarcoma.
- High TIL presence correlates with improved survival.
- NK or γ/δ T cell loss correlates with increased tumour pathogenicity.

Immune reactions against tumor cells

- T cell mediated (CD8+, CD4+Th1, NK)
- macrophage mediated
- immunoglobulin mediated (ADCC)
- network of cytotoxic cytokines

HOST DEFENCE AGAINST CANCER HAS MANY EFFECTORS





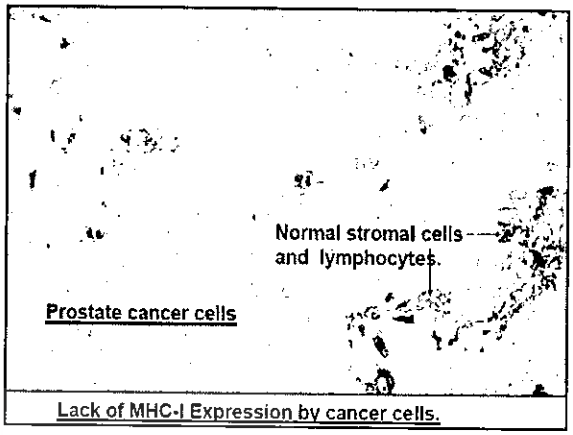
Tumor escape

- Over expression or down regulation of MHC-I.
- over expression of FcR
- deficiency of cytokine receptors
- production of different glycoproteins with masking effects

Fig. 20.9 CD4⁺ and CD8⁺ T cells in carcinoma of the breast. $CD4^+$ and $CD8^+$ cells were detected by the immunoalkaline phosphatase technique (pink stain) using monoclonal antibodies. The sections are counterstained with haematoxylin. CD4 (upper), and fewer CD8 cells (lower), were seen surrounding the tumour but few lymphocytes were within the tumour itself. Roitt et a

HOW CANCERS EVADE THE HOST RESPONSE

- Cancer cells grow faster than they can be killed.
- Reduced MHC expression evades CTL recognition.
- NK-cells have limited capacity for clonal expansion.
- Altered glycosylation masks TSA recognition.
- Immune complexes block cytotoxic cell activity.
- Immunosuppressive factors reduce host response.
- Angiogenic factors augment tumor cell nutrition.
- Cancer cells become immortal and evade killing.
- Cancer cells lose contact inhibition and requirement for attachment and can rapidly spread by blood or lymph to other sites (liver, lung, brain, etc).



Lack of MHC-I Expression by cancer cells.

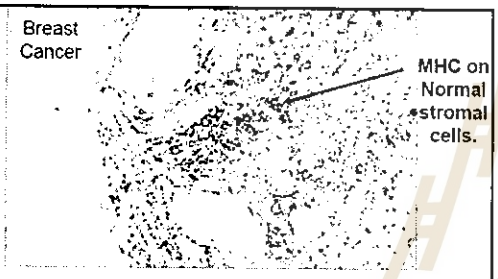
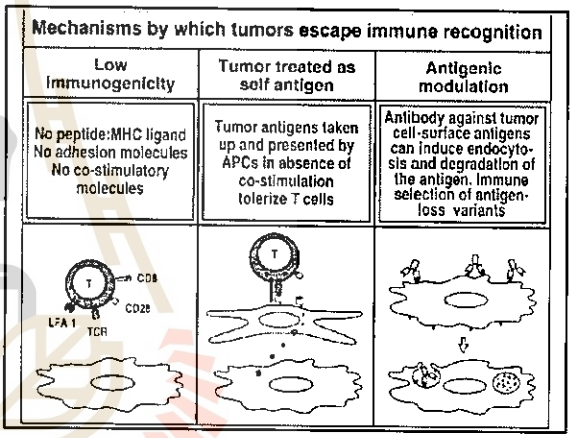
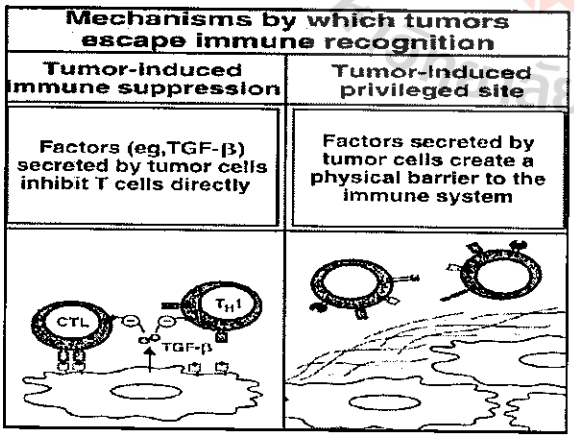
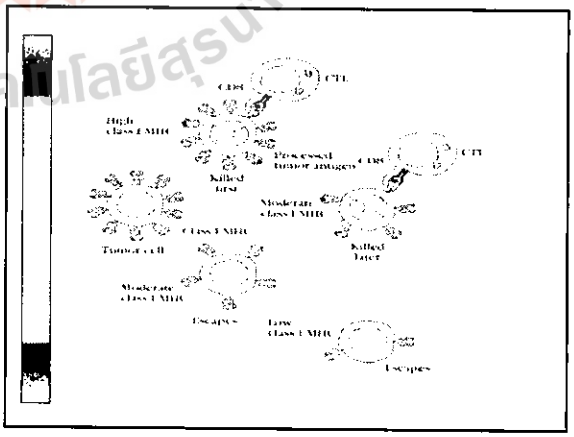


Fig. 20.13 Breast cancer tissue reactive with a monoclonal antibody to a monomorphic determinant of HLA class I antigens. Only stromal cells are stained (brown colour), as malignant epithelial cells fail to express normal MHC class I antigens. Some 50% of primary human cancers fall into this category. Aberrant class II expression may also occur on some tumours. (Indirect immunoperoxidase technique, counterstained with haematoxylin.)

Roitt, Brostoff Male







NK cell control of cancer in humans

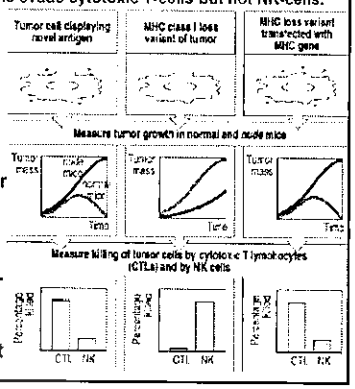
- NK / NKT cells in animal models destroy tumours with down-regulated Class I expression.
- Control of haematological malignancy after haplotype-mismatched BM/SC transplant
  Costello *et al* (2004) Trends Immunol.
- Maintenance of remission in acute leukaemias dependent upon $CD56^+/CD8\alpha^+$ NK cells
  Lowdell *et al* (2002) Br.J.Haematol.

NK-CELLS AND CYTOTOXIC T-CELLS KILL CANCER CELLS

MHC deficient tumor cells evade cytotoxic T-cells but not NK-cells.

•Cytotoxic T-cells can only kill tumor cells that express MHC-I antigens and the Tumor Specific Antigen (peptide).

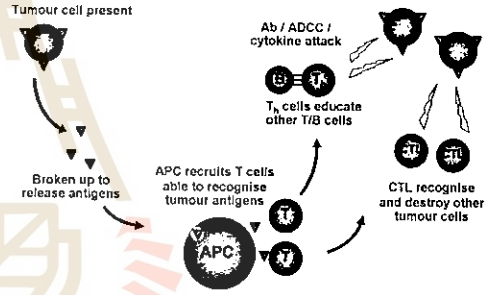
•NK-cells can kill tumor cells that lack MHC-I antigens but express other transformation markers. However, NK-cells have limited abilities to proliferate and mount a persistent attack on the cancer.



Antigens involved in tumour recognition

Tumour-specific antigens		Testes-specific antigens	
•Bcr-abl	(CML)	•MAGE 1-3	(melanoma)
•CDK-4 / β-catenin (melanoma)		•NY-ESO-1	(melanoma)
Differentiation antigens		**Tumour associated antigens**	
•Tyrosinase (TRP-1/2)		•MUC-1	(myeloma etc)
•Melan-A	(melanoma)	•α-fetoprotein	(many)
•Monoclonal Ab	(myeloma)	•Her-2/neu	(breast)
		•WT-1	(many)
		•myeloblastin	(leukaemias)
		•Survivin	(many)

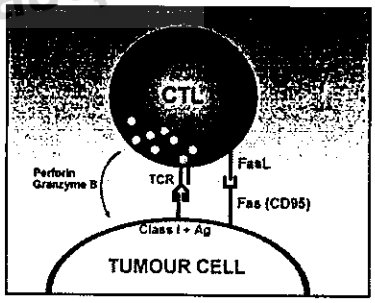
How does the adaptive IR target tumours?



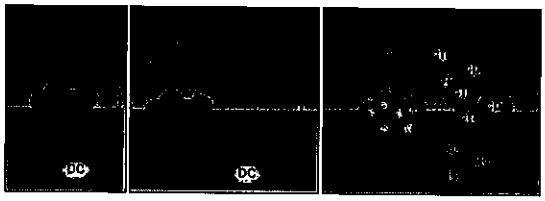
Effector mechanisms against cancer

- Monocyte / macrophage release lytic enzymes and phagocytose necrotic material
- Antibody against tumour antigens
- Induction of tumour-specific CTL and TIL
- Initiation of NK / CTL cytotoxic responses
- Release of cytokines / chemokines (TNFα, IFNs etc) and antiangiogenic factors

Direct CTL / NK attack



IR-Mediated Tumour Elimination



Innate IR recognises tumour cell establishment

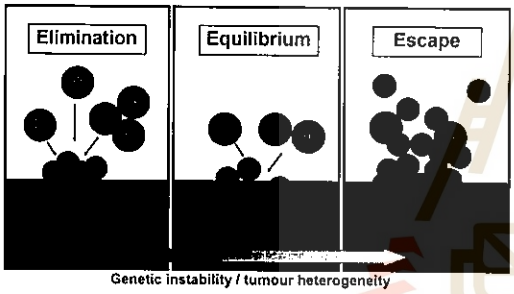
NK cells and other effectors recruited to site by chemokines, which also target tumour growth directly.

Tumour-specific T cells home to tumour site, along with macrophages and other effectors to eliminate tumour cells.

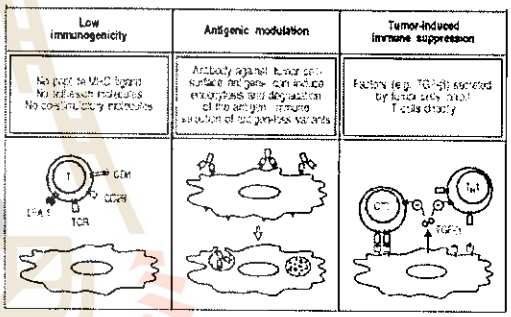
Immunoediting- *The Great Escape!*

- Strong evidence that IR controls and eradicates nascent cancer cells
- "Immunoediting" eventually produces low antigenicity tumour cells
- Pressure from immune system coupled with genomic instability selects for escape

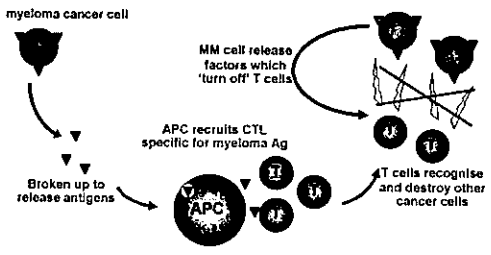
Three Es of Immunoediting



Evasion Mechanisms



How does MM evade the immune response?



HOW CAN WE HELP THE HOST RESPONSE?

- Detect cancers early when tumor is small.
- Develop tumor growth regulatory agents.
- Develop anti-angiogenic anti-tumor agents.
- Augment NK surveillance against cancer.
- Augment MΦ mediated tumor cytotoxicity.
- Augment the Tc-cell mediated cytotoxicity.
- Develop anti-tumor antibodies, ADCC.
- Block the malignant/invasive phenotype.

Possible immuno-therapies

- immunotargeting through the TAAs
- Immunomodulation
- tumor vaccines

Immunotherapy of tumors

active	non-specific	BCG, *Propionibacterium acnes*, levamisole, cytokine genes, *etc.*
	specific	killed tumor cells or their extract, recombinant antigens, idiotype, co-stimulatory molecule genes, *etc.*
passive	nonspecific	LAK cells, cytokines
	specific	antibodies alone or coupled to drugs, pro-drug toxins or radioisotope; bispecific antibodies; T-cells
	combined	LAK cells and bispecific antibody

* BCG: Bacillus Calmette-Guerin is a bovine strain of *Mycobacterium tuberculosis*

Non-specific active immunotherapy: biological response modifiers (BRMs)

type of BRM	examples	major effect
bacterial product	BCG, *P. acnes*, muramyl di-peptide, trehalose dimycolate	activate macrophages and NK cells (via cytokines)
synthetic molecules	pyran, poly I:C, pyrimidines	induce interferon production
cytokines	interferon-alpha, -beta, -gamma, IL-2, TNF	activate macrophages and NK cells

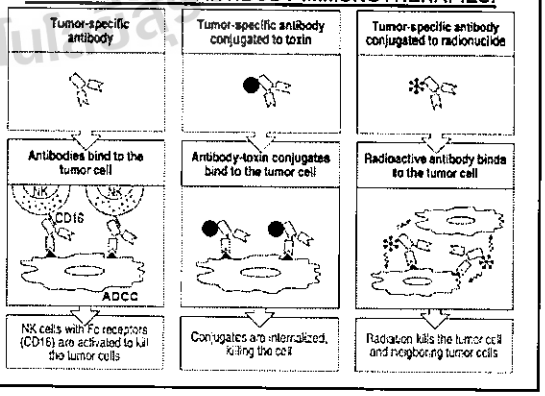
Cytokine therapy of tumors

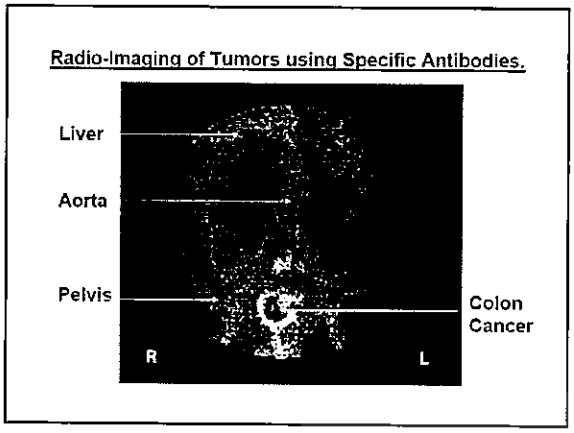
cytokine	tumor type and result	antitumor mechanism(s)
IFN-alpha, beta	remission of hairy cell leukemia, weak effect on some carcinomas	increased expression of class I MHC, possible cytostatic anti-tumor effect.
IFN-gamma	remission of peritoneal carcinoma of ovary; ineffective systemically	increased MHC antigens; macrophage, Tc and NK cell activation
IL-2	remission in renal carcinoma and melanoma	T-cell proliferation and activation, NK cells activation
TNF-alpha	can reduce malignant ascites	macrophage and lymphocyte activation

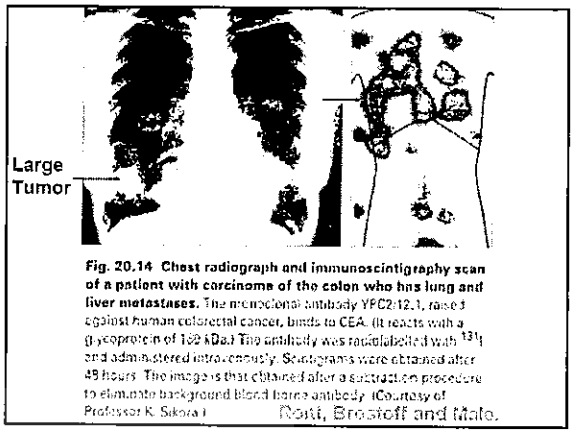
TUMOR SPECIFC ANTIGENS AND IMMUNOTHERAPY.

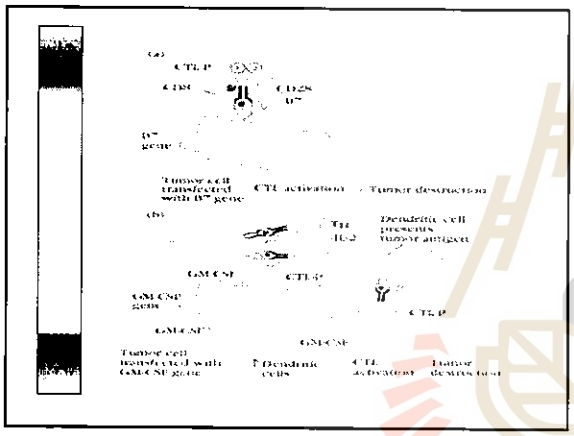
Tumor tissue origin	Type of antigen	Antigen	Tumor type
Lymphoma/ leukemia	Differentiation antigen	CD5 Idiotype CALLPATH-1 (CDw52)	T-cell lymphoma B-cell lymphoma T- and B-cell lymphoma
	B-cell signaling receptor	CD20	Non-Hodgkin's B-cell lymphoma
Solid tumors	Cell-surface antigens Glycoprotein Carbohydrate	CEA, mucin-1 Lewis Y CA-125 BrCa1	Epithelial tumors (breast, colon, lung) Epithelial tumors Ovarian carcinoma
	Growth factor receptor	Epidermal growth factor receptor p185HER2 IL-2 receptor	Lung, breast, head, and neck tumors Breast, ovarian tumors T- and B-cell tumors
	Stromal extracellular antigen	FAP-α Tenascin Metalloproteinases	Epithelial tumors Glioblastoma multiforme Epithelial tumors

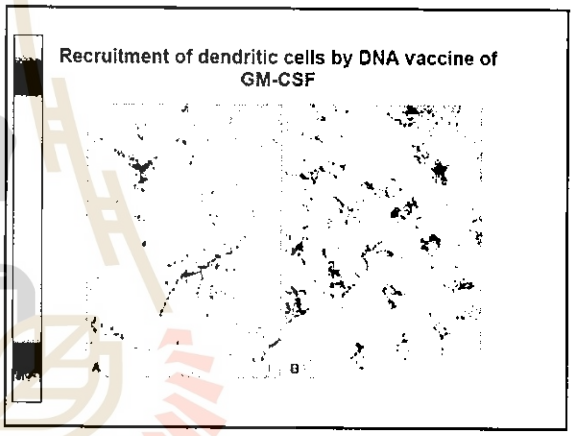
TUMOR SPECIFIC ANTIBODY IMMUNOTHERAPIES.

