면역학

감염체에 대한 숙주의 반응

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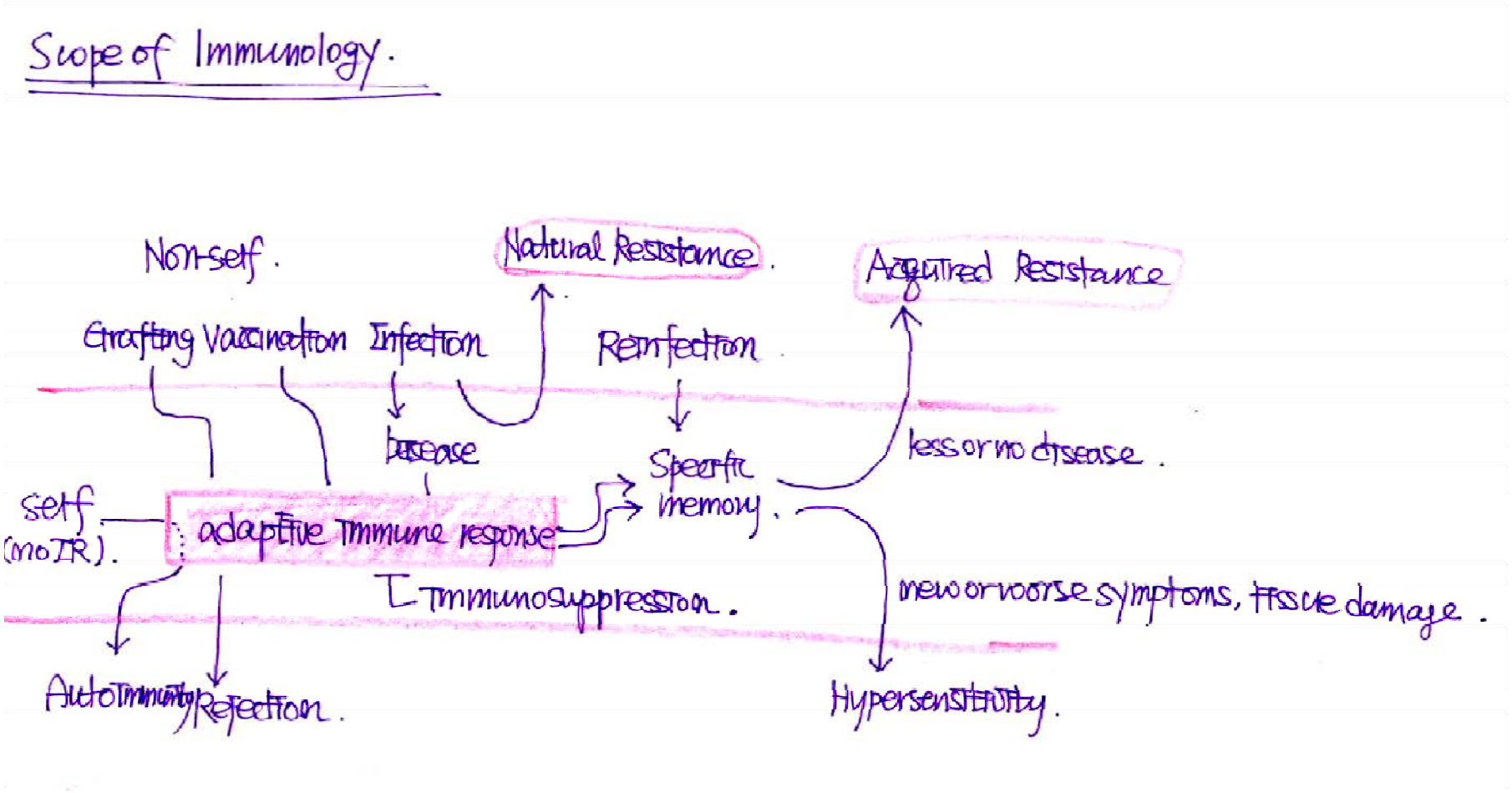
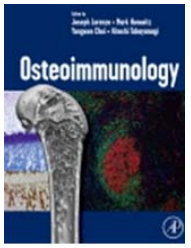
Scope of Immunology



From my undergraduate class note (1990)

공부할 내용

- **면역 반응 및 면역 체계**
  선천면역/적응면역 (세포성 면역/체액성 면역), 능동면역/수동면역
  면역세포 및 면역기관/조직
  보체 (C), 항원과 항체 (Ab)
  주조직 적합성 복합체 (MHC) 및 항원제시 (Ag presentation)
  항원 수용체 다양성 (TCR, BCR/IgR), co-receptors
- **면역 반응의 조절**
  싸이토카인 네트워크, Idiotypic network (= idiotype-anti-idiotype network)
  클론선택설
  T세포의 분화, 증식, 활성화
  B세포의 분화, 증식, 활성화
  면역관용 (immunologic tolerance): 면역관용원 (tolerogen)
- **면역의 실제 및 면역 질환**
  미생물에 대한 면역 및 백신 (감염에 대한 예방)
  종양면역, 줄기세포면역 (stem cell immunology), 골면역 (osteoimmunology)
  면역 질환: 과민반응, 이식면역, 자가면역, 면역결핍
  면역치료제: 치료용 mAb/백신/싸이토카인, 면역유전자 및 세포치료제, 면역억제제
- **면역 (항체) 기반 실험 기법**
  침전, 응집, 웨스턴블롯, RIA, ELISA, Immunofluorescence/FACS, 바이오칩



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Host Resistance and Immunity

- most pathogens (disease causing microbes)
  - must overcome surface barriers and reach underlying
  - overcome resistance by host
    - nonspecific resistance
    - specific immune response
- immune system
  - composed of widely distributed cells, tissues, and organs
  - recognizes **foreign** substances or microbes and neutralizes/destroys them (discrimination between self and non-self/foregin)
- immunity and immune response
  - ability of host to resist a particular disease or infection

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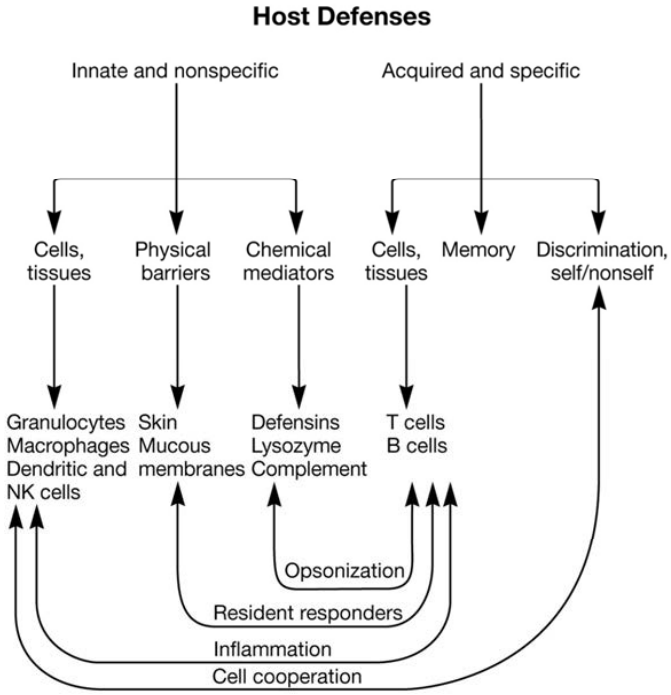
Immunity

- nonspecific immunity
  - aka. innate immunity and natural immunity
  - acts as *a first-line* defense
  - resistance to *any* microbe or foreign material
  - thus, lacks specificity and memory
- specific immunity
  - aka. acquired immunity, adaptive immunity
  - resistance to a *particular* foreign agent
  - thus, has **specificity** and **memory**
- the two types of responses *usually* **work together**



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Immunity

표 1-1 선천면역과 적응면역

	선천면역	적응면역
특 징		
특이성	미생물과 관련된 구조와 반응	미생물 항원 및 비미생물 항원과 반응
다양성	제한적	매우 다양함
기억력	없음	있음
자기세포와의 반응 여부	반응 안 함	반응 안 함
구성요소		
세포 및 화학 장벽	피부, 점막 상피조직; 항생 물질	상피세포 속의 임파구; 상피세포 표면에 분비된 항체
혈액 단백질	보체	항체
세포	탐식세포(대식세포, 호중구), NK 세포	임파구

표 3-1 선천면역계와 적응면역계

특 성	선천면역	적응면역
반응시간	수 분~수 시간으로 짧음	상대적으로 김. 수일
주요 구성 세포	탐식세포(단핵구, 대식세포. 호중구). NK 세포, 수지상세포	T 세포, B 세포, 항원제시세포
가용성분	항미생물 펩티드와 단백질	항체
특이성	병원체 관련 분자나 분자 구조에 대한 특이성	높은 특이성으로 미세한 분자구조의 차이를 구별
다양성	한정된 수의 배선에 암호화된 수용체 존재	매우 다양; 수용체 유전자의 유전적 재조합에 의해 아주 많은 수의 수용체 존재
자기 무반응성	완전; 숙주에는 미생물 특이 구조가 없음	아주 양호; 그러나 가끔 구별능력의 장애에 의해 자가면역질환이 생길 수 있음
기억반응	없음	지속적 기억에 의한 재감염에 대하여 신속하고 강한 반응을 보임

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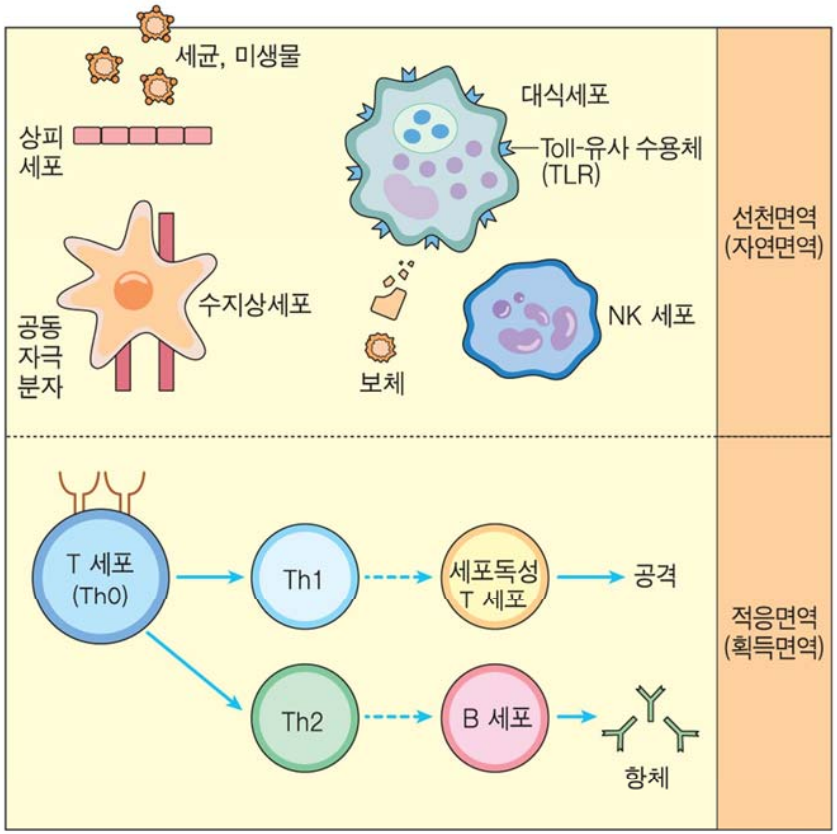
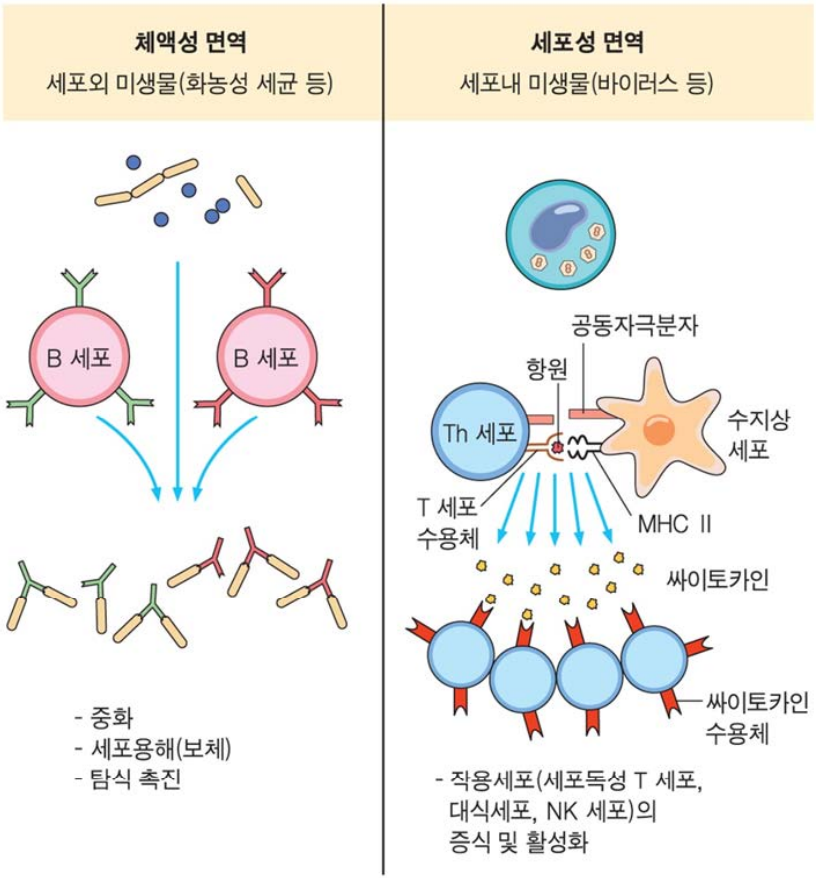
Immunity





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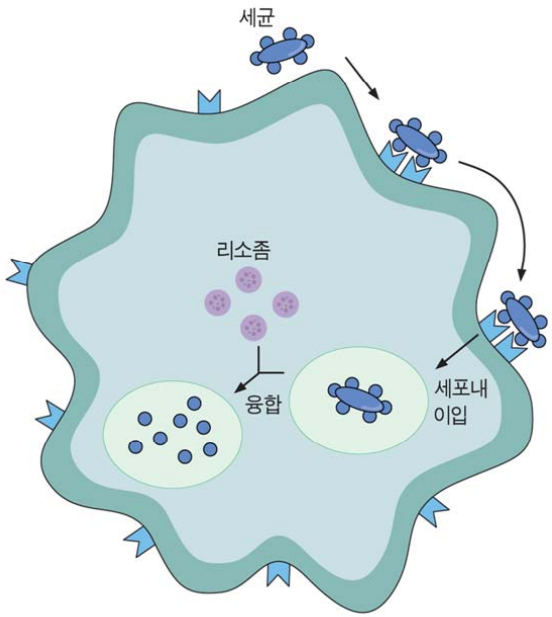
Antigens

- namely, **antibody generators**
- thus, invoke immune responses
  - presence of antigen in body ultimately results in B cell activation
    - antibodies bind to specific antigens, inactivating or eliminating them
    - other immune cells also become activated



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WBC of Innate and Adaptive Immunity

- white blood cells (WBCs) play a major role in the innate and specific responses
- **Hematopoesis (난황주머니 → 간, 비장 → 골수)**

  development of white blood cells in bone marrow of mammals
  - WBCs that mature prior to leaving bone marrow, e.g., macrophages (MΦ) and dendritic cells (DCs), become part of innate immune system and will respond to all antigens
  - WBCs that are not fully functional after leaving bone marrow become part of the adaptive immune response, e.g., B and T cells and could differentiate in response to specific antigens

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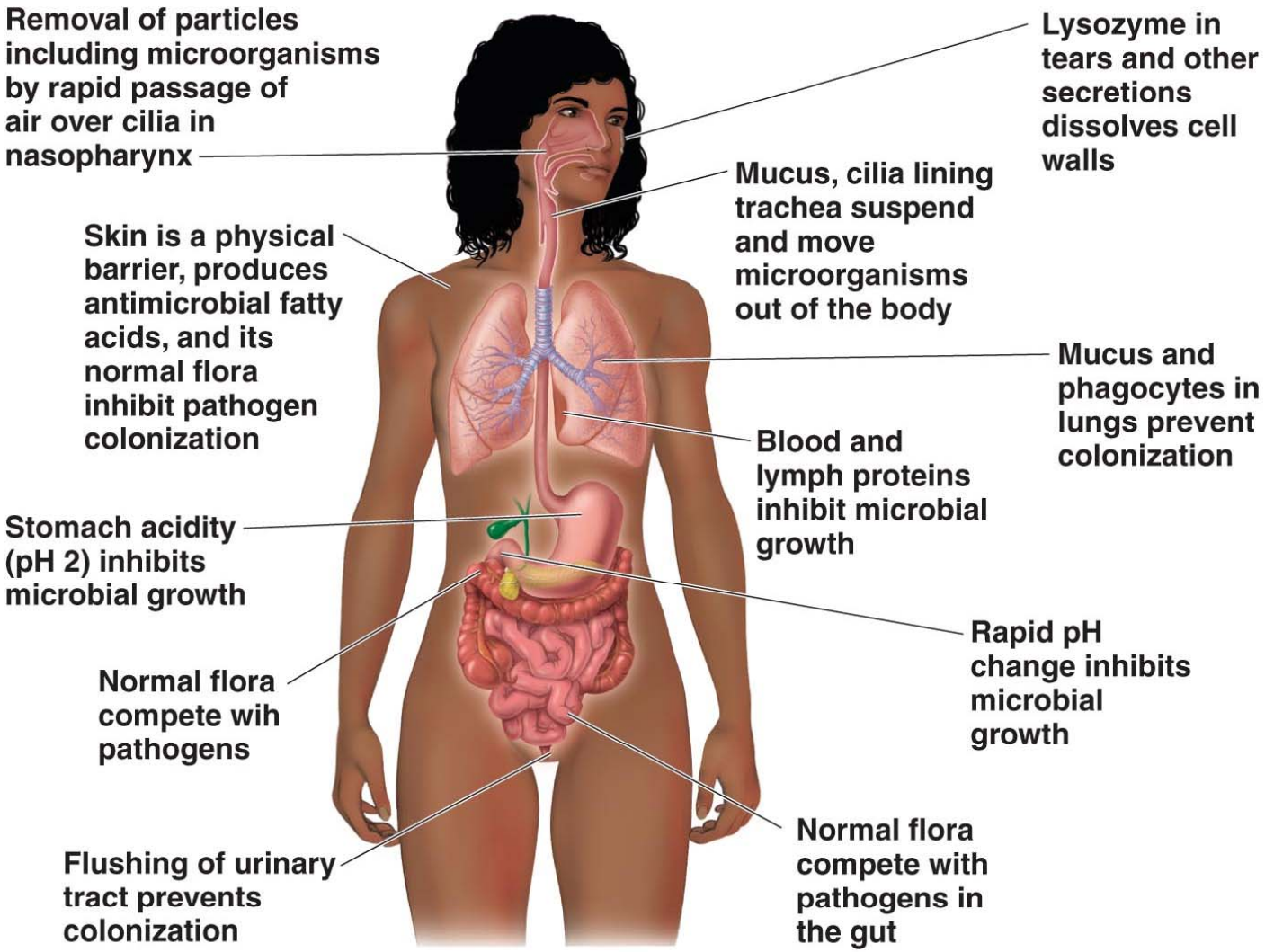
Physical, Chemical, and Anatomical Barriers to Infection



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선천면역의 방어체계

표 3-2 해부 · 물리적 방어체계

기관 또는 조직	방어기전
피부, 손톱	항미생물 펩티드, 피지 내 지방산, 물리적 분리 및 보호
눈	눈의 깜빡임, 안점막과 눈물
입과 식도	효소, 항미생물 펩티드, 위쪽으로의 액체 흐름에 의한 표면 세척
위	위산, 소화효소, 항미생물 펩티드, 장 내로의 액체 흐름
소장	소화효소, 항미생물 펩티드, 대장으로의 액체 흐름
대장	침입 세균에 경쟁하는 정상세균총, 액체나 대변의 직장 배출
코, 기도 및 폐	코 점막, 섬모에 의한 배출, 기침과 재채기에 의한 점액 배출, 폐포 대식세포
비뇨생식기	소변의 배출, 질의 pH

세포 종류	기 능
 호중구	탐식작용 활성산소 · 질소종 생산 항미생물 펩티드 생산
 대식세포	탐식작용 염증매개체 · 항원 전달 활성산소 · 질소종 생산
 수지상세포	항원제시 보조자극신호 활성산소종 생산 싸이토카인 분비
 NK 세포	바이러스 감염세포 사멸 대식세포 활성화 인터페론 분비

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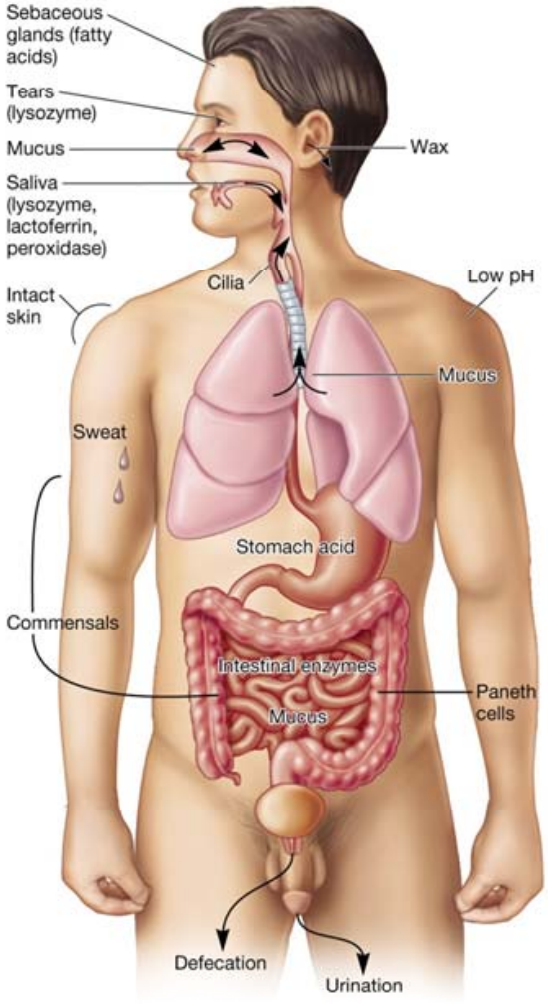
Physical/Mechanical Barriers

- along with host's secretions (flushing) these barriers are the first line of defense against microbes
- effectiveness impacted by:
  - direct factors
    - nutrition, physiology, fever, age, and genetics
  - indirect factors
    - personal hygiene, socioeconomic status, and living conditions



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Skin

- strong mechanical barrier to microbial invasion
  - keratin produced by keratinocytes in outer layer
- inhospitable environment for microbes
  - attached organisms removed by shedding of outer skin cells
  - pH is slightly acidic
  - high NaCl concentration
  - subject to periodic drying

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Mucous Membranes

- form protective covering that resists penetration and traps many microbes
- are often bathed in antimicrobial secretions which contain a variety of antimicrobial substances

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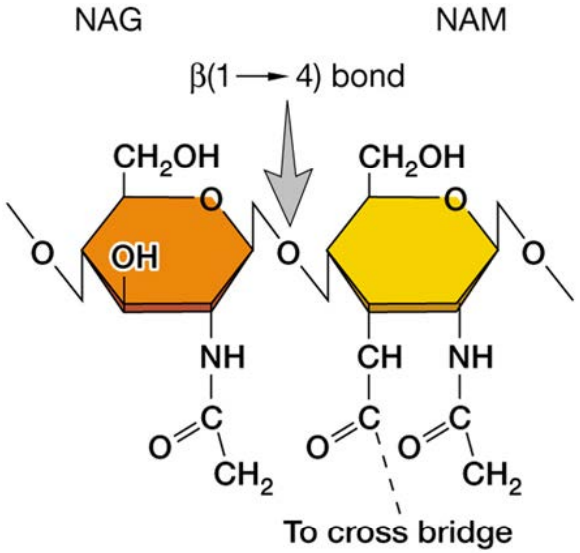
Antimicrobial Secretions

- lysozyme
  - hydrolyzes bond connecting sugars in peptidoglycan
- lactoferrin
  - secreted by activated macrophages and PMNs
  - sequesters iron from plasma
- lactoperoxidase
  - produces superoxide radicals



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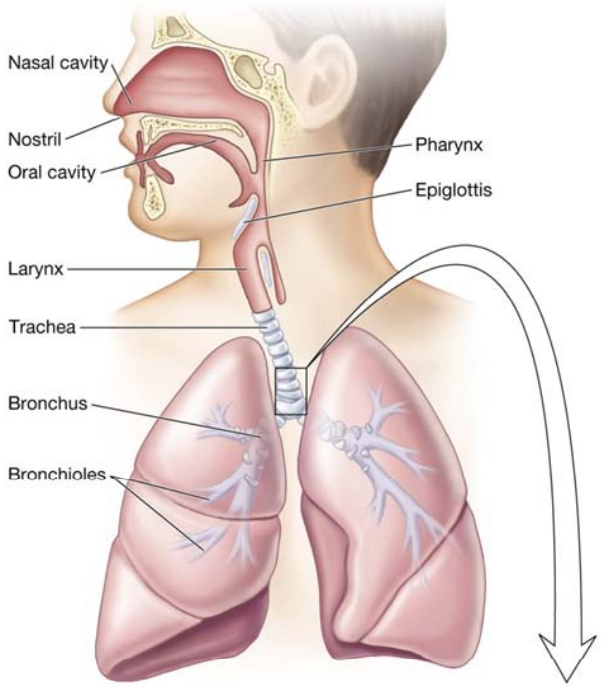
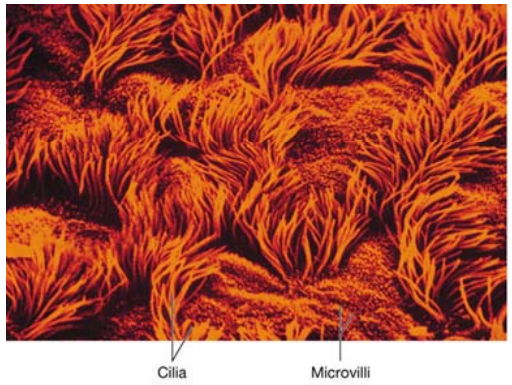
Respiratory System

- turbulent air flow deposits microbes onto mucosal surfaces
- mucociliary blanket
  - mucous secretions that traps microbes
  - once trapped, microbes transported away from the lungs (mucociliary escalator)
    - can be expelled by coughing or sneezing
    - salivation washes microbes to stomach
- alveolar macrophages
  - phagocytic cells in alveoli of lungs





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Gastrointestinal Tract

- stomach
  - gastric acid
- intestines
  - pancreatic enzymes
  - bile
  - intestinal enzymes
  - GALT (점막면역계)
  - peristalsis

- intestines
  - shedding of columnar epithelial cells
  - secretory IgA
  - normal microbiota
  - Paneth cells
    produce lysozyme

Genitourinary Tract

- unfavorable environment
  - low pH of urine and vagina
  - vagina has lactobacilli
  - urea and other toxic metabolic end products in urine
  - hypertonic nature of kidney medulla
- flushing with urine and mucus
- distance barrier of male urethra

The Eye

- mucus secreting epithelial membrane
- flushing action of tears
- lysozyme, lactoferrin, and secretory IgA in tears

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Chemical Mediators

- many already noted (e.g., gastric juices, lysozyme, urea)
- a variety of defensive substances such as defensins and other polypeptides are also found in blood, lymph, and other body fluids

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Antimicrobial Peptides

- cationic peptides
  - highly conserved through evolution
  - three classes whose biological activity is related to their ability to damage bacterial plasma membranes
- first class: linear, alpha-helical peptides that lack cysteine amino acid residues
  - e.g., cathelicidin, produced by a variety of cells
- second class: defensins
  - peptides that are open-ended, rich in arginine and cysteine, and disulfide linked
  - found in neutrophils, intestinal Paneth cells and intestinal and respiratory epithelial cells
- third class: larger peptides that are enriched for specific amino acids and exhibit regular structural repeats
  - e.g., histatin, present in human saliva and has anti-fungal activity

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Bacteriocins

- peptides produced by **normal microbiota**
- lethal to *related* species
- produced by gram-positive and gram-negative cells
- e.g., colicins produced by *E. coli*
- e.g., lantibiotics produced by gram-positive bacteria

표 3-4 항미생물 펩티드

종 류	생산동물	대상 미생물
알파-디펜신	사람(장의 호산구, 호중구)	세균
베타-디펜신	사람(상피, 기타 조직)	세균
카텔리시딘	사람, 소	세균
마가이닌	개구리	세균, 진균
세크로핀	누에	세균
드로소마이신	초파리	진균
스피니게린	흰개미	세균, 진균

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Cells of the Immune System

- each has ***specialized role*** in defending host
- leukocytes
  - aka. **white blood cells (WBCs)**
  - involved in both specific and nonspecific immunity
  - all arise from pluripotent stem cells

Table 33.4 Normal Adult Blood Count

Cell Type	Cells/mm^3	Percent WBC
Red blood cells	5,000,000	
Platelets	250,000	
White blood cells	7,400	100
Neutrophils	4,320	60
Lymphocytes	2,160	30
Monocytes	430	6
Eosinophils	215	3
Basophils	70	1

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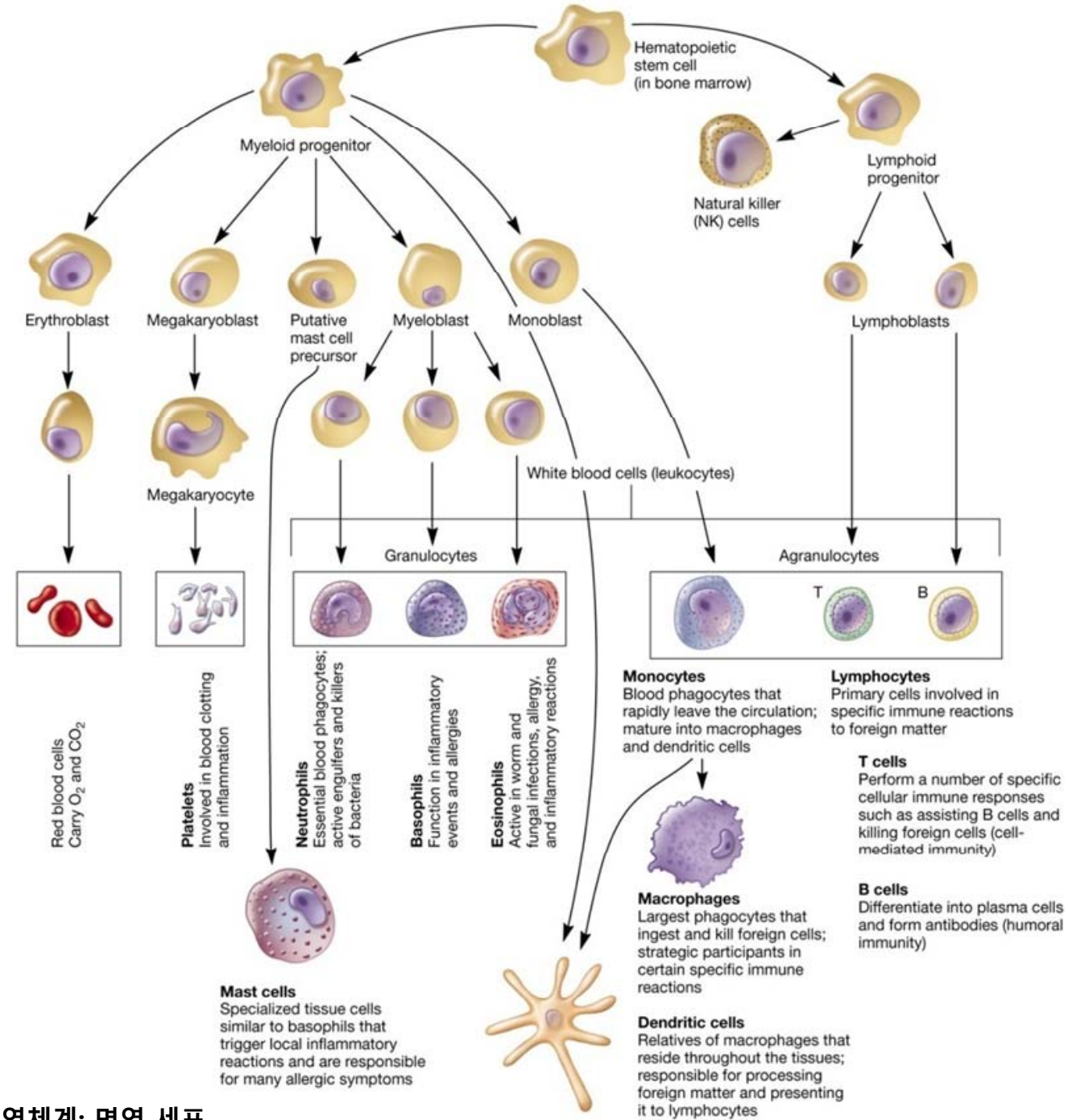
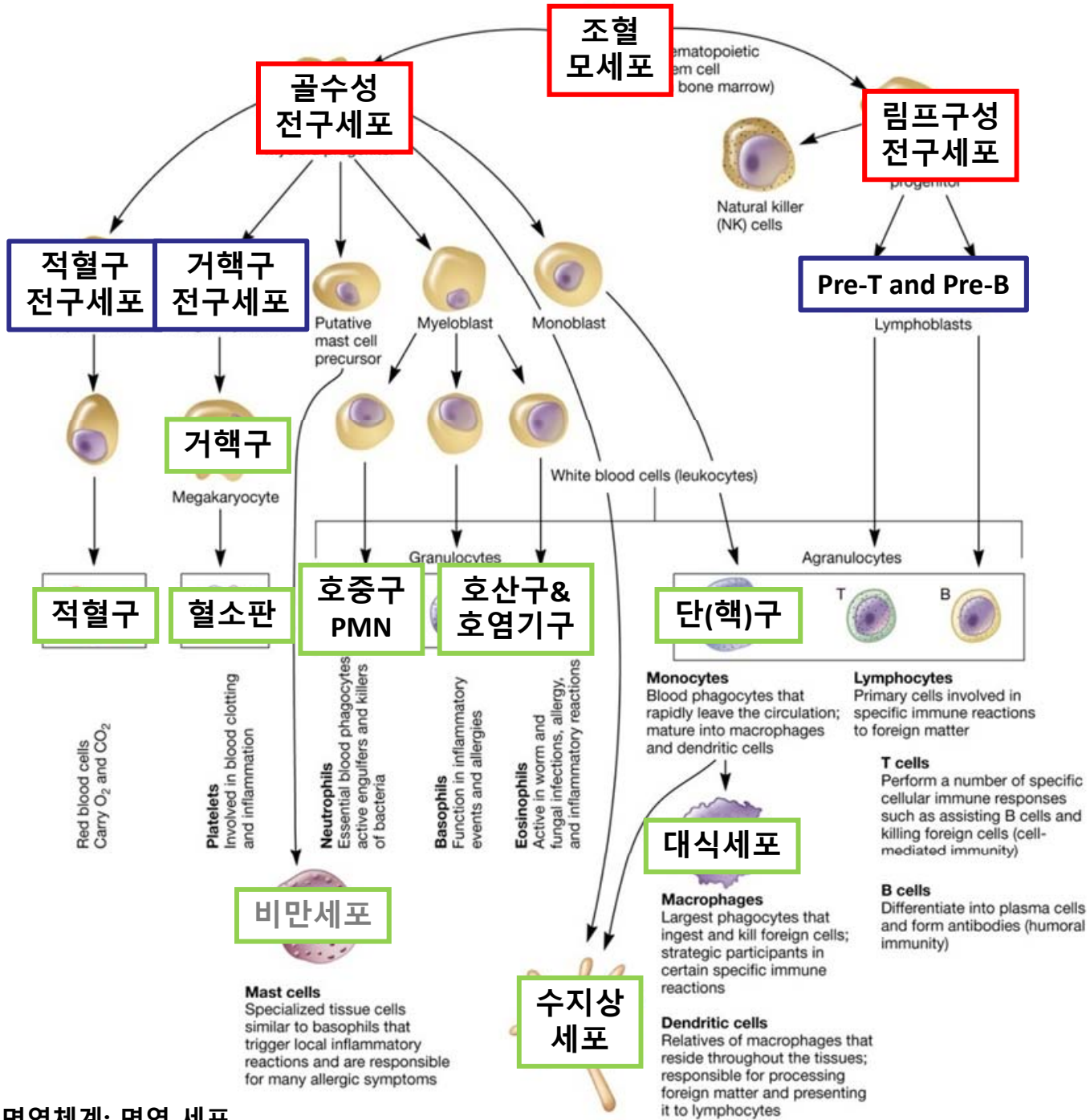
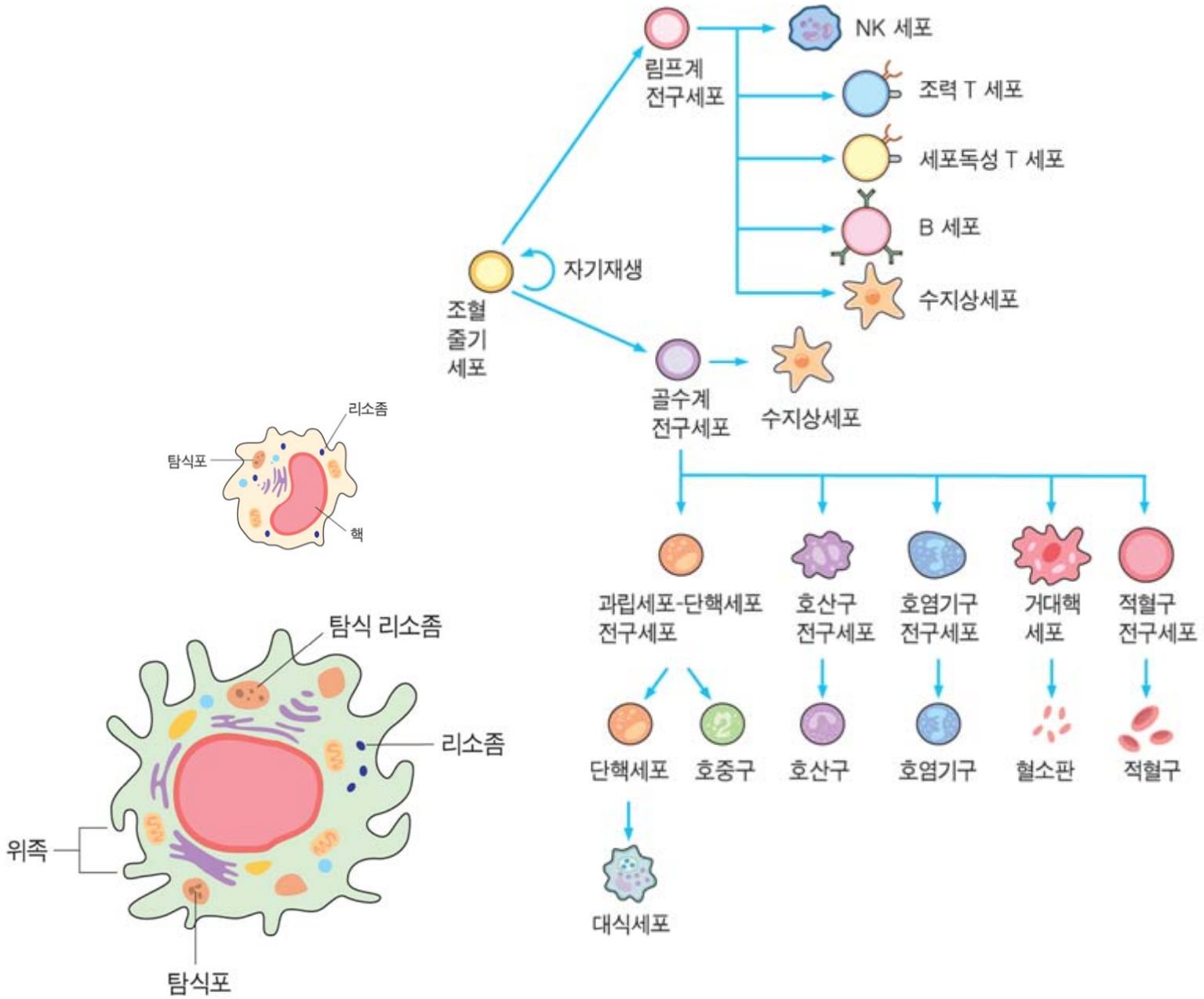