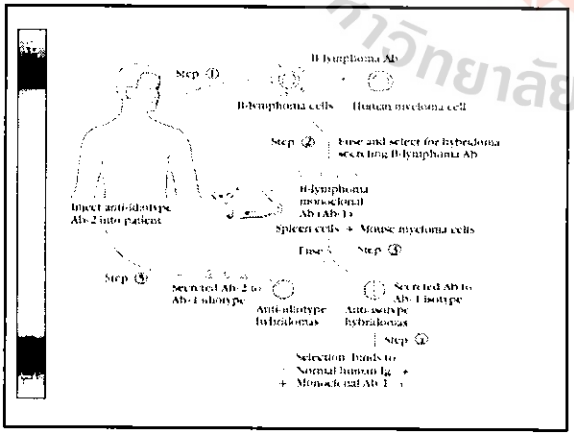


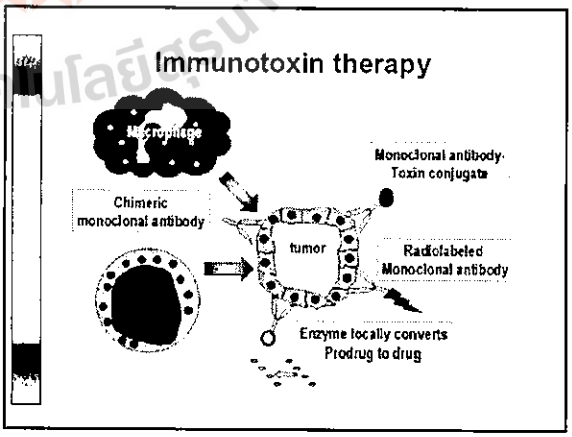




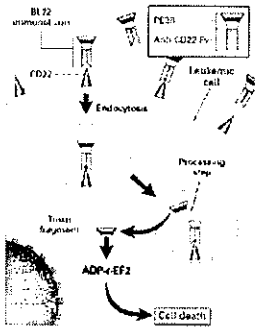








Immunotoxin therapy of hairy cell leukemia with BL22



FUTURE FOR CANCER

- Better early detection by immunoassays.
- Cancer specific drugs to regulate/slow growth.
- Cytokine therapies to optimize innate defenses.
- More specific antibody-based therapeutics.
- Cytokines to augment the host CMI response.
- Gene therapy to increase tumor immunogenicity
- Gene therapy to reverse tumor cell immortality.
- Better monitoring of the effect of treatment.
- *Development of vaccines to prevent cancers.*

The End For Now

Chapter 3.

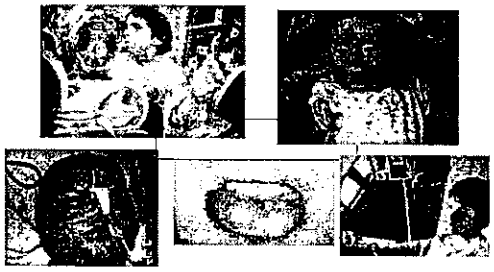
Diseases caused by Defective Immune Response 1 (Part A)

Immunodeficiency (Diseases caused by defective

By Asst. Prof. Dr. Wilairat Leeanansaksiri

1

What's Happen to Them ?



Can you help them ?

2

Immunodeficiency

Outline

1. Concise summarization of normal immune response
2. Concise summarization of normal immunity to infection
3. innate immunodeficiency and primary immunodeficiency diseases Adaptive Immunodeficiency
4. adaptive immunodeficiency and secondary immunodeficiency diseases

3

Immunodeficiency

Objectives

1. Understand and can explain normal immune response both innate and adaptive immunities
2. Understand and can explain capability of microbes to escape immune response
3. Understand and can explain innate immunodeficiency and primary immunodeficiency diseases
4. Understand and can explain adaptive immunodeficiency and secondary immunodeficiency diseases

4

Immunodeficiency

1. Primary Immunodeficiency
- Adaptive (Part A)
- Innate (Part B)

2. Secondary Immunodeficiency
- Adaptive (Part A)
- Innate (Part B)

5

1. Primary Immunodeficiency

- congenital Immunodeficiency (usually abnormal since birth)
- due to **genetic defect** leads to blocks in the maturation or functions of different components of the immune system
  - Innate immunity components defect e.g. phagocytosis, complement
  - Adaptive immunity components defect e.g. T- cells, B- cells

6

- Acquired Immunodeficiency
- **No genetic defect**
- **due to other defects** including infections, nutritional abnormalities, or treatment that cause loss or inadequate function of various components of the immune system e.g. immune suppressive drugs, HIV infection.

- Drug related
- Disease related
  - –Cancer
  - –AIDS
    - HIV
    - T helper cell as target

Loss or reduction of:

- Cell type
- Cell numbers
- Cell function

- Receptors
- Cell signaling
- Cytokine production
- Ig production
- Co stimulation impairment
- Intracellular killing
- Extravasation impairment

Part A : Adaptive immunity components defect e.g. T- cells, B- cells

Many diseases: SCID, CVID, etc.

- Drug related
- Disease related
  - – Cancer , AIDS

Part B : Innate immunity components defect e.g. Macrophage....

- **Chronic granulomatous disease**
- **Congenital agranulocytosis**
- **Leukocyte-adhesion deficiency**
- **Chediak-Higashi syndrome**

Part A
1. Primary Immunodeficiency

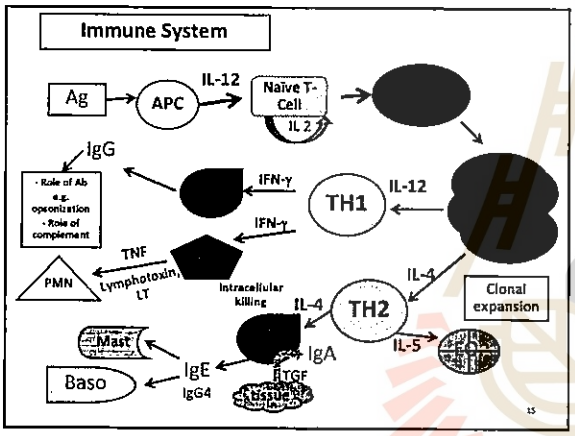
Defect in T and B cells

- Severe combined immunodeficiency (SCID)
- B cells
  - Agammaglobulinemia
  - Hypogammaglobulinemia
  - Specific Ig Deficiencies
- T cells
  - DiGeorge Syndrome
  - Wiskott Aldrich Syndrome

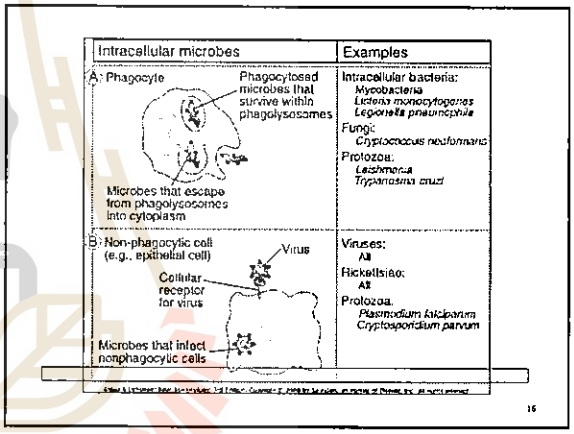
13

Review T and B cell in immune response

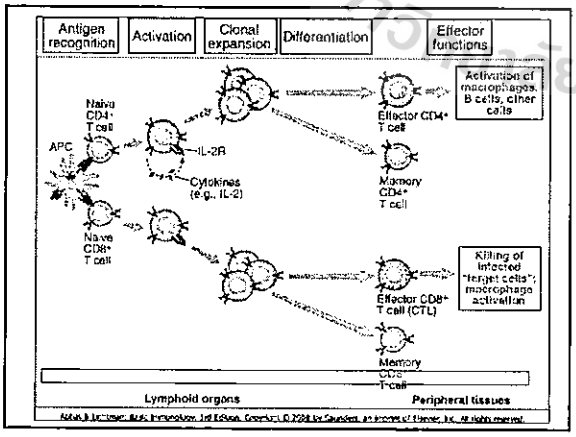
14

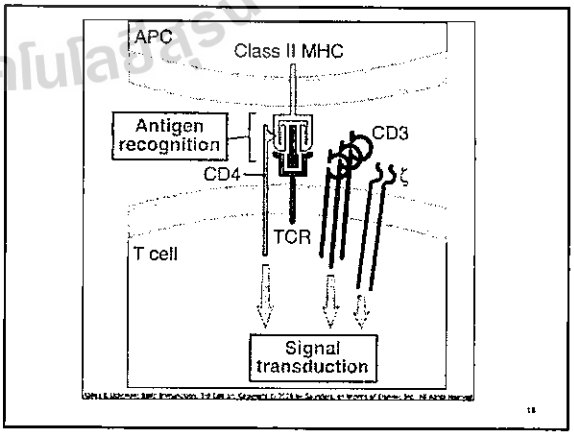


15

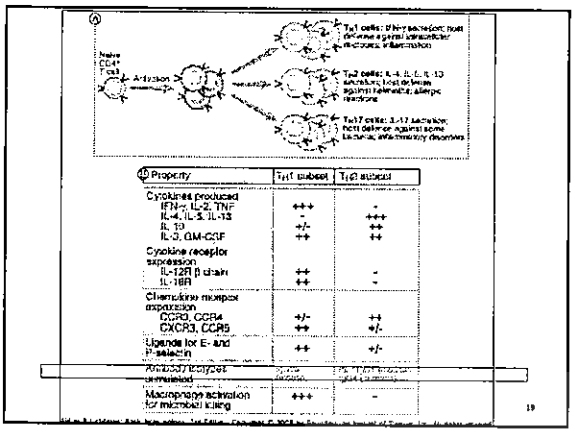


16



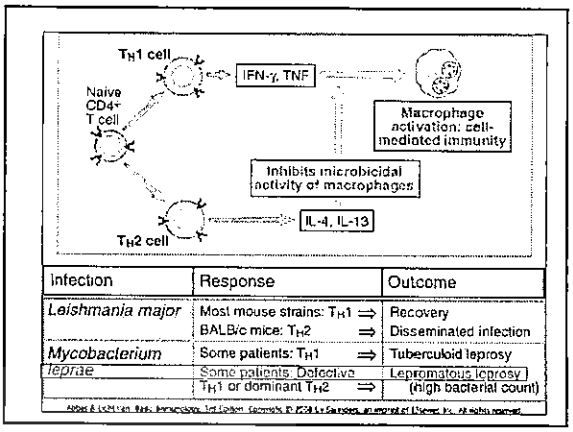


18

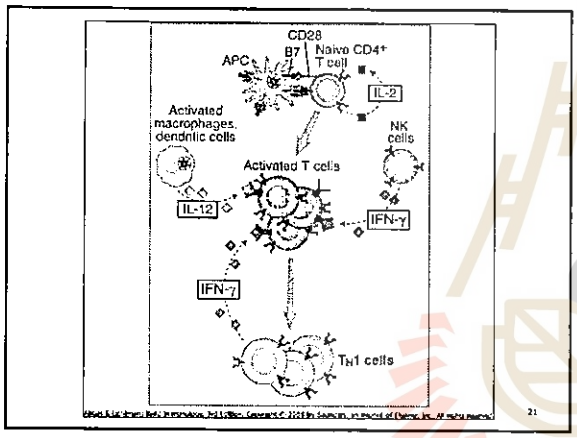


Property	T_{H1} subset	T_{H2} subset
Cytokines produced		
IFN-γ, IL-2, TNF	+++	-
IL-4, IL-5, IL-13	-	+++
IL-10	+/-	++
IL-3, GM-CSF	++	++
Cytokine receptor expression		
IL-12R β chain	++	-
IL-18R	++	-
Chemokine receptor expression		
CCR3, CCR4	+/-	++
CXCR3, CCR5	++	+/-
Ligands for E- and P-selectin	++	+/-
Antibody isotypes stimulated	[illegible]	[illegible]
Macrophage activation for microbial killing	+++	-

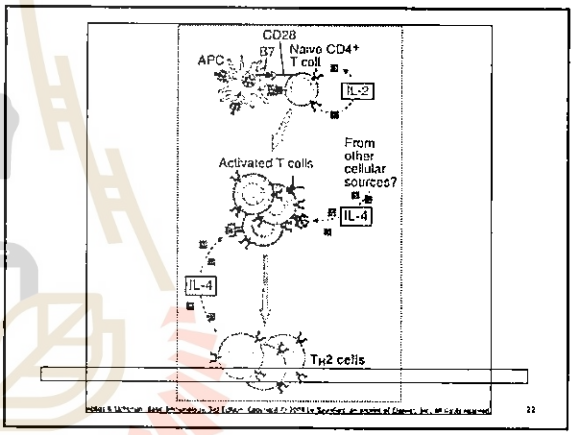
19



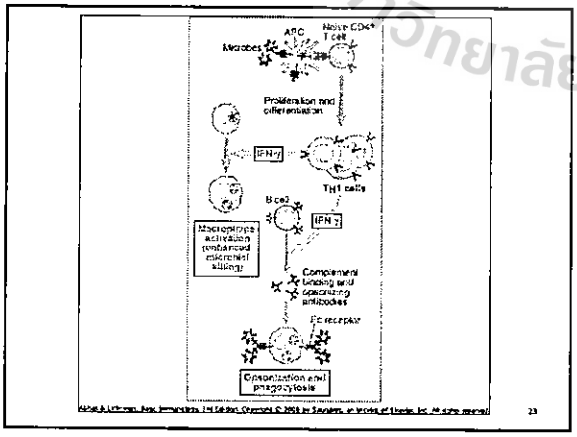
Infection	Response	Outcome
Leishmania major	Most mouse strains: T_H1 ⇒	Recovery
	BALB/c mice: T_H2 ⇒	Disseminated infection
Mycobacterium leprae	Some patients: T_H1 ⇒	Tuberculoid leprosy
	Some patients: Defective T_H1 or dominant T_H2 ⇒	Lepromatous leprosy (high bacterial count)