면역반응 및 면역체계: 면역 세포



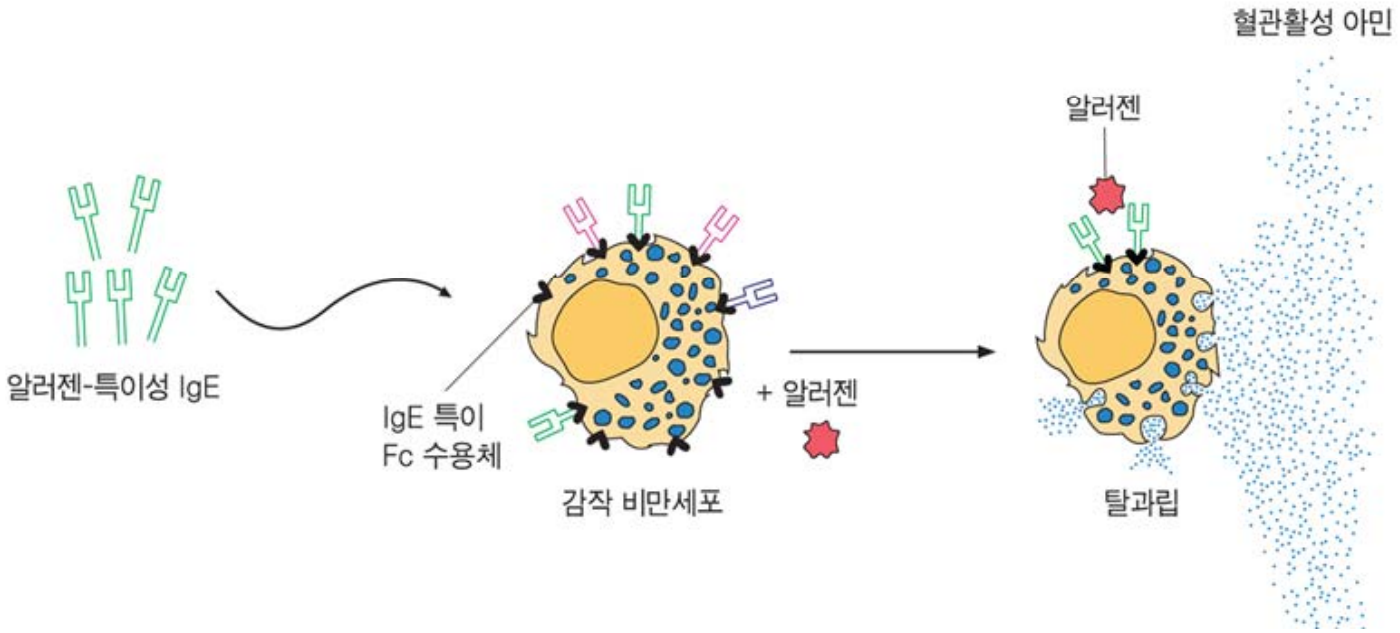
면역반응 및 면역체계: 면역 세포



면역반응 및 면역체계: 면역 세포

Mast Cells

- bone marrow-derived cells
- differentiate in blood and connective tissue
- contain **granules** containing histamine and other pharmacologically active chemicals
- play important role in development of **allergies (hypersensitivities)**



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Granulocytes

- Irregularly-shaped nuclei with two to five lobes
- cytoplasm has granules with reactive substances
  - kill microbes, enhance inflammation
- three types: **basophils**, eosinophils, **neutrophils (PMN)**

✓ **Basophils**

- stain bluish-black with basic dyes
- **nonphagocytic**
- release vasoactive mediators
  - e.g., histamine, prostaglandins, serotonin, and leukotrienes from granules
- play important role in development of allergies and hypersensitivities

✓ **Eosinophils**

- stain red with acidic dyes
- defend against protozoan and helminth parasites
- release cationic proteins and reactive oxygen metabolites
- may play a role in allergic reactions

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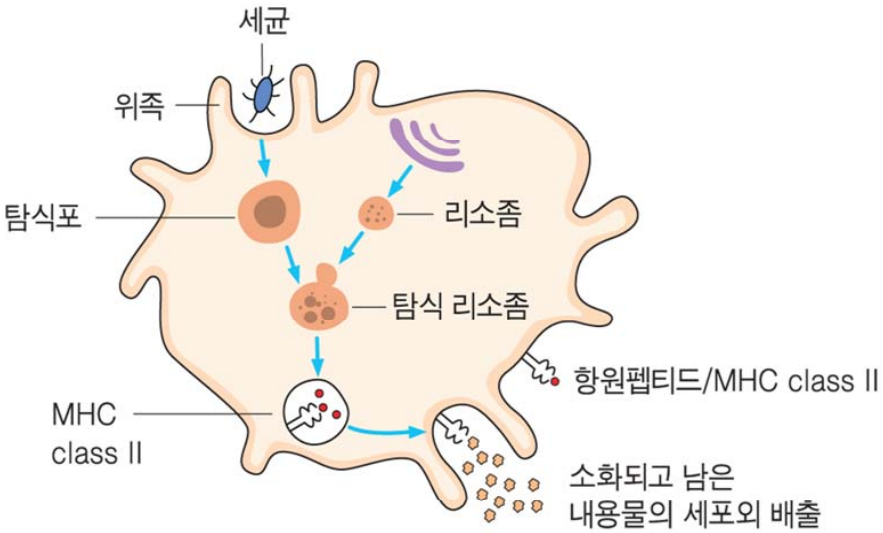
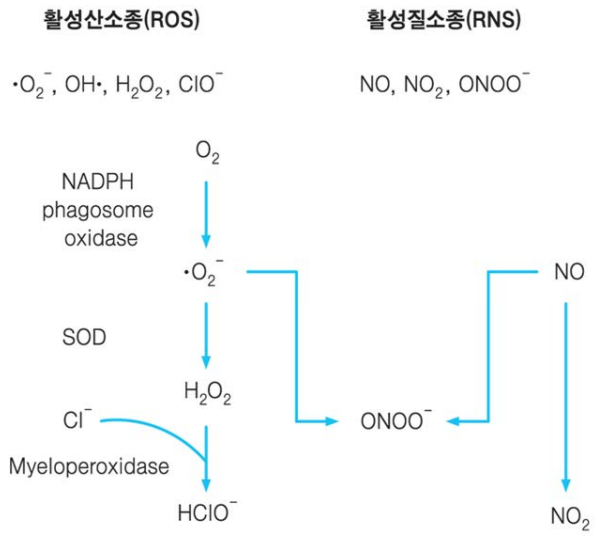
Granulocytes

✓ **<u>Neutrophils (= polymorphonuclear leukocytes. PMN)</u>**

- stain at neutral pH
- the most common leukocytes in the blood (60%)
- **highly phagocytic**
- circulate in blood then migrate to sites of tissue damage
- kill ingested microbes with lytic enzymes and reactive oxygen/nitrogen species (ROS and RNS) contained in primary and secondary granules





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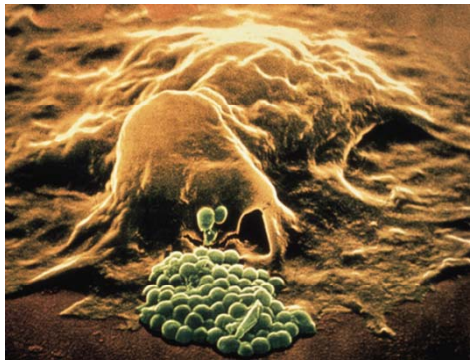
Monocytes and Macrophages

- **highly phagocytic cells**
- make up monocyte-macrophage system

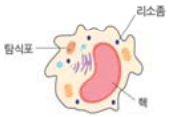


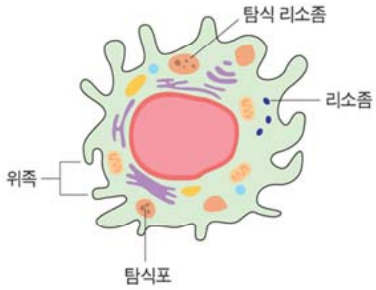
✓ **Monocytes**

- are mononuclear phagocytic leukocytes
- after circulating for ~8 hours, mature into macrophages

✓ **Macrophages (Mφ)**

- larger than monocytes, reside in **specific tissues**
- **highly phagocytic**
- have a variety of surface receptors including **PRR**s
  - recognize pathogen associated molecular patterns (PAMPs)
- named according to tissue in which they reside





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Dendritic Cells

- heterogeneous group of cells with **neuron-like *dendrites***
  - from lymphoid and myeloid lines
- present in small numbers in blood, skin, and mucous membranes of nose, lungs, and intestines
  - also express PRRs
  - contact, phagocytose, and **process antigens**

  → display foreign antigens on their surfaces (***antigen presentation***)

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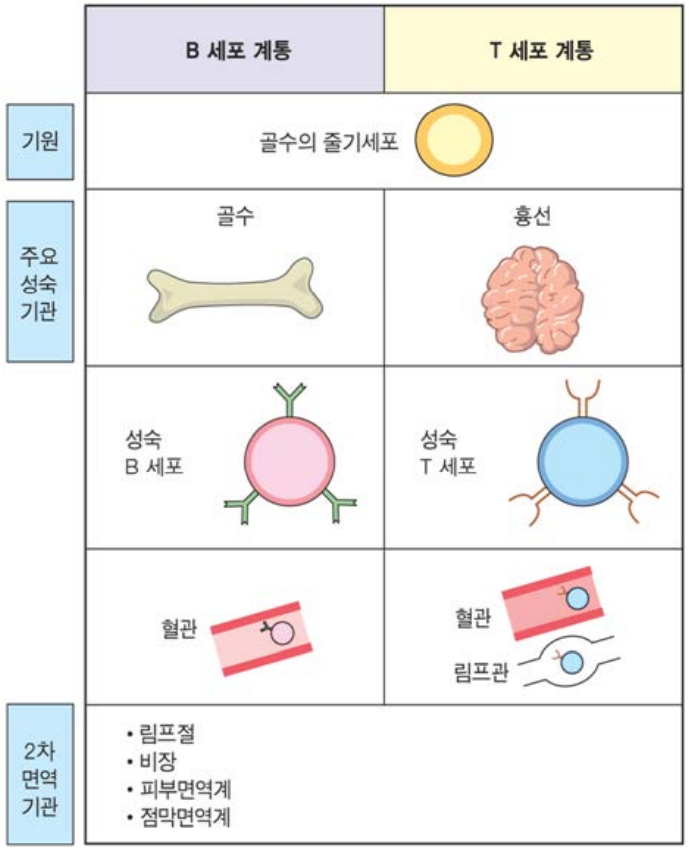
Lymphocytes

- major cells of the immune system
- the second common leukocytes in the blood (30%)
- include T, B, and NK (natural killer) cells
- B and T cells differentiate in bone marrow from stem cells
  - are only activated by binding of specific antigen onto lymphocyte surface receptors (**BCR** and **TCR**)
  - after activation, replication continues as lymphocytes circulate and enter lymphoid tissue
  - memory cells are activated lymphocytes that do not immediately replicate, but will do so later in host's life when antigen is again present



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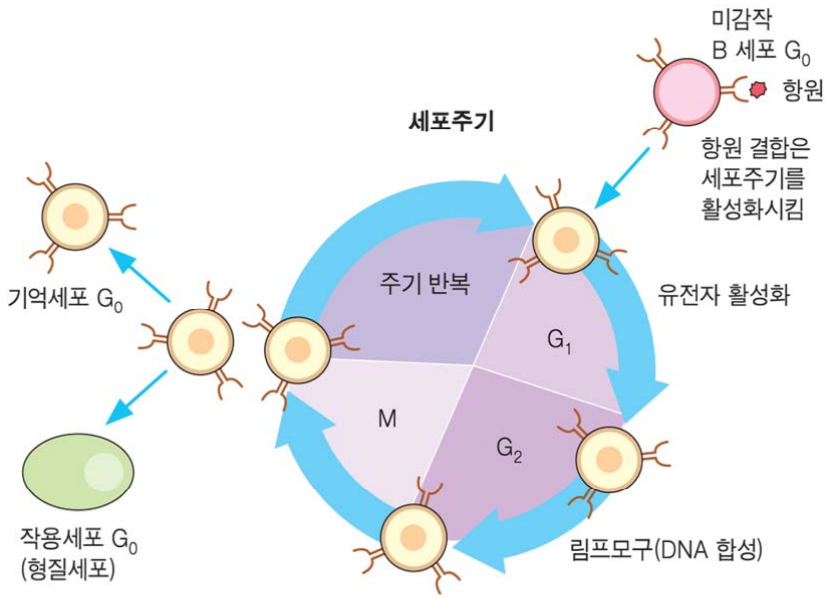
B and T Lymphocytes

✓ **B cells (B lymphocytes)**

- mature in bone marrow
- circulate in blood
- can settle in lymphoid organs
- after maturation and activation are called **plasma cells** and produce antibodies



✓ **T cells (T lymphocytes)**

- mature in thymus
- can remain in thymus, circulate in blood, or reside in lymphoid tissue
- like B cells, require antigen binding to surface receptors for activation and continuation of replication
- activated T cells differentiate into helper T cells (Th) and cytotoxic lymphocytes (T_C or CTLs)
- secrete cytokines, chemicals that have effects on other cells, are produced and secreted by activated T cells

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Natural Killer (NK) Cells

- small population of large non-phagocytic granular lymphocytes
  - important role in **innate** immunity
  - kill malignant cells and cells infected with pathogens by releasing granzymes (cytotoxic enzymes)
- *two* ways of recognizing target cells
  - bind to antibodies which coat infected or malignant cells (antibody-dependent cell-mediated cytotoxicity (**ADCC**)
  - recognizes cells that have *lost* their class I major histocompatibility (**MHC I**) molecule due to presence of virus or cancer

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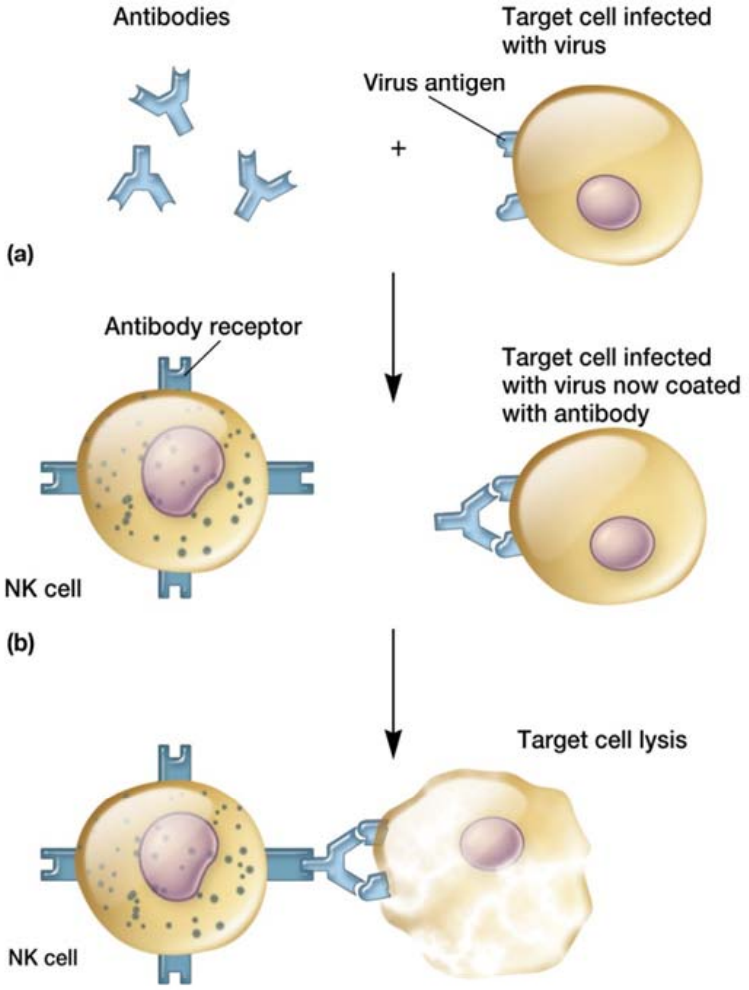
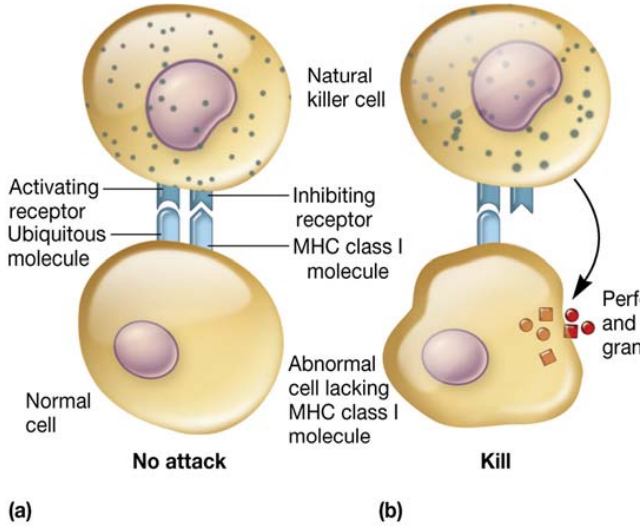
Natural Killer (NK) Cells





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Organs and Tissues of the Immune System

- primary organs and tissues
  - sites where lymphocytes mature and differentiate into antigen-sensitive mature B and T cells
- secondary organs and tissues
  - areas where lymphocytes may encounter and bind antigen
    - followed by proliferation and differentiation into fully mature effector cells

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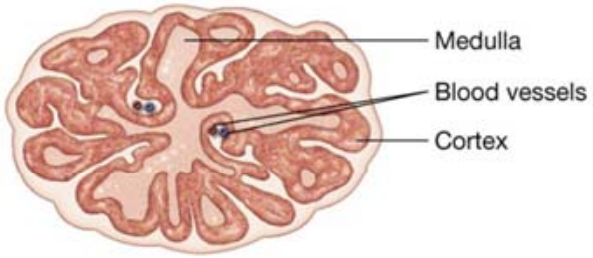
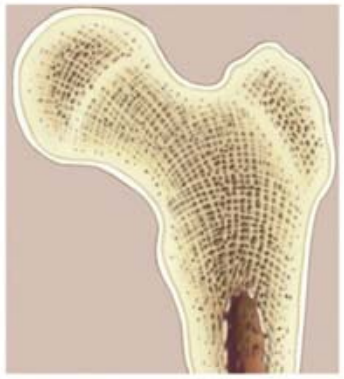
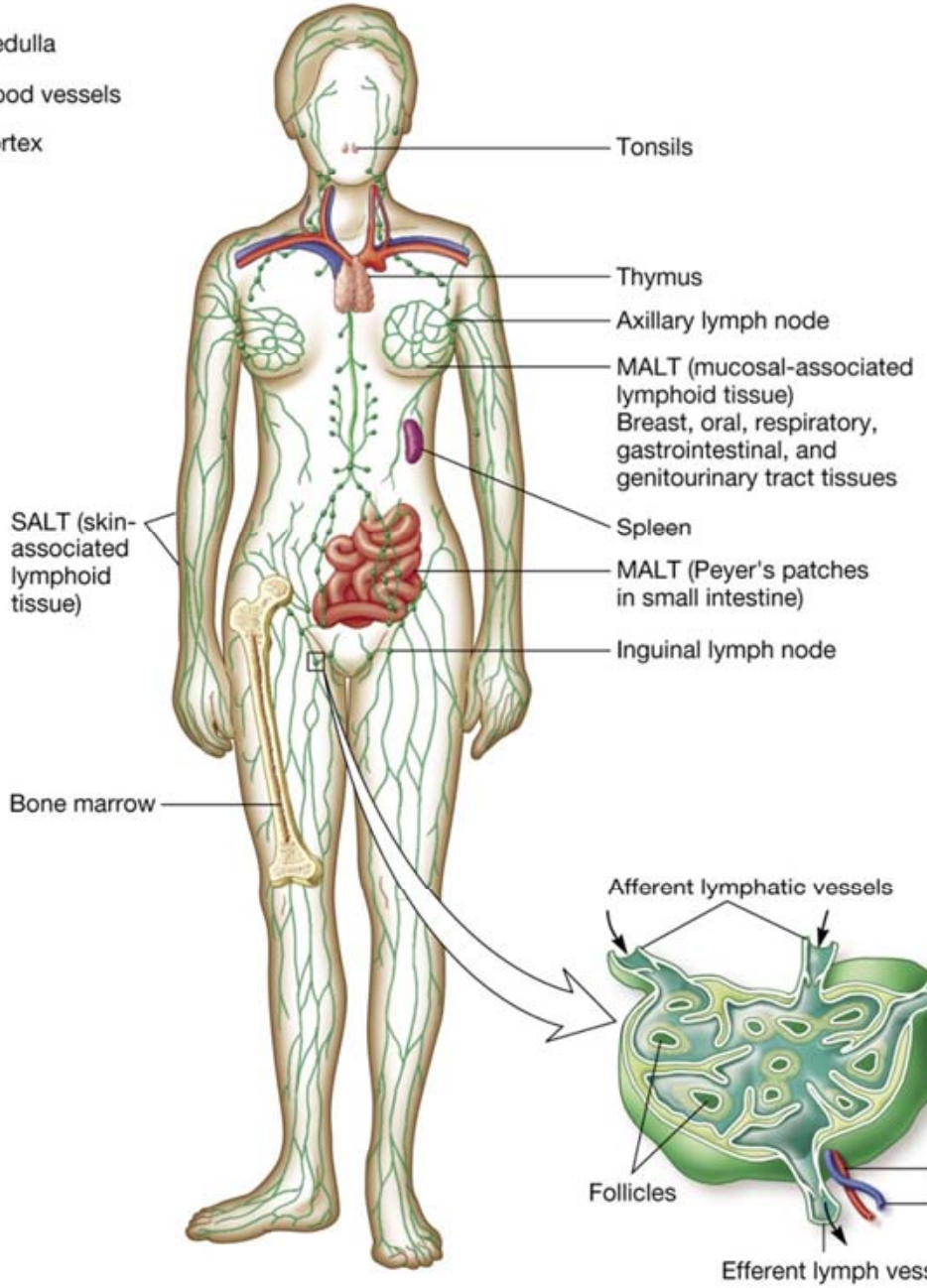


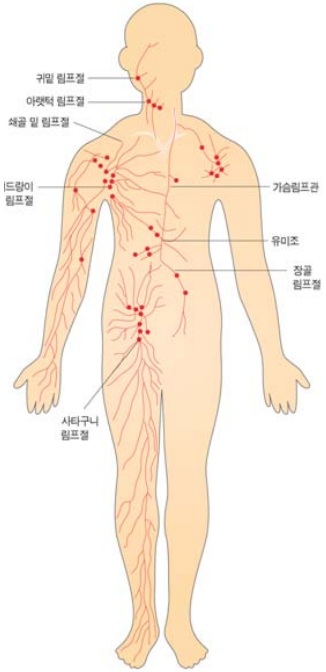
(a) Thymus (site of T-cell development)



(b) Bone marrow
(site of B cell development)



(c) Lymph node
(important site of T- and B-cell interaction with antigens and cells that present antigens)



면역반응 및 면역체계: 면역 기관

Primary Lymphoid Organs and Tissues

✓ **Thymus**

- precursor cells from bone marrow migrate here and proliferate
- thymic deletion removes T cells that recognize self antigens
- remaining cells become mature T cells
- enter bloodstream and recognize nonself antigens

✓ **Bone marrow**

- site of B cell maturation in mammals
- maturation involved removal of nonfunctioning and self-reactive cells

✓ Bursa of Fabricius (파브리시우스 낭, 활액낭)

- site of B cell maturation in birds

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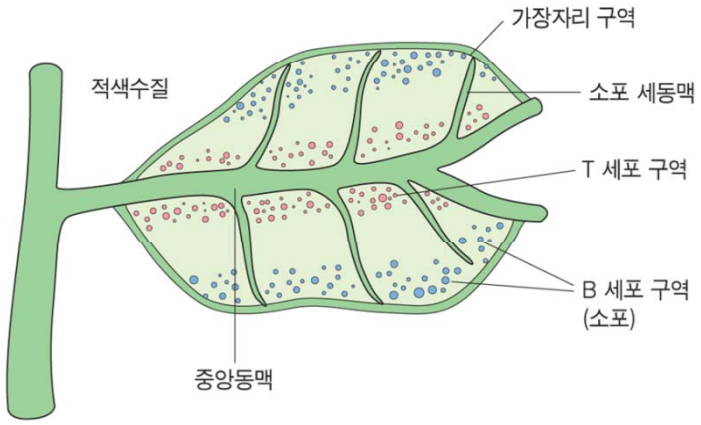
Secondary Lymphoid Organs and Tissues

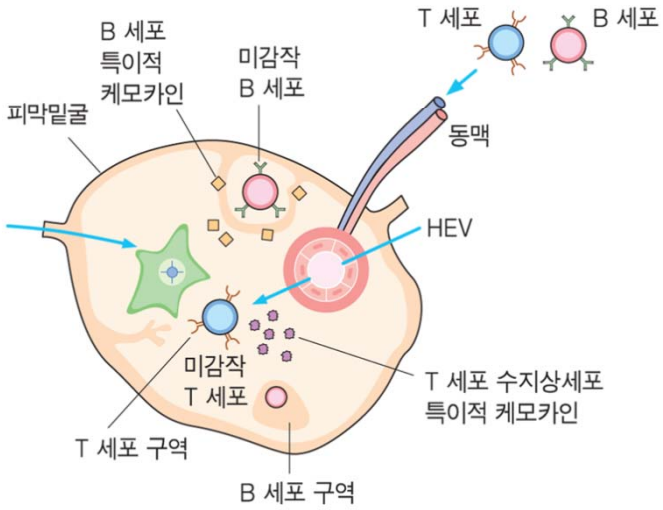
✓ **Spleen**

- most highly *organized* lymphoid organ
- filters blood
- macrophages and dendritic cells trap microbes and antigens
- present antigens to B and T cells
- most common way that lymphocytes become activated to carry out their immune functions



✓ **Lymph nodes**

- most highly organized lymphoid tissue
- filter lymph
- microbes and antigens trapped and phagocytosed by macrophages and dendritic cells
- B cells differentiate into memory and plasma cells within lymph nodes



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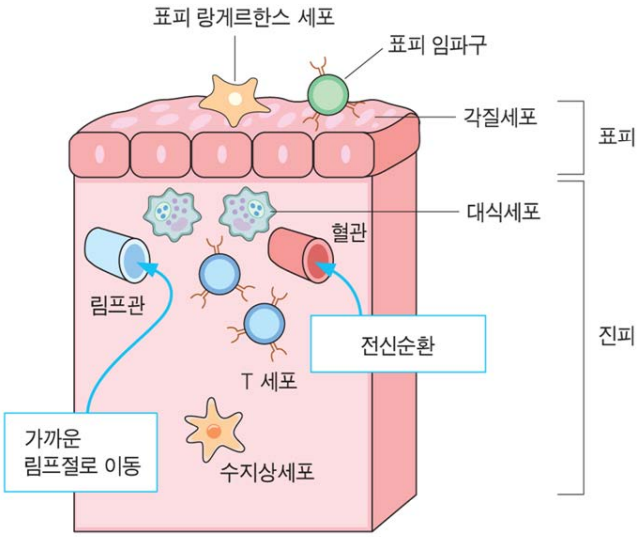
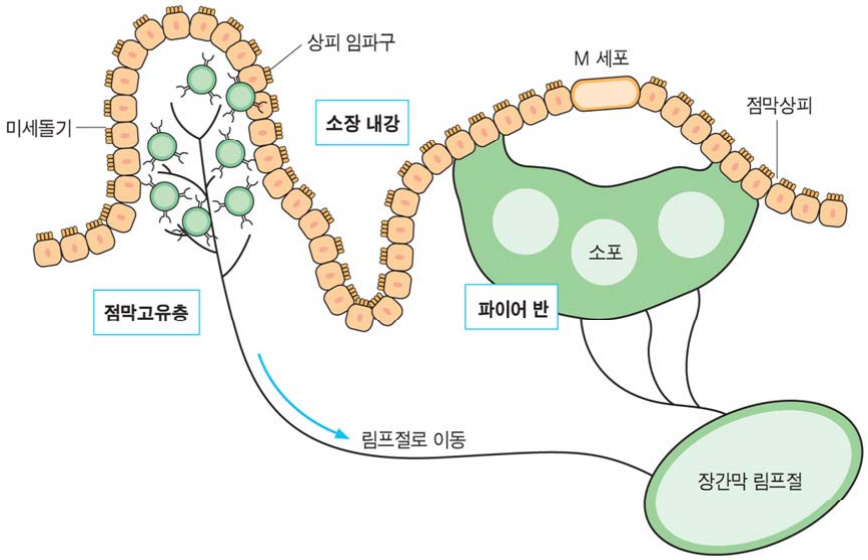
Secondary Lymphoid Organs and Tissues

✓ **Lymphoid tissues**

- located ***throughout*** **the body**
- serve as **interface** between innate and acquired host immunity
- act as areas of antigen sampling and processing
- some lymphoid cells are found closely associated with specific tissues such as skin-associated lymphoid tissue (SALT. 피부면역계) and mucous-associated lymphoid tissue (MALT. 점막면역계)





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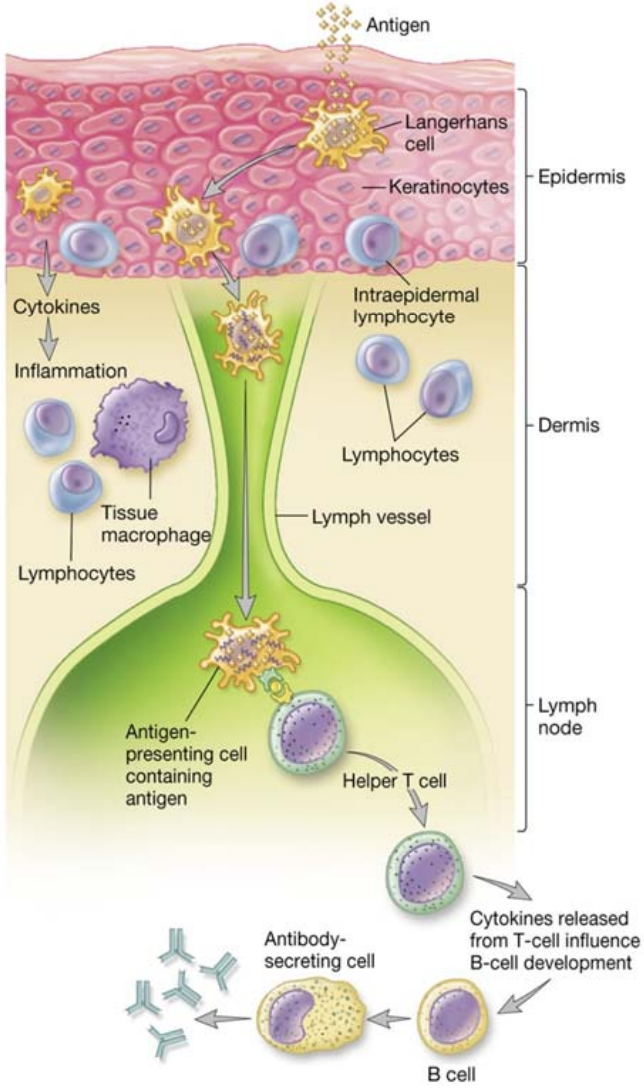
Skin-Associated Lymphoid Tissue (SALT)

contains specialized cells

- **Langerhans cells**
- dendritic cells that can phagocytose antigens
- differentiates into **interdigitating DCs** that present Ag to and activates T cells