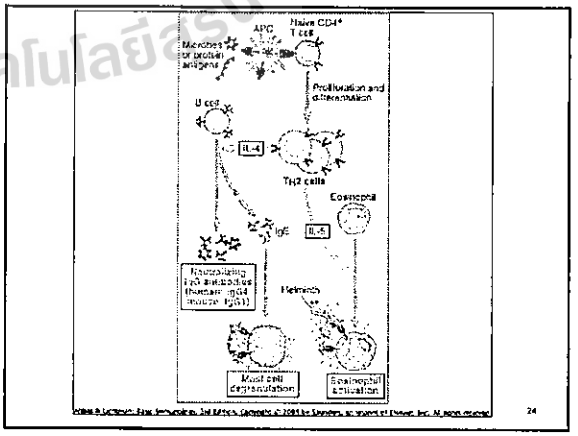
21



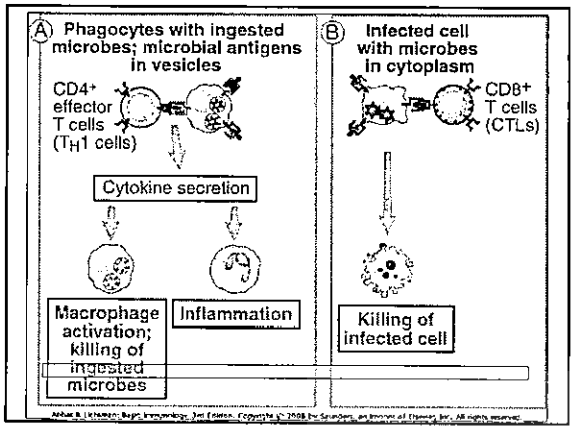
22

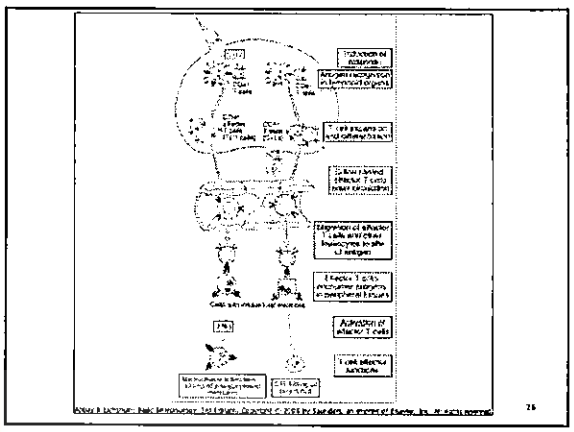


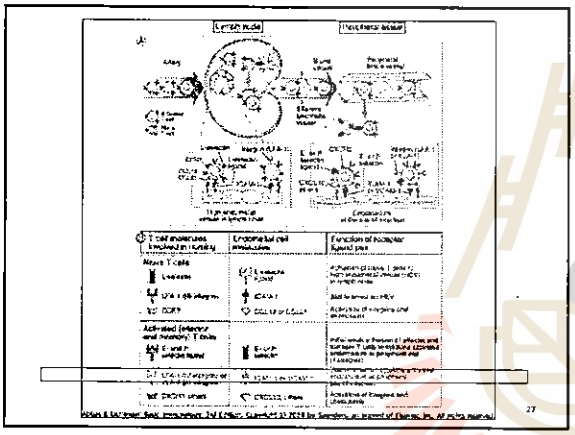
23

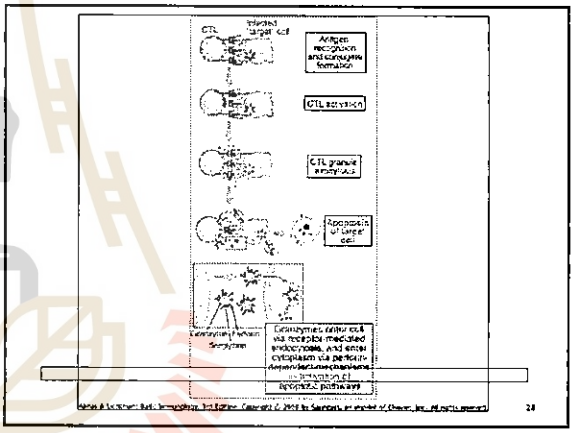


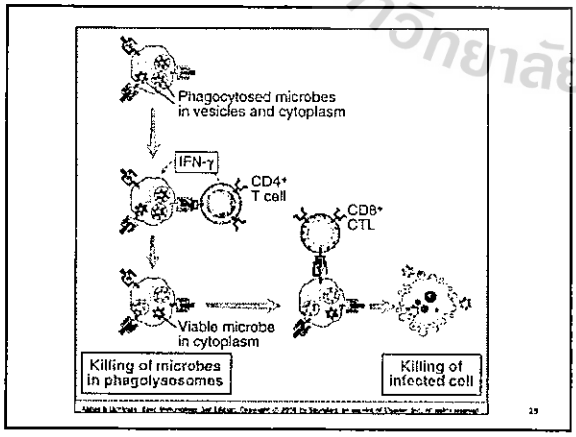
24

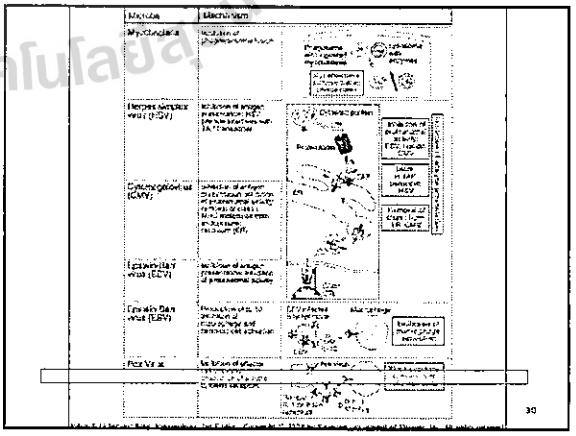


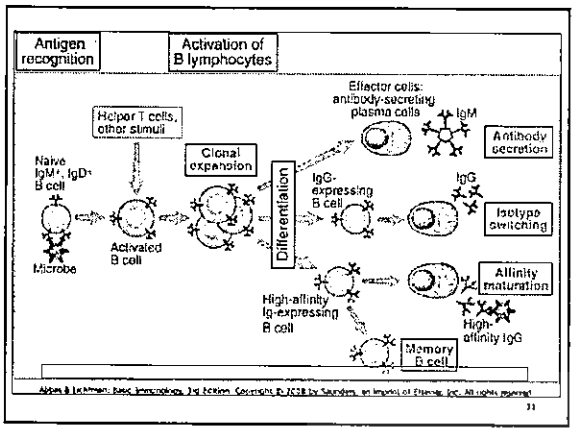


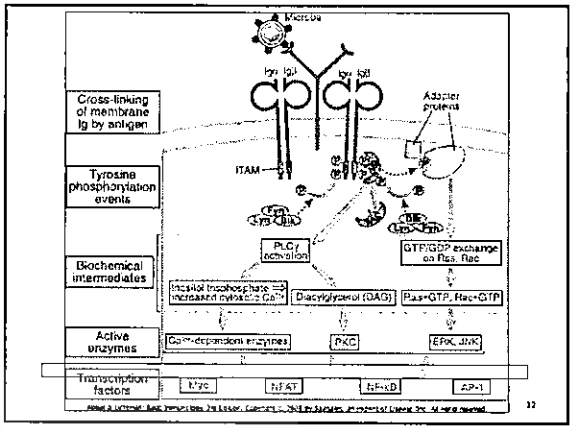


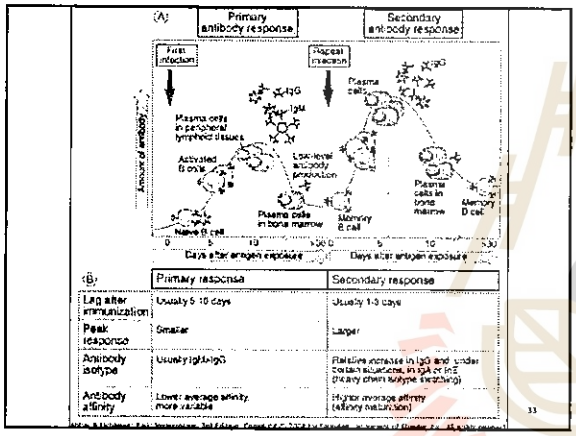


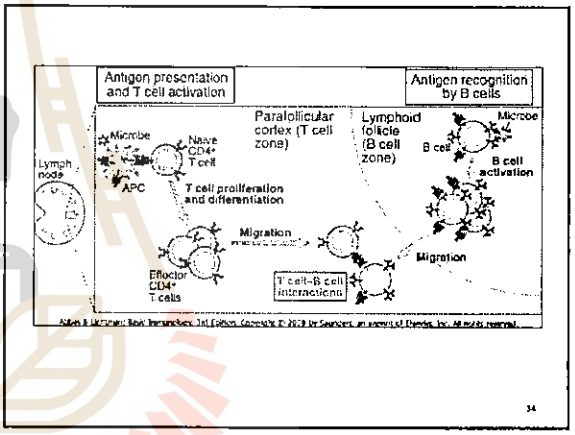


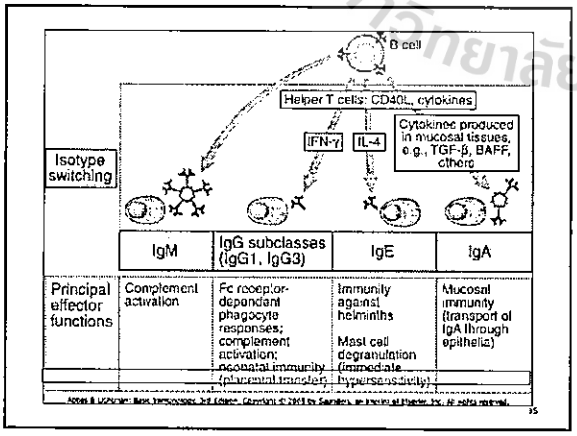


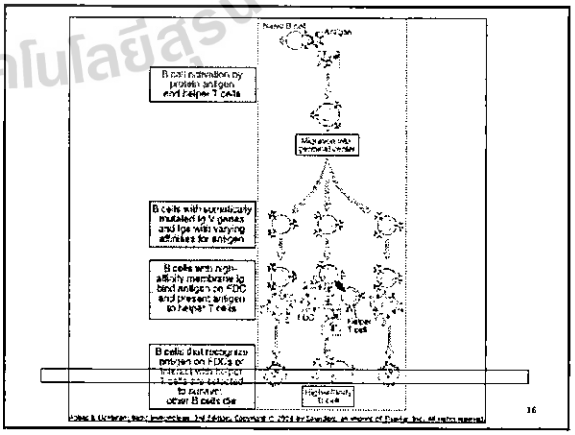

Antigen recognition
Activation of B lymphocytes
Helper T cells, other stimuli
Clonal expansion
Differentiation
Effector cells: antibody-secreting plasma cells
IgM
Antibody secretion
Naive IgM+, IgD+ B cell
Activated B cell
Microbe
IgG-expressing B cell
IgG
Isotype switching
Affinity maturation
High-affinity Ig-expressing B cell
High-affinity IgG
Memory B cell

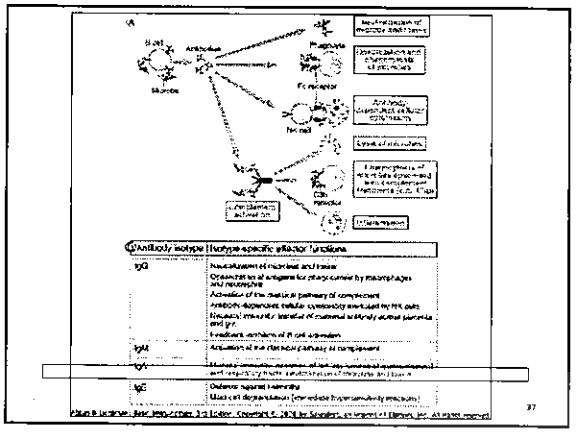

Cross-linking of membrane Ig by antigen
Tyrosine phosphorylation events
Biochemical intermediates
Active enzymes
Transcription factors
ITAM
Adaptor proteins
PLCγ activation
GTP/GDP exchange on Ras, Rac
Diacylglycerol (DAG)
Ras•GTP, Rac•GTP
PKC
ERK, JNK
NFAT
NF-κB
AP-1

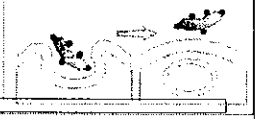

(A)
Primary antibody response
Secondary antibody response
First infection
Second infection
IgG
IgM
Plasma cells
Plasma cells in peripheral lymphoid tissues
Activated B cells
Isotype switching
Plasma cells in bone marrow
Memory B cell
Naive B cell
Amount of antibody
Days after antigen exposure
(B)
Primary response
Secondary response
Lag after immunization
Usually 5–10 days
Usually 1–3 days
Peak response
Smaller
Larger
Antibody isotype
Usually IgM/IgG
Antibody affinity
Lower; average affinity more variable
Higher average affinity (affinity maturation)


Antigen presentation and T cell activation
Antigen recognition by B cells
Paracortical cortex (T cell zone)
Lymphoid follicle (B cell zone)
Lymph node
Microbe
Naive CD4+ T cell
APC
T cell proliferation and differentiation
Migration
Effector CD4+ T cells
T cell–B cell interactions
B cell
B cell activation


B cell
Helper T cells: CD40L, cytokines
IFN-γ
IL-4
Cytokines produced in mucosal tissues, e.g., TGF-β, BAFF, others
Isotype switching
IgM
IgG subclasses (IgG1, IgG3)
IgE
IgA
Principal effector functions
Complement activation
Fc receptor-dependent phagocyte responses; complement activation; neonatal immunity (placental transfer)
Immunity against helminths
Mast cell degranulation (immediate hypersensitivity)
Mucosal immunity (transport of IgA through epithelia)


Naive B cell
Antigen
B cell activation by protein antigen and helper T cells
Migration into lymphoid follicle
B cells with somatically mutated Ig V genes and Ige with varying affinities for antigen
B cells with high-affinity membrane Ig bind antigen on FDC and present antigen to helper T cells
FDC
Helper T cell
B cells that recognize antigen on FDCs or interact with helper T cells are rescued
To survive, other B cells die
Memory B cell
Plasma cell



Mechanism of immune evasion	Example(s)	
Antigenic variation	Many viruses, e.g., influenza, HIV *Neisseria gonorrhoeae, E. coli, Salmonella typhimurium*	
Inhibition of complement activation	Many bacteria	
Resistance to phagocytosis	Pneumococcus	

Type of vaccine	Examples	Form of protection
Live attenuated, or killed, bacteria	BCG, cholera	Antibody response
Live attenuated viruses	Polio, rabies	Antibody response; cell-mediated immune response
Subunit (antigen) vaccines	Tetanus toxoid, diphtheria toxoid	Antibody response
Conjugate vaccines	*Haemophilus influenzae infection*	Helper T cell–dependent antibody response
Synthetic vaccines	Hepatitis (recombinant proteins)	Antibody response
Viral vectors	Clinical trials of HIV antigens in canary pox vector	Cell-mediated and humoral immune responses
DNA vaccines	Clinical trials ongoing for several infections	Cell mediated and humoral immune responses

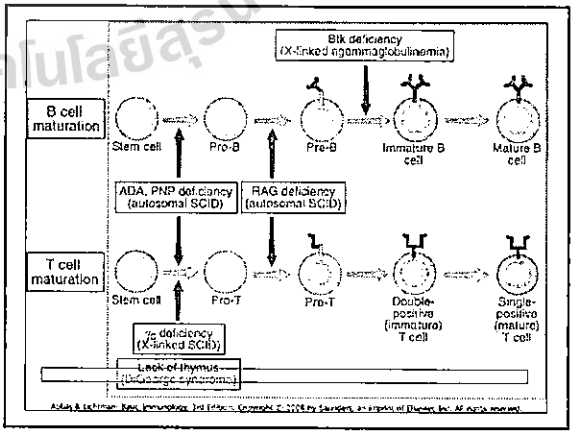
Primary Immunodeficiency

T- cells and B-cells Deficiency

1. Defects in Lymphocyte Maturation
2. Defects in Lymphocyte activation and Function

B cell most common (IgA def is #1)
T cells

Type of immunodeficiency	Histopathologic and laboratory abnormalities	Common infectious consequences
B cell deficiencies	Absent or reduced follicles and germinal centers in lymphoid organs Reduced serum Ig levels	Pyogenic bacterial infections
T cell deficiencies	May be reduced T cell zones in lymphoid organs Reduced DTH reactions to common antigens Defective T cell proliferative responses to mitogens *in vitro*	Viral and other intracellular microbial infections (e.g., *Pneumocystis jiroveci*, atypical mycobacteria, fungi) Virus-associated malignancies (e.g., EBV-associated lymphomas)
Innate immune deficiencies	Variable, depending on which component of innate immunity is defective	Variable; pyogenic bacterial infections



Severe combined immunodeficiency (SCID)

Disease	Functional deficiencies	Mechanism of defect
X-linked SCID	Markedly decreased T cells; normal or increased B cells; reduced serum Ig	Cytokine receptor common γ chain gene mutations, defective T cell maturation due to lack of IL-7 signals
Autosomal recessive SCID due to ADA, PNP deficiency	Progressive decrease in T and B cells (mostly T); reduced serum Ig in ADA deficiency, normal B cells and serum Ig in PNP deficiency	ADA or PNP deficiency leads to accumulation of toxic metabolites in lymphocytes
Autosomal recessive SCID due to other causes	Decreased T and B cells; reduced serum Ig	Defective maturation of T and B cells; genetic basis unknown in most cases; may be mutations in *RAG* genes

B cell immunodeficiencies

Disease	Functional deficiencies	Mechanism of defect
X-linked agammaglobulinemia	Decrease in all serum Ig isotypes; reduced B cell numbers	Block in maturation beyond pro-B cells, because of mutation in B cell tyrosine kinase
Ig heavy chain deletions	IgG1, IgG3, or IgG4 absent; sometimes associated with absent IgA or IgE	Chromosomal deletion at 14q32 (Ig heavy chain locus)

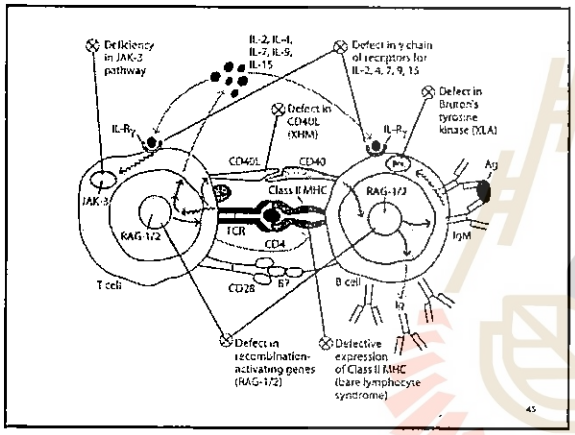
T cell immunodeficiencies

Disease	Functional deficiencies	Mechanism of defect
DiGeorge syndrome	Decreased T cells in blood; absent cell-mediated immunity	Anomalous development of 3rd and 4th branchial pouches, leading to thymic hypoplasia

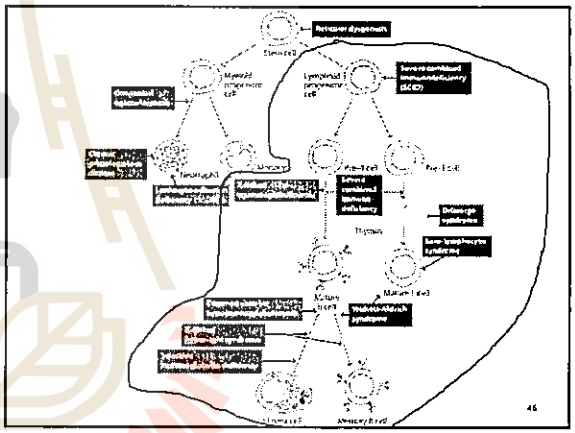
Primary Immunodeficiency

T- cells and B-cells Deficiency

44



45



46

Defects in lymphocyte Maturation

47

Both T and B-Cell Deficiency

SCID

48

SCID

- Various genetic defects
- No TCR or defective TCR
- Defective cell signaling
- Defective IL 2

- Recurrent infections
- Death at early age

49

SCID

- Stem cells defective or absent
- OR T helpers defective or absent
- OR thymus defective or absent (no T cell maturation
- B cells are affected because there's no T help
- ADA def: no T or B cells
- PNP (purine nucleoside phosphorolase): much more T cell
- Invasive infections and really serious viral infections; PCP

50

SCID

51

B-Cell Deficiency

52

(Selective) IgA deficiency

- Most common: 1 in 500?
- Related to CVID – can run in sibs
- Can evolve to normal or become increasingly deficient over years
- Have B cells, but they don't go on to form plasma cells.
- Allergy-type sxs and chronic mucosal infx

53

Common Variable Immune Deficiency (CVID)

Common Variable Immune Deficiency (CVID) is a disorder characterized by low levels of serum immunoglobulins (antibodies) and an increased susceptibility to infections. The exact cause of the low levels of serum immunoglobulins is usually not known. It is a relatively common form of immunodeficiency, hence, the word "common." The degree and type of deficiency of serum immunoglobulins, and the clinical course, varies from patient to patient, hence, the word "variable." In some patients, there is a decrease in both IgG and IgA; in others, all three major types (IgG, IgA and IgM) of immunoglobulins may be decreased. The clinical signs and symptoms also vary from severe to mild. Frequent and unusual infections may first occur during early childhood, adolescence or adult life. In the majority of patients, the diagnosis is not made until the 3rd or 4th decade of life. However, about 20% of patients have symptoms of disease or are found to be immunodeficient under the age of 16.