- **Intraepidermal lymphocytes**
- function as T cells



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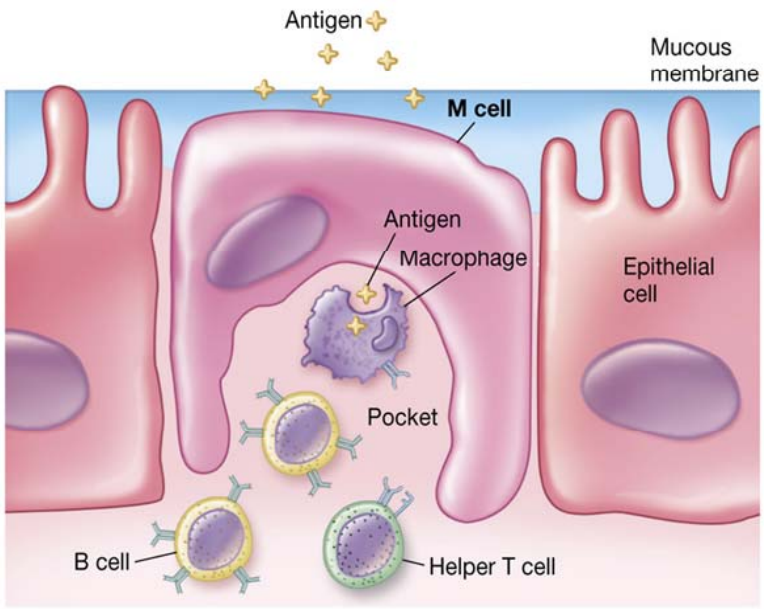
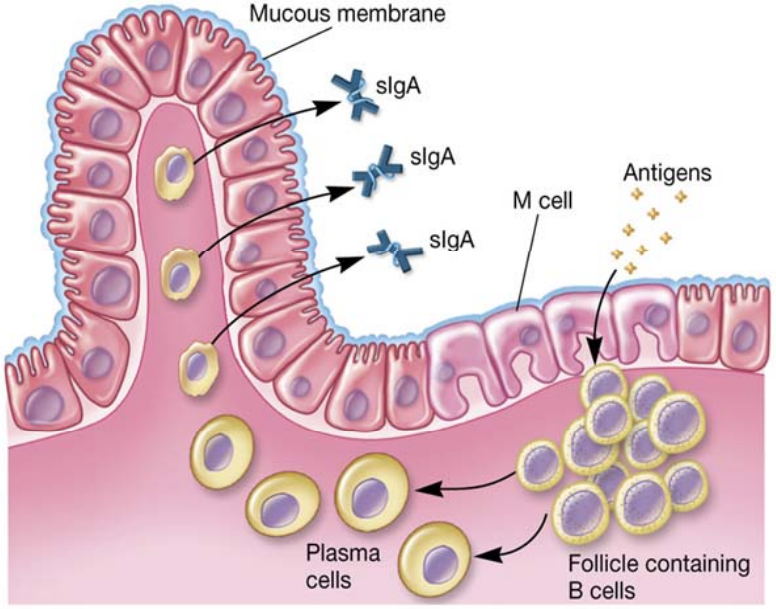
Mucosal-Associated Lymphoid Tissue (MALT)

specialized immune barrier LT

- bronchial-associated MALT (= BALT)
- gut-associated MALT (= GALT)
- urogenital system MALT





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The Complement System

- discovered by J. Bodet
- composed of >30 serum proteins produced in liver
- augments (or “complements”) the antibacterial activity of antibody
- three major activities:
  - defending against bacterial/viral infections
  - bridging innate and adaptive immunity
  - disposing of wastes

Table 33.1 Key Complement Proteins and Their Functions

	Protein	Function
COMPLEMENT ACTIVATION *(initiation through three unique pathways)*		
Alternative Pathway	C3	C3 is activated by repeating patterns within microbial structures and then is spontaneously cleaved into C3a and C3b; C3b binds to nearby membrane.
	Factor D	Factor D is activated by C3b to become an active enzyme, which activates Factor B.
	Factor B	Factor B adsorbs to C3b, forming a C3 convertase (protease).
	C3bBb	$\overline{C3bBb}$ (the active C3 convertase) cleaves additional C3 into its a and b fragments; C3b binds to the nearby membrane.
	Properidin	Properidin stabilizes the C3 convertase.
Lectin Pathway	Mannose-binding protein (MBP)	MBP binds to mannose on microorganisms, then recruits and binds plasma esterase to become mannose-associated serine protease (MASP).
	MASP	MASP (the active C3 convertase) cleaves C3 into C3a and C3b; C3b binds to the nearby membrane.
Classical Pathway	Antibody (Ab) formed by adaptive immune system upon subsequent exposure to antigen (Ag)	Ab binds to Ag on microorganism, causing three-dimensional shift in Ab structure, which reveals cryptic amino acids near carboxy-terminus (Fc region); the newly revealed amino acids attract plasma C1 protein.
	C1 (trimer of components q, r, and s)	C1q binds to Fc region of Ab-Ag complex; C1r,s binds plasma calcium.
	Ag-Ab-C1q,r,s-(Ca^{2+}) complex	Ag-Ab-C1-(Ca^{2+}) complex is an activated enzyme (called the C2/C4 esterase) that cleaves plasma C2 and C4 into their a and b fragments, respectively.
	C2a	C2a binds the Ag-Ab-C1-(Ca^{2+}) complex.
	C4b	C4b binds the Ag-Ab-C1-(Ca^{2+})-C2a complex, forming a C3 convertase (protease).
	Ag-Ab-C1-C2a-C4b	Ag-Ab-C1-C2a-C4b (the active C3 convertase) cleaves plasma C3 into its a and b fragments; C3b binds to the nearby membrane.
COMPLEMENT ACTION *(common effector pathway; convergence point for alternative, lectin, and classical activation pathways)*	C3b-membrane complex (stabilized C3b)	C3b stabilized in a membrane becomes an active C5 convertase (protease), cleaving plasma C5 into its respective a and b fragments.
	C5b	C5b binds plasma C6 and C7, forming a new, membrane-binding complex.
	C5b-C6-C7-membrane complex	Membrane-bound C5b-C6-C7 recruits plasma C8 and C9, which insert into the membrane adjacent to C5b-C6-C7.
	C5b-C6-C7-C8-C9 complex	C5b-C6-C7-C8-C9 complex forms transmembrane pore known as the membrane attack complex (MAC); MAC formation leads to cell lysis.

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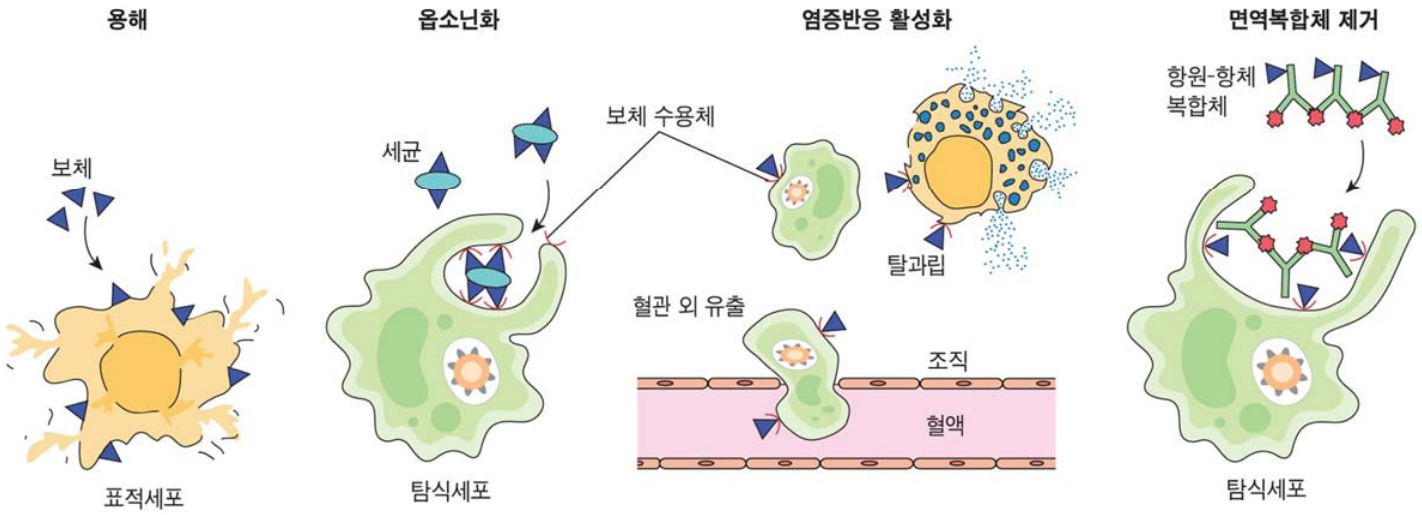
Functions of Complement Proteins

- **cytolysis**: puncture cell lysis of invading bacteria via MAC
- **opsonization**: opsonize the invading microbes in the circulating system
- **activation of inflammation**: recruit phagocytes / regulate immune cells
- **immune clearance**: remove Ag-Ab complexes to liver and spleen



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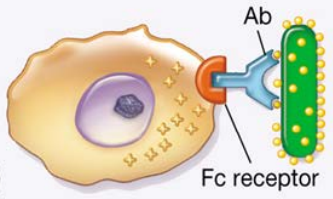
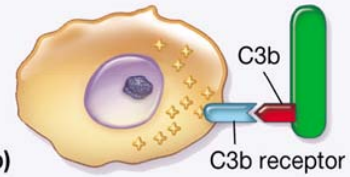
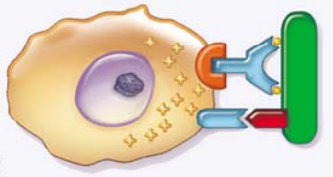
Opsonization

- process in which microbes are coated by serum components (= **opsonins** such as Ab, C3b, collectins, and bacteriotropin etc) in preparation for recognition/ingestion by phagocytes

cf. opsonins synergistically bind to microbial cells, *coating* them for phagocyte recognition

Phagocytic cell	Degree of binding	Opsonin
	+	Antibody
	+ +	Complement C3b
	+ + + +	Antibody and complement C3b

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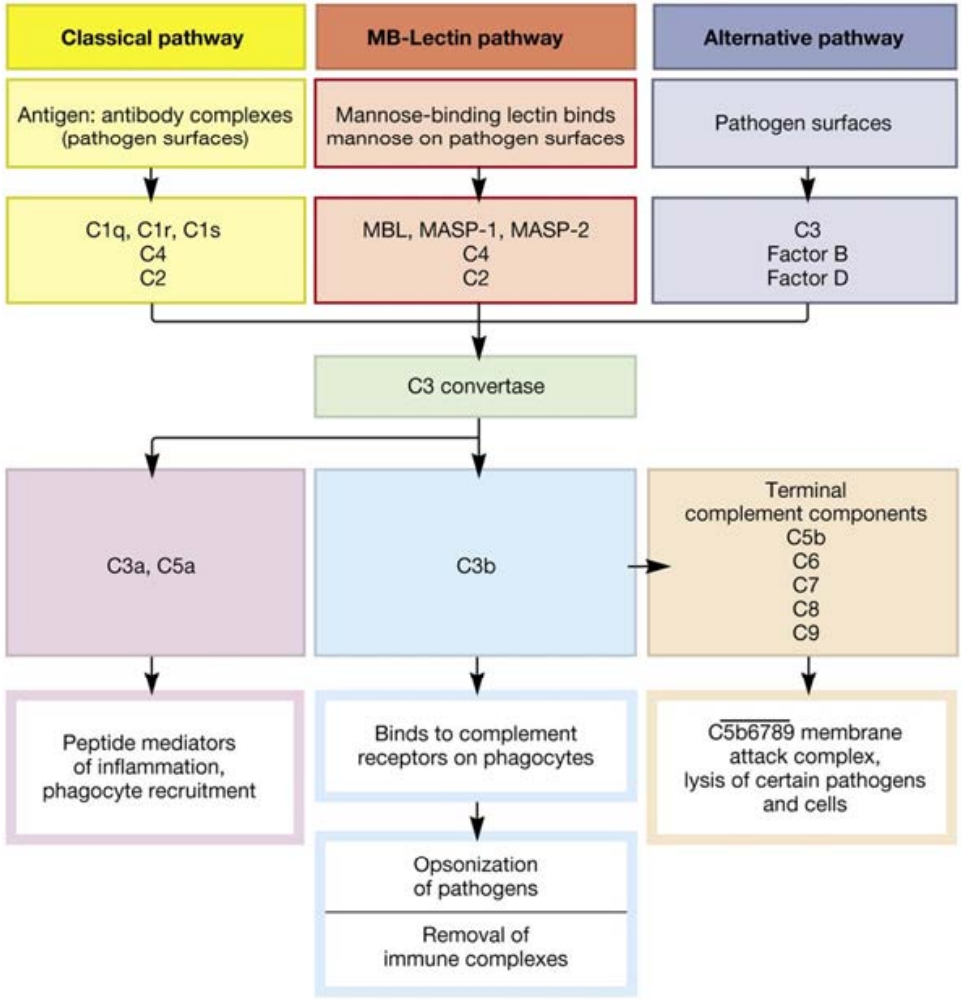
Complement Activation

- produced in inactive forms
- activated following enzymatic cleavage
- must be activated in cascade fashion
- three pathways of activation
  - classical
  - alternative
  - lectin



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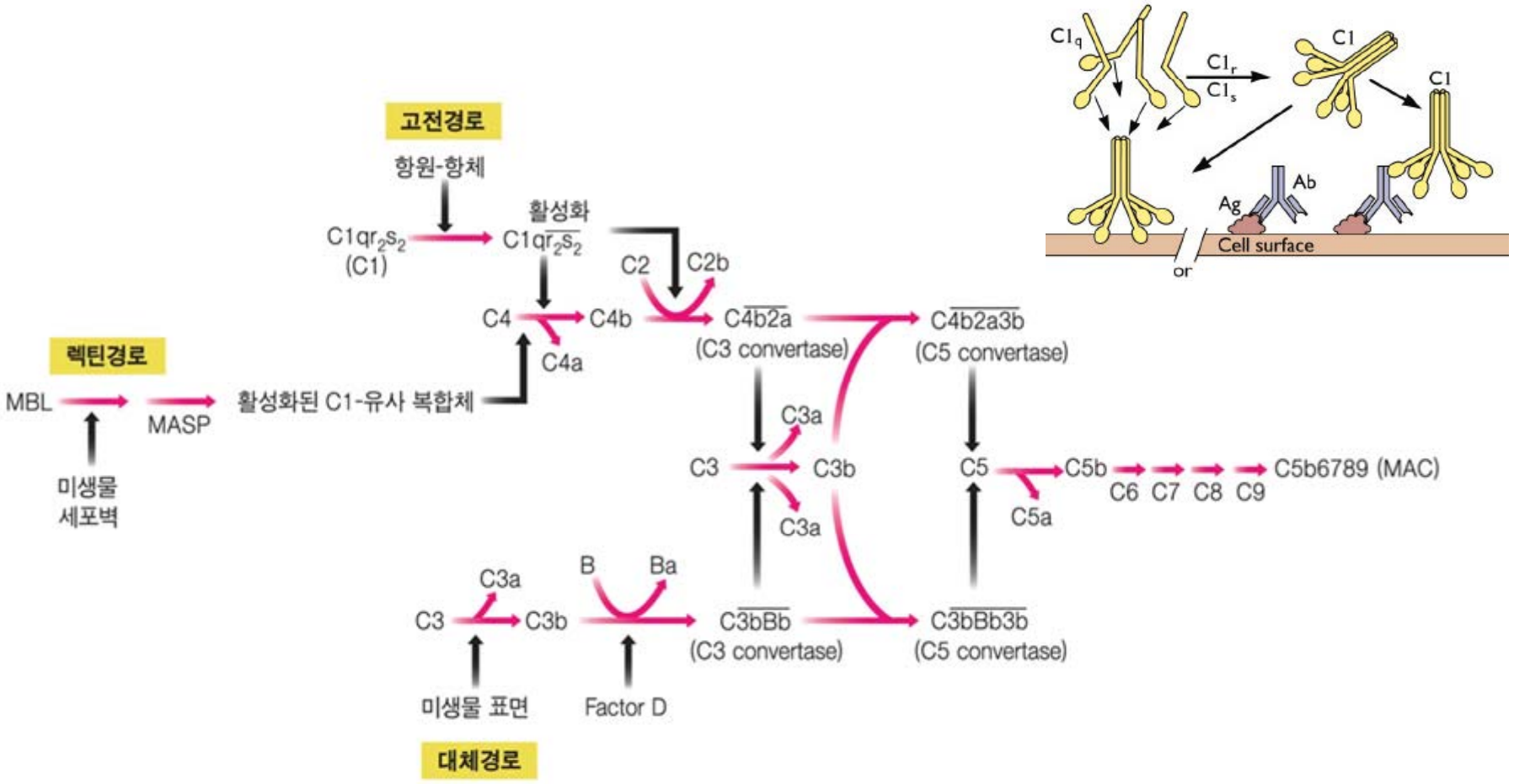
Complement Activation



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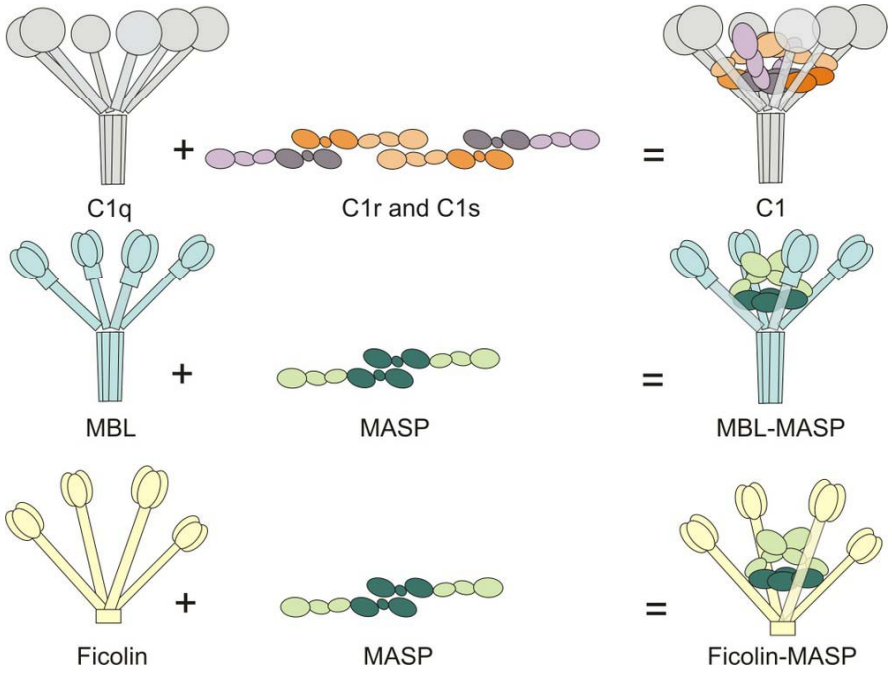
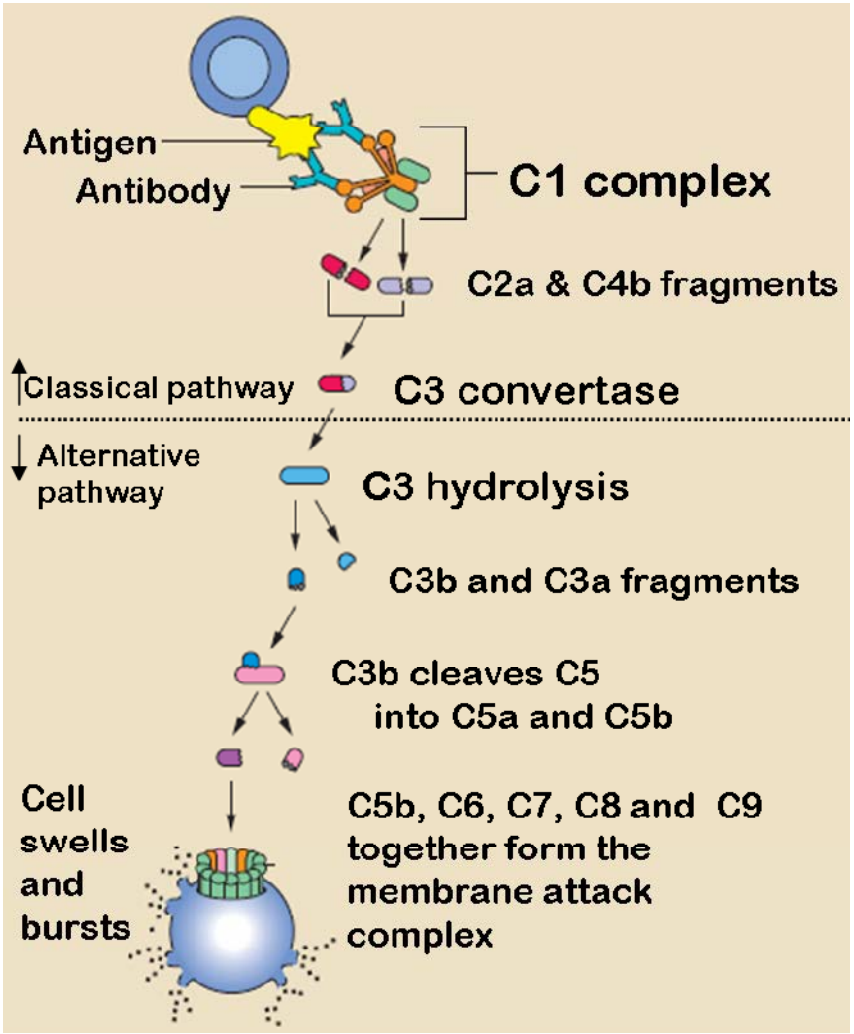
Complement Activation





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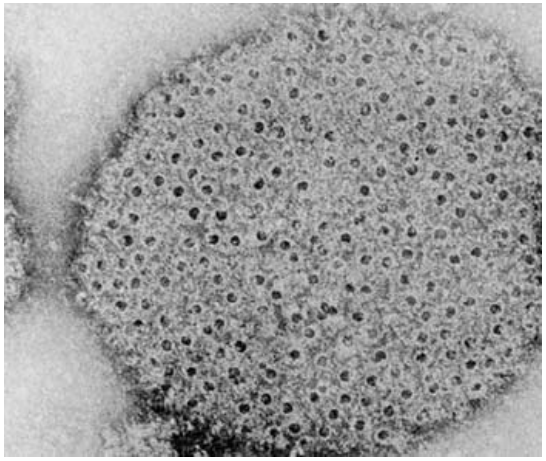
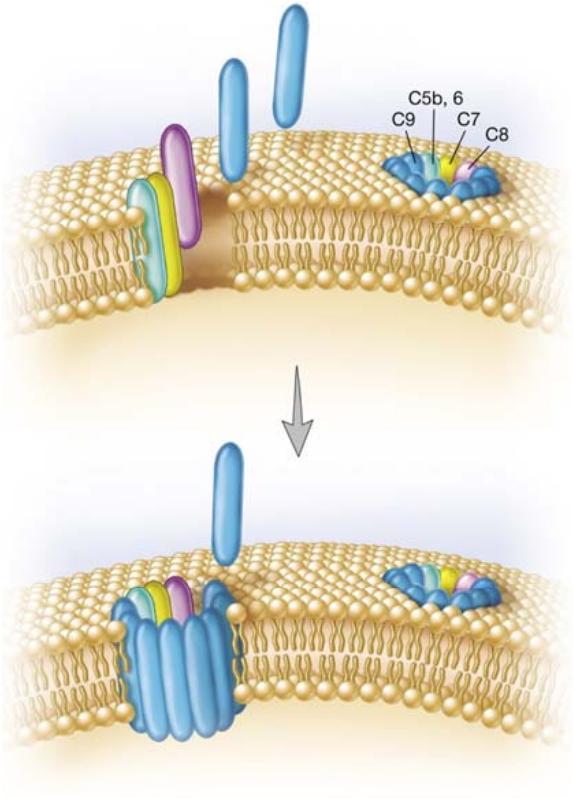
Classical Complement Pathway

- usually dependent on **antigen-antibody interactions**
  - this is part of **acquired immunity** and *not as fast as* other pathways
- produces cleavage products that participate in opsonization, chemotaxis, and the membrane attack complex (MAC)
- can also be activated in response to some microbial products

Alternative Complement Pathway

- involved in **nonspecific defenses** against intravascular invasion by bacteria and some fungi
- dependent on interaction of complement with repetitive structures on pathogen surfaces
- begins with activation of C3
- results in formation of MAC (membrane attack complex)





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Lectin Complement Pathway

- also called the mannan-binding lectin pathway
- begins with activation of C3 and lectin binding
- dependent on interaction of host mannose-binding protein (MBP) with pathogen surfaces
    - enhances phagocytosis

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Cytokines

- soluble proteins or glycoproteins (~25 kDa) that are released by one cell population that act as intercellular mediators or signaling molecules, whose production is induced by nonspecific stimuli (infection), inflammation, T cell-antigen interactions
- four families

  chemokines, hematopoietins, interleukins, tumor necrosis factor (TNF) family

Family	*Examples*	*Functions*
Chemokines	IL-8, RANTES[a], MIP (macrophage inflammatory protein)	Cytokines that are chemotactic and chemokinetic for leukocytes. They stimulate cell migration and attract phagocytic cells and lymphocytes. Chemokines play a central role in the inflammatory response.
Hematopoietins	Epo (erythropoietin), various colony-stimulating factors	Cytokines that stimulate and regulate the growth and differentiation processes involved in blood cell formation (hematopoiesis)
Interleukins	IL-1 to IL-18	Cytokines produced by lymphocytes and monocytes that regulate the growth and differentiation of other cells, primarily lymphocytes and hematopoietic stem cells. They often also have other biological effects.
Tumor necrosis factor (TNF) family	TNF-α, TNF-β, Fas ligand	Cytokines that are cytotoxic for tumor cells and have many other effects such as promoting inflammation, fever, and shock; some can induce apoptosis.

[a] RANTES: *R*egulated on *a*ctivation, *n*ormal *T* expressed and *s*ecreted; also called CCL5; member of the IL-8 cytokine superfamily.

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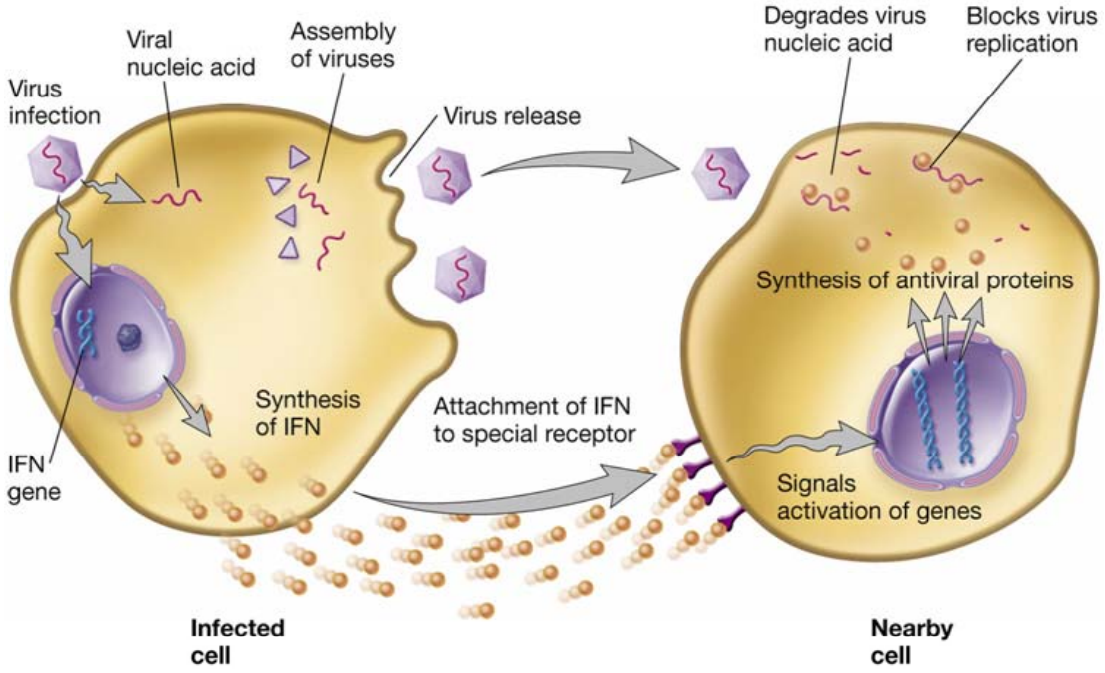
Interferons (IFNs)

- regulatory cytokines produced by some eukaryotic cells in response to viral infection
  - do not prevent virus entry into host cells, but defend against viruses by preventing viral replication and assembly
- also help to regulate the immune response



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Cytokines

- monokines: released from mononuclear phagocytes
- lymphokines: released from T lymphocytes
- interleukins: released from one leukocyte and act on another leukocyte
- colony stimulating factors (CSFs): stimulate growth and differentiation of immature leukocytes in bone marrow
- tumor necrosis factors (TNFs), interferons (IFNs)
- chemokines (8~12 kDa): affects migration of immunce cells

✓ **작용상의 특성**: 국소적/전신적 작용, 높은친화력 (pM), 다면성/중복성

- autocrine function: affect same cell responsible for their production
- paracrine function: affect nearby cells
- endocrine function: spread by circulatory system to distant target cells
- high affinity: 1~100 pM cf. 0.01~100 nM for Ag-Ab, 1 μM for MHC
- pleiotropy/redundancy → synergy/antagonism (IFN-γ vs IL-10 on Mφ)

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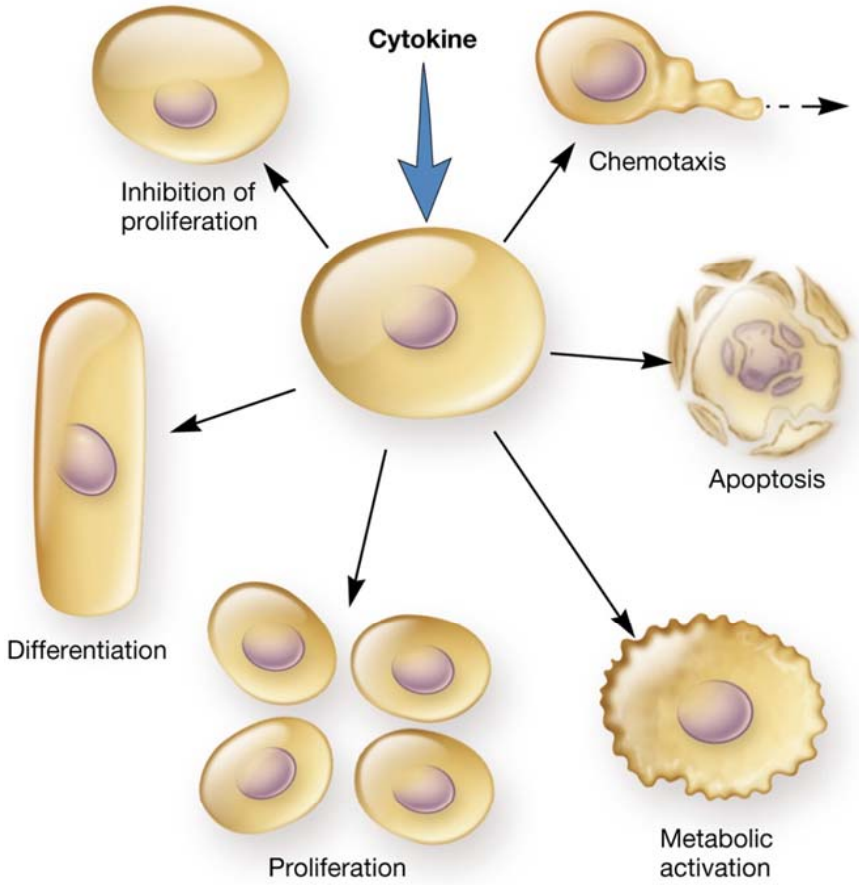
Biological Effects of Cytokines

- must bind to specific receptors on target cells
  - R (membrane receptor)
  - JAK (Janus kinase)
  - TYK (TY kinase)
  - STAT (singal transducer and activiator of transcription)

- many activities
  - differentiation
  - proliferation/anti-proliferation
  - apoptosis
  - chemotaxis/chemokinesis
  - metabolic activation

: 선천면역조절기능, 적응면역조절기능, 조혈조절기능



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Cytokines

Table 33.3 Examples of Cytokines Grouped by Function

	Cytokine[1]	Source	Role
Innate Resistance	IL-1	Macrophages, endothelial and epithelial cells	Upregulates inflammatory response, including fever
	IL-6	Macrophages, T cells, endothelial cells, and adipocytes	Upregulates acute phase response, including fever; stimulates neutrophil differentiation
	IL-23	Macrophages and dendritic cells	Upregulates inflammatory response via IL-17 from T cells; stimulates IL-1, IL-6, TNF, and chemokine production; enhances T-cell activation and memory response
	IL-27	Macrophages and dendritic cells	Enhances antigen recognition by T and B cells
	IFN-α	All somatic cells, especially macrophages	Upregulates RNase activity to control viral infection and tumor formation; upregulates inflammatory response, including antigen presentation
	TNF-α	Monocytes/Macrophages	Upregulates inflammatory response, including fever; stimulates acute-phase protein synthesis; induces tumor regression; mediates septic shock

Cytokines in Innate Immunity

- **TNF-α** (cachectin): antitumor cytokines (농도에 따라 다르게 작용)
- **IL-1**
- **IL-12**: CTL, NK activation; Th0 differentiation into Th1 cf. IL-4
- **IL-27**
- **IFN-α**, IFN-β: antiviral cytokines.