54

CVID

- Wastebasket dx for B cell + Ig deficient pt's
- Some have decreased total B cells,
  some decreased T-helpers,
  some increased T-suppressors.
- Low Ig's in any combination that includes "G." (G, G+A, G+A+M)
- Recurrent bacterial infections;
  - onset in infancy, at puberty, or even later.
  - Ears, nose, sinuses, bronchi, lungs.
  - Can have chronic lung dz.
- Enlarged neck and chest LN's;
  can have increased incidence of mycoplasma and/or chlamydia

55

CVID

Infusion Supplies: Intravenous immune globulin (IVIG) is the standard treatment for CVID.

56

Bruton's Agammaglobulinemia

X-Linked Agammaglobulinemia (XLA) was first described in 1952 by Dr. Ogden Bruton. This disease, sometimes called Bruton's Agammaglobulinemia or Congenital Agammaglobulinemia, was one of the first immunodeficiency diseases to be identified. XLA is an inherited immunodeficiency disease in which patients lack the ability to produce antibodies, proteins that make up the gamma globulin or immunoglobulin fraction of blood plasma.

57

Bruton's Agammaglobulinemia

- Recurrent pyogenic infections from infancy/early childhood: mucous membranes.
- Ears, sinuses, lungs, GI tract, bacteremias; also increased viral infections.
- Family history of affected lateral (maternal) male relatives
- No tonsils or palpable lymph nodes (they have nodes, but no B cell centers, so non-palpable.)
- Few mature B cells (unlike CVID)
  [Have pre-B's]

58

Bruton's Agammaglobulinemia

- Low levels of IgG
- B cell signal transduction affected
  - usually undetectable B cells in peripheral blood because B cell development is arrested at per-B cell stage
- Defective BCR
- Recurrent bacterial diseases starting at end of first year of life
- Short life span

59

XL Agammaglob, cont.

- Mutation in B cell specific protein (a tyrosine kinase – "BTK") in the proto-oncogenic src family (X q 22): abnormal kinase activity in B and pre-B
- Over 300 different mutations in BTK can result in this disease phenotype.
  - The most typical form has a mutation in the area of the protein for catalytic function.
  - Atypical forms have protein-protein interaction problems and are more subtle clinically.
  - Mouse model XID: N-terminal mutation (function unknown)

60

B cell (-) [AR] Agammaglob's

- μ heavy chain gene mutation
- λ 5/14.1 (surrogate light chain) mutation
- Ig α (B cell α Ag receptor) mutation
- B cell linker protein (BLNK) mutation

61

T Cell Immunodeficiencies

62

DiGeorge Syndrome

- Poorly developed or functioning thymus
- Associated with other developmental conditions
- Depression of T cell numbers
- Absence of T cell response
- Humoral response to T independent antigens only

63

DiGeorge Syndrome

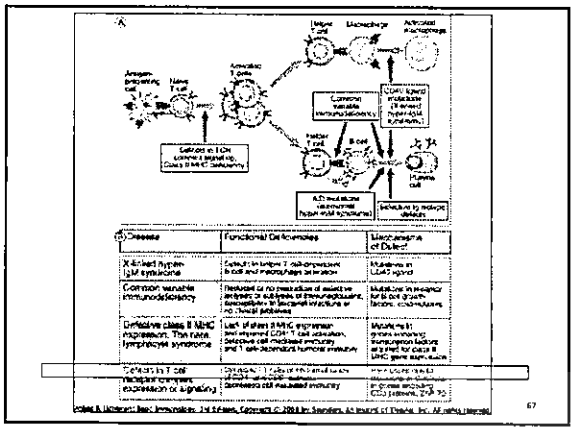
- Associated abnormalities of face, brain, thymus, parathyroid, heart/aorta (and platelets!)
- FISH for 11q22
- Hypocalcemia, seizures
- Extremely variable phenotype

64

DiGeorge Syndrome

Defects in lymphocyte Activation and Function

66



XL HyperIgM

- *in vivo*, no IgG, A, or E
- Can have the "no tonsils, no LN's" presentation
- B cells can make IgE with IL-4 and anti-CD40 *in vitro*
- Gene mutation at CD40L (it can't "hear from" the T cell)
- See also AR form

Hyper IgE

- Abscesses (staph), esp skin (boils) but also lung
- Lung abscesses progressing to giant cysts/pneumatocoeles.
- No diagnostic test; markedly elevated levels of IgE are even seen in atopic dermatitis

Lymphocyte Abnormalities Associated with Other Diseases

Wiskott-Aldrich Syndrome

- X linked disorder
- Affects platelet numbers/function
  - thrombocytopenia is one of crucial clue
- Affects T cell function
- Cytoskeleton of lymphocytes affected

- Lower amounts of IgM
- Increased susceptibility to certain bacterial infections

Wiskott-Aldrich Syndrome

- Eczema, thrombocytopenia; infections of ears, lungs, meninges. Opportunistic infections and bugs with capsular polysaccharide Ag's
- Poor response to polysaccharide antigens but normal IgG_2
  (So look for Ab's, not IgG subclasses)
- Xp11.22-11.23
- WASP gene binds lots of signaling molecules

Wiskott-Aldrich Syndrome

73

From the CBC

- Normal Absolute Lymphocyte Count (ALC):
  - excludes T cell defects, AIDS
  - excludes congenital and acquired neutropenias and LAD (increased ANC)
- Normal platelets:
  - excludes Wiscott Aldrich Syndrome (WAS)
- No Howell-Jolly bodies: no asplenia

74

Screening for B cell defects

- IgA: most common
- IgG and IgM: agammaglobulinemia
- Isohemaglutinins:
  - IgM to blood group(s): get if Ig's are low to see if production failure vs. loss
- Antibody titers to immunizations
- AGE NORM'S: IgG and A are not at adult levels until age 7
- Check flow: if no B cells, usually = Bruton

75

IgG subclasses

- No good age norm's
- Lows can be transient
- Poorly correlated with disease
- BUT, can be a harbinger of CVID
- Best test: immunize with protein then polysaccharide vaccines; check serum before and after. If they respond, they're okay.

76

T cell defects

- Mucocutaneous candida, chronic diarrhea, PCP, FTT, disseminated CMV/VZV/HSV
- Examples: SCID, CVID, AIDS
- ALC usually low, though can be normal in DiGeorge
  - (NOTE: Adult ALC > 1000; NB ALC ≥ 4000)
- Candida skin test: kids should respond by age 9 mos; a normal response virtually rules out T cell problems.
- Can also check flow, do mitogen/antigen stim, assay cytokines

77

Part A
2. Secondary Immunodeficiency

- Acquired Immunodeficiency
- **No genetic defect**
- **due to other defects** including infections, nutritional abnormalities, or treatment that cause loss or inadequate function of various components of the immune system e.g. immune suppressive drugs, HIV infection.

78

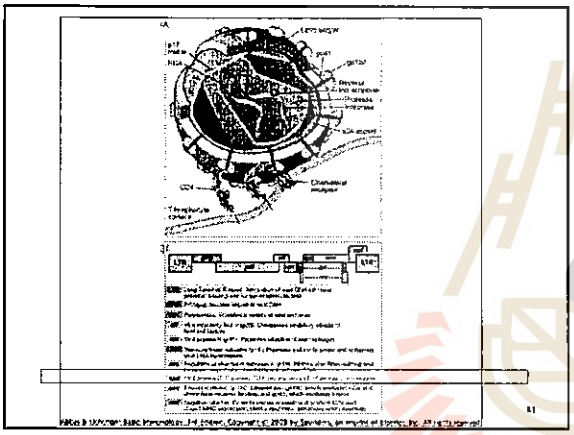
Part A
2. Secondary Immunodeficiency

- Drug related
- Disease related
  - –Cancer
  - –AIDS
    - HIV
    - T helper cell as target

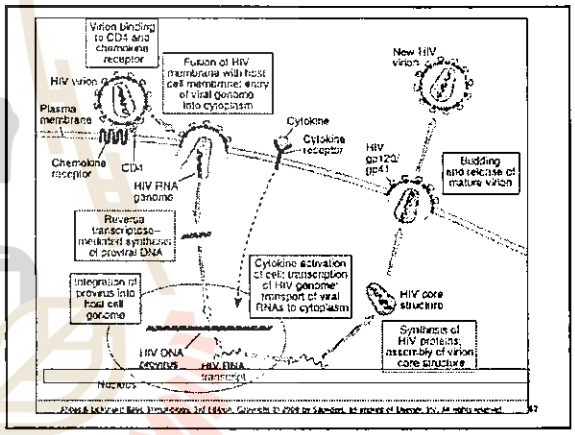
79

Cause	Mechanism
Human immunodeficiency virus infection	Depletion of CD4+ helper T cells
Irradiation and chemotherapy treatments for cancer	Decreased bone marrow precursors for all leukocytes
Involvement of bone marrow by cancers (metastases, leukemias)	Reduced site of leukocyte development
Protein-calorie malnutrition	Metabolic derangements inhibit lymphocyte maturation and function
Removal of spleen	Decreased phagocytosis of microbes

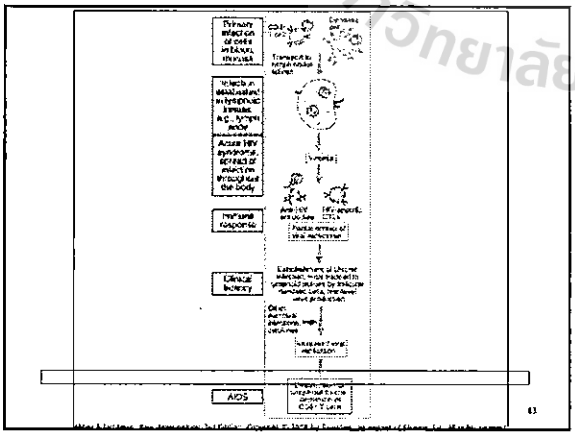
80



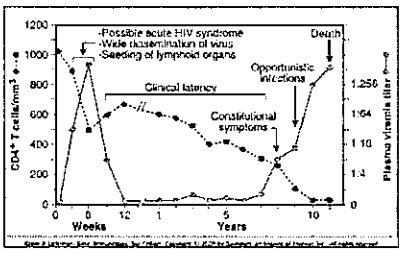
81



82



83



84

4.Laboratory evaluation.

1. Complete blood count .(total & differential).

2. Evaluation of antibody responses.
   A. determination of serum immunglobulins.

   B. measure specific antibody responses:

   -To polysaccharide antigens.
   (measure isohemagglutinins.)

   - To protein antigens .
   (measure antibodies to tetanus .)

3. Determination of T & B cell counts.
   (by flow cytometry)

4. Determination of the complement components. C3, C4 .
   - assess functional activity by CH50.

5. Assess phagocyte function.
   - phagocytosis & respiratory burst.

6. Carrier detection & prenatal diagnosis . (important for genetic counseling .)

From the CBC

- Normal Absolute Lymphocyte Count (ALC):
  - excludes T cell defects, AIDS
  - excludes congenital and acquired neutropenias and LAD (increased ANC)
- Normal platelets:
  - excludes Wiscott Aldrich Syndrome (WAS)
- No Howell-Jolly bodies: no asplenia

Screening for B cell defects

- IgA: most common
- IgG and IgM: agammaglobulinemia
- Isohemaglutinins:
  - IgM to blood group(s): get if Ig's are low to see if production failure vs. loss
- Antibody titers to immunizations
- AGE NORM'S: IgG and A are not at adult levels until age 7
- Check flow: if no B cells, usually = Bruton

IgG subclasses

- No good age norm's
- Lows can be transient
- Poorly correlated with disease
- BUT, can be a harbinger of CVID
- Best test: immunize with protein then polysaccharide vaccines; check serum before and after. If they respond, they're okay.

T cell defects

- Mucocutaneous candida, chronic diarrhea, PCP, FTT, disseminated CMV/VZV/HSV
- Examples: SCID, CVID, AIDS
- ALC usually low, though can be normal in DiGeorge
  - (NOTE: Adult ALC > 1000; NB ALC ≥ 4000)
- Candida skin test: kids should respond by age 9 mos; a normal response virtually rules out T cell problems.
- Can also check flow, do mitogen/antigen stim, assay cytokines

Analysis of lymphocytes in umbilical cord blood during gestation

* Help to diagnose immunodeficiency In pregnancies at risk .

* Bone marrow or stem cell transplantation may be applied before birth .

Therapy of immunodeficiency.

1. IVIG .(IV infusion of immunoglobulin.)
   For : a. agammaglbulinaemia .
   b. CVI. c. WAS.

2. Periodic antibiotic treatment.

3. Bone marrow transplantation .
   For : a. SCID . b. WAS.

4. Enzyme replacement .
   For : a. ADA deficiency.

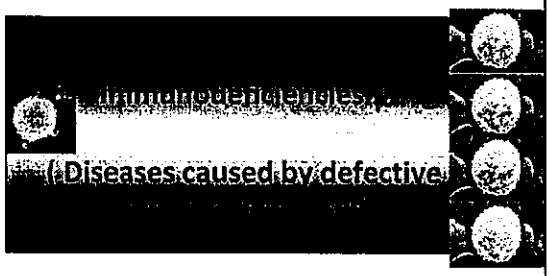
5. G-CSF.(colony stimulating factor)
   For : neutropenia .

6. Thymus transplantation .
   For : DiGeorge syndrome.

7. IFN – gamma .
   For : CGD.

Chapter 4.

Diseases caused by Defective Immune Response 2 (Part B)



By Asst. Prof. Dr. Wilairat Leeanansaksiri

What's Happen to Them ?

Can you help them ?

Immunodefeiciency

Outline

1. Concise summarization of normal immune response
2. Concise summarization of normal immunity to infection
3. innate immunodeficiency and primary immunodeficiency diseases Adaptive Immunodeficiency
4. adaptive immunodeficiency and secondary immunodeficiency diseases

Immunodefeiciency

Objectives

1. Understand and can explain normal immune response both innate and adaptive immunities
2. Understand and can explain capability of microbes to escape immune response
3. Understand and can explain innate immunodeficiency and primary immunodeficiency diseases
4. Understand and can explain adaptive immunodeficiency and secondary immunodeficiency diseases

Immunodeficiency

1. Primary Immunodeficiency
- Adaptive (Part A)
- Innate (Part B)

2. Secondary Immunodeficiency
- Adaptive (Part A)
- Innate (Part B)

Part B : Innate immunity components defect e.g. Macrophage....

- Chronic granulomatous disease
- Congenital agranulocytosis
- Leukocyte-adhesion deficiency
- Chediak-Higashi syndrome

1. Primary Immunodeficiency

- congenital Immunodeficiency (usually abnormal since birth)
- due to **genetic defect** leads to blocks in the maturation or functions of different components of the immune system
  - Innate immunity components defect e.g. phagocytosis, complement
  - Adaptive immunity components defect e.g. T- cells, B- cells

[redacted]

Loss or reduction of:

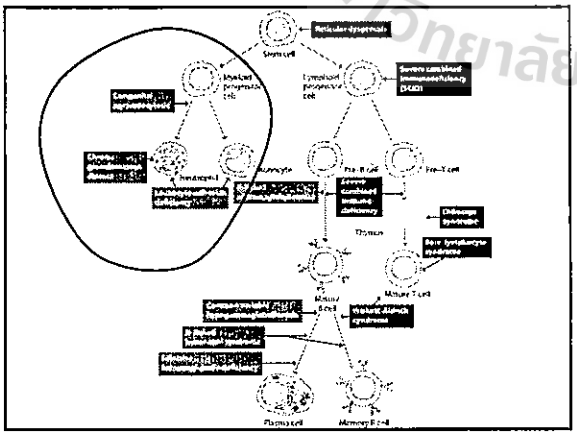
- Cell type
- Cell numbers
- Cell function

[redacted]

- Receptors
- Cell signaling
- Cytokine production
- Ig production
- Co stimulation impairment
- Intracellular killing
- Extravasation impairment

1. Primary Immunodeficiency

- **Defect in innate immunity**
  - **Chronic granulomatous disease**
  - **Congenital agranulocytosis**
  - **Leukocyte-adhesion deficiency**
  - **Chediak-Higashi syndrome**
- **Defect in T and B cells**
  - **Severe combined immunodeficiency (SCID)**
  - **B cells**
    - **Agammaglobulinemia**
    - **Hypogammaglobulinemia**
    - **Specific Ig Deficiencies**
  - **T cells**
    - **DiGeorge Syndrome**
    - **Wiskott Aldrich Syndrome**



Primary Immunodeficiency
Pathogens

- Humoral defects
  - Capsulated bacteria
    - *S. pneumoniae*
    - *H. influenzae*
    - *N. meningitidis*
    - *S. aureus*
  - Enteroviruses
  - mycoplasma
- Neutrophil defects
  - *S. aureus, Candida, Aspergillus*
- Cell-mediated
  - intracellular bacteria
    - *Mycobacteria, Salmonella, Listeria, Legionella*
  - Viruses
    - Herpes, Respiratory & Enteric viruses
  - Fungi & protozoa
    - *Candida, Aspergillus, Pneumocystis, Cryptococcus, Cryptosporidium, Toxoplasma*

Congenital Infections

- Toxoplasmosis
- Rubella
- CMV
- HSV
- Hepatitis B, HIV
- Parvovirus B19
- Syphilis
- Ophthalmia neonatorum

- Seek expert advice on management & diagnosis
- Prevention
  - Vaccination
    - rubella, hep B
  - Treatment
    - Antimicrobial (anti-retrovirals, syphilis, acyclovir, spiramycin for toxo, silver nitrate eye drops etc.)
    - Other (intra-uterine blood transfusion for B19)
  - Screening (syphilis, HIV, hep B), Vigilance , Avoidance (e.g. of slapped cheek syndrome)

Primary Immunodeficiency
Management

- Correct defect
  - Immunoglobulin, cytokines
  - BMT
  - Gene therapy?
- Early aggressive antibiotic treatment
- Prophylaxis
  - Daily co-trimoxazole
  - Penicillin if complement deficiency
  - Flucloxacillin in some neutrophil disorders

Secondary Immunodeficiency

- Acquired Immunodeficiency
- **No genetic defect**
- **due to other defects** including infections, nutritional abnormalities, or treatment that cause loss or inadequate function of various components of the immune system e.g. immune suppressive drugs, HIV infection.

Secondary Immunodeficiency

- Drug related
- Disease related
  - Cancer
  - AIDS
    - HIV
    - T helper cell as target

CORE

A. Laboratory tests to assess immune function

(1) T cell: Enumeration (flow cytometry), functional assays (mitogen response, MLR, DTH skin tests)

(2) B cell: Enumeration, circulating antibody levels

(3) Macrophage: Enumeration, functional assays (nitroblue tetrazolium)

(4) Complement: Direct measurement of complement components, complement hemolysis assay

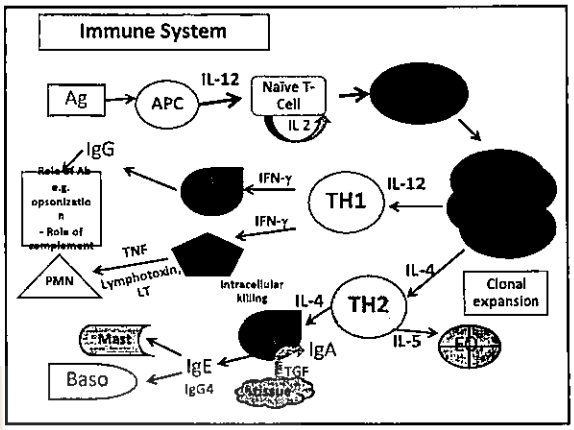
1. Innate Immunodeficiency

- **Chronic granulomatous disease**
- **Congenital agranulocytosis**
- **Leukocyte-adhesion deficiency**
- **Chediak-Higashi syndrome**
- **Phagocyte Defect**
- **Complement Defect**

Normal and Abnormal of Innate Immunity

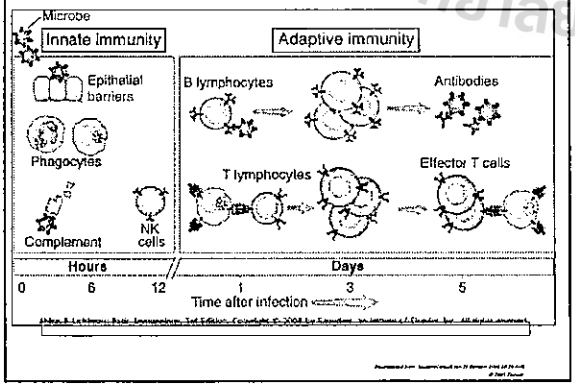
Outline

1. Normal innate immunity
2. Role of immunity in infectious diseases
3. How microbes escape immune response ?
4. Diseases associates with immunodeficiency in innate immunity



Role of the immune system	Implications
Defense against infections	Deficient immunity results in increased susceptibility to infections; exemplified by AIDS Vaccination boosts immune defenses and protects against infections
The immune system recognizes and responds to tissue grafts and newly introduced proteins	Immune responses are barriers to transplantation and gene therapy
Defense against tumors	Potential for immunotherapy of cancer

Disease	Maximum number of cases (year)	Number of cases in 2004	Percent change
Diphtheria	206,939 (1921)	0	-99.99
Measles	894,134 (1941)	37	-99.99
Mumps	152,209 (1968)	236	-99.90
Pertussis	265,269 (1934)	18,957	-96.84
Polio (paralytic)	21,269 (1952)	0	-100.0
Rubella	57,686 (1969)	12	-99.98
Tetanus	1,560 (1923)	26	-98.33
Haemophilus influenzae type b infection	~20,000 (1984)	16	-99.92
Hepatitis B	26,611 (1985)	6,632	-75.08



Third line of DEFENSE

Specific Defense Mechanisms

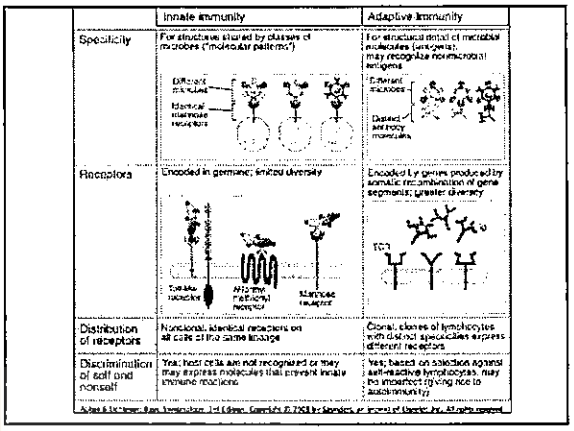
How to deal with specific & recurring invasion?

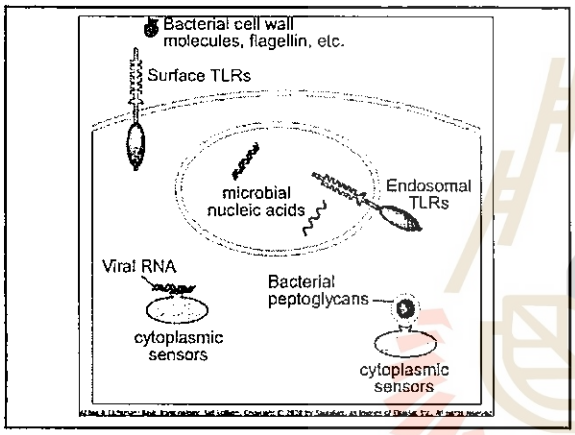
MEMORY

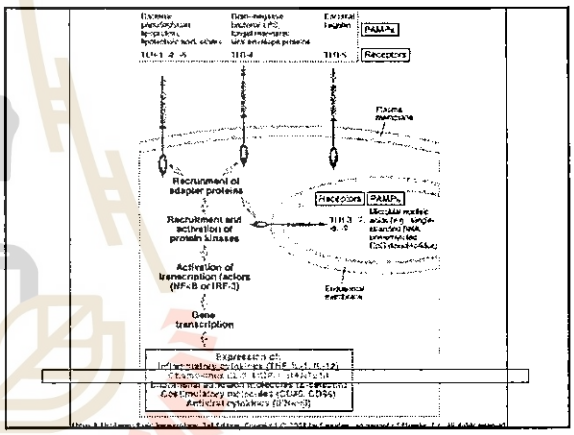
3rd line of defense
ACQUIRED IMMUNITY
Slower responses to specific microbes

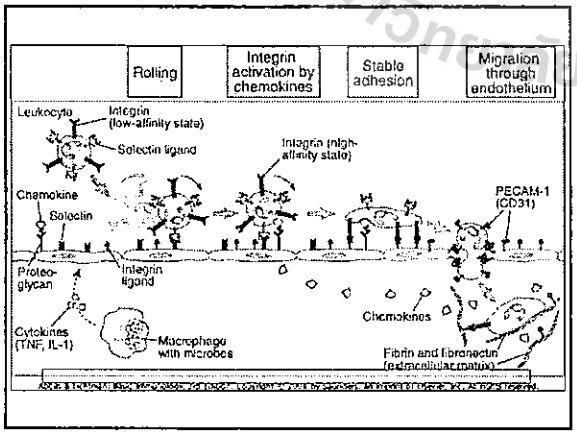
- Humoral response (antibodies: B-lymphocytes)
- Cell-mediated response (cytotoxic T-lymphocytes)

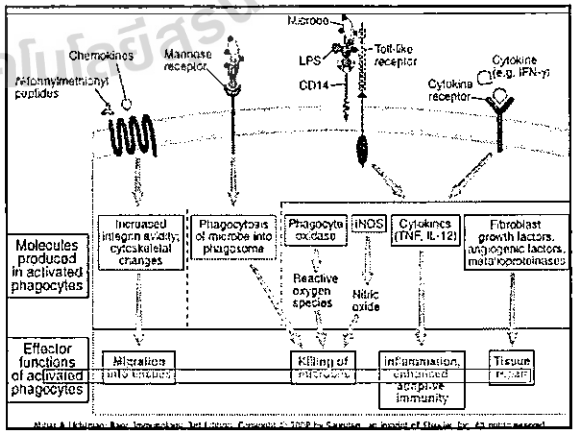
	Innate	Adaptive
Characteristics		
Specificity	For structures shared by groups of related microbes	For antigens of microbes and for nonmicrobial antigens
Diversity	Limited; germline-encoded	Very large; receptors are produced by somatic recombination of gene segments
Memory	None	Yes
Nonreactivity to self	Yes	Yes
Components		
Cellular and chemical barriers	Skin, mucosal epithelia; antimicrobial chemicals	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Blood proteins	Complement, others	Antibodies
Cells	Phagocytes (macrophages, neutrophils), natural killer cells	Lymphocytes

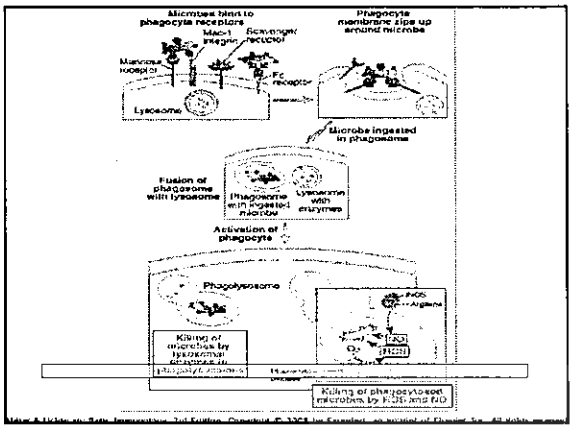


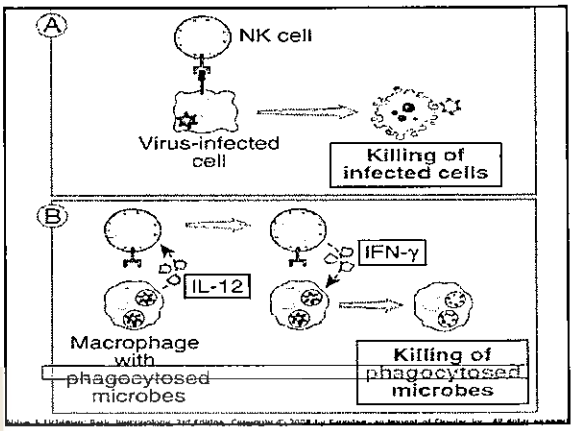


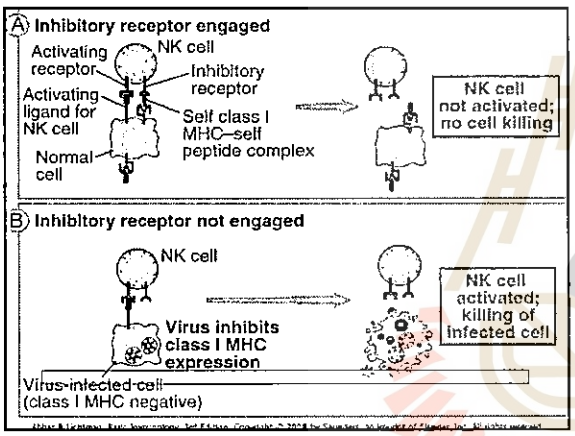


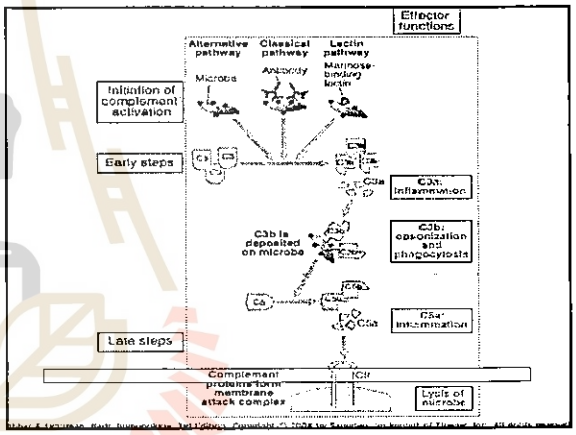


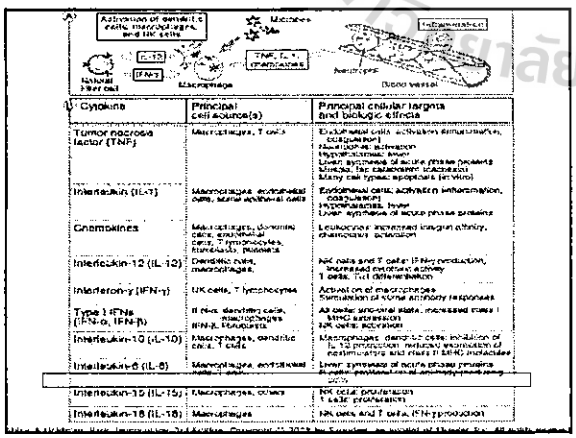






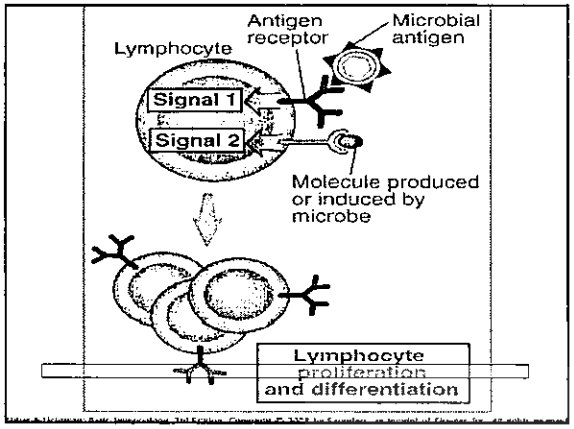


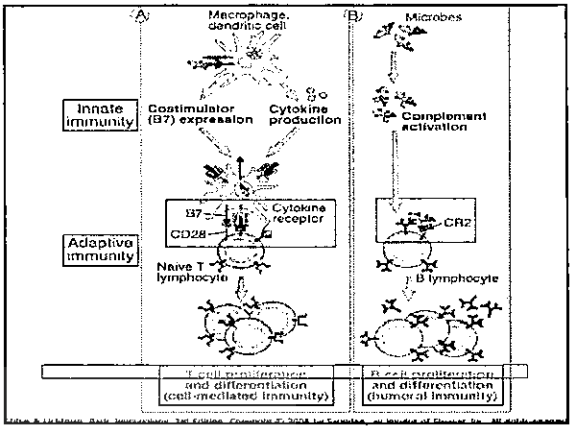


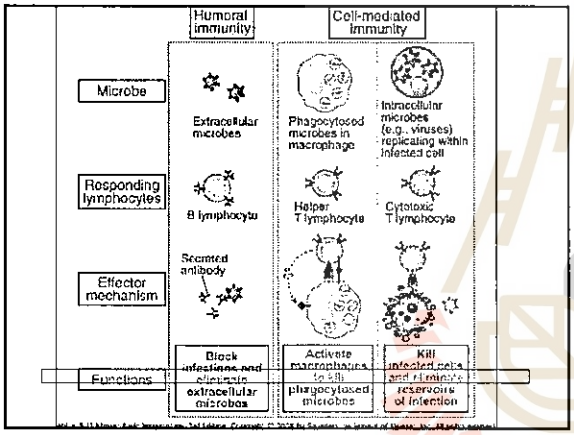


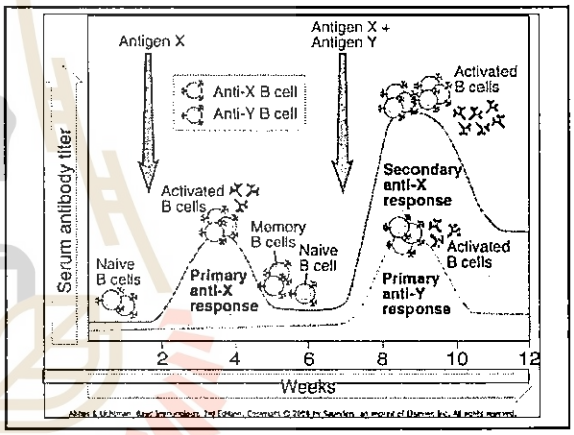
Cytokine	Principal cell source(s)	Principal cellular targets and biologic effects
Tumor necrosis factor (TNF)	Macrophages, T cells	Endothelial cells: activation, inflammation, coagulation Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute phase proteins Shock, fat catabolism, cachexia Many cell types: apoptosis (at high doses)
Interleukin-1 (IL-1)	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation, inflammation, coagulation Hypothalamus: fever Liver: synthesis of acute phase proteins
Chemokines	Macrophages, dendritic cells, endothelial cells, T lymphocytes, fibroblasts, platelets	Leukocytes: increased integrin activity, chemotaxis, activation
Interleukin-12 (IL-12)	Dendritic cells, macrophages	NK cells and T cells: IFN-γ production, increased cytotoxic activity T cells: Th1 differentiation
Interferon-γ (IFN-γ)	NK cells, T lymphocytes	Activation of macrophages Stimulation of some antibody responses
Type I IFNs (IFN-α, IFN-β)	Most cells, especially dendritic cells, macrophages	All cells: antiviral state, increased class I MHC expression NK cells: activation
Interleukin-10 (IL-10)	Macrophages, dendritic cells, T cells	Macrophages, dendritic cells: inhibition of IL-12 production, reduced expression of costimulators and class II MHC molecules
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	Liver: synthesis of acute phase proteins B cells: proliferation and antibody secretion
Interleukin-15 (IL-15)	Macrophages, others	NK cells: proliferation T cells: proliferation
Interleukin-18 (IL-18)	Macrophages	NK cells and T cells: IFN-γ production