✓ **Pro-inflammatory cytokines (mostly, type I-Th1)**

IL-1, IL-12 (APCs); TNF-α, IL-6 / IFN-γ, TNF-β (Th1); IL-5 (Th2)

✓ **Anti-inflammatory cytokines (type II-Th2)**

IL-10 / IL-4, IL-13

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Cytokines

Table 33.3 Examples of Cytokines Grouped by Function

	Cytokine[1]	Source	Role
Adaptive Immunity	IL-2	T cells (autocrine process)	Stimulates growth and differentiation of T cells and NK cells; promotes antibody secretion from B cells
	IL-4	T-cell subset (and putatively basophils)	Induces differentiation of a T-cell subset; stimulates production of antibody, especially antibody associated with allergies; inhibits IL-12 production
	IL-5	T-cell subset and mast cells	Stimulates growth of B cells; enhances antibody secretion; activates eosinophils
	IL-12	Macrophages, dendritic cells, and a T-cell subset	Stimulates growth and function of T-cell subsets, stimulates killing functions of NK cells and cytotoxic lymphocytes
	IL-17	T-cell subset	Monocyte and neutrophil chemokine; induces pro-inflammatory cytokines IL-6, TNF-α, IL-1, chemokines, and prostaglandins from various cells
	IFN-γ	T-cell subset and NK cells	Enhances phagocytic functions of macrophages; upregulates cytolytic function of NK cells; stimulates antiviral functions
	TNF-β (lymphotoxin)	Numerous somatic cells	Upregulates T- and B-cell development; activates neutrophils; lyses tumor cells; upregulates CSF-2 and CSF-3

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Cytokines in Adaptive Immunity

✓ **림프구 분화, 활성화, 증식**

- **IL-2** (TCGF): T cell mitogen, low stability in the blood
- **IL-4** (BCGF): Th2 from Th0, inhibitor of TNF and IL-1
- TGF- β: inhibition of Mφ activation

✓ **염증반응 활성화** (Pro-inflammatory cytokines)

- IFN-γ
- IL-5
- TNF-β (= Lympotoxin alpha LTA)

✓ **Th1** cytokines (**IL-2**, **IFN-γ**, **TNF**) vs. **Th2** cytokines (**IL-4**,**-5**,**-10**,-13)

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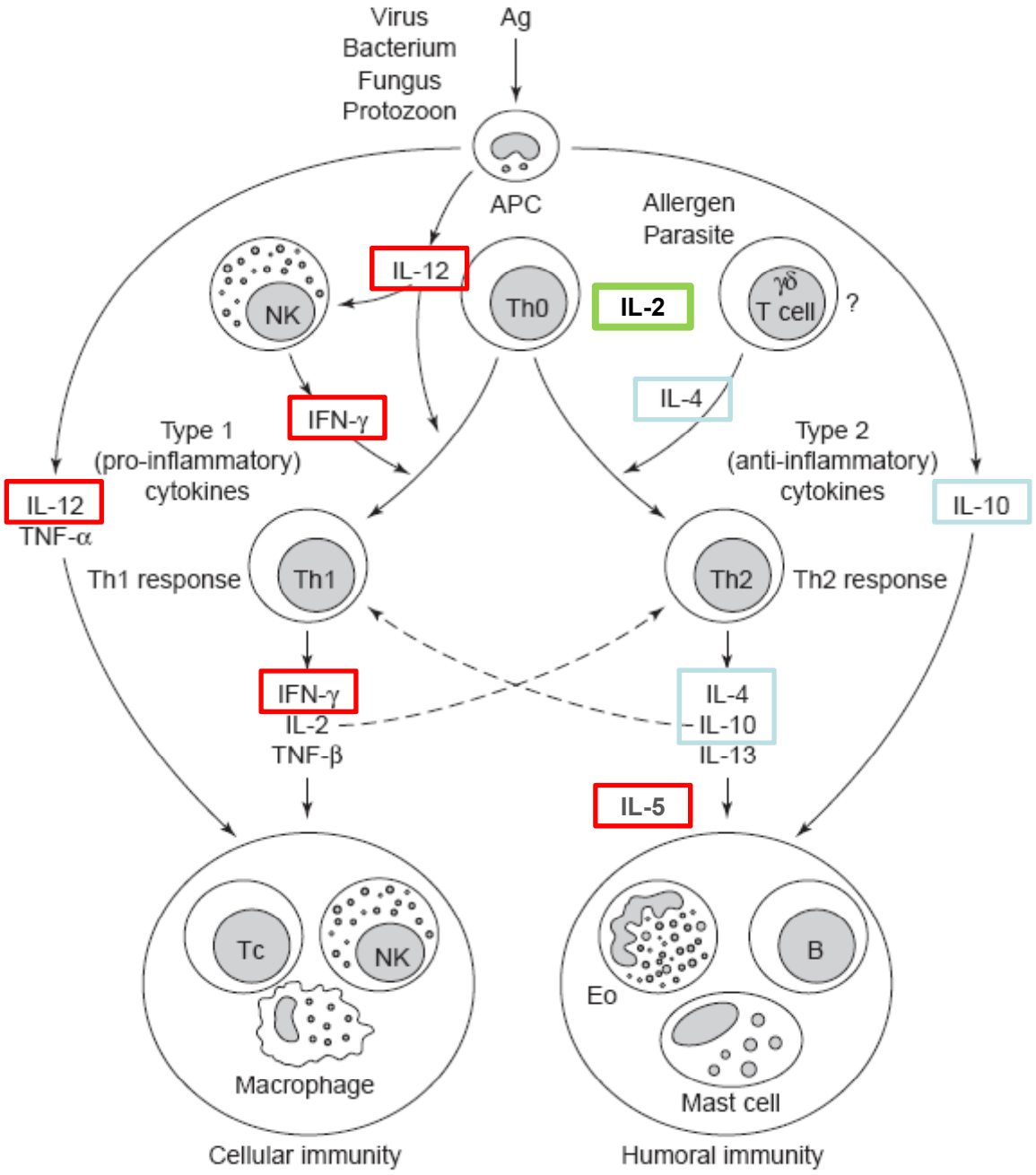

T세포의 분화/증식/활성화와 주요 Cytokines

Cytokines

Table 33.3 Examples of Cytokines Grouped by Function

	Cytokine[1]	Source	Role
Hematopoiesis	IL-3	Basophils and activated T cells	Stimulates pluripotent hematopoietic stem cells to become myeloid progenitor cells; stimulates myeloid cell proliferation
	IL-7	Bone marrow and thymic stromal cells, dendritic and epithelial cells, and hepatocytes	Stimulates pluripotent hematopoietic stem cells to become lymphoid progenitor cells; stimulates lymphoid cell proliferation
	CSF-1	Osteoblasts	Induces hematopoietic stem cells to proliferate and differentiate into monocytes/macrophages; promotes monocyte survival
	CSF-2	Macrophages, T cells, endothelial and mast cells, and fibroblasts	Induces hematopoietic stem cells to proliferate and differentiate into granulocytes and monocytes
	CSF-3	Numerous cells and tissues	Induces hematopoietic stem cells to proliferate and differentiate into neutrophils; stimulates neutrophil function and survival

1 Cytokine: IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; CSF, colony-stimulating factor

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Cytokines in Hematopoiesis

- **IL-3 (multi-CSF)**
- IL-7
- M-CSF (CSF-1)
- **GM-CSF (CSF-2)**
- G-CSF (CSF-3)
- **EPO**

표 5-3 조혈작용에 관여하는 싸이토카인

싸이토카인	생성부위	표적부위
인터루킨-3	T 세포	미성숙전구세포, 비만세포
인터루킨-7	다양한 조직의 기조직 세포	미성숙 림프구
인터루킨-11	골수 기조직 세포	거핵구 세포
적혈구생성소 (erythropoietin)	신장	적혈구
M-CSF	대식세포, 내피	호중구세포
GM-CSF	T 세포, 대식세포, 내피세포, 골수 기조직 세포	미성숙전구세포
SCF/c-kit	골수 기조직 세포	미성숙전구세포, 비만세포

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2015 M/DEET 문항

35. 다음은 보조 $CD4^+$ T 세포의 분화에 필요한 사이토카인의 역할을 알아보기 위한 실험이다.

<자료>

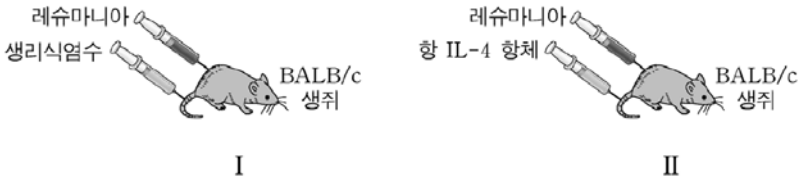
○ 레슈마니아(*Leishmania major*)를 제거하는 데 제1형 보조 $CD4^+$ T 세포(Th1)의 면역반응이 중요하다.

○ BALB/c 생쥐는 레슈마니아 감염에 감수성이 있다.

<실험 과정>

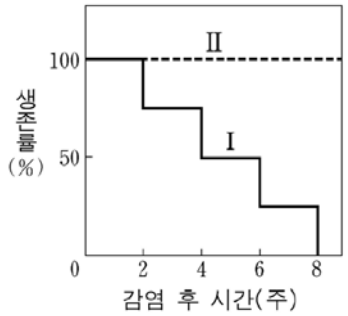
(가) BALB/c 생쥐를 두 그룹 Ⅰ과 Ⅱ로 나눈다.

(나) Ⅰ의 생쥐에 레슈마니아를 감염시키고, Ⅱ의 생쥐에 레슈마니아와 IL-4에 대한 항체(항 IL-4 항체)를 함께 주입한다.



(다) 감염 후 2주 간격으로 8주 동안 Ⅰ과 Ⅱ의 생존률을 측정한다.

<실험 결과>



이에 대한 설명으로 옳은 것만을 <보기>에서 있는 대로 고른 것은? [2.5점]

<보 기>

ㄱ. IL-4가 제2형 보조 $CD4^+$ T 세포(Th2)의 분화를 유도한다.

ㄴ. Ⅰ에서 레슈마니아에 대한 Th2 반응이 Th1 반응보다 우세하게 일어난다.

ㄷ. IFN-γ 생성은 Ⅱ가 Ⅰ보다 낮다.

① ㄱ ② ㄴ ③ ㄷ ④ ㄱ, ㄴ ⑤ ㄴ, ㄷ

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Cytokine Signaling

- cytokine receptors and signal transducers

IL-1	IL-1R	MyD88/IRAK	TRAF6	NF-κB/AP-1
IL-2	IL-2R	JAK1/JAK3		STAT5
IL-6	IL-6R	JAK1		STAT3
TNF-α	TNFR	TRADD	TRAF2/FADD	NF-κB/AP-1/??
IFN-α/-β	IFNAR	JAK1/TYK2		STAT2
IFN-γ	IFNGR	JAK1/JAK2		STAT1
IFN-γ	OprF	??		Quorum sensing
	Pseudomonas aeruginosa			

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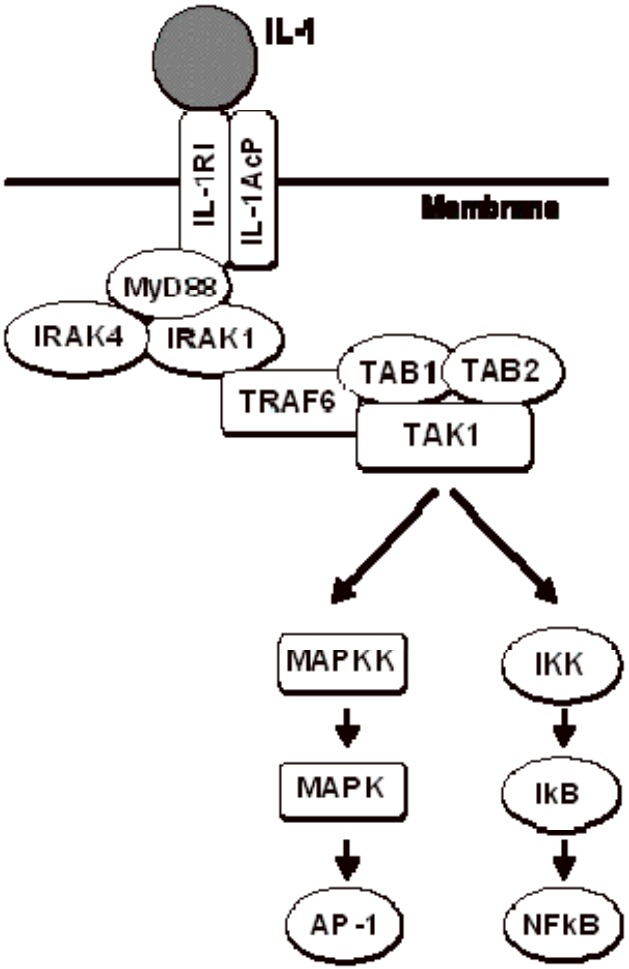
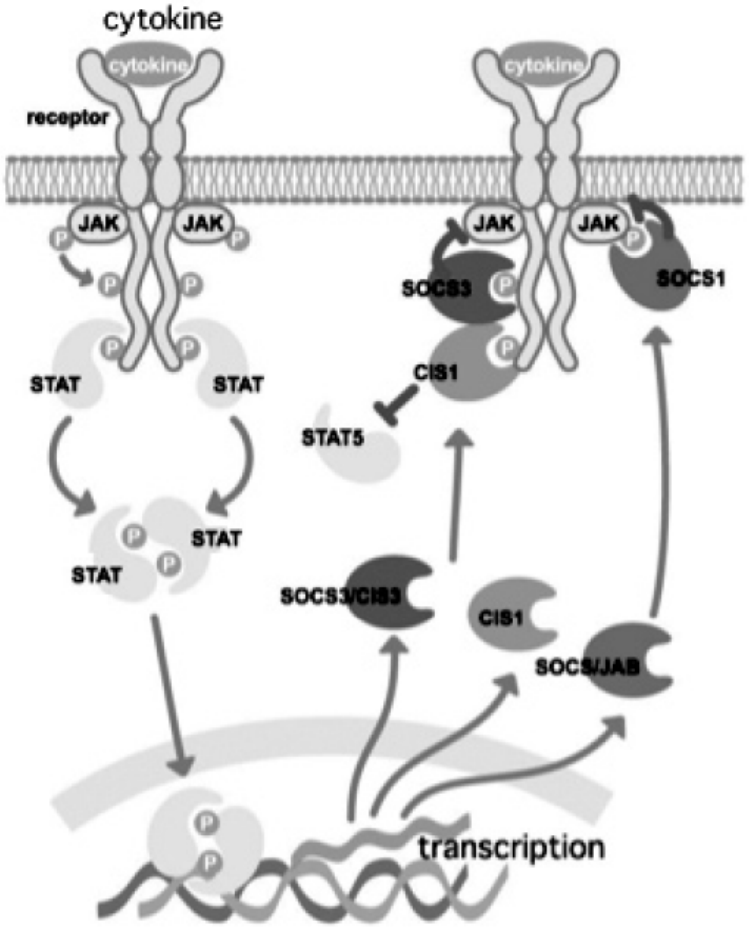
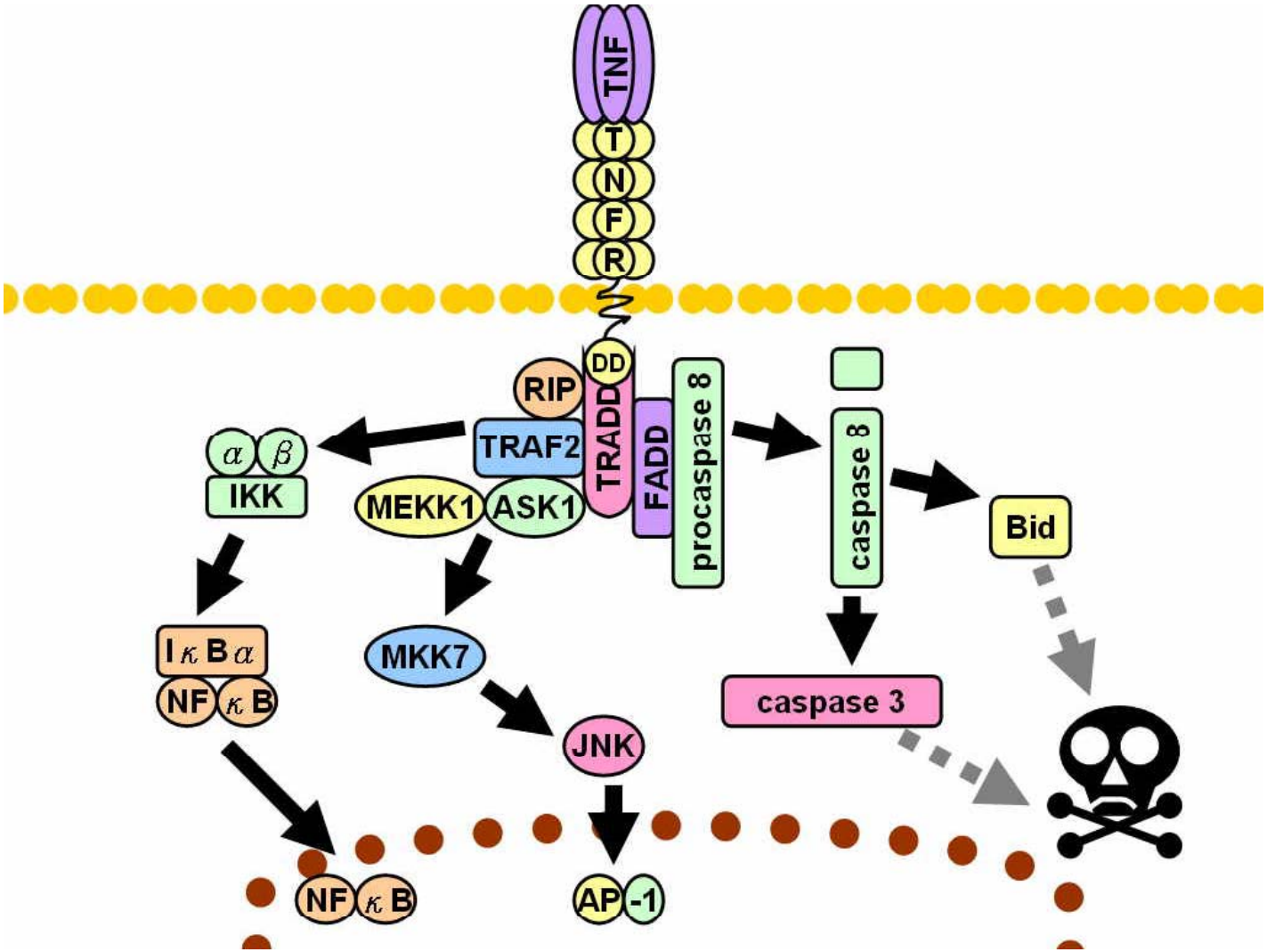
Cytokine Signaling: IL-1 and others





Cytokine Signaling: TNF



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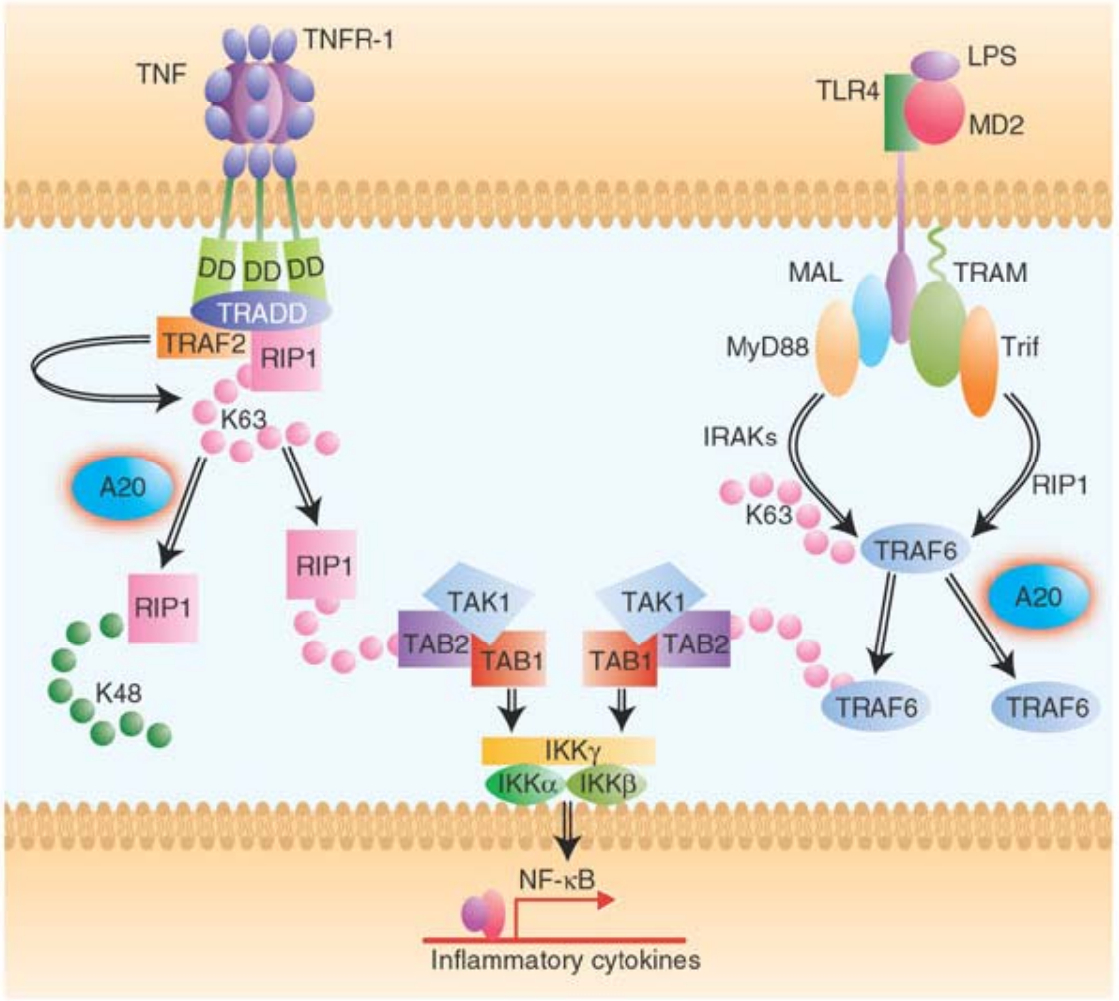
TNF and TLR Signaling



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INFγ-OprF Interaction

Recognition of Host Immune Activation by *Pseudomonas aeruginosa*

Licheng Wu,[1] Oscar Estrada,[1] Olga Zaborina,[1] Manjeet Bains,[4] Le Shen,[2] Jonathan E. Kohler,[1] Nachiket Patel,[1] Mark W. Musch,[3] Eugene B. Chang,[3] Yang-Xin Fu,[2] Michael A. Jacobs,[5] Michael I. Nishimura,[1] Robert E. W. Hancock,[4] Jerrold R. Turner,[2] John C. Alverdy[1]*

It is generally reasoned that lethal infections caused by opportunistic pathogens develop permissively by invading a host that is both physiologically stressed and immunologically compromised. However, an alternative hypothesis might be that opportunistic pathogens actively sense alterations in host immune function and respond by enhancing their virulence phenotype. We demonstrate that interferon-γ binds to an outer membrane protein in *Pseudomonas aeruginosa*, OprF, resulting in the expression of a quorum-sensing dependent virulence determinant, the PA-I lectin. These observations provide details of the mechanisms by which prokaryotic organisms are directly signaled by immune activation in their eukaryotic host.

cytotoxic to epithelial cells (*4*). Finally, the expression of PA-I (*lecA*) is dependent on the quorum-sensing (QS) signaling system (*5*), a core system of virulence gene regulation that controls multiple virulence genes in *P. aeruginosa*.

We considered that immune elements might directly activate the virulence of *P. aeruginosa*. As a physiologically relevant in vitro source of such immune factors, supernatants from antigen-stimulated T cells, which express an array of cytokines (*6*), were evaluated for their ability to increase PA-I expression in *P. aeruginosa* strain PLL-EGFP/27853, a PA-I-GFP reporter (*7*) that was readily available and verified in a previous report by our laboratory (*8*). PA-I expression was increased by supernatant from activated T cell cultures, as assessed by enhancement of fluorescence in the PA-I-GFP fusion reporter strain (Fig. 1A), but not in controls. To determine whether this effect was due to specific cytokines, the reporter strain was individually exposed to human IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α). Of these, only IFN-γ induced a significant increase in PA-I expression

Science 2005

Endogenous Pyrogens

- cytokines that elicit *fever* in the host
- fever
  - in adults
    - oral temperature >98.6˚F (37˚C)
    - rectal temperature >99.5˚F (37.5˚C)
  - most common cause of fever is *microbial infection* or *bacterial toxins*
- in most cases, directly triggers fever production
  - e.g. IL-1, IL-6, TNF-α produced by Mφs in response to microbes
- after release, pyrogens → hypothalamus and induce production of prostaglandins which reset hypothalamus to a higher temperature

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How Fever Augments Host's Defenses

- stimulation of leukocytes so that they can destroy pathogens
- enhances immune system activity
- enhances **microbiostasis** by decreasing available **iron** to microbes
  - hypoferremia – decreased iron availability
  - hyperferremia – increased iron availability (enhances virulence)

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Liver Response: Acute Phase Proteins

- macrophage activation by bacteria

→ cytokine release

→ liver stimulation

→ **acute phase protein** production

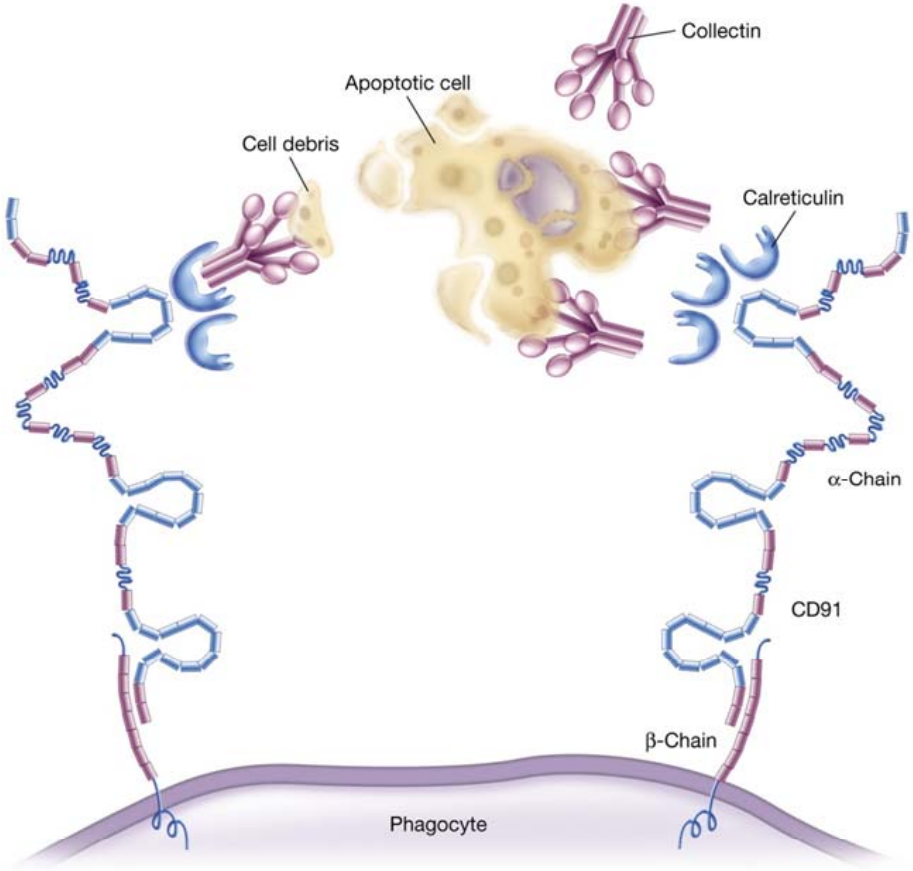
includes C-reactive protein (**CRP**), mannose-binding lectin (**MBL**), surfactant proteins A and B (**SP-A** and **SP-D**)

- bind ***bacterial surfaces*** and act as

Classical pathway activator: CRP+C1q

Lectin pathway activator: MBL

Collectins: SP-A, SP-D, C1q



Collectins (= Molecular scavengers with collagen-like motif)

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Positive Acute Phase Proteins

Protein	Immune system function
C-reactive protein	Opsonin on microbes
Serum amyloid P component	Opsonin
Serum amyloid A	Recruitment of immune cells to inflammatory sites Induction of enzymes that degrade extracellular matrix
Complement factors	Opsonization, lysis and clumping of target cells. Chemotaxis
Mannan-binding lectin (MBL)	Mannan-binding lectin pathway of complement activation
Fibrinogen, prothrombin, factor VIII, von Willebrand factor	Coagulation factors, trapping invading microbes in blood clots. Some cause chemotaxis
Plasminogen	Degradation of blood clots
Alpha 2-macroglobulin	Inhibitor of coagulation by inhibiting thrombin. Inhibitor of fibrinolysis by inhibiting plasmin
Ferritin	Binding iron, inhibiting microbe iron uptake
Hepcidin	Stimulates the internalization of ferroportin, preventing release of iron bound b y ferritin within intestinal enterocytes and macrophages
Ceruloplasmin	Oxidizes iron, facilitating for ferritin, inhibiting microbe iron uptake
Haptoglobin	Binds hemoglobin, inhibiting microbe iron uptake
Orosomucoid	Steroid carrier (= Alpha-1-acid glycoprotein, AGP)
Alpha 1-antitrypsin	Serpin, downregulates inflammation
Alpha 1-antichymotrypsin	Serpin, downregulates inflammation

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Phagocytosis

- process by which phagocytic cells (monocytes, Mφs, DCs, and PMNs) **recognize**, **ingest**, and eventually **kill** extracellular microbes

- **two mechanisms** for recognition of microbe by phagocyte
  - opsonin-independent (**non-opsonic**) recognition
  - opsonin-dependent (**opsonic**) recognition

- phagocytic efficiency can be *greatly increased* by **opsonization**

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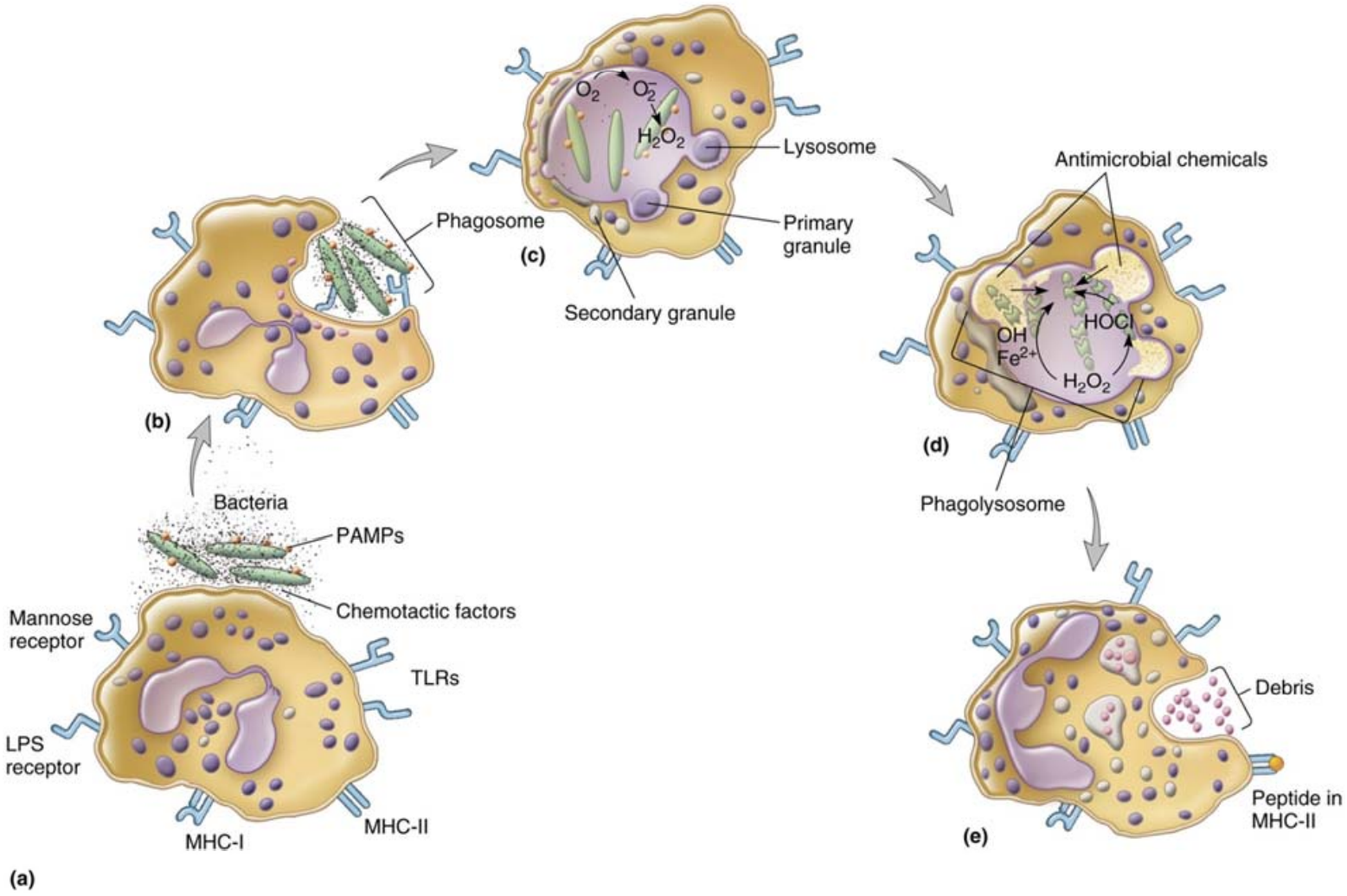
Phagocytosis



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Pathogen Recognition (PR)

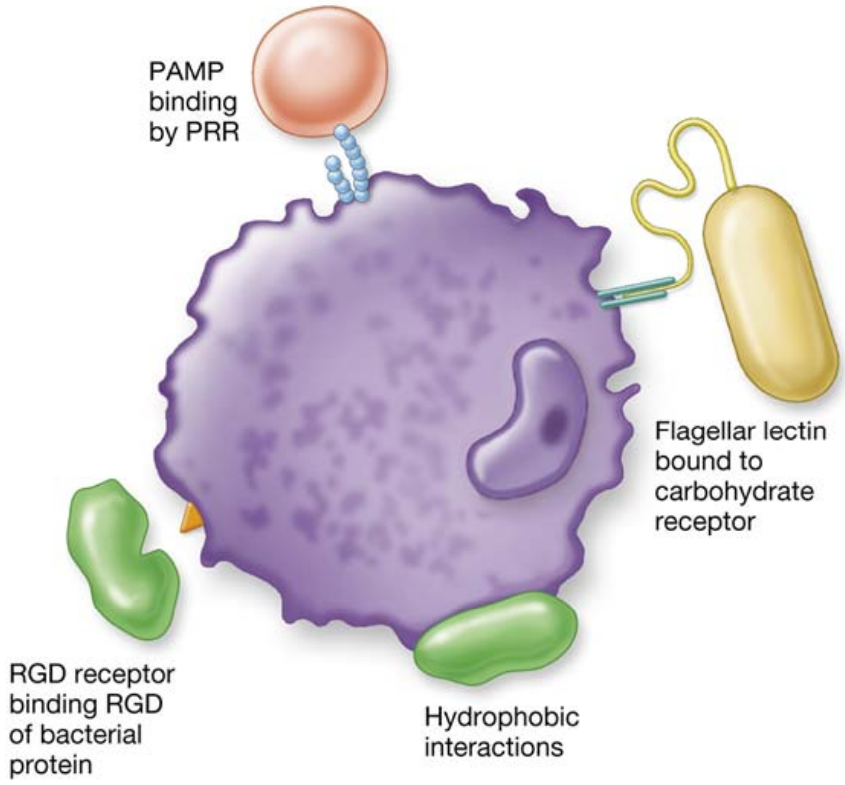
- opsonin-independent mechanism
- involves *nonspecific* and *specific* **receptors** on phagocytic cells

  common pathogen components are **nonspecifically** recognized to activate phagocytes

  signaling mechanism involved afterwards

- <u>four major types</u>

- hydrophobic interactions
- **lectin-carbohydrate** interactions
- **protein-protein** interactions (RGD motif)
- **PAMP-PRR** interactions



PR by Mϕ

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Pathogen-Associated Molecular Patterns (PAMPs)

- conserved microbial molecular structures that occur in patterns
- PAMPs are unique to microbes, not present in host

  lipopolysaccharide (LPS) of gram-negative bacteria

  peptidoglycan of gram-positive bacteria

  glucan of fungi
- **PAMPs** recognized by **PRRs** on phagocytes such as **collectins** and **TLRs** (Toll-like receptors)

✓ **TLRs**

- a class of PRRs that function exclusively as signaling receptors
- recognize and bind unique PAMPs of viruses, bacteria, or fungi

  the binding triggers an ***evolutionarily ancient signal*** and is communicated to the host cell nucleus which initiates the host response
- TLRs are **highly conserved** in metazoans

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TLR4 is the MMTV Receptor?!