Mechanism of immune evasion	Organism (example)	Mechanism
Resistance to phagocytosis	Pneumococci	Capsular polysaccharide inhibits phagocytosis
Resistance to reactive oxygen species in phagocytes	Staphylococci	Production of catalase, which breaks down reactive oxygen intermediates
Resistance to complement activation (alternative pathway)	*Neisseria meningitidis*	Sialic acid expression inhibits C3 and C5 convertases
	Streptococci	M protein blocks C3 binding to organism, and C3b binding to complement receptors
Resistance to antimicrobial peptide antibiotics	*Pseudomonas*	Synthesis of modified LPS that resists action of peptide antibiotics









Disease	Functional Deficiencies	Mechanisms of Defect
Chronic granulomatous disease	Defective production of reactive oxygen species by phagocytes	Mutations in genes encoding components of the phagocyte oxidase enzyme, most often cytochrome b558
Leukocyte adhesion deficiency-1	Absent or deficient expression of β2 integrins causing defective leukocyte adhesion–dependent functions	Mutations in gene encoding the β chain (CD18) of β2 integrins
Leukocyte adhesion deficiency-2	Absent or deficient expression of leukocyte ligands for endothelial E- and P-selectins, causing failure of leukocyte migration into tissues	Mutations in gene encoding a protein required for synthesis of the sialyl-Lewis X component of E- and P-selectin ligands
Complement C3 deficiency	Defect in complement cascade activation	Mutations in the C3 gene
Complement C2, C4 deficiency	Deficient activation of classical pathway of complement leading to failure to clear immune complexes and development of lupus-like disease	Mutations in C2 or C4 genes
Chédiak-Higashi syndrome	Defective lysosomal function in neutrophils, macrophages, and dendritic cells, and defective granule function in natural killer cells	Mutation in a gene encoding a lysosomal trafficking regulatory protein

Defects in phagocytic cells

Type of defect/name of syndrome	Associated infectious or other diseases
Leukocyte adhesion deficiency	Widespread pyogenic bacterial infections
Chronic granulomatous disease	Intracellular and extracellular infection, granulomas
G6PD deficiency	Defective respiratory burst, chronic infection
Myeloperoxidase deficiency	Defective intracellular killing, chronic infection
Chediak–Higashi syndrome	Intracellular and extracellular infection, granulomas

Primary phagocyte deficiencies (symptoms, description of defect, current therapy)

- (1) Neutropenia
- (2) Chronic Granulomatous Disease
- (3) Leukocyte Adhesion Deficiency

Phagocyte deficiencies:

QUANTITATIVE OR QUALITATIVE .

Quantitative defects:

1. Congenital agranulocytosis :

Kostmann syndrome .

Defect in the gene inducing G-CSF (granulocyte colony stimulating factor).

Features: pneumonia ,otitis media, gingivostomatitis perineal abscesses.

Management:

Respond to G-CSF therapy.

Qualitative defects :

1.Defect in response to chemotactic agents.

2.Defect in intracellular killing.

A . Defect in chemotaxis:

Leukocyte adhesion deficiency (LAD.)

2 types.

*LAD type 1: defect in gene encoding CD18. (B integrin .)

B.Defect in intracellular killing:

1.Chronic granulomatous disease:

X-LINKED. (75%)

AUTOSOMAL RECESSIVE .(25%).

DEFECT: in the oxidative complex .

(responsible for producing superoxide radicals .)

FEATURES:

Extreme susceptibility to infections.

Granulomatous inflammation.

(chronic T-cell stimulation.)

2. Glucose -6- phosphate dehydrogenase deficiency . (G6-P-D). (no resp.burst.)

3. myeloperoxidase deficiency .

(no resp. burst).

4. Chediak - Higashi syndrome: defect in formation of phagolysosome .

Associated with:

abnormal platelet function.

partial albinism .

Chronic Granulomatous Disease

Defect in mutation in enzyme phagocyte oxidase, which catalyzes the production of microbicidal reactive oxygen intermediates in lysosome

↓

Result:

Neutrophils and macrophages that phagocytose microbes unable to kill the microbes

Immune events:

Immune try to compensate

↓

Try to get more phargocytes to the site of infection by activate more T-cells

↓

Accumulation of phagocytes
Resembles granulomas

↓

Disease : chronic granulomatous disease

CGD

These defects leave patients vulnerable to severe recurrent bacterial and fungal infections and chronic inflammatory conditions such as gingivitis (swollen inflamed gums), enlarged lymph glands, or tumor-like masses called granulomas. While not malignant, granulomas can cause serious problems by obstructing passage of food through the esophagus, stomach, and intestines as well as blocking urine flow from the kidneys and bladder.

Rhinoscleroma

Rhinoscleroma is a chronic granulomatous disease of the upper respiratory tract caused by a gram-negative organism, Klebsiella rhinoscleromatis. It is uncommon in the US, but is endemic in parts of Central and South America, Egypt, Africa, and Eastern Europe. Mucopurulent discharge characterizes the rhinitic stage, followed by mucosal thickening during florid stage. The disease resolves with marked fibrosis.

Rhinoscleroma is rare in the US, but occurs in regions of poor living conditions that foster the spread of the bacteria. Three clinical stages define the disease: 1) rhinitic 2) florid, and 3) fibrotic. Symptoms vary with the location of the infection. Nasal cavity (septum) is the most common site, but other sites of infection include: paranasal sinuses, orbit, larynx, tracheobronchial tree, and middle ear. Tetracycline is the treatment of choice with excellent prognosis. However, significant airway obstruction requires surgical excision.

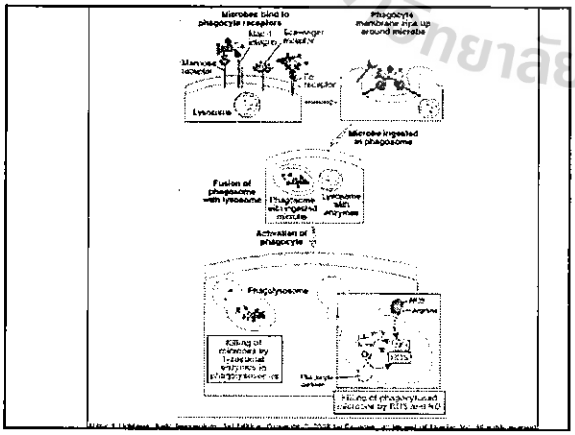
Suppurative lymphadenitis in chronic granulomatous disease

CGD

Suppurative lymphadenitis is a common feature of CGD,

CGD occur in many

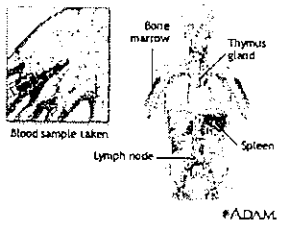
organs in response to chronic inflammation. which develop in many organs in response to chronic inflammation. organ



Chronic Granulomatous Disease

- Short arm of the X chromosome
- NBT (nitro blue tetrazoleum):
  - feed to PMN's with a particle (bacteria, latex).
    If the hexose monophosphate path is nl, the dye is reduced (turns purple).
    Heparin interferes. High false (-) rate.
- Respiratory burst assay:
  - non-fluorescing dye to PMN's; addition of particle makes it fluoresce. A quantitative test – can pick up carriers.
- Poor phagocytosis; poor peroxidase production
- Infections with non-peroxidase-producing org's: staph, serratia
- Abscesses of lung, LN; also infx of skin, liver, bone

Nitroblue tetrazolium test



Nitroblue tetrazolium test is a blood test that measures the ability of the immune system to convert the colorless nitroblue tetrazolium (NBT) to a deep blue. This test is performed as a screen for chronic granulomatous disease (CGD). If an individual has CGD, the white cells in their blood will not turn blue when exposed to the NBT

Agranulocytosis

a severe reduction in the number of leukocytes (basophils, eosinophils, and neutrophils). Neutropenia results, whereby the body is severely depleted in its ability to defend itself. Fever, prostration, and bleeding ulcers of the rectum, mouth, and vagina may be present. The acute disease may be an adverse reaction to a medication or the result of the effect of radiation therapy or chemotherapy on bone marrow.

Agranulocytosis

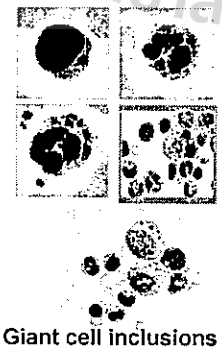
Oral lesions are ulceronecrotic, involving the gingivae, tongue, buccal mucosa, or lips. Regional lymphadenopathy and lymphadenitis are prevalent.

Chediak-Higashi syndrome

Defect lysosomal function in neutrophils, Macrophages, and dendritic cells, and defective granule function in NK cells

Molecular defect
mutation in gene encoding a lysosomal trafficking regulatory protein

Chediak-Higashi syndrome



Giant cell inclusions

Leukocyte-adhesion deficiency

This 10-month-old patient with severe leukocyte adhesion deficiency type I (LAD I) developed a cervical adenitis caused by *Klebsiella pneumoniae*. Following incision and drainage, wound healing took 4 months.

LAD type 1:

- 3.TYPES:

*CD18+CD11a- leukocyte function associated molecule (LFA-1).

CD18+CD11b- complement receptor (CR3).

CD18+CD11c- complement receptor (CR4).

LFA-1 mediate tight adhesion of leukocytes to the endothelium .

WITH DEFECT IN LFA-1:

- Leukocytes are trapped in the circulation.
- Leukocyte count can reach 100,000 cells per mm3.
- Abscesses do not suppurate.

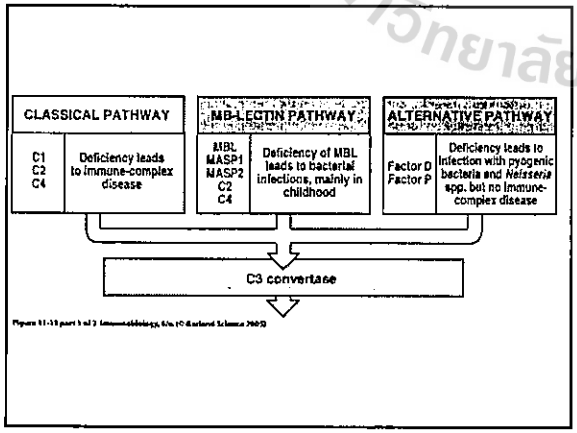
LAD type 2:

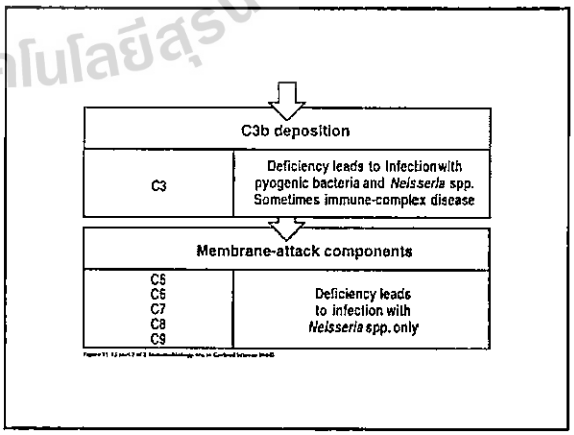
- Defect in Sialyl lewis protein (ligand for E- selectin).

Leukocytes cannot attach to endothelium.

Defective chemotaxis .

Name of deficiency syndrome	Specific abnormality	Immune defect	Susceptibility
Phagocyte deficiencies	Many different	Loss of phagocyte function	Extracellular bacteria and fungi
Complement deficiencies	Many different	Loss of specific complement components	Extracellular bacteria especially *Neisseria* spp.





Deficiencies in the pathways of complement activation

Complement protein	Effects of deficiency	
C1, C2, C4	Immune-complex disease	Clearance of immune (Ab-Ag) complex
C3	Susceptibility to capsulated bacteria	Opsonin
C5–C9	Only effect is susceptibility to *Neisseria*	Membrane attack
Factor D, properdin (factor P)	Susceptibility to capsulated bacteria and *Neisseria* but no immune-complex disease	Enhances alternative path
Factor I	Similar effects to deficiency of C3	Supplies C3
DAF, CD59	Autoimmune-like conditions including paroxysmal nocturnal hemoglobinuria	Prevent host cell destruction

Complement deficiencies :

Deficiency of all complement components have been described C1-C9.

1. Deficiency of C1, C2 & C4.
(classical pathway)
lead to immune-complex diseases which can cause significant pathology in autoimmune diseases.

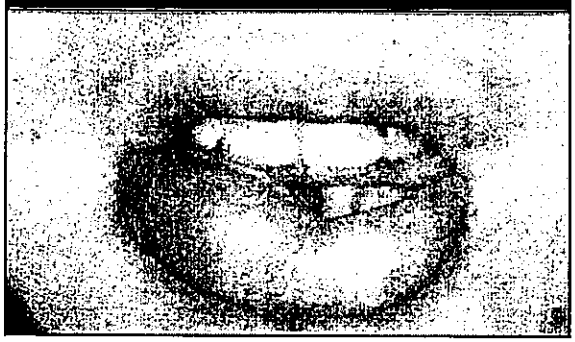
Complement Deficiencies and Disease. Classical Pathway.

Pathway Component	Disease	Mechanism
C1INH	Hereditary Angioedema	Overproduction of C2b (prokinin)
C1, C2, C4	Predisposition to SLE	Opsonization of immune complexes help keep them soluble; deficiency results in increased precipitation in tissues and inflammation

4. Deficiency of membrane - attack complex.
(C5 - C9)
Lead to infection with N.meningitides and N.gonorrhea .

5. Deficiency of C3.
Lead to infections with pyogenic bacteria.
impaired clearance of immune-complexes. .

C1 - inhibitor deficiency: hereditary angioedema



Complement Deficiencies and Disease. Lectin Pathway

Pathway Component	Disease	Mechanism
MBL	Susceptibility to bacterial infections in infants or immunosuppressed	Inability to initiate lectin pathway

2. Deficiency of mannose - binding lectin. (lectin pathway)

*MBL , C2, & C4.

Lead to bacterial infections mainly in Early childhood .

3. Deficiency of Factor D & Factor P. (alternative pathway).

Lead to infection with pyogenic bacteria .

Complement Deficiencies and Disease. Alternative Pathway cont.

Pathway Component	Disease	Mechanism
Properdin (X-linked)	Susceptibility meningococcal meningitis	Lack of opsonization of bacteria
Factors H or I	C3 deficiency and susceptibility to bacterial infections	Uncontrolled activation of C3 via alternative pathway resulting in depletion of C3

Complement Deficiencies and Disease. Alternative Pathway

Pathway/Component	Disease	Mechanism
Factors B or D	Susceptibility to pyogenic (pus-forming) bacterial infections	Lack of sufficient opsonization of bacteria
C3	Susceptibility to bacterial infections	Lack of opsonization and inability to utilize the membrane attack pathway
C5, C6, C7 C8, or C9	Susceptibility to Gram-negative infections	Inability to attack the outer membrane of Gram-negative bacteria

Diseases (other than I.D.), caused by complement defects.

1.Loss of control proteins.
(decay accelerating factor, DAF, & CD59.)
Lead to destruction of R.B.C., which result in paroxysmal nocturnal hemoglobinuria .

2.C1 esterase inhibitor deficiency (C1 inhibitor.) result in excess of vasoactive mediators (kinins).
Causes : Hereditary angioneurotic edema.
*Recurrent attacks of subepithelial swellings involving the larynx & intestinal mucosa.
(may be fatal)

Clinical approach to suspected immunodeficiency.

1.History.

* Infections of unusual frequency, chronicity or severity .

* Family history of infectious problems.

Consanguinity should be investigated (inter-family marriages).

2.Physical examination.

* Absence of tonsils.
* Partial albinism.
* Telangiectasia .(bleeding capillaries).

3.Radiologic evaluation .

* Absence of thymic shadow .
* Pneumatocele (hyper IgE syndrome)

4.Laboratory evaluation.

1. Complete blood count .(total & differential).

2. Evaluation of antibody responses.
   A. determination of serum immunglobulins.

   B. measure specific antibody responses:

   -To polysaccharide antigens.
   (measure isohemagglutinins.)

   - To protein antigens .
   (measure antibodies to tetanus .)

3. Determination of T & B cell counts.
(by flow cytometry)

4. Determination of the complement components. C3, C4 .
- assess functional activity by CH50.

5. Assess phagocyte function.
- phagocytosis & respiratory burst.

6. Carrier detection & prenatal diagnosis . (important for genetic counseling .)

Analysis of lymphocytes in umbilical cord blood during gestation

* Help to diagnose immunodeficiency In pregnancies at risk .

* Bone marrow or stem cell transplantation may be applied before birth .

Laboratory tests to assess immune function

- Macrophage: Enumeration, functional assays (nitroblue tetrazolium)
- Complement: Direct measurement of complement components, complement hemolysis assay

Determination of the complement components. C3, C4 .
- assess functional activity by CH50.

Assess phagocyte function.
- phagocytosis & respiratory burst.

Phagocytic cell defects

- Skin infections without underlying skin disease
- Abscesses of skin, liver, lung, nodes
- Examples: CGD, LAD
- Check flow (NK cells, CD11/CD18 [LAD-1], CD15 [LAD-2, aka Sialyl Lewis X – VERY rare)

Complement problems

- CH50 assay is the screen; need all the other levels to be normal for it to be normal
- Complement spontaneously activates
  - blood that has been sitting around is inappropriate for testing
- CH50 levels should turn up VERY low – like 11

Therapy of immunodeficiency.

1. IVIG.(IV infusion of immunoglobulin.)
   For : a. agammaglbulinaemia .
   b. CVI. c. WAS.

2. Periodic antibiotic treatment.

3. Bone marrow transplantation .
   For : a. SCID . b. WAS.

4. Enzyme replacement .
   For : a. ADA deficiency.

5. G-CSF.(colony stimulating factor)
   For : neutropenia .

6. Thymus transplantation .
   For : DiGeorge syndrome.

7. IFN – gamma .
   For : CGD.

Chapter 5.

Immunological Tolerance and Autoimmunity

Immunological Tolerance and Autoimmunity

Self-Nonself Discrimination in the Immune System and its Failure

Asst. Prof. Dr. Wilairat Leeanansaksiri

Why we need immune system?

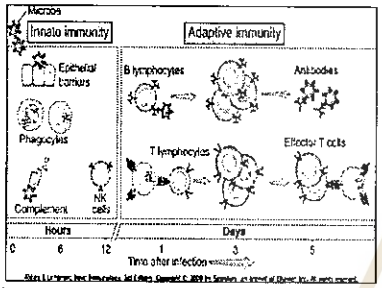
- **Immune system recognizes many microbes :**
  = Immune protection

but not against one's own Ags

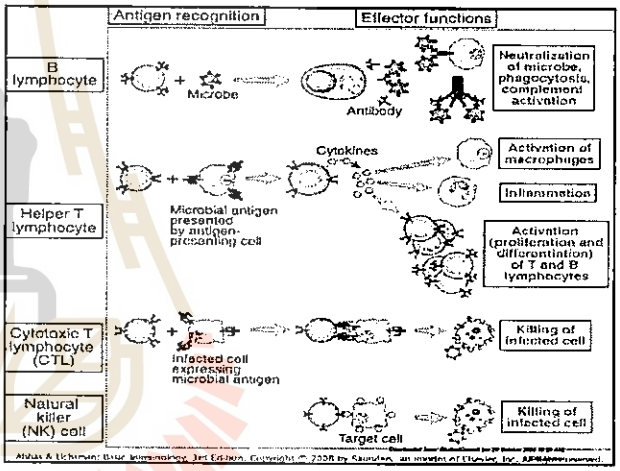
Mechanism : immunological tolerance

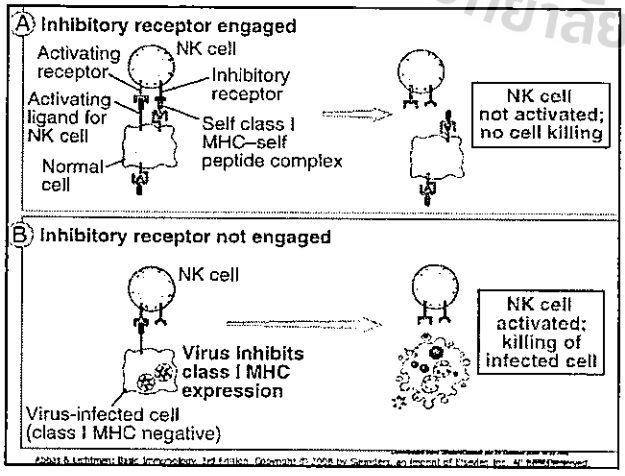
Recognize self Ag → **Autoimmunity** → **Autoimmune diseases**

Main cells are Lymphocytes; T and B Mostly by T-cells



Lymphocyte receptors are constantly being generated to self and readily accessible to self-Ag must prevent immune response to self-Ag ability to discriminate between self and non-self failure results in autoimmunity = autoimmune disease





Immunological Tolerance

- Lack of response to Ag that is induced by exposure of lymphocytes to these Ag
- 3 possible outcomes
  1. lymphocytes activated – elicit a response
  2. functionally inactivated or killed – tolerance
     - Ag is said to be tolerogenic
  3. Ag-specific lymphocyte that don't act – ignorance
- Self-Ag are either ignored or tolerogenic
  - outcome is determined by Ag-specific lymphocyte, nature of Ag and how it is presented

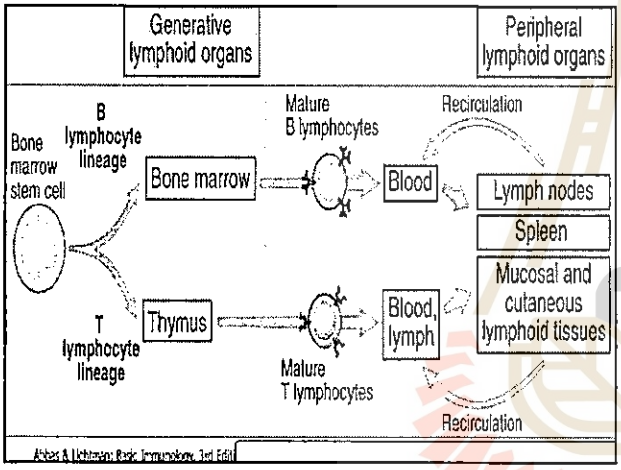
Tolerance is Important

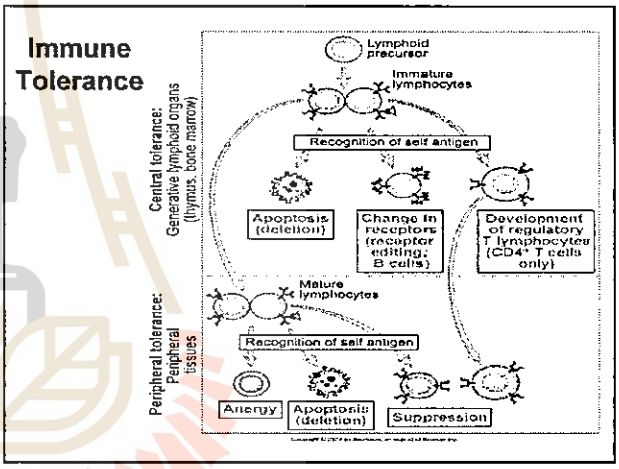
- Self-Ag usually (normally) induces tolerance
- Learn to induce tolerance and can then use to prevent or control unwanted immune response
  - used for allergy, autoimmune disease and prevent rejection of organ transplants
  - may be necessary for gene therapy also

Immunotolerance

1.Central Tolerance

2.Peripheral Tolerance





Immunological Tolerance

- Different self-Ag may induce developing lymphocyte
  - encounter Ag in generative lymphoid organ – Central Tolerance
    - see Ag in the BM and thymus
  - encounter Ag in peripheral tissues – Peripheral Tolerance
    - all other self-Ag tolerance is done peripherally
- Don't know the numbers of lymphocytes that are involved in either process or those that are ignored

The mechanism of tolerance 1

Central tolerance

The majority of forbidden clones get destroyed by apoptosis (T cells in the thymus, B cells in the bone marrow).

There are a number of proofs that the deletion of forbidden clones is not complete. Autoreactive T and B clones are always present in healthy individuals. So an additional, so called peripheral tolerance mechanism must exist.

The mechanism of tolerance 2

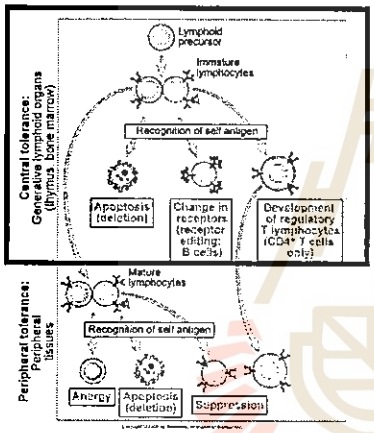
Tolerance is achieved by at least two levels of protection

Central	Peripheral	Suppression (peripheral 2)
deletion	deletion anergy ignorance	suppressor cells? anti-idiotypes

Central Tolerance

Immune Tolerance

1. Central Tolerance



Central T-Cell Tolerance

- Immature T-cells in thymus recognize self-Ag in the thymus and die by apoptosis
  - T-cells with receptors to many Ag (foreign/self)

Central T-Cell Tolerance

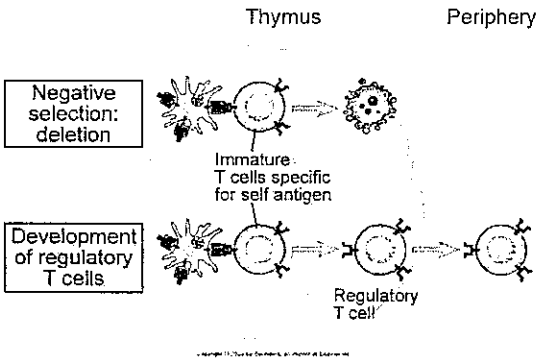
- Self-Ag on MHC and immature T-cell bind strongly – triggers apoptosis signals
  - dies before maturation
  - negative selection – principle mechanism of central tolerance
  - Ag that trigger negative selection are usually in higher concentration than Ag that cause positive selection
    - Ag such as plasma proteins and common cellular proteins may actually ve expressed on the epithelial cells in the thymus
  - negative selection may protect from wide range variety of self-protein Ag – T-cells against self-Ag are deleted before making peripheral response

Tolerance in CD4+ T-cells

- Helper cells control virtually all immune responses to protein Ag
  - if non-responsive to self-proteins it is enough to stop autoimmune cell-mediated and humoral immune response to these Ag
- Failure to develop tolerance may cause autoimmunity

Regulatory T-Cells

- T-cells in thymus surviving negative selection will mature and move on – self reactive CD4+ and CD8+ T-cells
- Some immature T-cells recognize self-Ag develop into regulatory T-cells move to peripheral lymphatics
  - not sure what separates this from negative selection induction



Peripheral T-Cell Tolerance

- Induced when mature T-cells recognize self-Ag in the peripheral tissues that are not expressed in the thymus
  - leads to functional inactivation (anergy) or death or when self-reactive lymphocytes are suppressed by regulatory T-cell
  - may prevent autoimmunity in situations were central tolerance is incomplete – back-up mechanism

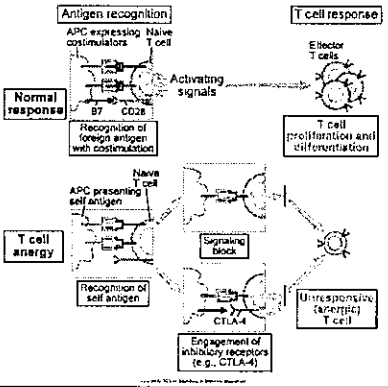
Peripheral tolerance

Central	Peripheral	Suppression (peripheral 2)
deletion	deletion anergy ignorance	suppressor cells? anti-idiotypes

Anergy

- Functional inactivation of T-cells when recognize Ag without adequate levels of co-stimulators (2nd signals) needed for full T-cell activation
  - need at least 2 signals to proliferate and differentiate into effector cells
    - SIGNAL 1 – Ag
    - SIGNAL 2 – co-stimulators on professional APC's, B7 proteins
- Self-Ag on APC and find T-cell which recognizes Ag (signal 1) but no necessary 2nd signal
  - leads to anergy (may also induce no response)

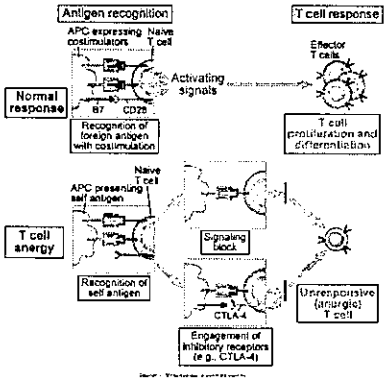
Formation of Anergy



Anergy (cont)

- **T-cell may express a molecule called CTLA-4 (CD52) or PD-1 (programmed cell death protein -1) which is a high affinity receptor for B7 that delivers inhibitory signals to T-cells**
  - inactivates the T-cell
  - not sure how cell chooses CTLA-4 or CD28 to bind to the B7
  - may be enough B7 for inhibitory receptor rather than to activate the T-cells

Formation of Anergy



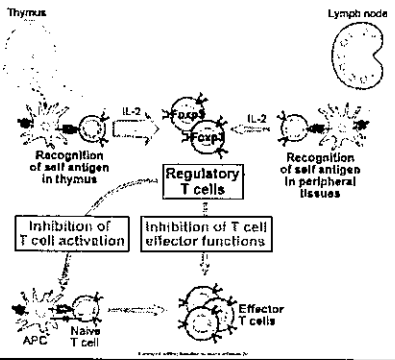
Immune Suppression

- **On encounter with self-Ag some reactive T-cells may develop into regulatory cells**
  - **functions to prevent or suppress the activation of other, potentially harmful self-reactive lymphocyte**
- **Regulatory T-cells may develop in the thymus or in peripheral lymphoid organs**
  - **most are CD4+ express high levels of CD25 – the α chain of the IL-2 receptor**
    - **know little about mechanisms of regulation**

T-cell Inhibition of Immune Response

- **Some produce TGFβ and IL-10 – Block activation of lymphocytes and macrophages**
  - **may interact to suppress other lymphocytes or APC's directly – cytokines not involved**
- **Some evidence in animal models**
  - **T-cells depleted of CD25 and put into mouse with no lymphocytes – disseminated autoimmune in multiple organs**

Suppression of Immune Response

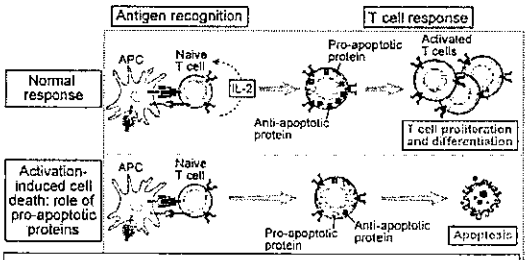


Deletion

- **Repeated activation of mature T-cell by self-Ag or recognition of self-Ag without 2nd signals will trigger pathways of <u>apoptosis</u> that result in elimination (deletion) of the self-reactive lymphocytes**
- **There are 2 likely mechanisms**
  - **induce pro-apoptosis proteins**
  - **death receptor e.g. fas receptor-fas ligand**

Mechanism #1

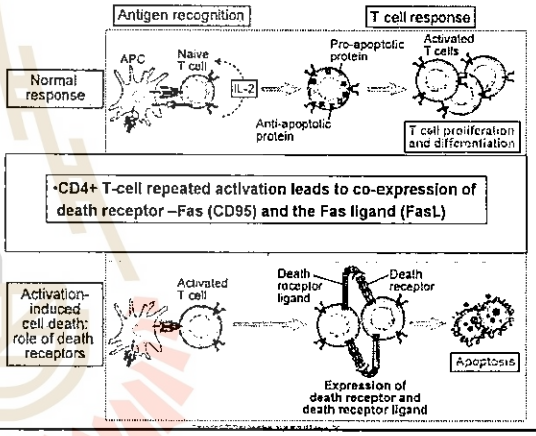
- Postulated mechanism of induced cell death – Ag recognition induces production of **pro-apoptotic proteins** in T-cells from the mitochondria
  - usually counteracted by anti-apoptotic proteins made by costimulation
  - not Fas-mediated



•Ag recognition induces production of pro-apoptotic protein in T-cells from the mitochondria

Mechanism #2

- **CD4+ T-cell repeated activation leads to co-expression of death receptor –Fas (CD95) and the Fas ligand (FasL)**
  - FasL binds Fas on same cell or adjacent cell that cause activation = apoptosis
  - internal death program – from self-Ag present through life – causing repeated stimulation
  - microbes are different because likely not to be persistant
  - T-cell IL-2 (growth factor) potentiates Fas-mediated apoptosis
    - can initiate and terminate response, not sure how 2 opposing actions occur



Self vs. Foreign Ag

Feature of antigen	Tolerogenic self antigens	Immunogenic foreign antigens
	Tissue	Microbe
Presence in generative organs	Yes (some self antigens); high concentrations induce negative selection and regulatory T cells (central tolerance)	No; microbial antigens are concentrated in peripheral lymphoid organs
Presentation with second signals (costimulation, innate immunity)	No; deficiency of second signals may lead to T cell anergy or apoptosis	Yes; typically seen with microbes; second signals promote lymphocyte survival and activation
Persistence of antigen	Long-lived (throughout life); prolonged TCR engagement may induce anergy and apoptosis	Usually short lived; immune response eliminates antigen

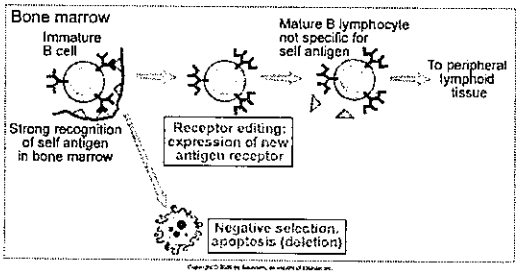
B-Cell Tolerance

- Self-polysaccharides, lipids and nucleic acids are T-cell independent so must make B-cells self-tolerant
  - don't want autoantibody production
  - similar to T-cell tolerance
- Protein Ag can also induce tolerance in B-cells
  - systemic lupus erythematosus – auto-antibody disease thought to be caused by defective tolerance in B and T-helper cells

Central B-Cell Tolerance

- **Immature B-cell interact strongly with self-Ag in bone marrow**
  - either killed (negative selection) or change receptor specificity (receptor editing)
- **Negative selection – similar to T-cell negative selection**
  - remove cells that have high affinity receptor to abundant and widely expressed cell membrane or soluble self-Ag

B-Cell Negative Selection



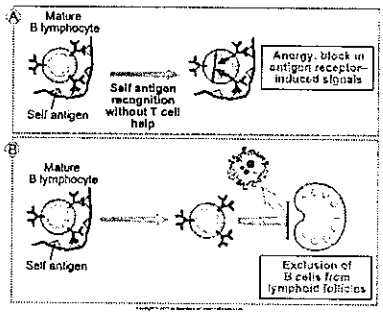
2nd Mechanism

- **May reactivate Ig gene recombination machinery and make a new Ig light chain – unites with original heavy chain with a new receptor for a different Ag**
  - called Receptor Editing NOT isotype switching
- **Not sure how many undergo wither mechanism or why 1 or the other is used**
  - no good example of failure of B-cell central tolerance causing autoimmunity

Peripheral B-Cell Tolerance

- **Mature B-cell encounter high concentration of self-Ag in peripheral lymphoid tissue – become anergic and cannot respond to that self-Ag**
- **B-cell recognize Ag and do not receive T-cell help (absent or tolerant) – B-cell becomes anergic**
  - T-cell independent Ag activate B-cells when signal is strong enough
- **B-cells that are anergic – leave follicle and can't return – may die because not receiving survival signals**

Peripheral Tolerance



Definition of autoimmunity

The immune system mounts an attack against the tissues of its own host without a clear reason.