Successful Transmission of a Retrovirus Depends on the Commensal Microbiota

Melissa Kane,[1] Laure K. Cas
Alexander V. Chervonsky,[2]†

To establish chronic infections,
Many retroviruses, including m
through mucosal surfaces rich i
stimulates a state of unrespons
microbiota, as antibiotic-treate
offspring. MMTV-bound bacteri
interleukin-6 (IL-6)–dependent
on the interaction with the mic
reveal the fundamental import

Successful pathogens hav
to counteract the immu
to use established immu

tection is dependent on the adaptor molecule that signals downstream of most TLRs, expressed by myeloid differentiation primary response gene 88 (MyD88) (*1*–*3*). Retroviruses employ various mechanisms of immune evasion (*4*, *5*), however,

Intestinal Microbiota Promote Enteric Virus Replication and Systemic Pathogenesis

Sharon K. Kuss,[1] Gavin T. Best,[1] Chris A. Etheredge,[1*] Andrea J. Pruijssers,[2,3]
Johnna M. Frierson,[3,4] Lora V. Hooper,[1,5,6] Terence S. Dermody,[2,3,4] Julie K. Pfeiffer[1]†

Intestinal bacteria aid host health and limit bacterial pathogen colonization. However, the influence of bacteria on enteric viruses is largely unknown. We depleted the intestinal microbiota of mice with antibiotics before inoculation with poliovirus, an enteric virus. Antibiotic-treated mice were less susceptible to poliovirus disease and supported minimal viral replication in the intestine. Exposure to bacteria or their *N*-acetylglucosamine–containing surface polysaccharides, including lipopolysaccharide and peptidoglycan, enhanced poliovirus infectivity. We found that poliovirus binds lipopolysaccharide, and exposure of poliovirus to bacteria enhanced host cell association and infection. The pathogenesis of reovirus, an unrelated enteric virus, also was more severe in the presence of intestinal microbes. These results suggest that antibiotic-mediated microbiota depletion diminishes enteric virus infection and that enteric viruses exploit intestinal microbes for replication and transmission.

Enteric viruses encounter up to 10^{14} bacteria in the mammalian intestine (*1*). It is unclear whether commensal microorganisms affect enteric viruses. Poliovirus is an enteric human pathogen transmitted by the fecal-oral route and serves as a model for enteric virus infections (*2*). Orally acquired poliovirus undergoes a primary replication cycle in the gastrointestinal tract before dissemination. Poliovirus occasionally disseminates from the intestine to the central nervous system, which results in paralytic poliomyelitis days to weeks after initial infection in the gastrointestinal tract. A key question is whether microbiota influence viral replication in the gastrointestinal tract to augment systemic dissemination.

To investigate the effect of intestinal microbiota on poliovirus infection, mice susceptible to

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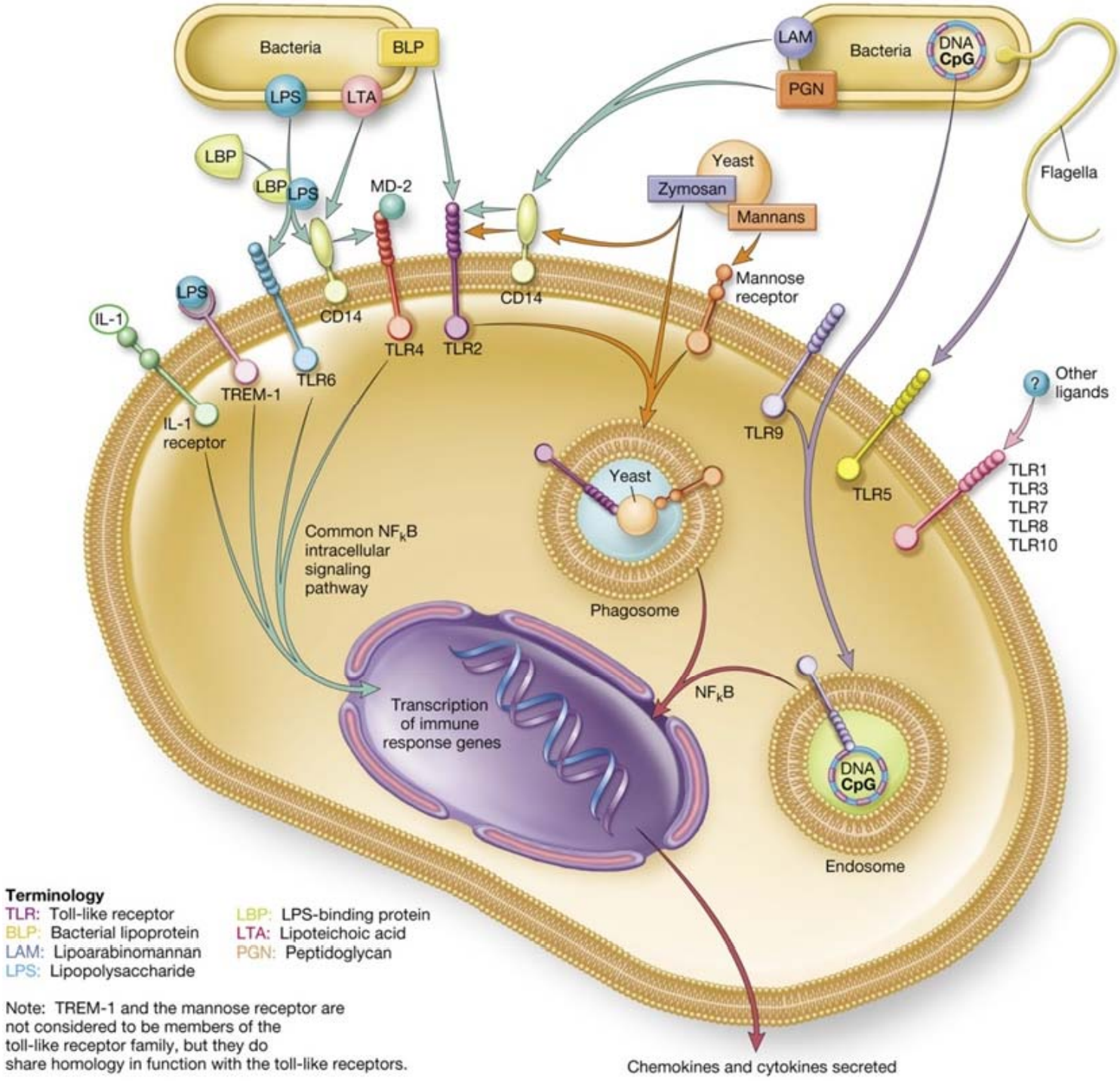
†To whom correspondence should be addressed. E-mail: julie.pfeiffer@utsouthwestern.edu

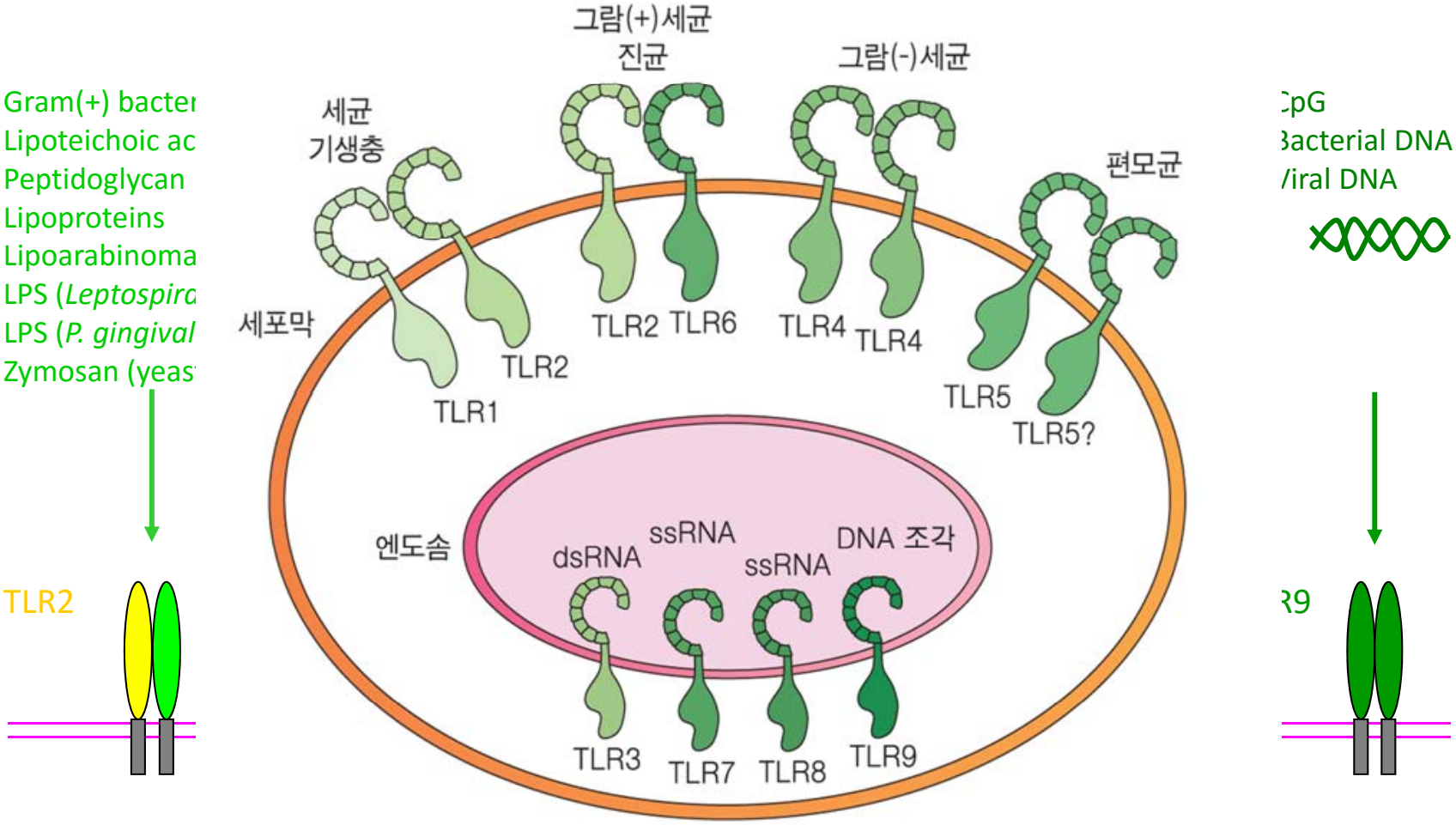


Terminology

TLR: Toll-like receptor
BLP: Bacterial lipoprotein
LAM: Lipoarabinomannan
LPS: Lipopolysaccharide
LBP: LPS-binding protein
LTA: Lipoteichoic acid
PGN: Peptidoglycan

Note: TREM-1 and the mannose receptor are not considered to be members of the toll-like receptor family, but they do share homology in function with the toll-like receptors.

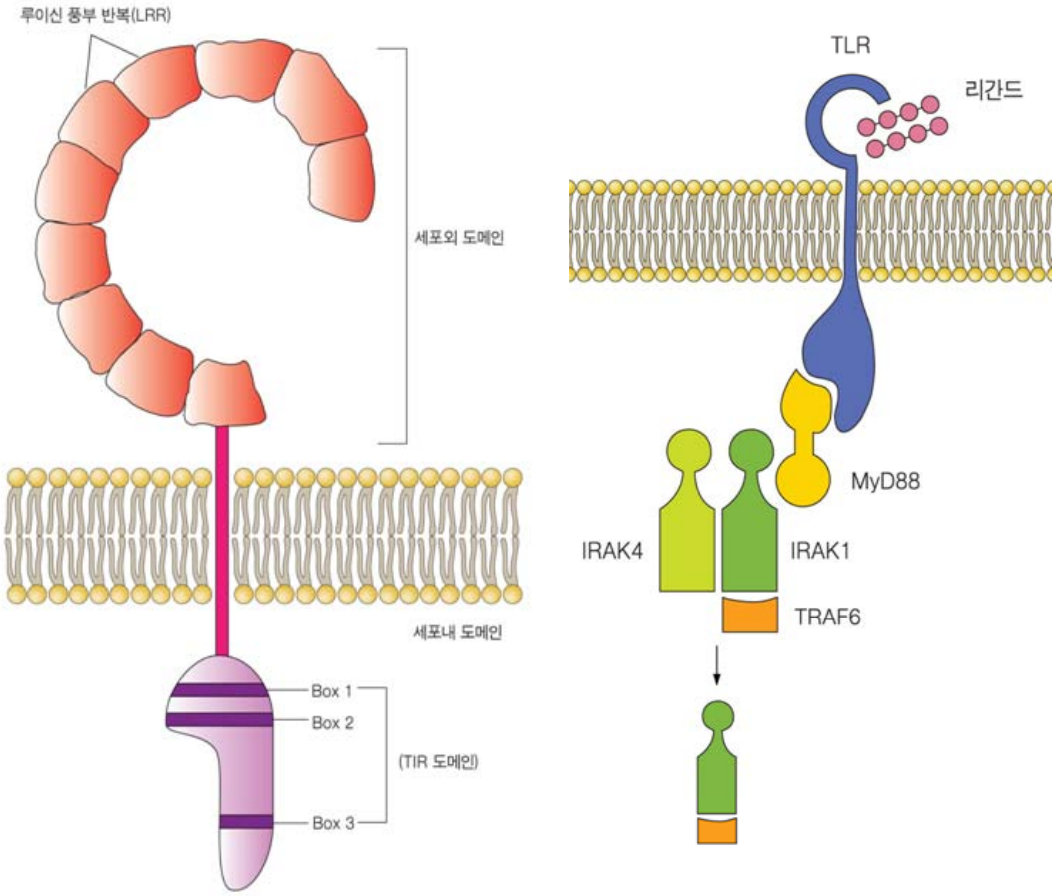
TLR Recognizes Structurally Conserved Pathogen-Associated Molecular Pattern (PAMP)



Drosophila (9 Tolls), Human (10 TLRs), Murine (11 TLRs), and **Nematodes (1 Tol)**
Arabidopsis (85 TIR-NB-LRR resistance genes)

Toll-like Receptors (TLRs)

표 3-3	Toll-유사 수용체	
종 류	리간드	대상 미생물
TLR1	Triacyl lipopeptide	세균
TLR2	Peptidoglycan	그람양성세균
	GPI-linked protein	트리파노소마
	Lipoprotein	마이코박테리아
	Zymosan	효모, 진균
TLR3	dsRNA	바이러스
TLR4	LPS	그람음성세균
	F-protein	RSV
TLR5	Flagellin	세균
TLR6	Diacyl lipopeptide	마이코박테리아
	Zymosan	효모, 진균
TLR7	ssRNA	바이러스
TLR8	ssRNA	바이러스
TLR9	CpG unmethylated dinucleotide	세균 DNA
	Dinucleotide	
	Herpes virus	Herpes virus
TLR10,12, 13	미상	미상
TLR 11	Profilin	세균



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Cori Bargmann
(Rockefeller University)

Article

Nature **438**, 179-184 (10 November 2005) | doi:10.1038/nature04216

Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*

Yun Zhang[1], Hang Lu[1] and Cornelia I. Bargmann[1]

Food can be hazardous, either through toxicity or through bacterial infections that follow the ingestion of a tainted food source. Because learning about food quality enhances survival, one of the most robust forms of olfactory learning is conditioned avoidance of tastes associated with visceral malaise. The nematode *Caenorhabditis elegans* feeds on bacteria but is susceptible to infection by pathogenic bacteria in its natural environment. Here we show that *C. elegans* modifies its olfactory preferences after exposure to pathogenic bacteria, avoiding odours from the pathogen and increasing its attraction to odours from familiar nonpathogenic bacteria. Particular bacteria elicit specific changes in olfactory preferences that are suggestive of associative learning. Exposure to pathogenic bacteria increases serotonin in ADF chemosensory neurons by transcriptional and post-transcriptional mechanisms. Serotonin functions through MOD-1, a serotonin-gated chloride channel expressed in sensory interneurons, to promote aversive learning. An increase in serotonin may represent the negative reinforcing stimulus in pathogenic infection.

▲ Top

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Correspondence to: Cornelia I. Bargmann[1] Correspondence and requests for materials should be addressed to C.I.B. (Email: cori@rockefeller.edu).

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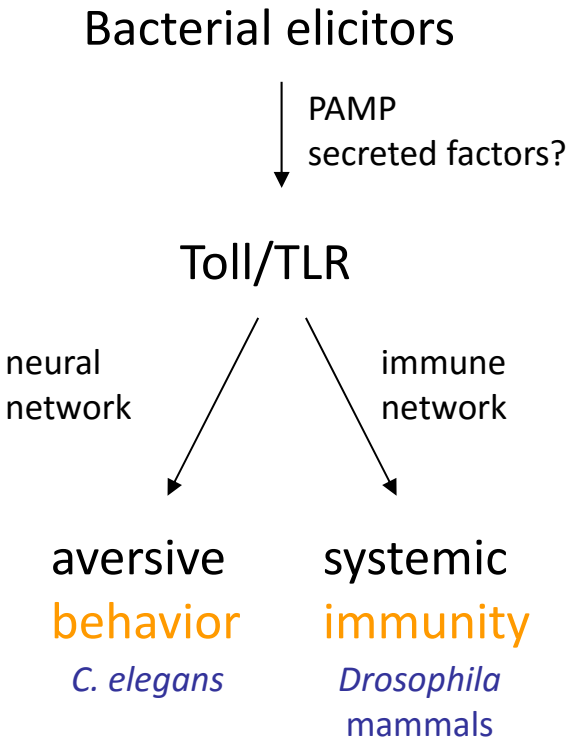

cf. *C. elegans* has PCD/killing system for defense.

Drosophila and mammals have local/epithelial immunity as well.

Toll and TLR signaling pathway

- **Ligand processing machinery**: PAMP generation
  involving multiple proteases and protease inhibitors (called serpins)

- **PRRs**: PAMP recognition
  ← indirectly by Toll (e.g. via PGRPs) or directly by TLRs

- **Receptor adaptor complex** : signal transduction to MAPK and/or I-κB
  (MyD88, IRAK/Pelle (kinase), TRAF2/6 etc.)
  I-κB degradation by I-κB phosphorylation and ubiquitination

- **NF-κB trasncription factor**: directing transcription of immunity factors such as cytokines, AMPs, and antibodies etc.

  cf. sometimes, apoptosis.

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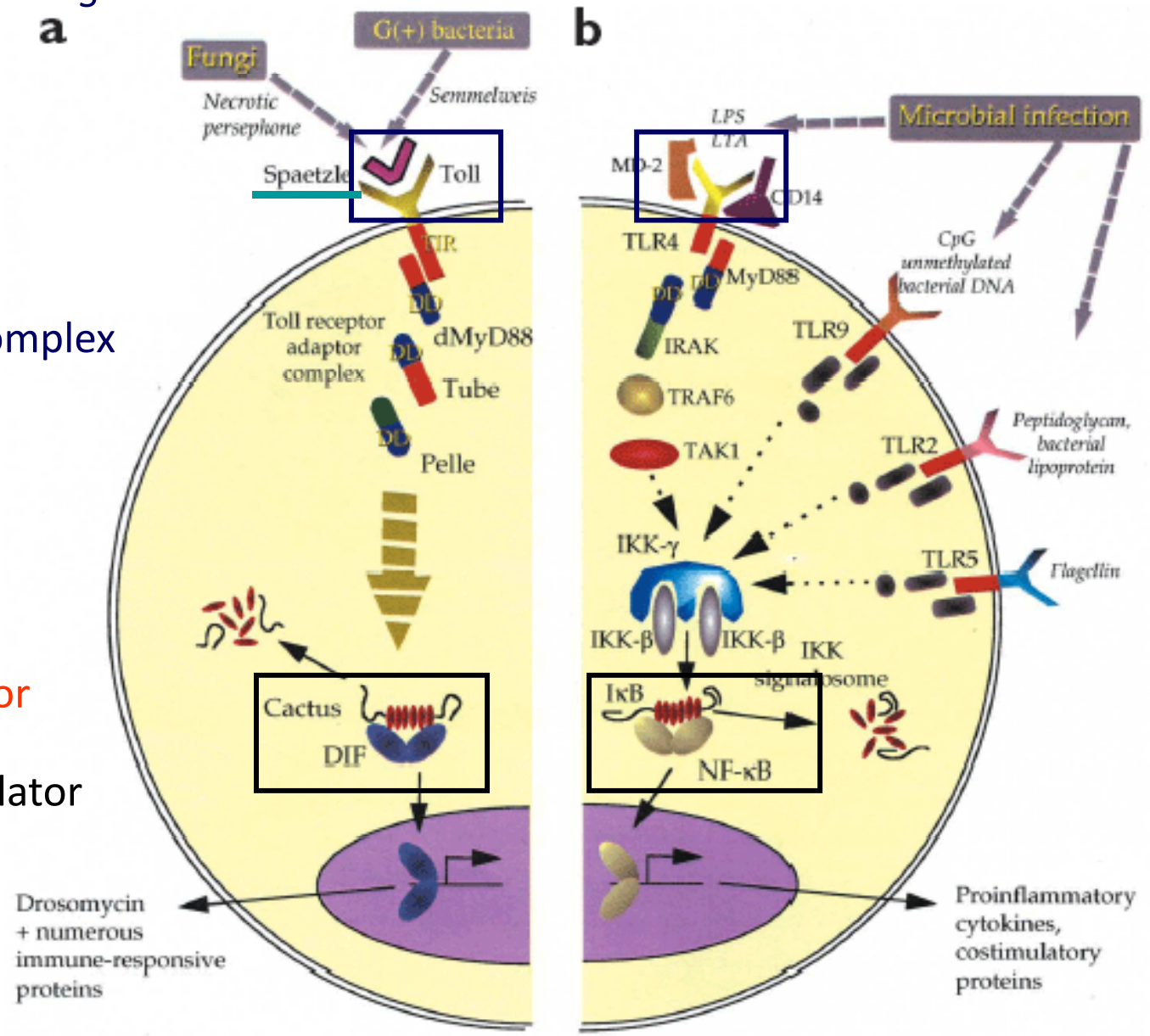
Ligand processing

Receptor

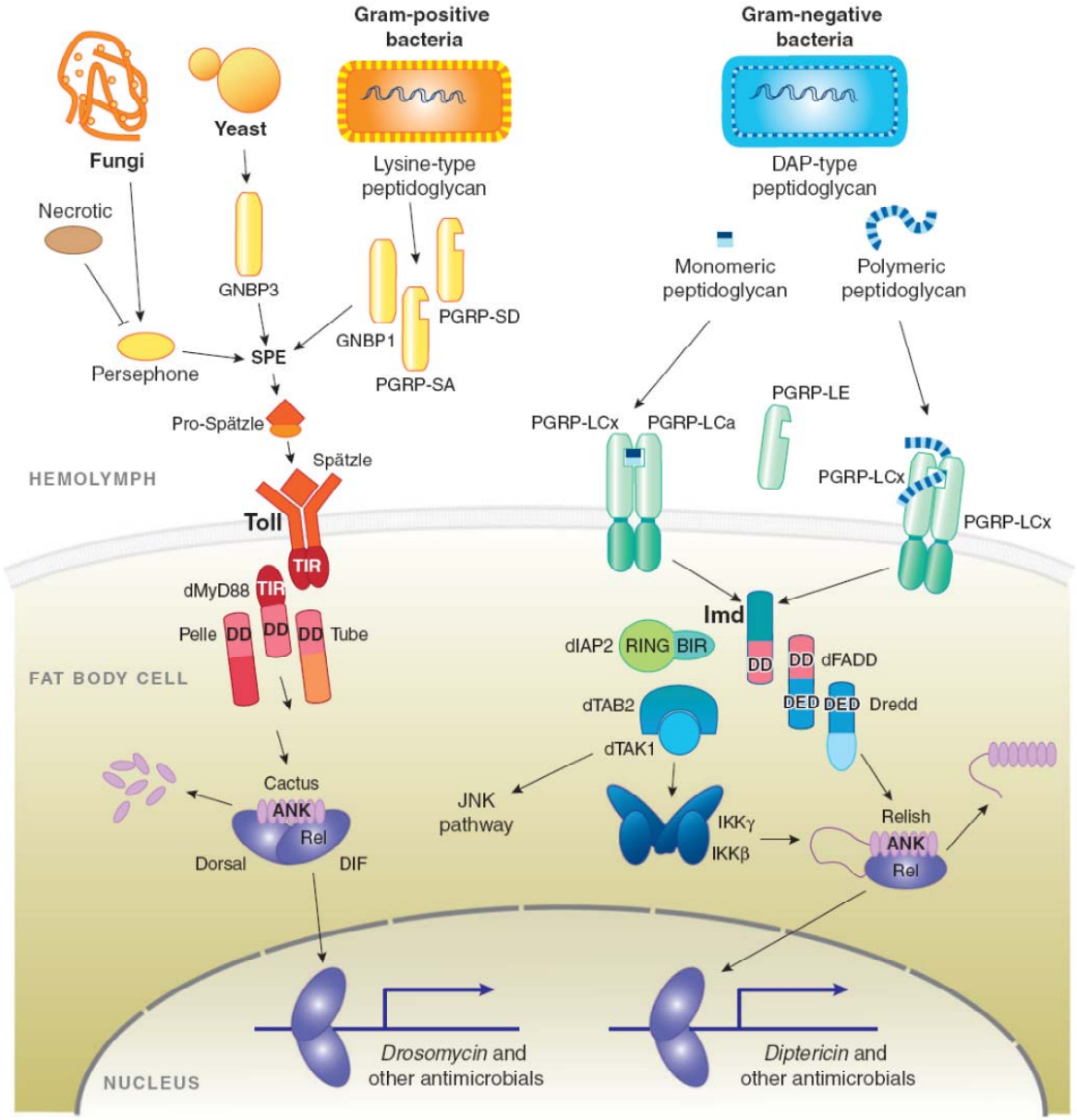
Adaptor complex

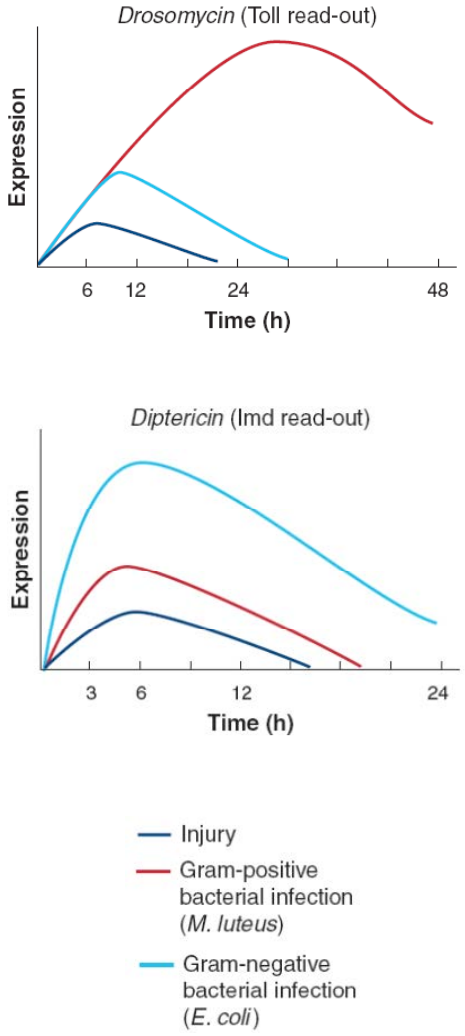
I-kB inhibitor

NF-kB regulator



Lemaitre and **Hoffman**. 2007. Annu. Rev. Immunol. 25: 697-743





Evolution of Immunity

표 3-5 선천면역계의 종간 공유

분 류		선천면역	적응면역	침입유도효소 등 효소계	탐식작용	항미생물펩티드	패턴인식 수용체	이식거부	T 및 B 세포	항체
식물		+	-	+	-	+	+	-	-	-
무척추	Porifera	+	-	?	+	?	?	+	-	-
	Annelids	+	-	?	+	?	?	+	-	-
	Arthropods	+	-	+	+	+	+	?	-	-
척추	Elasmobranchs	+	+	+	+	+	+	+	+	+
	Teleost fish	+	+	+	+	가능	+	+	+	+
	Amphibians	+	+	+	+	+	+	+	+	+
	Reptiles	+	+	+	+	?	+	+	+	+
	Birds	+	+	+	+	?	+	+	+	+
	Mammals	+	+	+	+	+	+	+	+	+

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Intracellular Digestion

- after binding, microbes or components in the phagosome can be internalized and fused with a lysosome to become a **phagolysosome**
  - **respiratory burst**
  - **an arsenal of toxic materials** such as

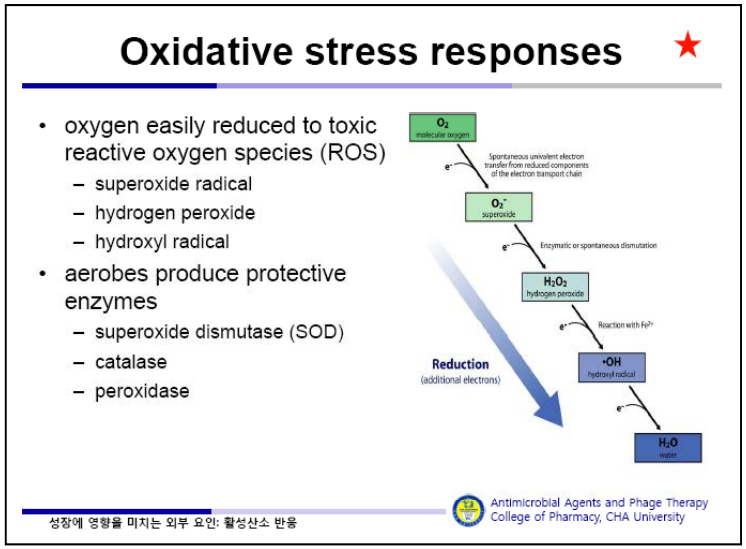
    hydrolases (lysozyme, phospholipase A_2, RNase, DNase, protease)

    ROI (reactive oxygen intermediates. ROS)

    RNI (reactive nitrogen oxides intermediates. RNS)

Table 33.5 Formation of Reactive Oxygen Intermediates

Oxygen Intermediate	Reaction
Superoxide ($O_2^{-}\bullet$)	$NADPH + 2O_2 \xrightarrow{\text{NADPH oxidase}} 2O_2^{-}\bullet + H^+ + NADP^+$
Hydrogen peroxide (H_2O_2)	$2O_2^{-}\bullet + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$
Hypochlorous acid (HOCl)	$H_2O_2 + Cl^- \xrightarrow{\text{Myeloperoxidase}} HOCl + OH^+$
Singlet oxygen ($^1O_2$)	$ClO^- + H_2O_2 \xrightarrow{\text{Peroxidase}} {}^1O_2 + Cl^- + H_2O$
Hydroxyl radical ($\bullet OH^-$)	$O_2^{-}\bullet + H_2O_2 \xrightarrow{\text{Peroxidase}} 2\bullet OH^- + O_2$



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Exocytosis and Antigen Presentation

- process to **expel** microbial fragments after they have been digested
  - phagolysosome unites with cell membrane
  - results in *extracellular release* of microbial fragments

- process to **present** microbial fragments on the APC surface
  - pass fragments from phagolysosome to **endoplasmic reticulum**
  - peptide components of fragments combine with glycoproteins which ultimately **become part of cell membrane (*outward*)**
  - important process since it allows *wandering* lymphocytes to be activated
  - ***links*** nonspecific and specific immune responses

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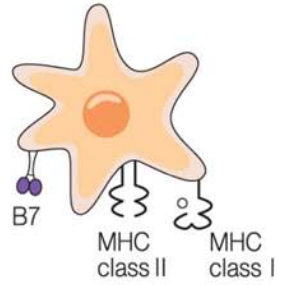
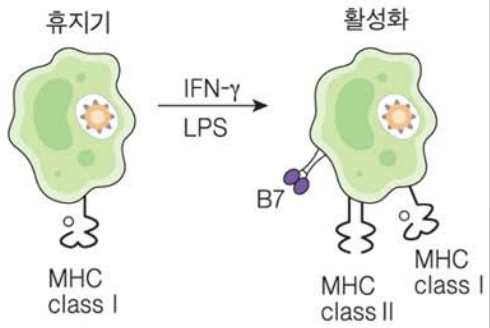
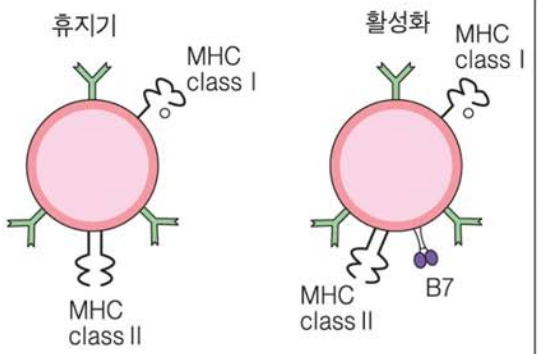
Antigen Presentation

	수지상세포(DC)	대식세포(MΦ)		B 세포	
					
항원 섭취	+++	탐식작용 +++		항원특이수용체 ++++	
MHC class II 발현	조직 DC: 저발현 림프조직 DC: 고발현	세균 및 싸이토카인에 의한 발현 유도(− → +++)		항원자극에 의한 증가 (+++ → ++++)	
공동자극 활성	++++	B7 분자에 의한 유도(+++)		B7 분자에 의한 유도(− → +++)	
항원 제시	펩티드, 바이러스, 알러젠	입자성 항원, 세포 내외 병원체		용해성 항원, 독소, 바이러스	
T 세포 활성	미감작 T 세포, 작용 T 세포, 기억 T 세포	—	작용 T 세포, 기억 T 세포	작용 T 세포, 기억 T 세포	미감작 T 세포, 작용 T 세포, 기억 T 세포
장소	림프조직, 결합조직, 상피조직	림프조직, 결합조직		림프조직, 말초혈관	

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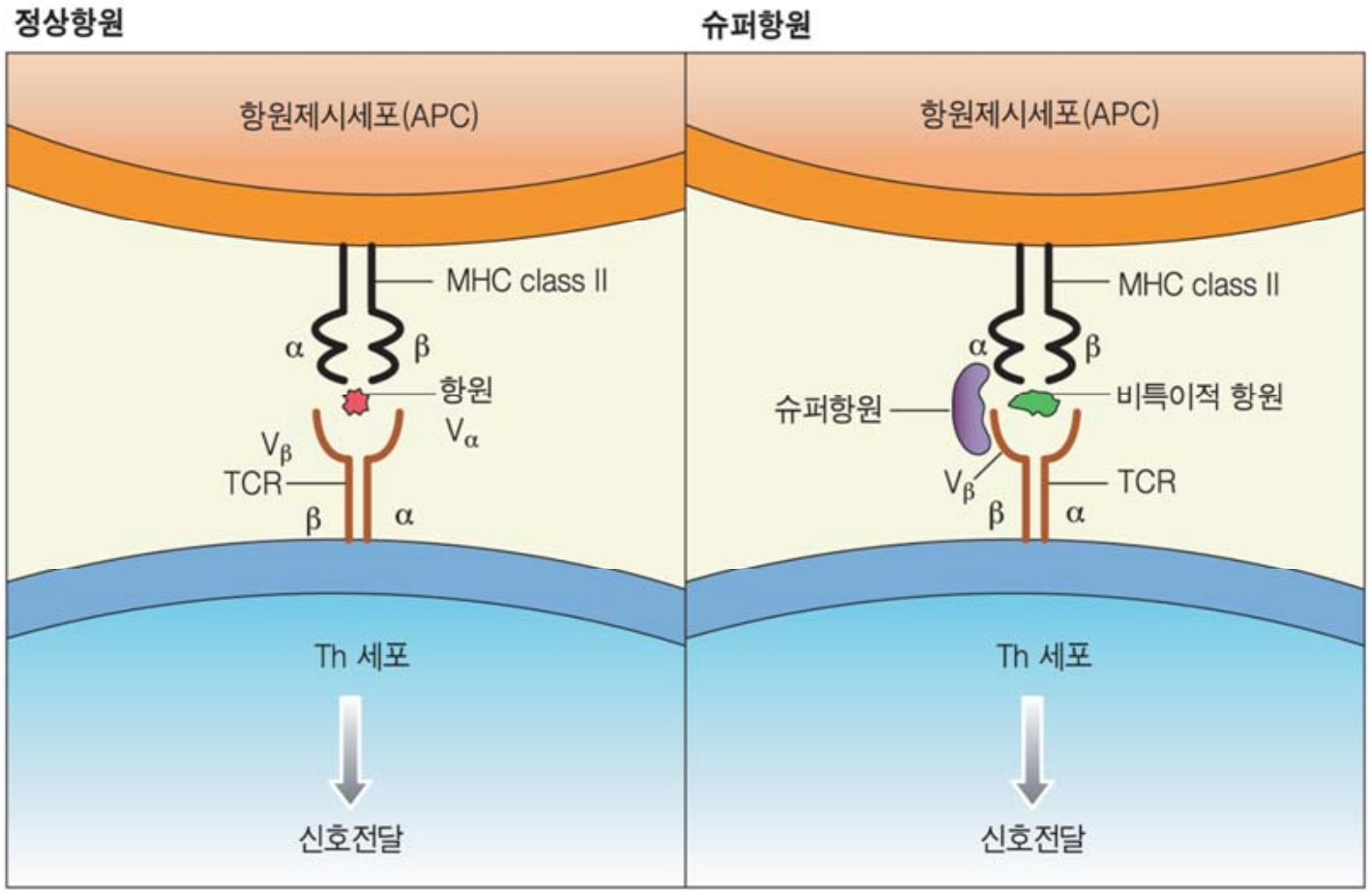
Antigen Presentation?



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Inflammation

- nonspecific response to tissue injury
  - can be caused by **pathogen** as well as **physical trauma**
  - inflammation is the response of body to injury or cell death
  - acute inflammation vs chronic inflammation (depending on time span)

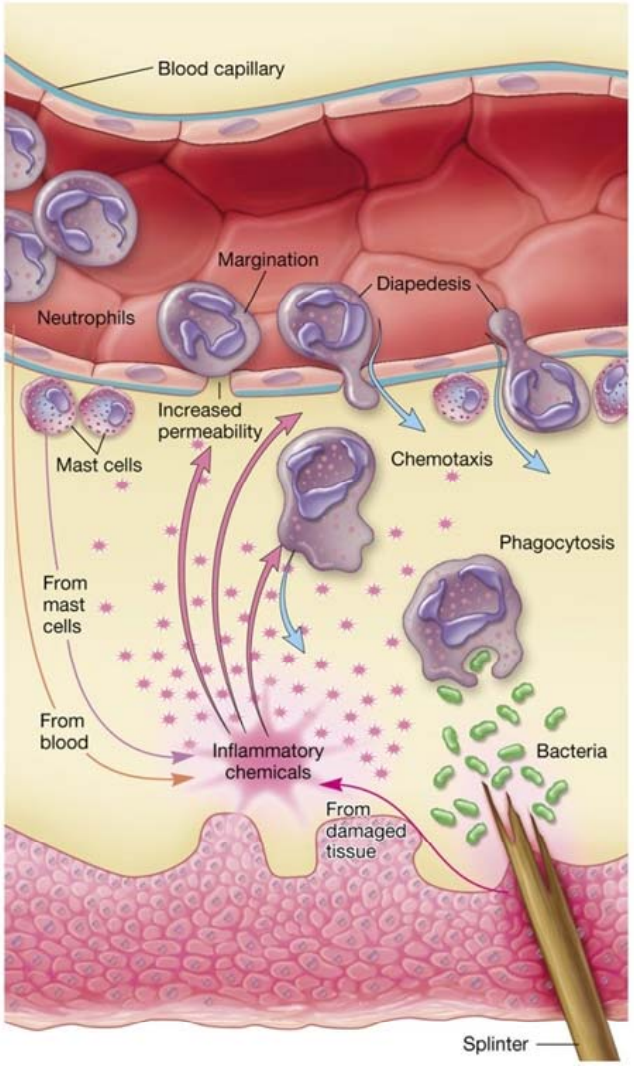
- four cardinal signs of inflammation
  - **redness (rubor)**
  - **warmth (calor)**
  - **pain (dolor)**
  - **swelling (tumor)**
  - resulting in functional loss or failure (*functio laesa*)

Acute Inflammatory Response

- the release of **inflammatory mediators** from injured/infected tissue cells initiates *a cascade of events* which result in the signs of inflammation

- <u>chemical mediators produced</u>
  selectins cell adhesion molecules on activated capillary *endothelial cells*
  Integrins adhesion receptors on *neutrophils*
  chemotaxins chemotactic factors released by *injured cells*

- <u>events initiated</u>
  extravasion (분출)/diapedesis (누출)
  margination (변연)



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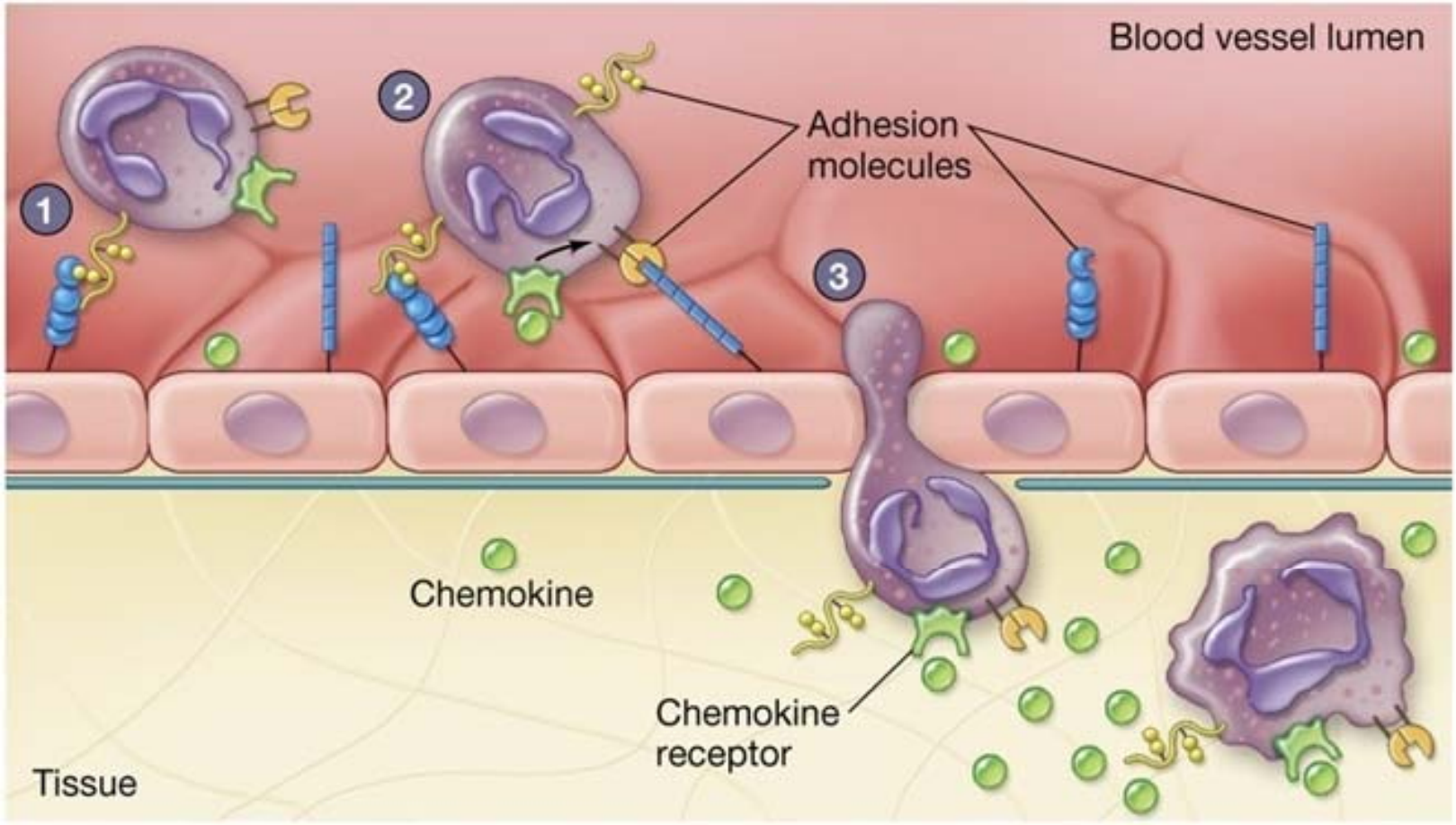
Acute Inflammatory Response



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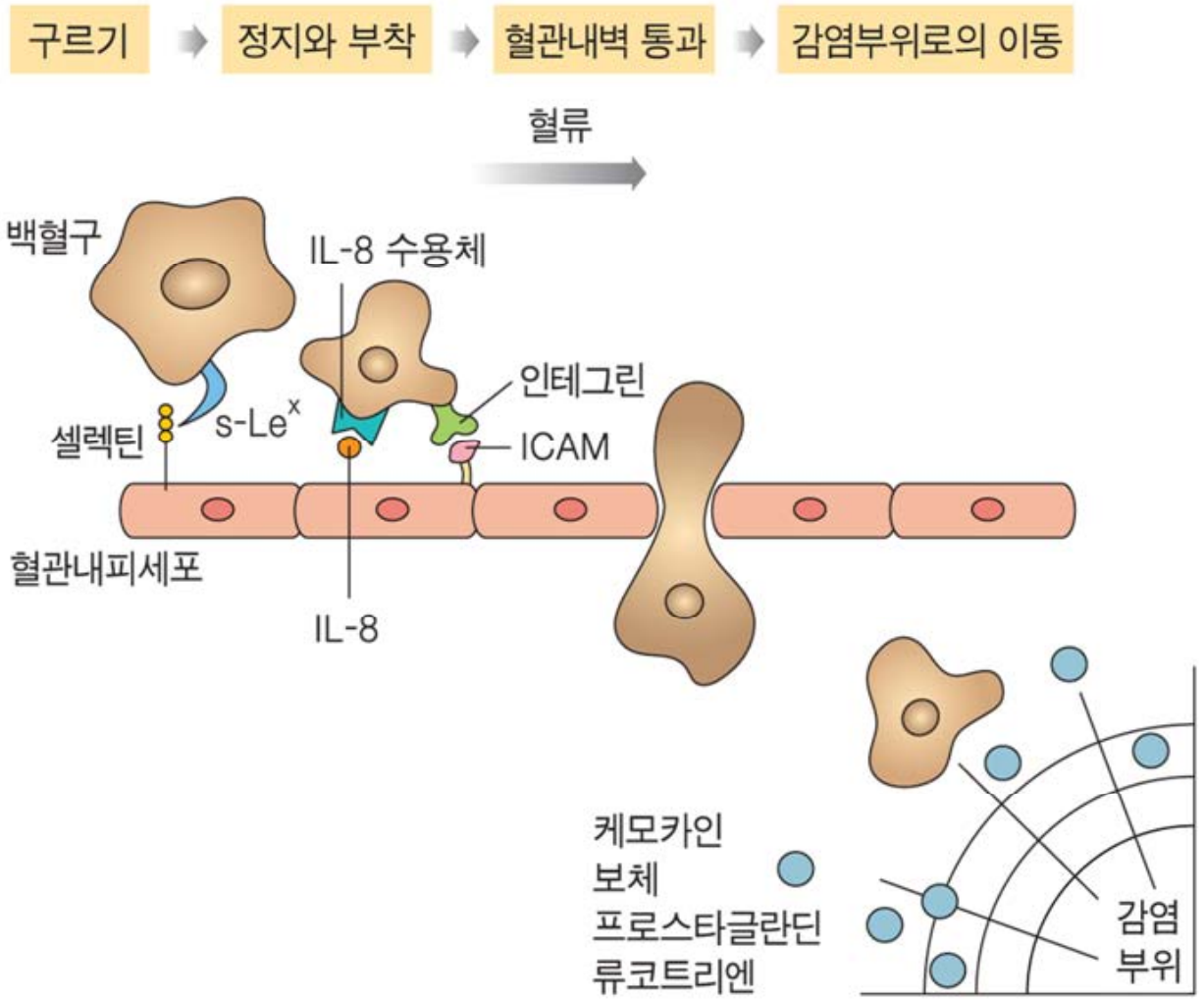
Acute Inflammatory Response



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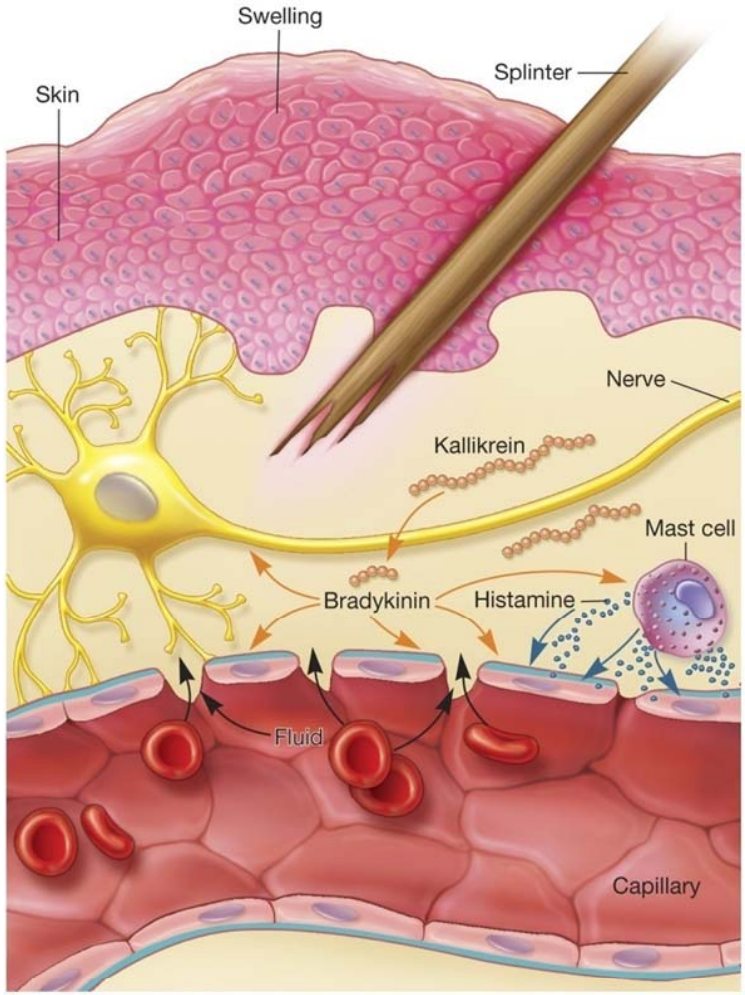
Acute Inflammatory Response

- tissue injury causes release of kalikrein and various other mediators that increase capillary dilation and increased blood flow bring more antimicrobial factors and leukocytes that kill pathogens
- fibrin clot may restrict pathogen movement
- phagocytes accumulate in inflamed area and destroy pathogens
- bone marrow is stimulated by numerous released chemicals to release neutrophils and increase rate of granulocyte production



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Chronic Inflammation

- slow process
- involves formation of **new connective tissue**
- usually causes **permanent** **tissue damage**
- dense infiltration of lymphocytes and macrophages at site of inflammation (resulting in **granuloma**)
  - walled off area
  - formed when phagocytic cells can't destroy pathogen

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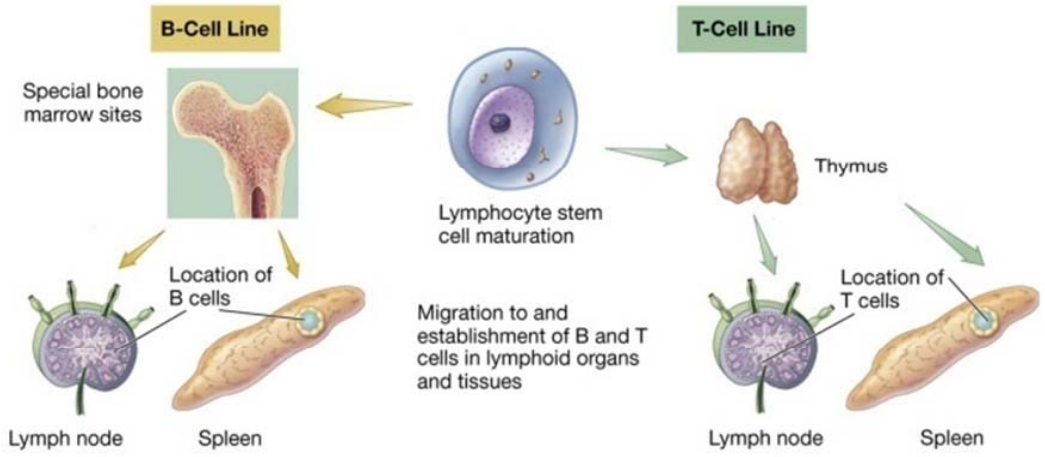
Overview of Specific (Adaptive) Immunity

- three major characteristics
  - ***discrimination*** between self and non-self
  - ***specific*** and **diversified *response*** to each of a trillion of non-selves
  - ***memory*** of non-self as well as self

- three major functions
  - ***recognize*** non-self
  - ***respond to*** non-self
    - effector response
      - eliminates or renders foreign material harmless
    - anamnestic response
      - upon second encounter with same pathogen immune system mounts a faster and more intense response
  - ***remember*** non-self

Types of Specific Immunity

- humoral immunity
  - also called antibody-mediated immunity
  - based on antibody activity
- cellular immunity
  - also called cell-mediated immunity
  - based on action of specific kinds of T lymphocytes



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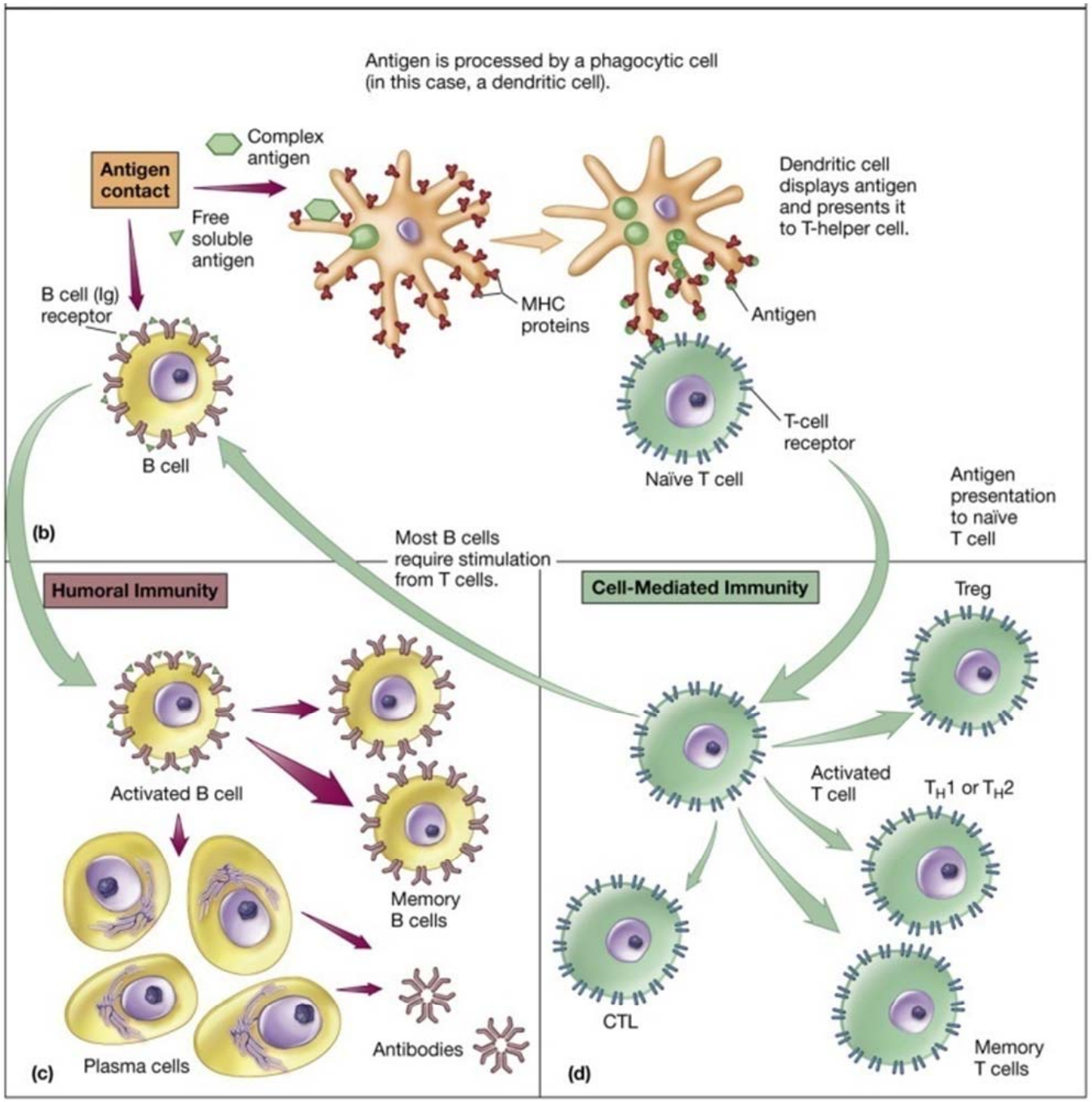

면역반응 및 면역체계: 적응 면역의 과정

Antigens and Epitopes

- self and non-self substances that elicit an immune response and react with products of that response
- most are large, complex molecules

- antigenic determinant sites (epitopes)
  - site on antigen that reacts with specific antibody or T cell receptor
  - valence is number of epitopes and determines number of antibodies that can combine with antigen at one time
- antibody affinity

  strength with which antibody binds to its antigen at a given Ag-binding site

- avidity of antibody

  overall antigen-binding at all antigen binding sites

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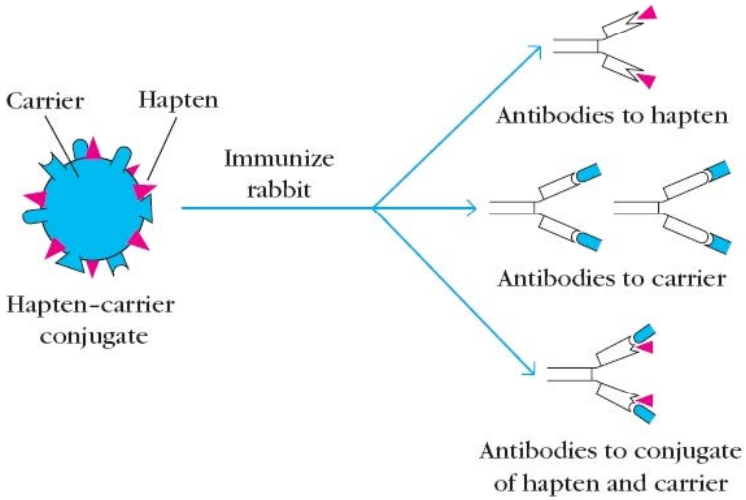
Haptens

- small organic molecules
- not antigenic but may become antigenic when bound to larger carrier molecule
  - e.g. penicillin, common agents (fluoroscein, biotin, digoxygenin, DNP etc)
  - may elicit hapten-specific and carrier-specific responses



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti-BSA
Hapten–carrier conjugate (DNP-BSA)	Anti-DNP (major) Anti-BSA (minor) Anti-DNP/BSA (minor)

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Types of Specific Immunity

Acquired Immunity

Natural immunity
is acquired through the normal life experiences of a human and is not induced through medical means.

Artificial immunity
is that produced purposefully through medical procedures (also called immunization).

Active immunity
is the consequence of a person developing his or her own immune response to a microbe.

Infection

Passive immunity
is the consequence of one person receiving preformed immunity made by another person.

Maternal antibody

Active immunity
is the consequence of a person developing his or her own immune response to a microbe.

Vaccination

Passive immunity
is the consequence of one person receiving preformed immunity made by another person.

Immune globulin therapy

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Types of Specific Immunity

- naturally acquired active immunity
  - type of specific (adaptive) immunity a host develops after exposure to foreign substance or after transfer of antibodies or lymphocytes from an immune donor
- naturally acquired passive immunity
  - transfer of antibodies, e.g., mother to fetus across placenta, mother to infant in breast milk
- artificially acquired active immunity
  - results from vaccination – intentional exposure to a foreign material
- artificially acquired passive immunity
  - preformed antibodies or lymphocytes produced by one host are introduced into another host
  - e.g., gamma globulin, bone marrow transplant

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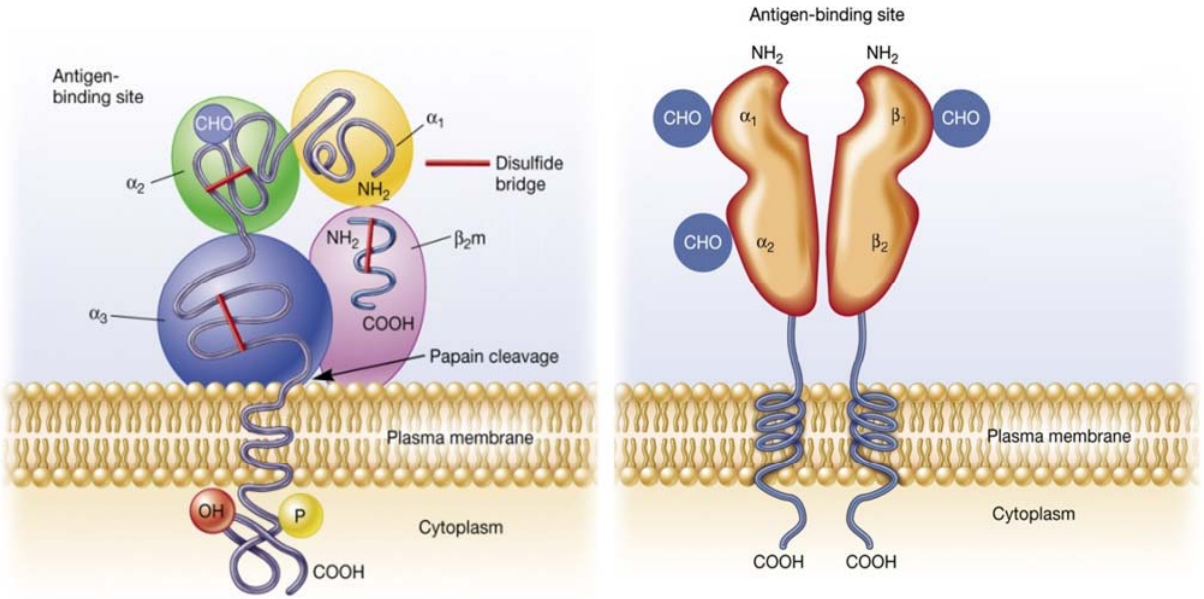
Recognition of Foreignness

- distinguishing between self and non-self is essential for the proper functioning of the immune system
  - this allows for selective destruction of invading pathogens without destruction of host tissues
  - involves **major histocompatibility complex** (MHC)



MHC class I (좌)와 MHC class II (우)

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Major Histocompatibility Complex (MHC)

- collection of genes that code for self/non-self recognition potential of a vertebrate
- in humans, called human leukocyte antigen (HLA) complex
  - on chromosome 6
  - three classes of MHC molecules
  - one paternal allele and one maternal allele

  cf. in mice, called histocompatibility-2 (H-2) complex encoded on chr.17

- class I molecules (MHC I) found on **almost all types** of cells
  - important for *organ transplantation* and *viral infection*
- class II molecules (MHC II) found **only** on **professional APC**s
  - required for *T cell communication* to Mφs, DCs, B cells, (*Basophiles*)
- class III molecules (MHC III) include secreted proteins not required for self/non-self recognition

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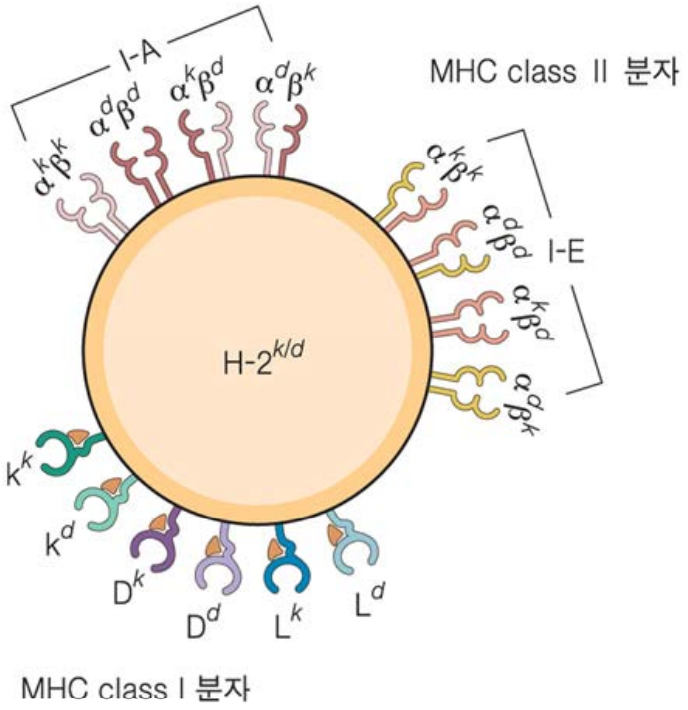
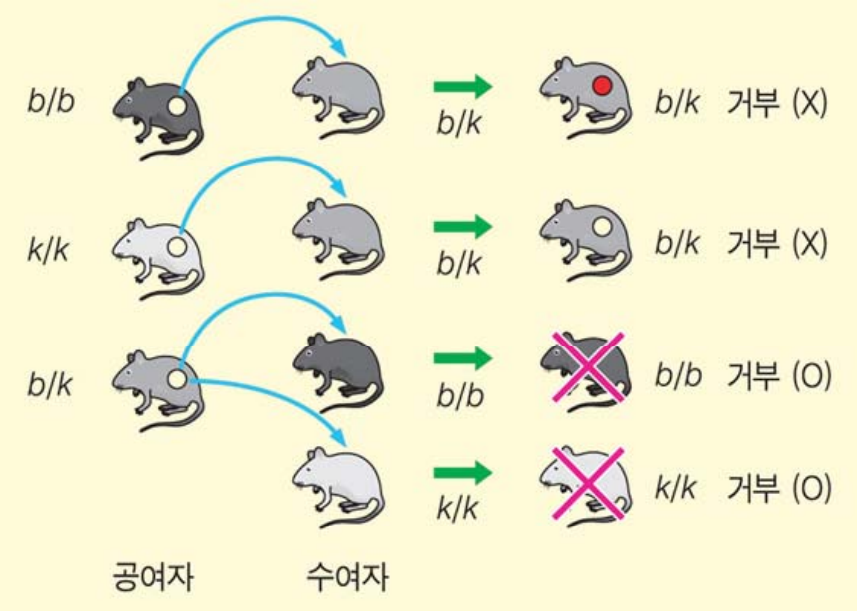
Major Histocompatibility Complex (MHC)

- Co-dominance phenotype in MHC complex
  - Rejection of heterozygotic graft by homozygotic parents





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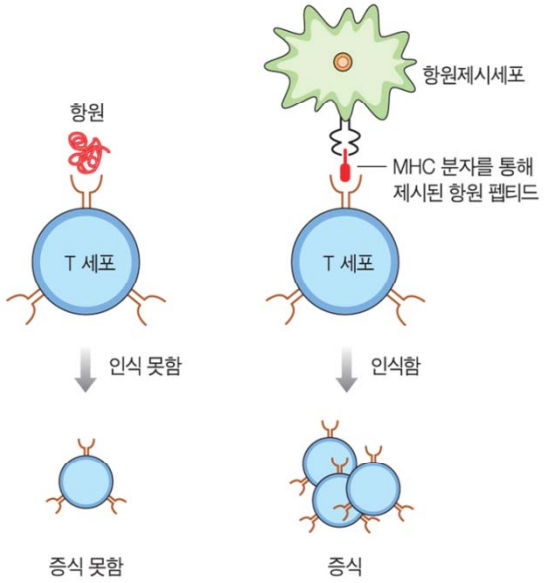
Major Histocompatibility Complex (MHC)

표 7-2

특징
T 세포는 직접 (다른 분자는
T 세포는 세포 인식함
$CD4^+$ T 세포는 항원을, $CD8^+$ 내에 존재하는



T세포의 MHC-제한을 증명한 실험

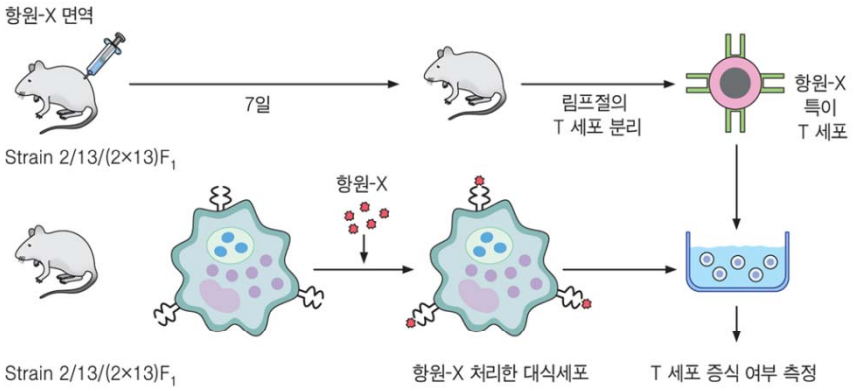


표 7-1 MHC class I 및 class II 분자의 특징

특징	MHC class I	MHC class II
폴리펩티드 사슬	α 폴리펩티드 사슬, β_2-마이크로글로불린	α 폴리펩티드 사슬, β 폴리펩티드 사슬
펩티드 결합 부위	α1/α2	α1/β1
펩티드 결합 부위에 결합하는 펩티드 크기	8~10개 아미노산	13~18개 아미노산
펩티드 결합 부위의 특징	두 개 끝이 닫혀 있음	두 개 끝이 열려 있음
명명		
사람	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP
마우스	H-2K, H-2D, H-2L	I-A, I-E

항원-X 특이 T 세포	항원-X 처리한 대식세포		
	Strain 2	Strain 13	Strain (2×13)F_1
Strain 2	+	−	+
Strain 13	−	+	+
Strain (2×13)F_1	+	+	+

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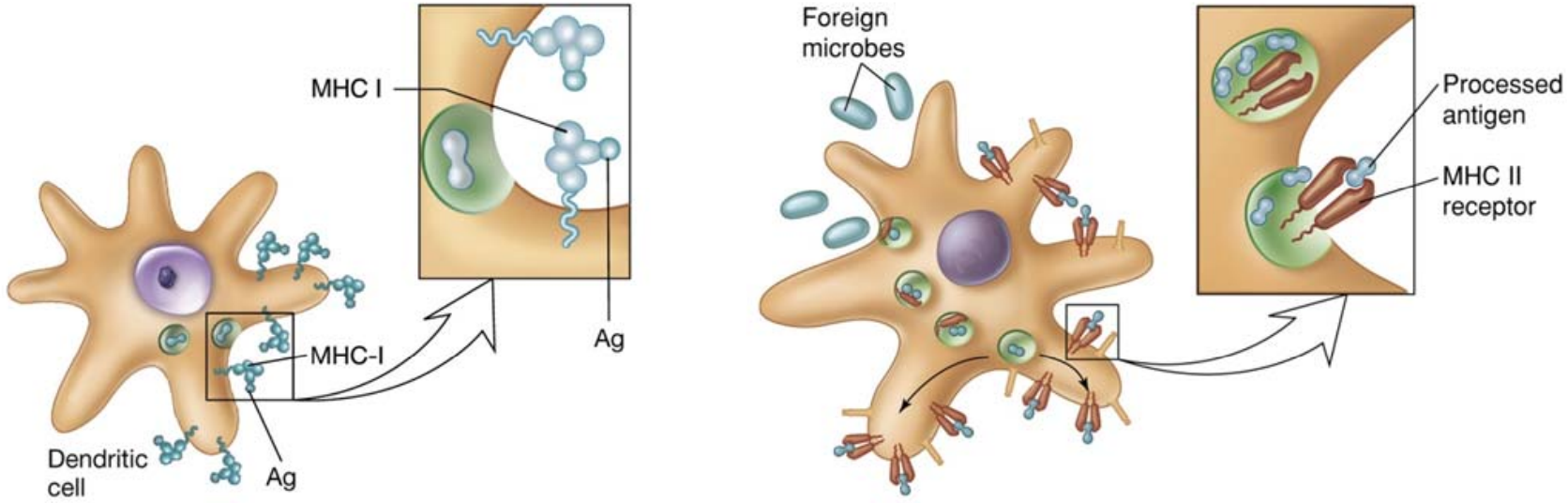
MHC and Antigen Processing

- *endogenous/cytoplasmic* antigen processing

  MHC I binds to antigen peptides that originate in the **cytoplasm** and present antigen to $CD8^+$ T cells

- *exogenous/vesicular* antigen processing

  MHC II binds to antigen fragments that come from outside the cell and present to $CD4^+$ T helper (Th0) cells



MHC-I/CD8 vs MHC-II/CD4

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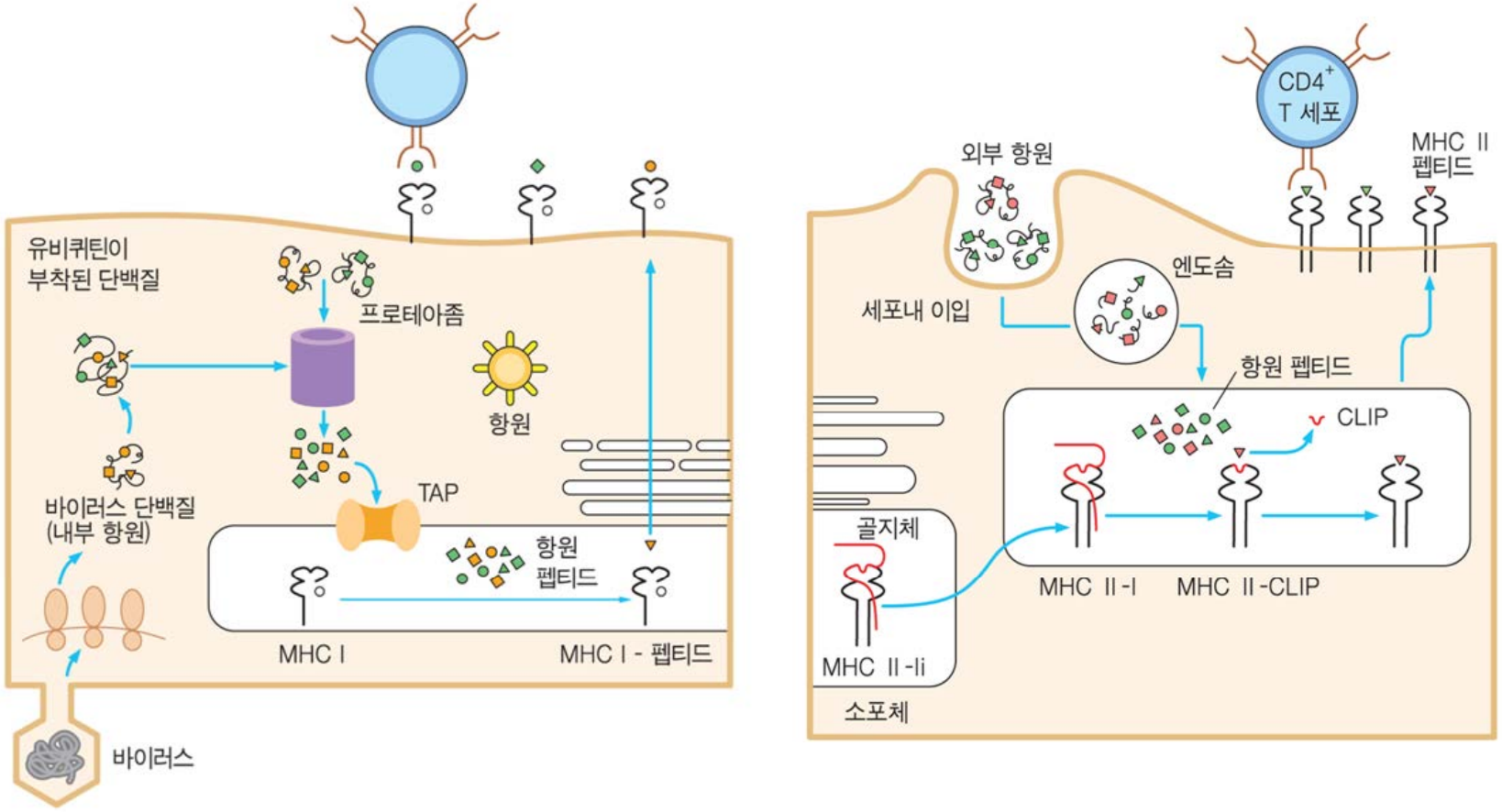
MHC and Antigen Processing



※ 만약 endosome 내의 항원이 cytosol로 빠져나오는 경우는?