Implicit statements:

- If we know the reason of the immune reaction, it is not called autoimmunity (e.g. viral infection)
- The immune system can distinguish between self and non-self (dogma)
- The immune system will not attack tissues recognized as self (the concept of tolerance)

The frequency of autoimmune diseases

- 4-5% of the population affected
- Highest prevalence (cca. 1-1%):
  - Autoimmune diseases of the thyroid (Graves disease, Hashimoto thyreoiditis)
  - Rheumatoid arthritis (RA)
- A few dozens of rare diseases also belong to this group

Clinical classification of autoimmune diseases

organ specific

- Graves (Basedow) dis.
- Hashimoto thyreoiditis
- Pernicious anemia
- Addison's disease
- DM type 1
- Myasthenia gravis
- Guillain-Barré sy.

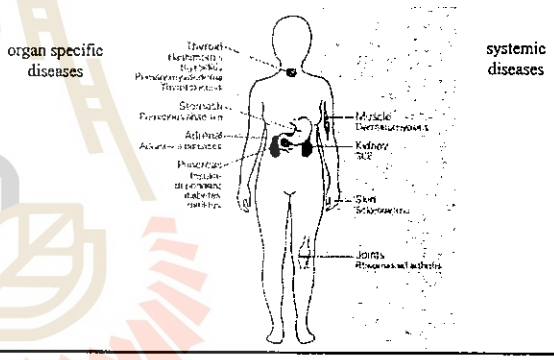
systemic

- SLE
- RA
- Scleroderma
- Dermatomyositis
- Vasculitis

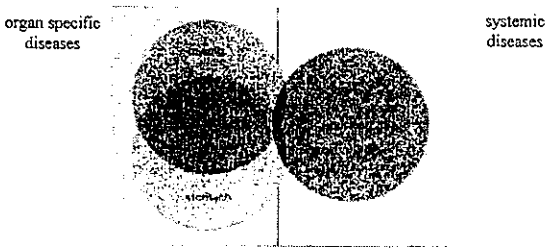
Significance of autoimmune diseases

- Potentially fatal disease (e.g. DM type 1, pernicious anemia)
- Lifelong treatement is necessary
- They cause severe, progressive inflammatory reactions (the systemic ones)

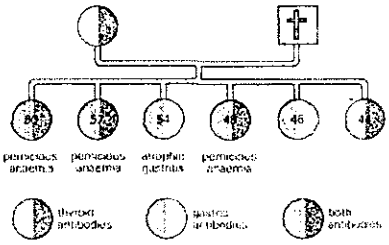
Frequently affected organs



Combined occurrance of autoantibodies



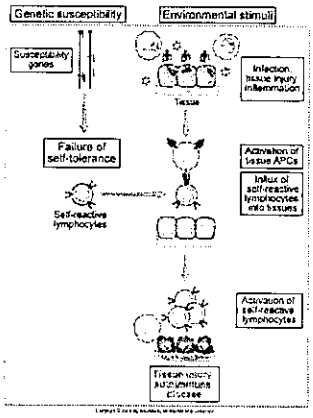
Occurrence of autoantibodies in a family



Presence of an autoantibody by itself doesn't necessarily cause clinical symptoms!

Autoimmunity

- Immune response against self-Ag – can cause disease
  - 1-2% of population has autoimmune disease
    - even when no true evidence of immune response to self-Ag
- Several factors in disease development
  - inheritance of susceptibility genes that contribute to failure of self-tolerance
  - environmental triggers such as infection may activate self-reactive lymphocytes



- Human autoimmune disease has no known etiology despite some good animal models
- Heterogeneous and multifactorial – disease may occur well after the autoimmune response is initiated
  - Ab ot self-Ag or activate T-cells react with self-Ag

Human Autoimmune Disease

- Still don't know the cause
- May involve 3 factors
  - autoimmune disease in humans usually are heterogeneous and multifactorial
  - self-Ag that are inducers and targets of autoimmune reactions often are unknown
  - diseases may manifest clinically long after the autoimmune reactions have been initiated

Genetic Factors

- Multiple genes can predispose but MHC genes are most important – saw with identical twins one has autoimmune, the other is more apt to get that a random person
  - genome scanning techniques and animal breeding indicate autoimmune disease is linked to multi-gene loci – MHC
- Many autoimmune disease in humans and inbred animals – linked to some MHC alleles
- Association between HLA alleles and autoimmune disease – line of evidence that T-cells important role in these disorders
  - May increase chance but not the cause, requires other things to happen
    - MHC may be involved because they are ineffective Ag presenters leading to defective negative selection or peptide in MHC fail to stimulate regulatory T-cells
- Some non-HLA alleles are associated with autoimmune disease
  - many are just large chromosomal segments and gene not yet identified

Autoimmune Disease and HLA Alleles

Evidence	Examples		
	Disease	MHC allele	Relative risk
"Relative risk" of developing an autoimmune disease in individuals who inherit particular HLA allele(s) compared with individuals lacking these alleles	Ankylosing spondylitis	HLA-B27	90
	Rheumatoid arthritis	HLA-DR4	4
	Type 1 diabetes mellitus	HLA-DR3/DR4	25
	Pemphigus vulgaris	HLA-DR4	14
Animal models: breeding studies establish association of disease with particular MHC alleles	Type 1 diabetes mellitus (nonobese diabetic mouse strain)	$I\text{-}A^{g7}$	

Non-HLA Genes and Autoimmune Disease

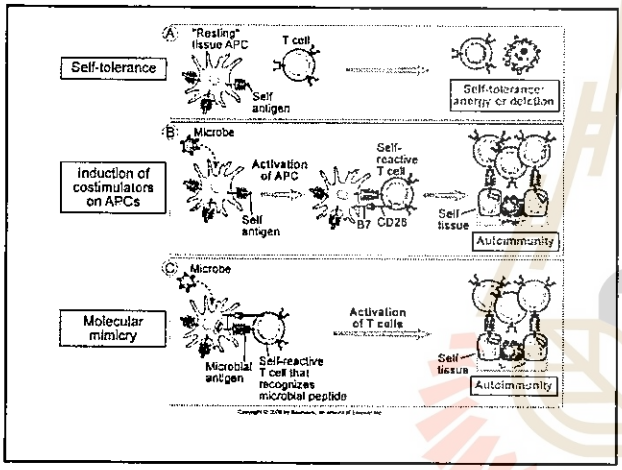
Gene(s)	Disease association	Mechanism
AIRE	Autoimmune polyendocrine syndrome	Defective expression of tissue antigens and elimination of self-reactive T cells in the thymus
Complement proteins (C2, C4)	Lupus-like disease	Defective clearance of immune complexes? Defects in B cell tolerance?
Fas, FasL	Lpr, gld mouse strains; human ALPS	Defective elimination of self-reactive lymphocytes
$Fc_\gamma RIIb$	Lupus-like diseases	Defective feedback inhibition of B cell activation
Foxp3 *	X-linked polyendocrinopathy and enteropathy (IPEX)	Deficiency of regulatory T cells
IL-2; IL-2Rα/β	Several autoimmune diseases (increased risk with polymorphisms)	Deficiency of regulatory T cells
NOD-2 *	Crohn's disease (inflammatory bowel disease)	Defective resistance or abnormal responses to intestinal microbes?
PTPN22 *	Several autoimmune diseases	Abnormal tyrosine phosphatase regulation of lymphocyte activation?

Role of Infections

- Infections may activate self-reactive lymphocytes and lead to development of autoimmune disease
  - autoimmune disease often preceded by infectious prodromes (early symptoms of disease) – can do in animal models
- Infections can contribute to autoimmunity

Mechanisms

- Induce local innate response – may lead to increased expression of costimulators/ cytokines by APC – break anergy, promote survival and activation
- Molecular mimicry – infection may make peptides that are similar to self-Ag and cross-react with them – immune response will attack self-Ag and cause problems
- Infection can cause tissue damage releasing Ag that is usually are not exposed to immune response
  - eye and testis – now seen as foreign



Clinical classification of autoimmune diseases

organ specific	systemic
• Graves (Basedow) dis. • Hashimoto thyreoiditis • Pernicious anemia • Addison's disease • DM type 1 • Myasthenia gravis • Guillain-Barré sy.	• SLE • RA • Scleroderma • Dermatomyositis • Vasculitis

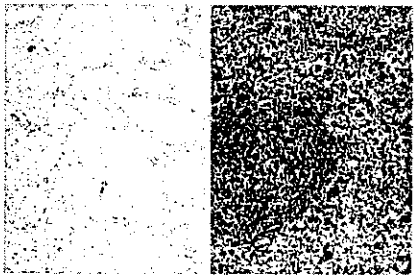
Hashimoto thyreoiditis 1

Clinical picture

enlargement of the thyroid gland

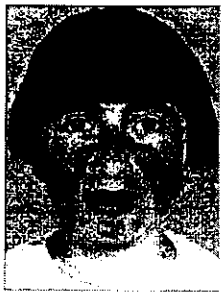
Hashimoto thyreoiditis 2

Histological picture of the thyroid



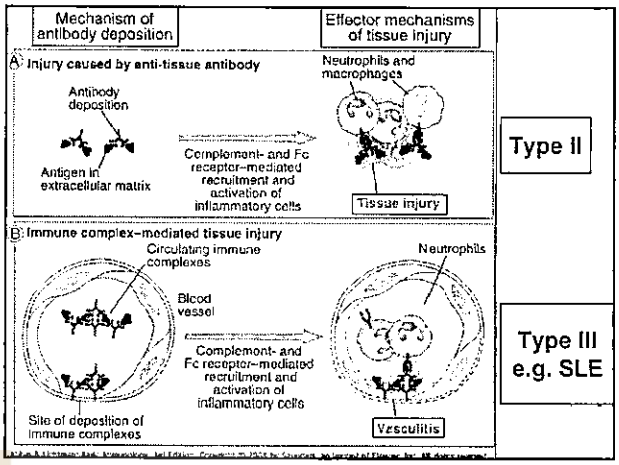
healthy | Hashimoto thyreoiditis

SLE: systemic lupus erythematodes



A frequent and most typical symptom of the disease is a butterfly-shaped erythema on the cheeks. It is caused by photosensitivity.

The tissue damage occurs by the **type 3 hypersensitivity reaction.** The symptoms are very variable, depending where the circulating immune complexes get deposited, causing an inflammatory reaction.



Human Immune Complex Diseases

Immune complex disease	Antibody specificity	Clinicopathologic manifestations
Systemic lupus erythematosus	DNA, nucleoproteins, others	Nephritis, arthritis, vasculitis
Polyarteritis nodosa	Hepatitis B virus surface antigen	Vasculitis
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigen(s)	Nephritis
Serum sickness (clinical and experimental)	Various protein antigens	Systemic vasculitis, nephritis, arthritis
Arthus reaction (experimental)	Various protein antigens	Cutaneous vasculitis

SLE
Causes/risk factors

inherited/genetic

- MHC I, II
- complement
- apoptosis
- CTLA-4
- TNF-α

acquired

- infection (molecular mimicry)
- fetal/neonatal infection
- haptens (drugs)

Inherited/genetic factors

Susceptibility to autoimmune disease was linked to more than two dozens of genes in mice experiments. Only a few examples are presented here.

The role of MHC I, II alleles

Antigens get presented associated with MHC, so the efficiency of the presentation of a particular antigen (and the possibility of an autoimmune reaction) may be determined by MCH alleles.

- In most autoimmune diseases, certain MHC alleles were found to be risk factors
- Some alleles can be protective (e.g. in DM type 1)
- Some alleles are risk factors in certain races only

The role of complement

The first few members of the complement system (C1, C4, C2, C3) play an important role in the elimination of immune complexes. Immune complexes carrying C3b are bound to RBCs, get taken up by the RES, where they are degraded.

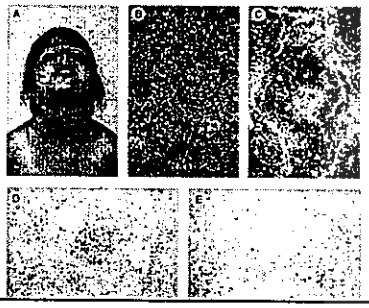
Congenital deficiency of C1,C2,C3,C4 frequently leads to autoimmune diseases (the pathomechanism of the tissue damage is type 3 hypersensitivity reaction).

The role of apoptosis

A mutation in the genes regulating apoptosis can cause autoimmunity

ALPS: Autoimmune lymphoproliferative syndrome

A rare congenital disease: chronic, nonmalignant proliferation of lymph nodes, splenomegaly, large number of double negative ($CD3^+$, $CD4^-$, $CD8^-$) ly, autoimmune phenomena.



A: lymphadenopathy

B-E: lymph node

B: HE staining
- hyperplasia,
- plasmocytosis

C: $CD3^+$ staining

D: $CD4^+$ staining

E: $CD8^+$ staining

Ann Intern Med (1999) 130: 591

ALPS

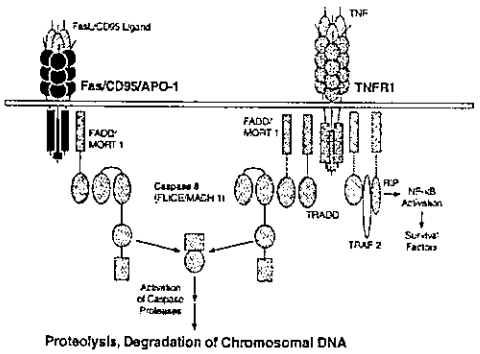
Pathogenetic factors

- Mutation in the Fas/CD95 gene
- Overexpression of IL-10

Autoimmune phenomena

- Autoantibodies
  - positive Coombs test
  - anticardiolipin, antinuclear antibodies
- Cytopenias of autoimmune origin
  - RBC (AIHA)
  - platelet (ITP)
  - neutrophil

The signaling system of apoptosis



The role of CTLA-4

CTLA-4 = cytotoxic-T-lymphocyte-associated protein 4 (CD 52). A receptor protein on the surface of T cells, through which activated T cells can get deactivating signals.

An inherited mutation of the gene, which causes slight changes in the function of the receptor is associated with the following diseases:

- Autoimmune diseases of the thyroid
- DM type 1
- Primary biliary cirrhosis

Acquired/environmental factors

- infection
  (superantigens, molecular mimicry)
- fetal/neonatal infections
- haptens (e.g. drugs)

Molecular mimicry 1

If an antigen of a microbe is identical or very similar to an antigen of the body *(molecular mimicry)*, then infection by the microbe can activate clones which are originally autoreactive or capable of cross-reacting with the self antigen.

For:

- The outbreak autoimmune diseases is frequently preceded by a viral infection
- sounds possible

Against:

- Infections are very common, autoimmunity is not

Molecular mimicry 2

Molecular mimicry is implicated in the pathogenesis of the following diseases (no direct proof is available yet in any of them):

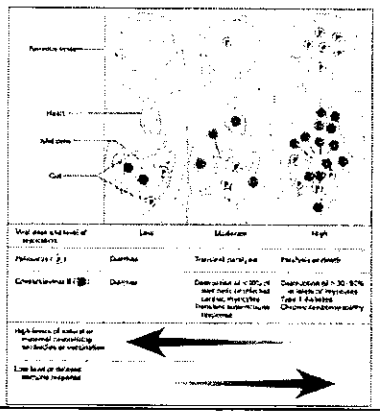
Disease	Pathogen, Ag	Autoantigen
Rheumatic fever	Streptococcus	cardiac myosin
Guillain-Barré sy.	Campylobacter jejuni lipopolysaccharide	myelin ganglioside (GM1)
DM type 1	Coxsackie virus P2-C protein	GAD (glutaminic acid decarboxilase)
Multiple sclerosis	EBV, influenza virus A, human papilloma virus	myelin basic protein (MBP)

Guillain-Barré syndrome

An acute demyelinating polyneuropathy causing paralysis. The paralysis is typically "ascending" (starts at the feet, and spreads upwards).

Many cases are preceded by *Campylobacter jejuni* infection (especially of serotype O:19). Antibodies against ganglioside (GM1) appear in the blood as a result of the infection.

The role of fetal/neonatal infections



If the titer of maternal Ig-s is low, the cytopathogenic and immune mediated damaging effects of the infection can lead later to autoimmune disease (e.g. DM type 1).

Zinkernagel

This theory can explain why there is a parallel increase of DM with better hygienic standards.

NEJM (2001) 345: 1331

The role of haptens

Many drugs cause hemolytic anemia, thrombocytopenia, neutropenia, or SLE-like disease with an autoimmune mechanism.

Many autoimmune disease shows geographical variation.

The role of gliadin in the development of celiac disease is also proven.

Therapeutical possibilities

Classic approach:
general inhibition of inflammation, immunosuppression

New methods:

Inhibition of TNF-α: RA, Crohn disease
Inhibition of IL-10: SLE
Destroy the immune system, then transplant allogenic stem cells: severe SLE

The End