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Cluster of Differentiation Molecules (CDs)

- membrane proteins on lymphocytes and other cells
  - indicates **cellular differentiation** status
  - have specific roles in *intercellular communication*
  - used to identify and differentiate between leukocyte subpopulations
- CD3, **CD4**, **CD8**, CD14, CD19/CD22, **CD28**, CD34, **CD40**, **CD40L**

Table 34.1 Functions of Some Cluster of Differentiation (CD) Molecules

Molecule	Function
CD1 a, b, c	MHC class I–like receptor used for lipid antigen presentation
CD3 δ, ε, γ	T-cell antigen receptor
CD4	MHC class II coreceptor on T cells, monocytes, and macrophages; HIV-1 and HIV-2 (gp120) receptor
CD8	MHC class I coreceptor on cytotoxic T cells
CD11 a, b, c, d	α-subunits of integrin found on various myeloid and lymphoid cells; used for binding to cell adhesion molecules
CD19	B-cell antigen coreceptor
CD34	Stem cell protein that binds to sialic acid residues
CD45	Tyrosine phosphatase common to all hematopoietic cells
CD56	NK cell and neural cell adhesion molecule

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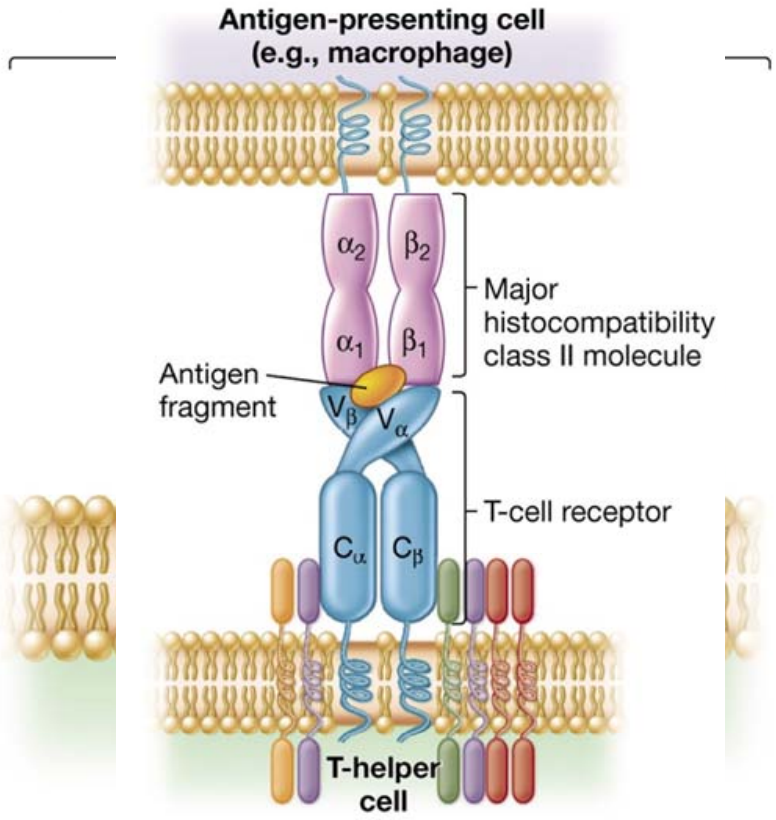
T-Cell Biology

- originate from **CD34⁺ stem cells** in the BM but mature in thymus
- have major role in B cell activation (i.e. humoral immunity) (Th2)
- major players in cell-mediated immune response (CMI) (CTL and Th1)

✓ **<u>T cell receptors (TCRs)</u>**

- reside in the plasma membrane surface
- recognize and bind antigen
- antigen must be presented by antigen-presenting cells (APCs)



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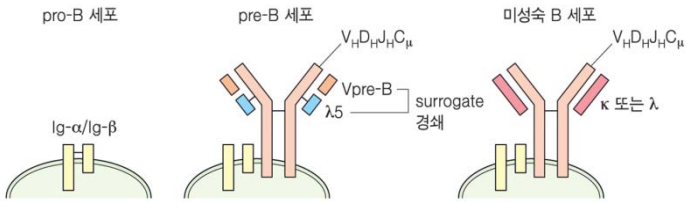
T Cell Differentiation

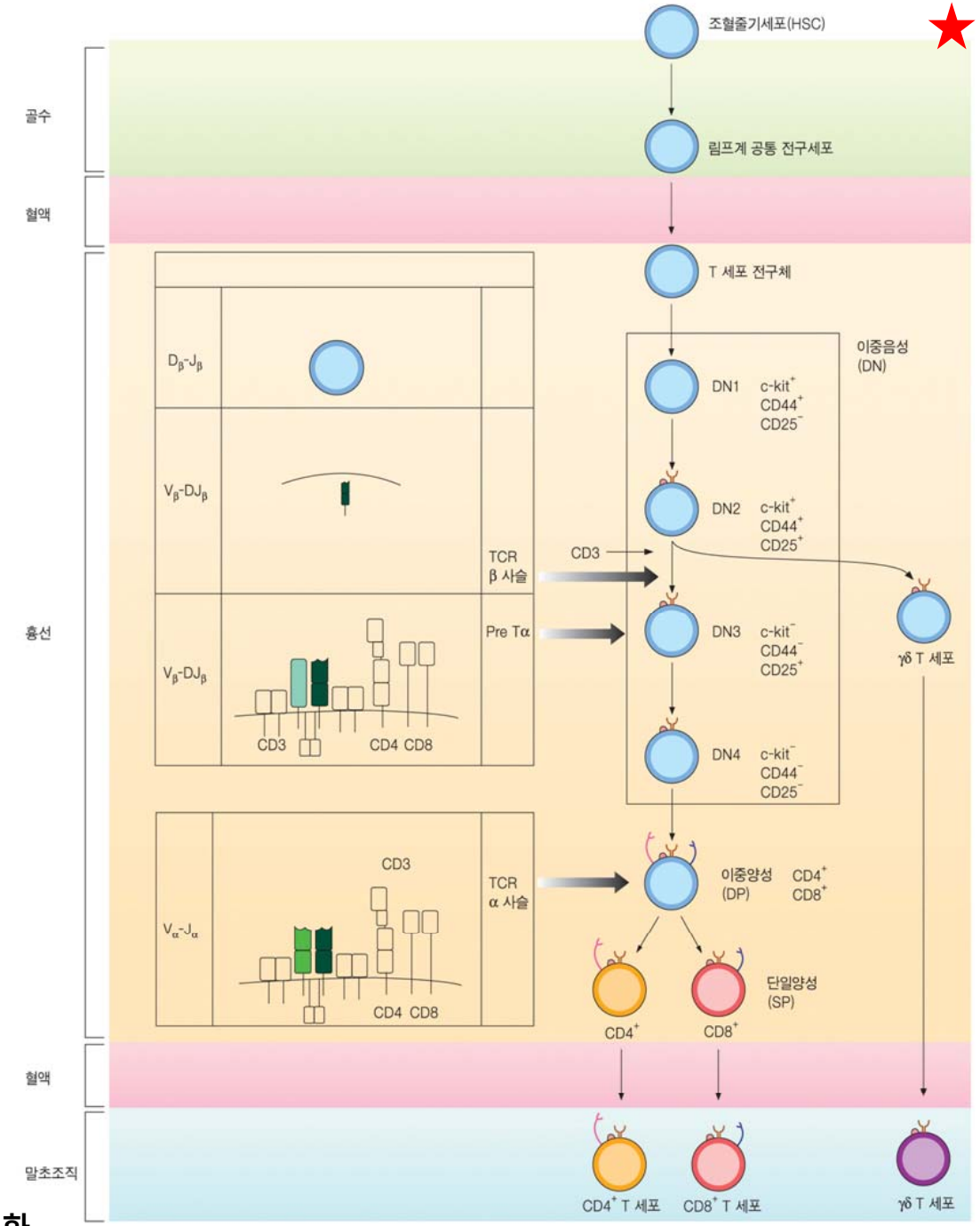
- HSC: CD34⁺ stem cell (BM)
- Lymphoid progenitor

- T lymphoblast (= progenitor T)
- DN (double-negative)
- DP (double-positive)
- SP (single-positive): immature T

<u>cf. B cell differentiation</u>

- HSC: CD34⁺ stem cell (BM)
- Lymphoid progenitor
- B lymphoblast (= progenitor B)
- Pre-B
- Immature B

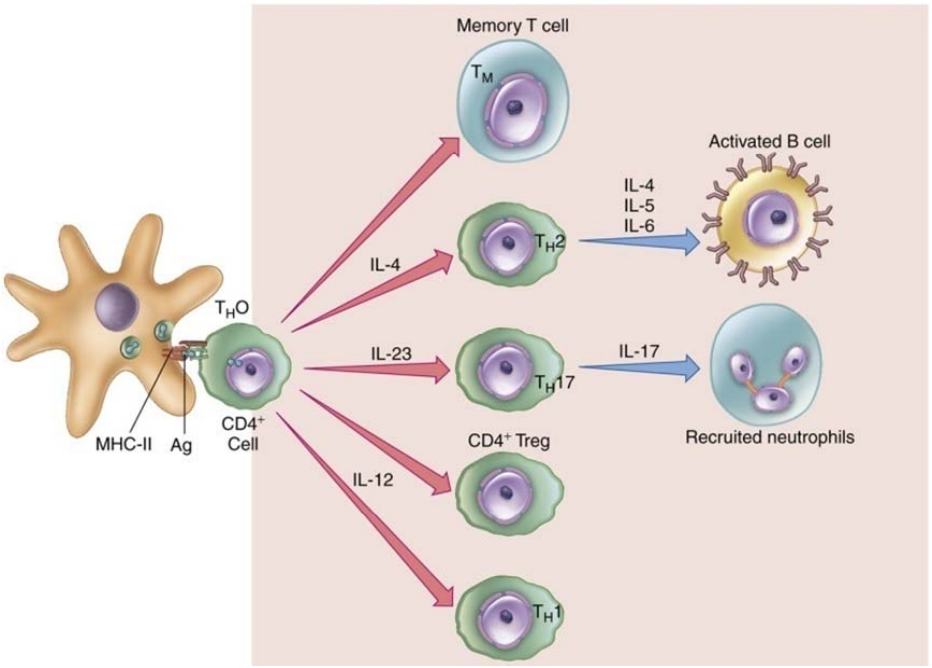


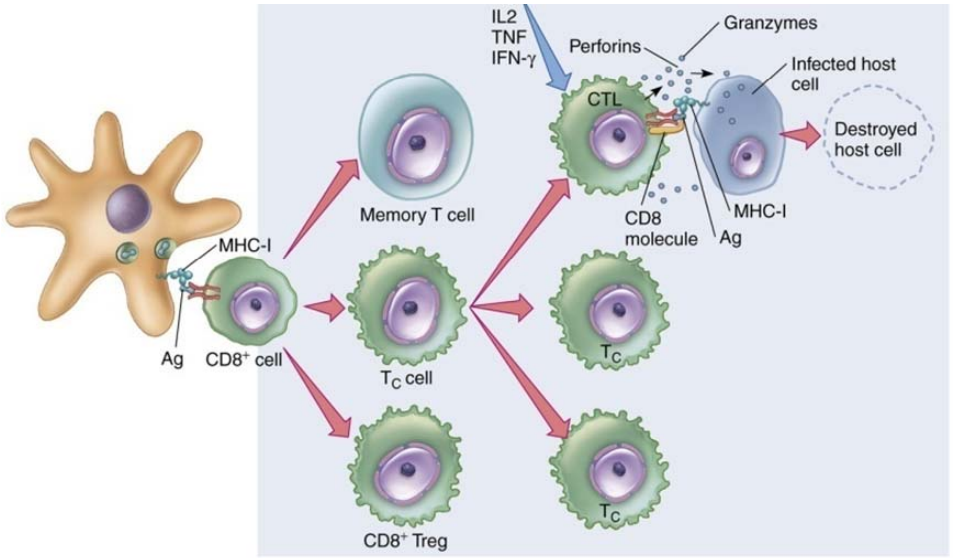


면역 반응의 조절: TCR, T-세포의 분화, 증식, 활성화

Types of T Cells

- mature T cells are naïve until they are activated by Ag presentation
- once activated they proliferate into **effector** and **memory** cells
  - effector cells carry out specific functions to protect host
  - three types: T helper cells (Th), cytotoxic T cells (Tc), and regulatory T cells (Treg)





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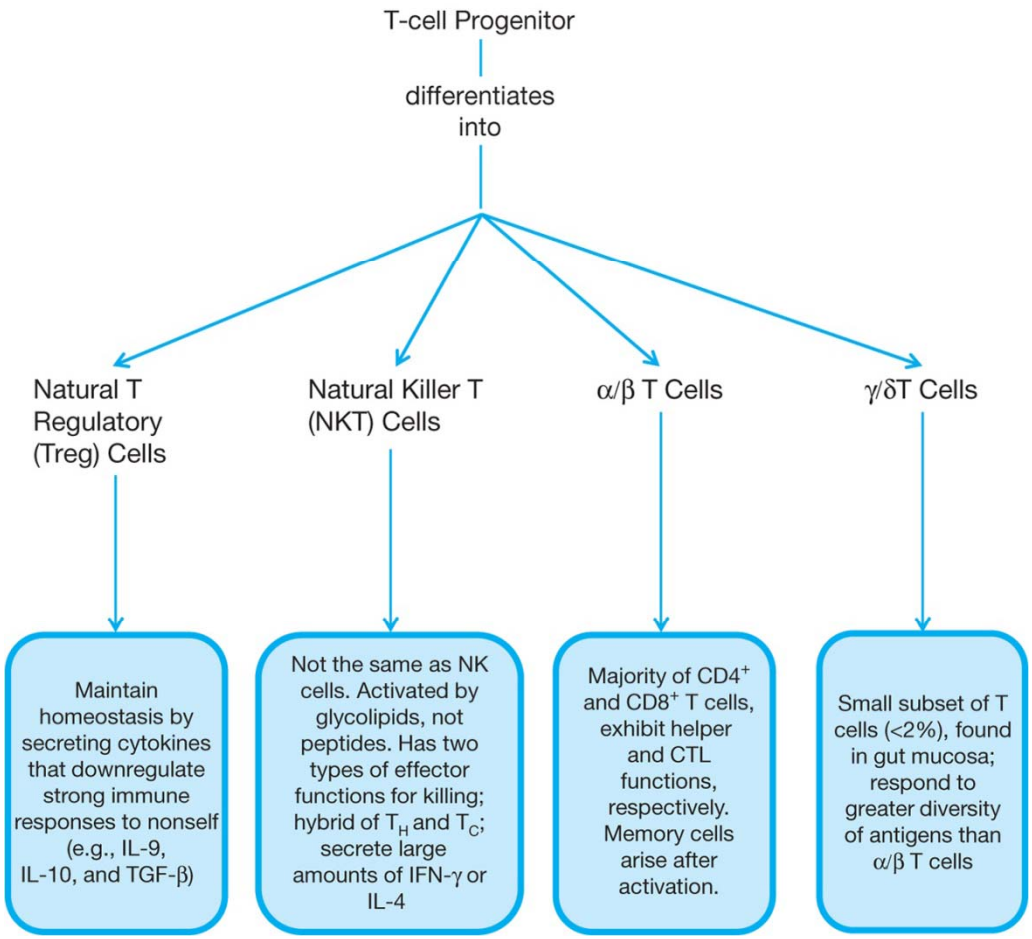
Types of T Cells

- mature T cells are naïve until they are activated by Ag presentation
- once activated they proliferate into effector cells and memory cells
  - effector cells carry out specific functions to protect host
  - **Four types**

    Helper T cells (Th)
    Regulatory T cells (Treg)
    Cytotoxic T cells (CTL or Tc)
    Natural killer T cells (NKT)



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T Helper (Th) Cells

- aka. $CD4^+$ T cells
- activated by antigen presentation with class II MHC
- subdivisions of T helper cells
  - Th0: undifferentiated (= precursor) T cells
  - Th1 (help CMI), Th2 (help Ab), **Th17** (IL-17, antibacterial?) vs. Treg

- **Th1 cells**
  - promote cytotoxic T lymphocyte activity and activate macrophages
  - mediate inflammation and delayed hypersensitivity (DTH) by producing a specific set of cytokines (IL-2, IFN-γ, TNF-β)
- **Th2 cells**
  - stimulate antibody responses and defend against helminth parasites
  - involved in promoting allergic reactions
  - produce a specific set of cytokines (IL-4, IL-5, IL-6, IL-10, IL-13)

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Tc (CTLs) and Treg Cells

✓ **Cytotoxic T (Tc) cells**

- a subset of $CD8^+$ T cells activated by Ag presented on MHC I
- once activated, CTLs can kill target cells that have the same Ag-MHC I combination that originally activated the CTL
- after bind target, CTL kills target cell via the perforin pathway and CD95 pathway

✓ **Regulatory T (Treg) cells**

- derived from 10% of $CD4^+$ T cells and 2% of $CD8^+$ T cells
- **IL-9, IL-10, and TGF-β** induce regulatory function by **inhibiting** Th cell function

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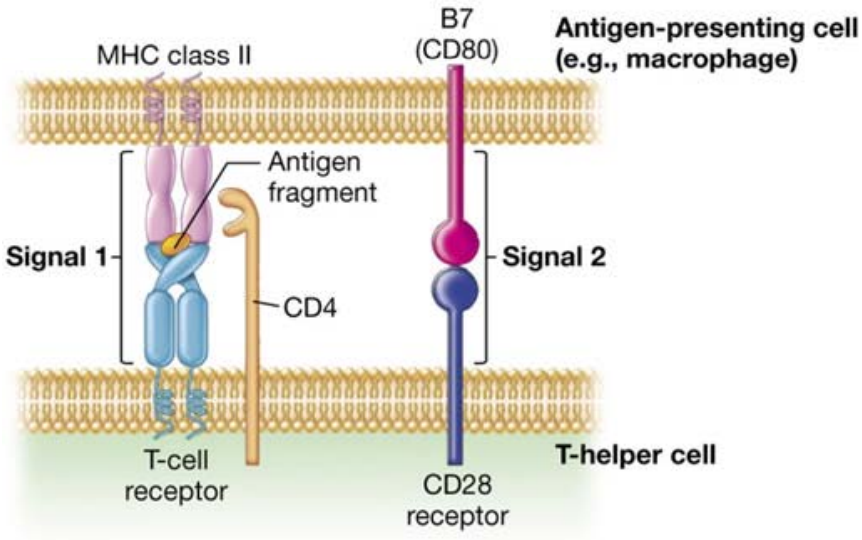
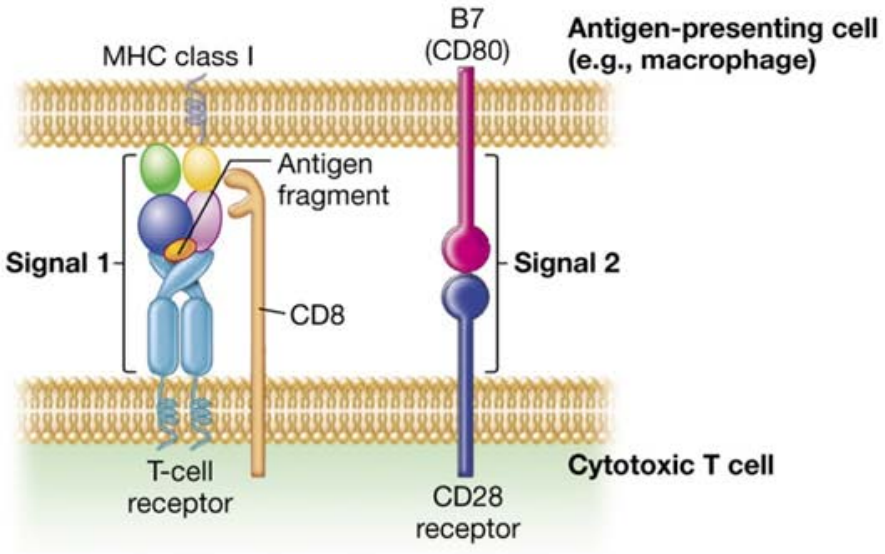
T Cell Activation

- requires binding a specific antigen
  - occurs through Ag presentation which bridges MHC of the APC to the TCR of the T cell
  - ***a second signal through CD28-B7*** is required for lymphocyte proliferation, differentiation, and for specific cytokine gene expression
  - if no second signal present, T cell becomes ***anergic*** (clonal anergy)





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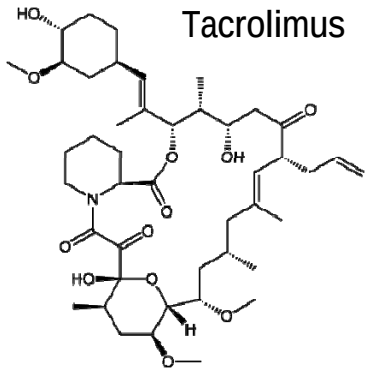
Signal Transduction in T Cell Activation

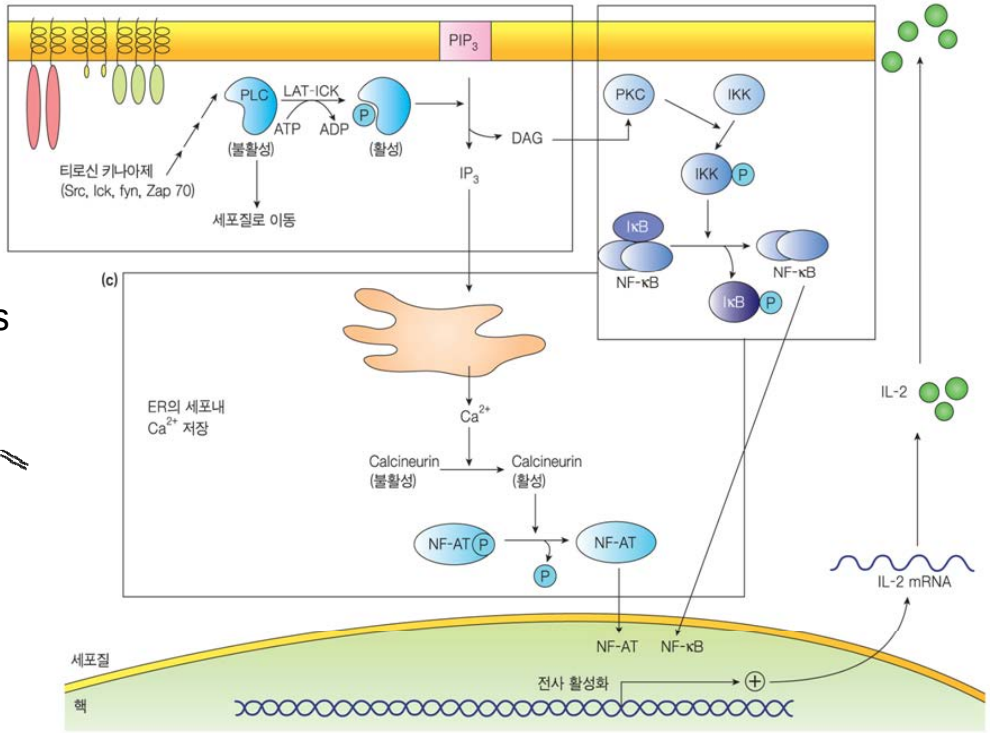
- TCR-associaated kinases (Src, Lck, Fyn, ZAP-70)
- PLC
- $PIP_2 = IP_3 + DAG$

- Calcineurin
- NF-AT

- PKC
- IKK
- I-κB/NF-κB

- Ras signaling
- Raf
- MEK (MAPKK)
- MAPK (ERK, JNK, p38)
- AP-1





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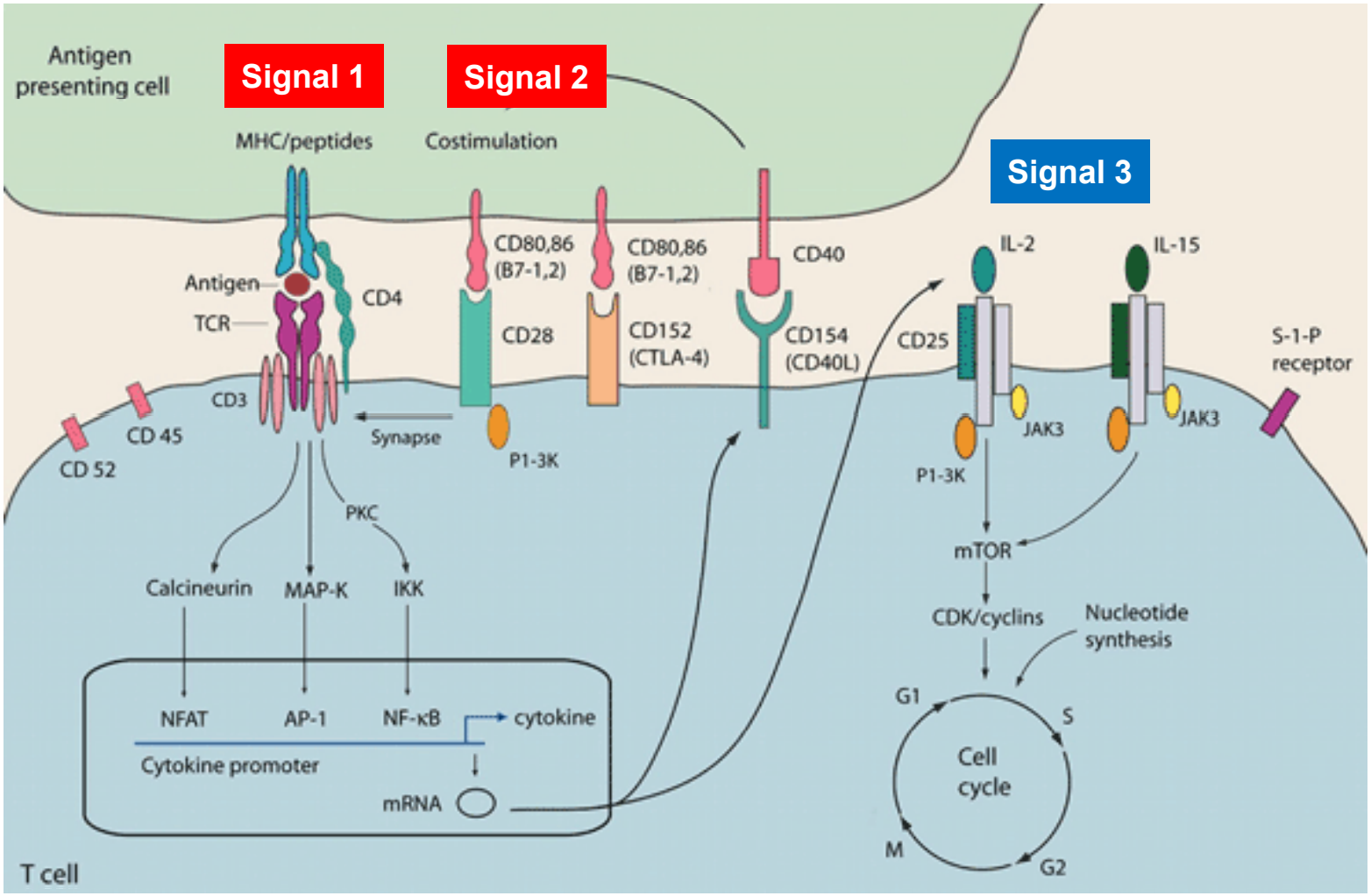
Steps in T cell activation



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Immunosuppressants

SELECTIVE INHIBITORS OF CYTOKINE PRODUCTION AND FUNCTION

Cyclosporine NEORAL, SANDIMMUNE
Everolimus ZORTRESS
Sirolimus RAPAMUNE
Tacrolimus PROGRAF

IMMUNOSUPPRESSIVE ANTIMETABOLITES

Azathioprine IMURAN
Mycophenolate mofetil CELLCEPT
Mycophenolate sodium MYFORTIC

ANTIBODIES

Alemtuzumab CAMPATH
Antithymocyte globulins ATGAM, THYMOGLOBULIN
Basiliximab SIMULECT
Daclizumab ZENAPAX
Muromonab-CD3 ORTHOCLONE OKT3

ADRENOCORTICOIDS

Methylprednisolone MEDROL
Prednisolone ORAPRED, PRELONE
Prednisone DELTASONE

Purine biosynthesis
de novo pathway (신생경로): 분열중인 세포에서 활발 (억제 타겟)
salvage pathway (회피경로)

책에서 다루는 면역억제제

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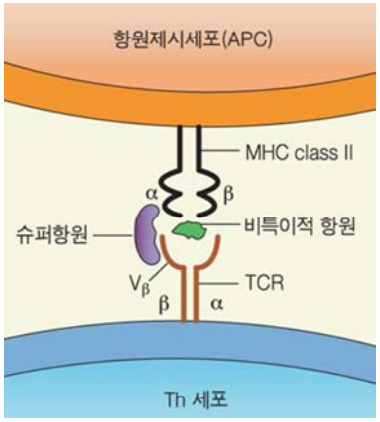
Superantigens

- bacterial and viral proteins that stimulate stronger immune response than normal antigens by **"tricking" T cells** into activation although they have not been triggered by a specific antigen
- stimulate T cells to proliferate ***nonspecifically***

- contribute to microbial pathogenicity
- stimulate release of massive quantities of cytokines from T cells
  may result in circulatory shock and multi-organ failure

- examples
  - staphylococcal enterotoxin B (SEB)
    - causes food poisoning
    - is on the Select Agent List as a potential agent of terrorism
  - toxic shock syndrome toxin (TSST1)
  - mouse tumor virus (MTV) superantigen
  - putative proteins from EBV and rabies virus



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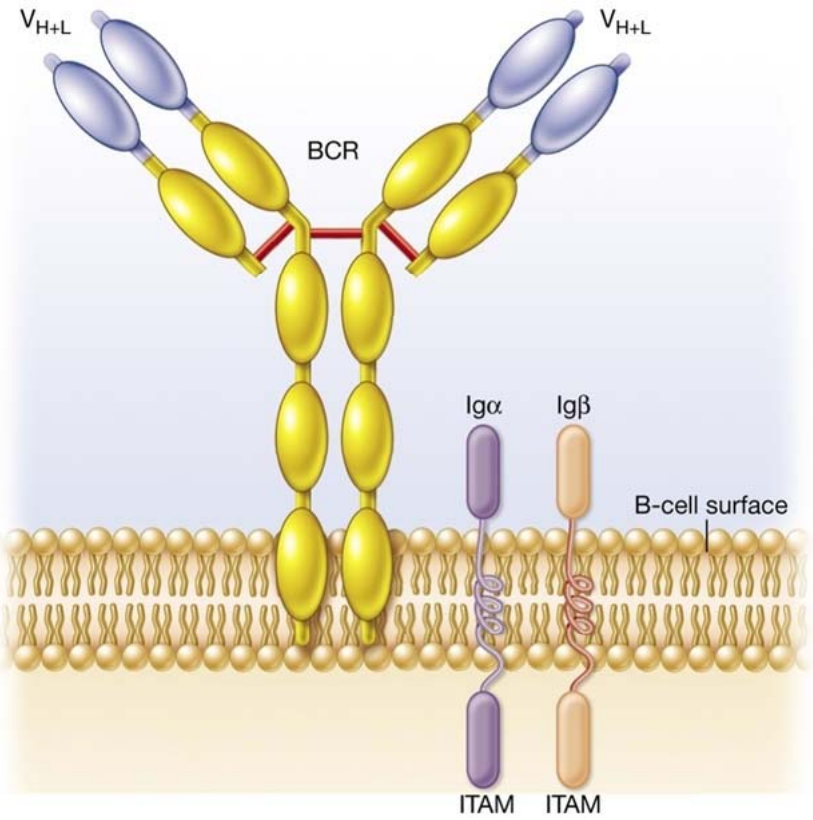
B-Cell Biology

- B cells must be activated by a specific antigen to continue mitosis then replicate and differentiate into plasma cells which secrete antibodies
- B cells have immunoglobulin receptors (IgRs) for the specific antigen that will activate that particular B cell lineage
  - these receptors associate with other proteins and are called B-cell receptors (BCRs)
- interaction with that Ag is communicated to the nucleus via a signal transduction pathway similar to that described for T cells



BCR: monomeric IgM + coreceptors (Igα and Igβ)

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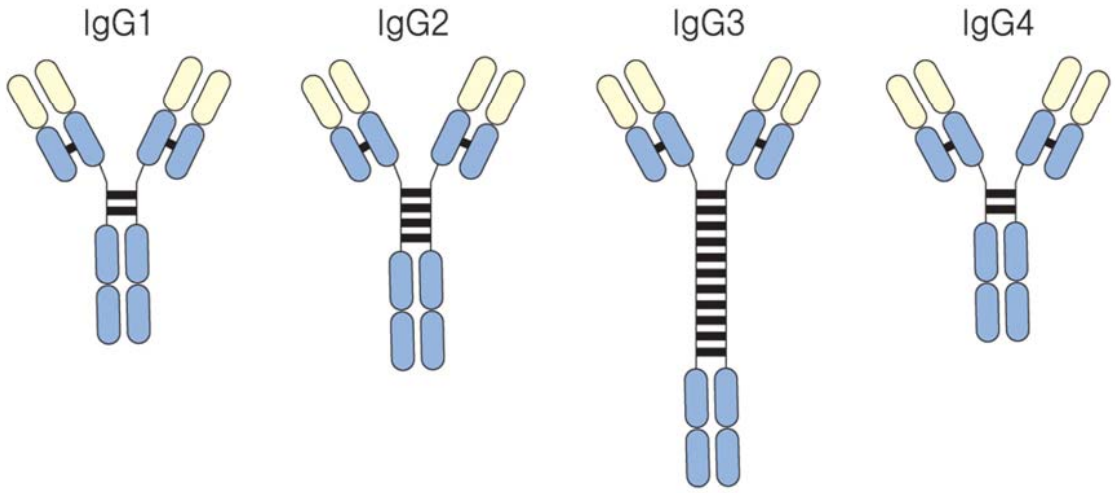
Antibodies (Abs)

- antibody
  - immunoglobulin (Ig)
  - glycoprotein made by activated B cells (plasma cells)
  - serves as antigen receptor (IgR) on B cell surface (BCR)
- found in blood serum, tissue fluids, and mucosal surfaces of vertebrate animals
  - an antibody can recognize and bind antigen that caused its production



IgG subtypes

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Table 34.2 Physicochemical Properties of Human Immunoglobulin Classes

	IMMUNOGLOBULIN CLASSES				
Property	**IgG[1]**	**IgM**	**IgA[2]**	**IgD**	**IgE**
Heavy chain	γ_1	μ	α_1	δ	ε
Mean serum concentration (mg/ml)	9	1.5	3.0	0.03	0.00005
Percent of total serum antibody	80–85	5–10	5–15	<1	<1
Valency	2	5(10)	2(4)	2	2
Mass of entire molecule (kDa)[3]	146	970	160[3]	184	188
Placental transfer	+	−	−	−	−
Half-life in serum (days)[4]	23	5	6	3	2
Complement activation					
Classical pathway	++	+++	−	−	−
Alternative pathway	−	−	+	−	−
Induction of mast cell degranulation	−	−	−	−	+
% carbohydrate	3	7–10	7	12	11
Major characteristics	Most abundant Ig in body fluids; neutralizes toxins; opsonizes bacteria	First to appear after antigen stimulation; very effective agglutinator; expressed as membrane-bound antibody on B cells	Secretory antibody; protects mucous membranes	Present on B-cell surface; B-cell recognition of antigen	Anaphylactic-mediating antibody; resistance to helminths

1 Properties of IgG subclass 1.
2 Properties of IgA subclass 1.
3 sIgA = 360 − 400 kDa.
4 Time required for half of the antibodies to disappear.

Antibodies (Abs)

표 4-4	인간 항체의 분자적 성질 및 생물학적 활성	
항체의 종류	아형 (subtype)	기 능
IgG	IgG1 ~ IgG4	옵소닌화, 보체의 활성화, 항체 의존 세포독성, 신생아의 면역
IgA	IgA1, IgA2	주로 이량체로 존재하며 점막면역을 담당
IgM	없음	미감작 B 세포의 수용체 역할, 보체의 활성화
IgD	없음	미감작 B 세포의 수용체 역할
IgE	없음	기생충에 대한 방어 작용, 급성 과민반응을 담당

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Immunoglobulin Structure

- all immunoglobulin molecules have the same basic structure
  - **four polypeptide chains**
    - two identical heavy chains → **isotypes** (동형)
    - two identical light chains
    - heavy and light chains connected to each other by **disulfide bonds**
  - both chains contain *two different* regions
    - constant (C) regions (CL and CH)
    - variable (V) regions (VL and VH)

- four chains are arranged in form of a flexible Y with a hinge region
  - stalk of Y is the crystallizable fragment (Fc)
    - composed of only constant region
  - top of Y is two antigen binding fragments (Fab)
    - composed of both constant and variable regions

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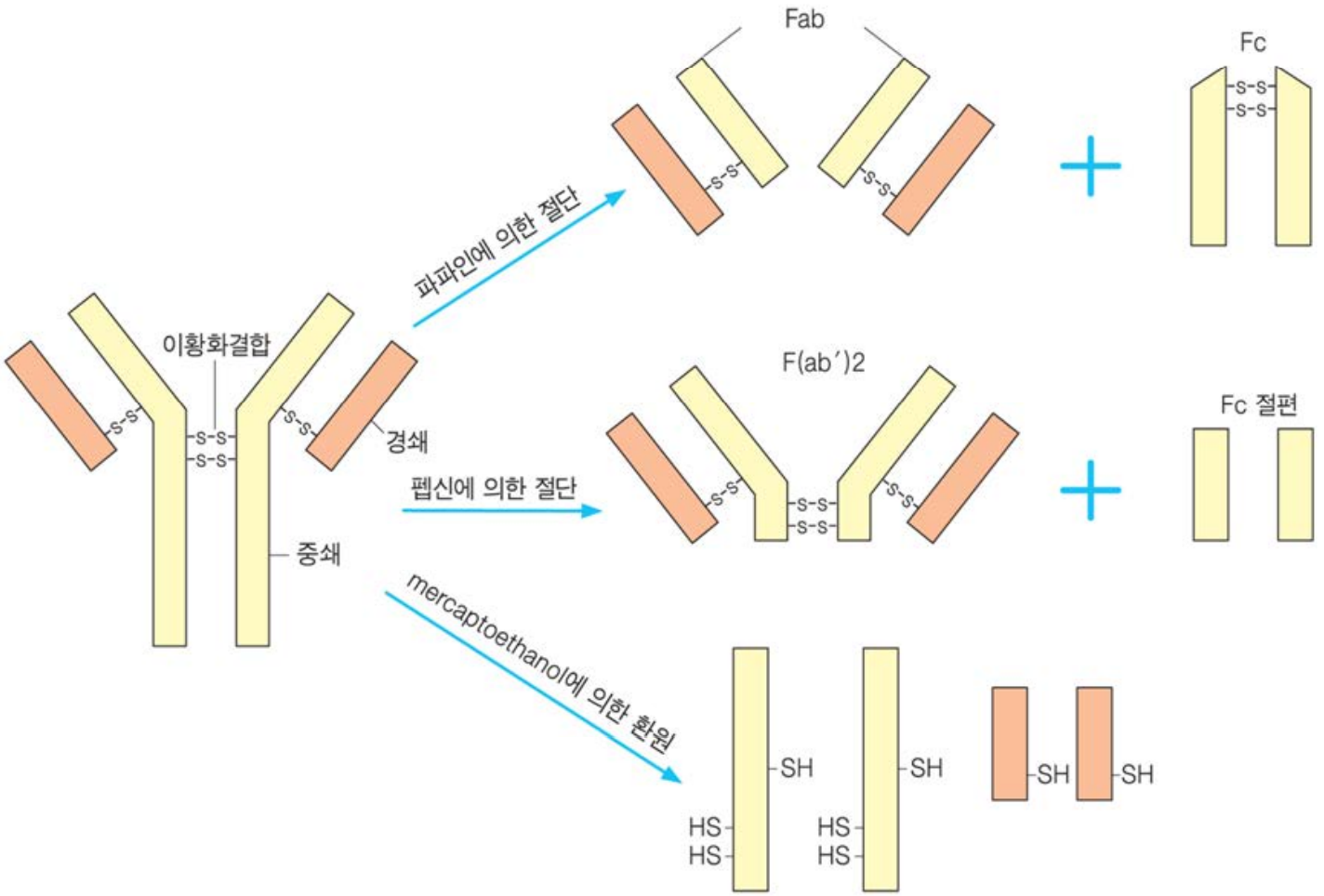
Immunoglobulin Structure



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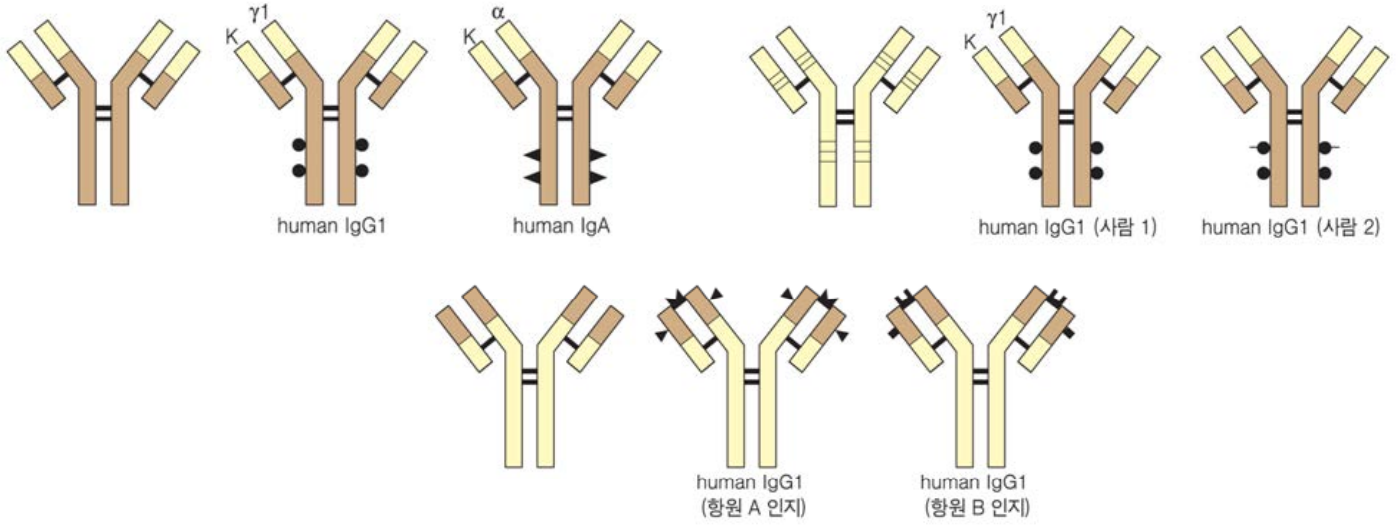
Immunoglobulin Types

- **Isotypes** (동형): heavy chain/light chain의 C영역에 따라 구분
  α1, α2, γ1, γ2, γ3, γ4, δ, ε, μ / κ, λ의 총 16가지 isotype이 있음
- **Allotypes** (동종형): 같은 isotype 내에서 polymorphism에 따라 구분
  α2 heavy chain의 경우, α2m(1), α2m(2)의 2 가지 allotype이 있음
- **Idiotypes** (이디오타입): heavy chain/light chain의 V영역에 따라 구분
  같은 IgG1이라도 인식항원이 다르면 이디오타입이 다를 수 있음



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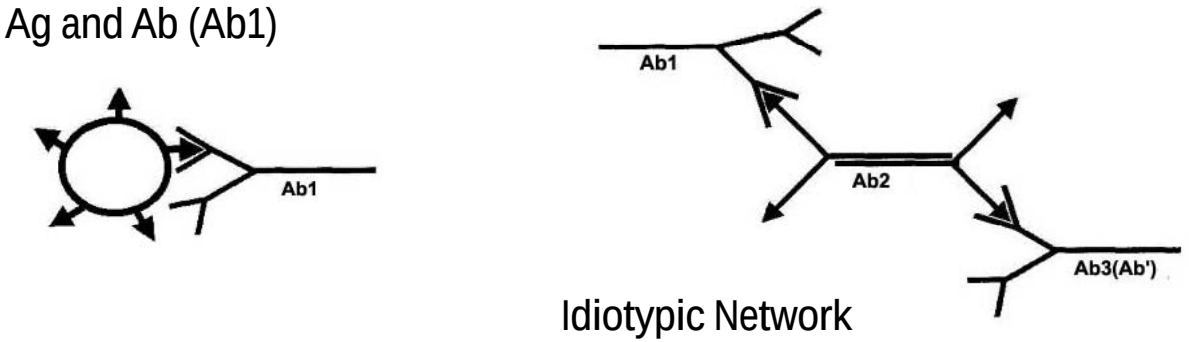
Idiotypic Network

- ✓ **aka. Idiotype-anti-idiotype network**
- Ag triggers Ab production (Ab1).
- Ab1 not only react against the Ag, but also serve as idiotypic (Id) Ag.
- Thus, Ab1 triggers the production of Ab2, an anti-idiotypic Ab to Ab1.
- Likewise, Ab2 serves to induce Ab3, and the process continues.
- At each step, the Ab concentration to provoke formation of the next Ab is less than in the preceding step. (언젠가는 끝남??)



cf. Therapeutic application of Ab2 rather than Ag??

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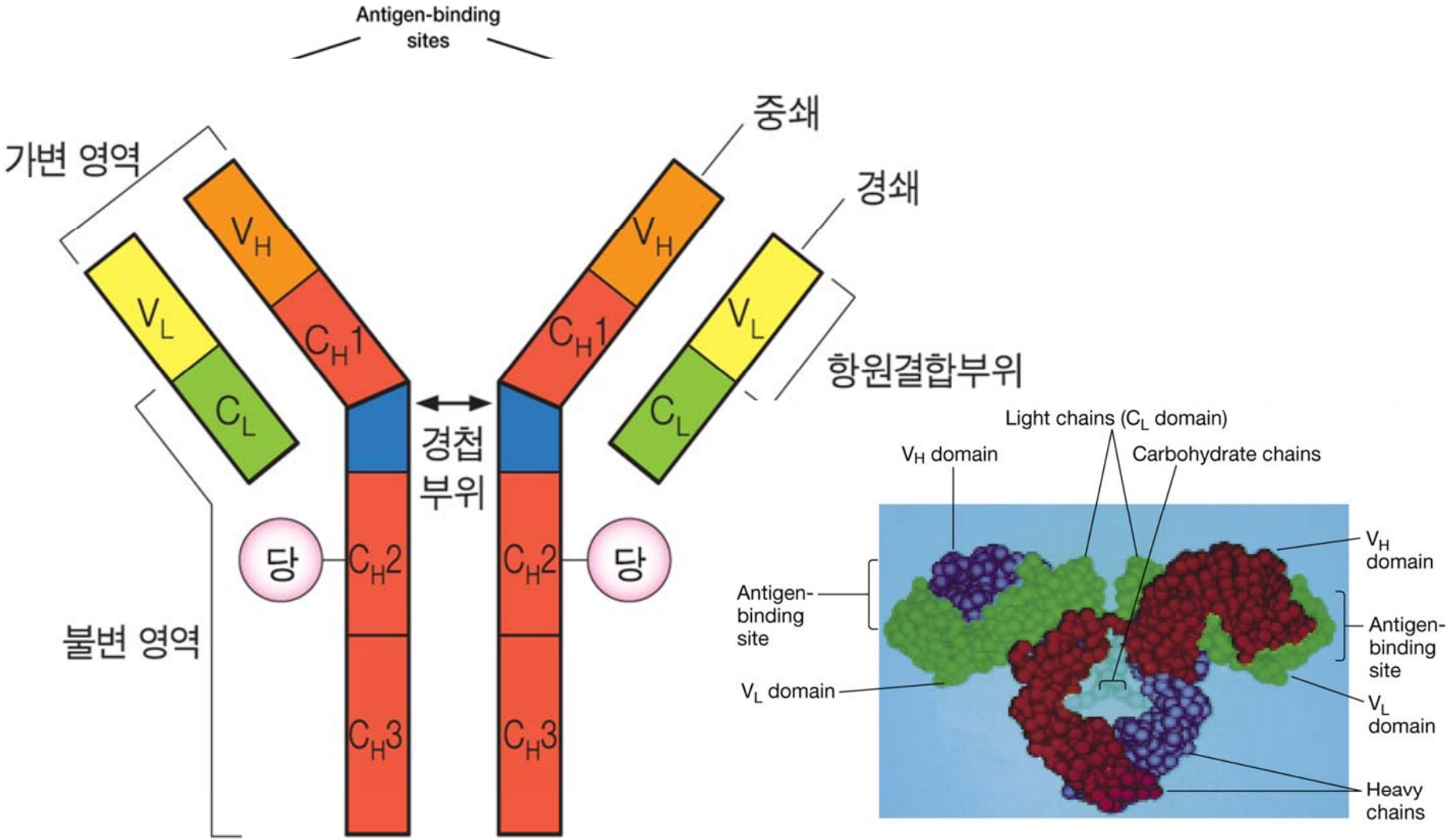
Immunoglobulin Structure



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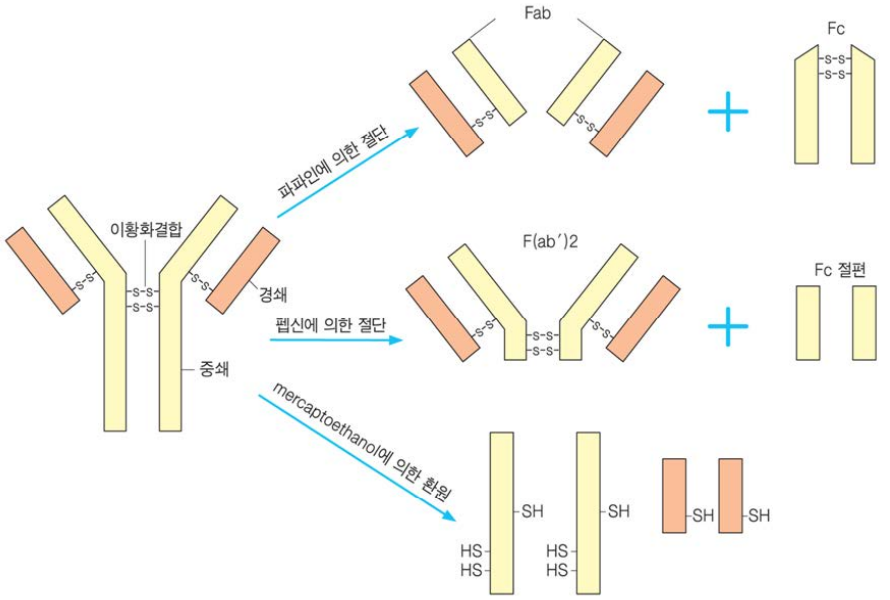
Immunoglobulin Function

- Fab binds antigen specifically
  - marks antigen for immunological attack
  - activates nonspecific defense mechanisms that can destroy antigen
    - e.g., opsonization for enhanced phagocytosis

- Fc mediates binding to:
  - host tissue
  - various cells of immune system
  - first component of complement system



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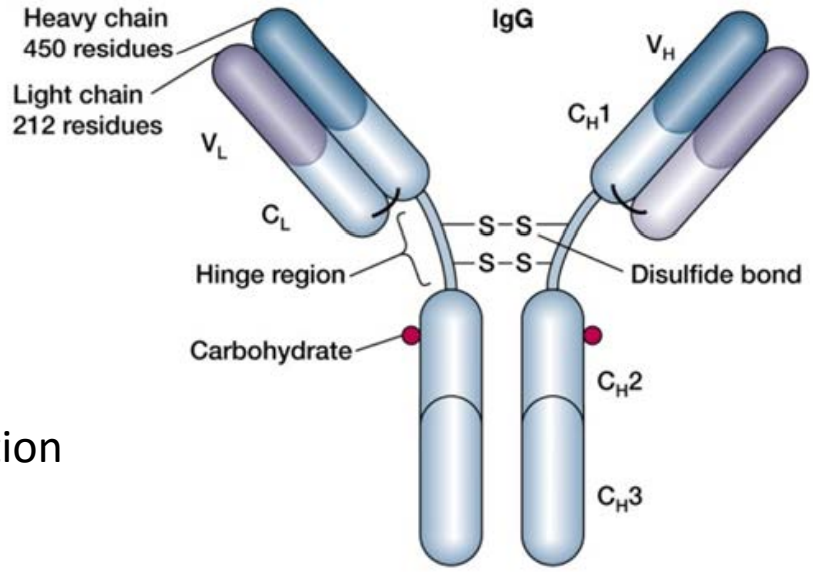
Immunoglobulin Classes

- IgG
  - **80%** of serum immunoglobulin
  - opsonization, neutralization, **complement activation**
  - **placenta transfer** for natural passive immunity to neonate
- IgA, secretory IgA (sIgA)
  - **monomers** and **dimers**
  - **secreted** across mucosal surfaces
  - tears, saliva, breast milk, MALT
- IgM
  - **pentamer** arranged in pinwheel
  - **first Ig** in all immune responses
  - **agglutination**, **complement activation**
- IgD
  - **part of the BCR complex**
  - signals B cells to start antibody production



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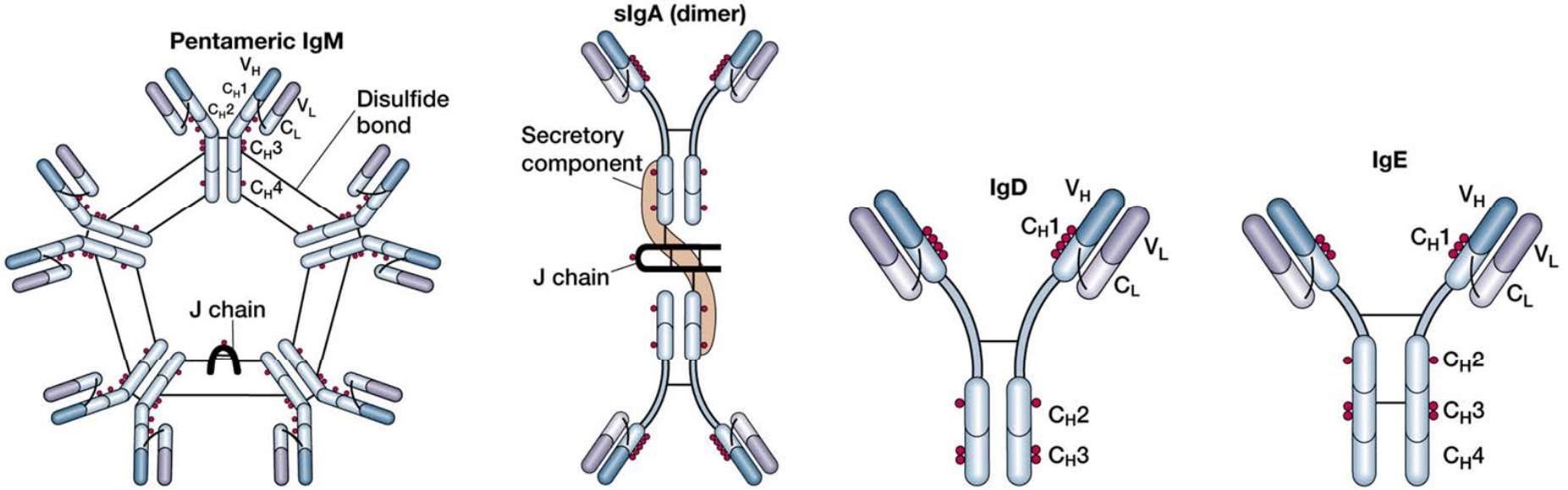
Immunoglobulin Classes

- IgE
  - lowest Ig serum level, elevated in parasitic infection and allergic reactions
  - opsonization (binds to receptors on dendritic cells and macrophages)
  - **mast cells** bind Fc portion and are activated to degranulate vasoactive granules when Fab portion binds allergens



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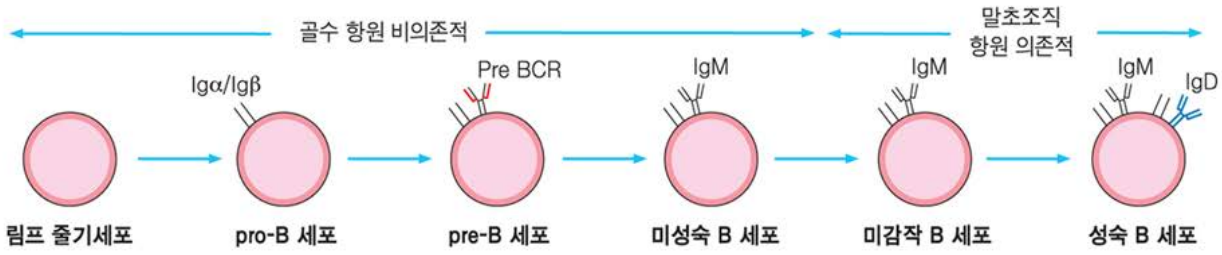
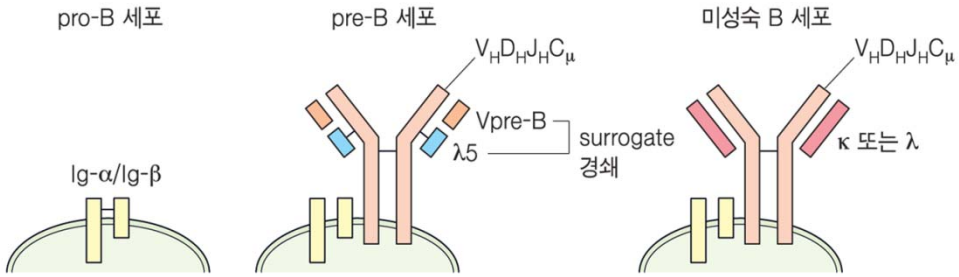
B Cell Differentiation



H 사슬 유전자	germ-line	D_HJ_H	$V_HD_HJ_H$			
L 사슬 유전자	germ-line	surrogate Vpre-B와 λ5 germ-line κ와 λ	surrogate Vpre-B와 λ5 germ-line κ와 λ	V_LJ_L		
RAG-1/2	−	+	+	−	−	−
TdT	−	+	−	−	−	−
세포막 Ig						
중쇄	−	−	μ			μ + δ
경쇄	−	surrogate 경쇄	surrogate 경쇄	κ와 λ		



B세포 분화 및 IgR 발현

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B Cell Activation

- immunological roles
  - proliferate and differentiate into plasma cells (which respond to antigens by producing antibodies) and memory cells
  - internalize antigen and act as antigen-presenting cells

✓ **B cell activation**

- leads to proliferation and differentiation into plasma cells
  - some cytokines produced by helper T cells can act on B cells and assist in growth and differentiation
- typically antigen-specific
- two mechanisms for antigen-specific activation
  - T (or thymus)-dependent (TD): 단백질
  - T-independent (TI): 중합체 (TI-1, 다당류, 당지질; TI-2, 핵산, 단백질중합체)

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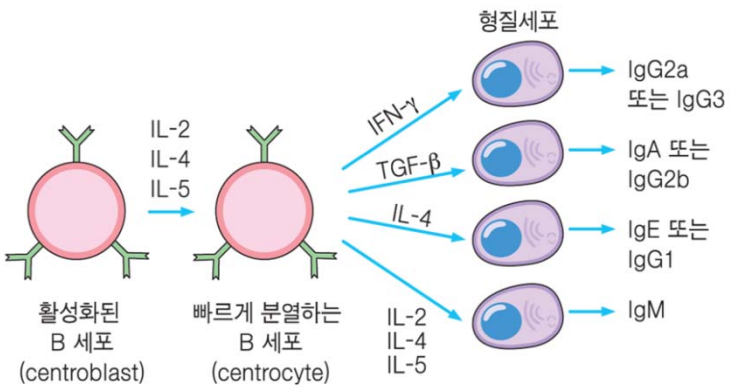
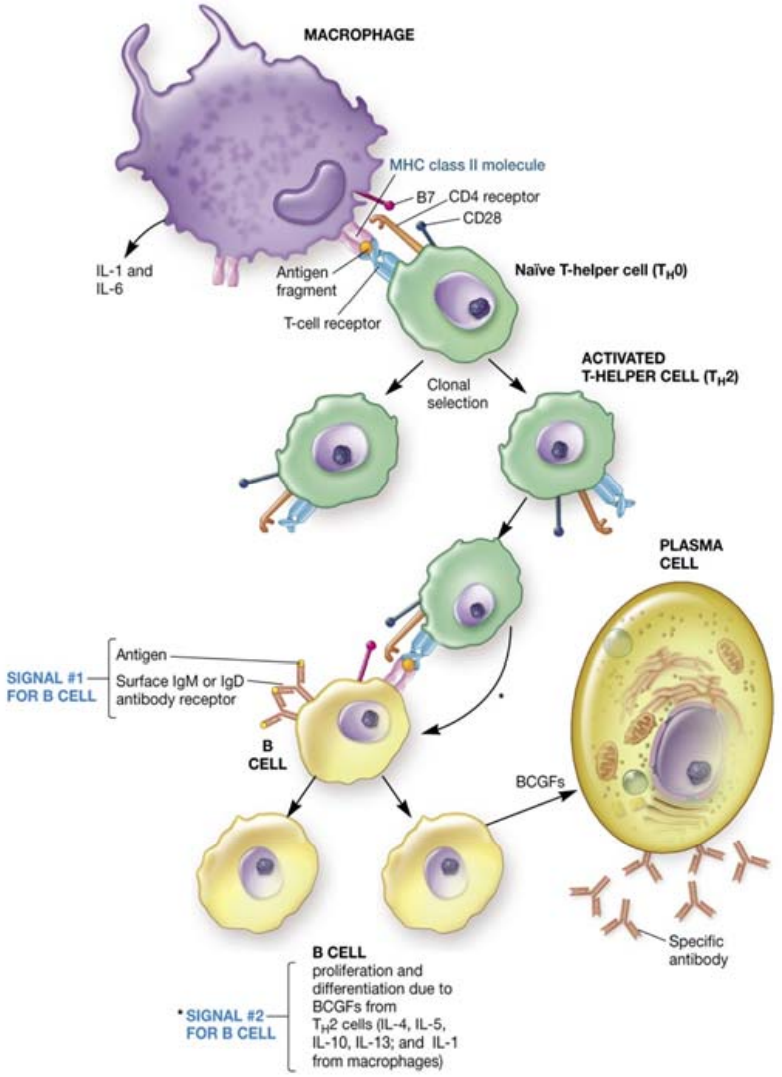
B Cell Activation by TD Antigens

- Two signals are required
  - BCR-Ag interaction
  - MHC/Ag-CD4 (Th2)
  - B7-CD28
  - CD40-CD40L: optional
- B cell differentiates into plasma cell and memory cell
- **Isotype**s are switched upon cytokines with CD40-CD40L interaction





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B Cell Activation by TD Ag

B) BCR-Ag interaction (**bridging**)

B) BCR-mediated endocytosis of Ag

B) B7 expression increased

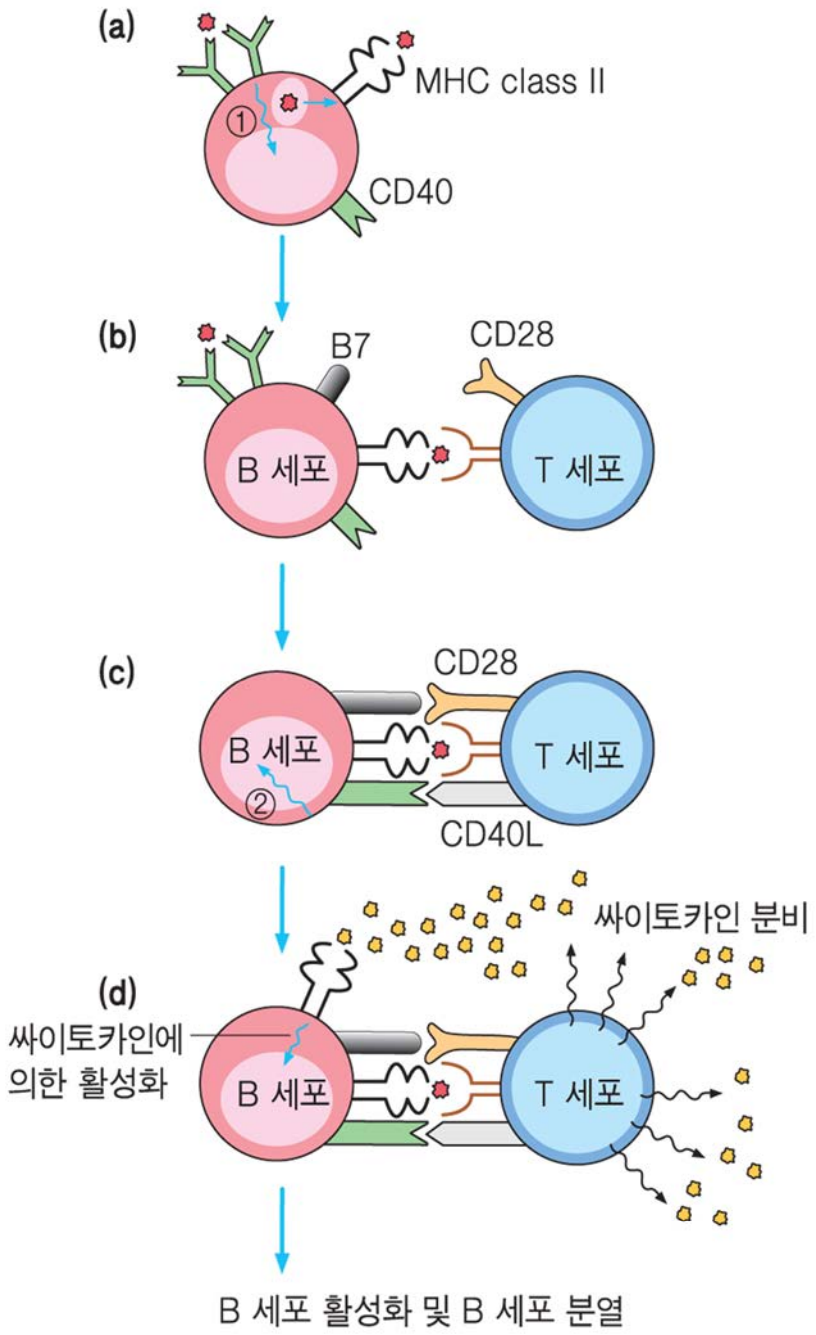
B) Endocytosed Ag presented on MHC II

- B7-CD28 interaction activates Th2

Th2) cytokine production and CD40L expression increased

- cytokines activate B

- CD40L-CD40 interaction mediates class switching



면역 반응의 조절: BCR, B-세포의 분화, 증식, 활성화

B Cell Activation by TI Antigens

- T-independent antigens (TI-1 and TI-2 antigens)
  - **polymeric** antigens with large number of identical epitopes (e.g. LPS, pilin)
  - LPS actually binds to TLR4 rather than BCR (thus, polyclonal B cell activator)
  - V regions can be bridged by a pilin/flagellin molecule

- less effective than T-dependent B cell activation
  - no affinity maturation
  - no memory B cells formed
  - no isotype switching observed

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Antibody Kinetics

- Antibody synthesis and secretion can also be evaluated as a function of time
  - monomeric IgM is the BCR for antigen whereas after B cell activation, pentameri c IgM is secreted (in plasma cells)
  - class/isotype switching

    change in Ab class secreted by plasma cells under the influence of Th2 cells

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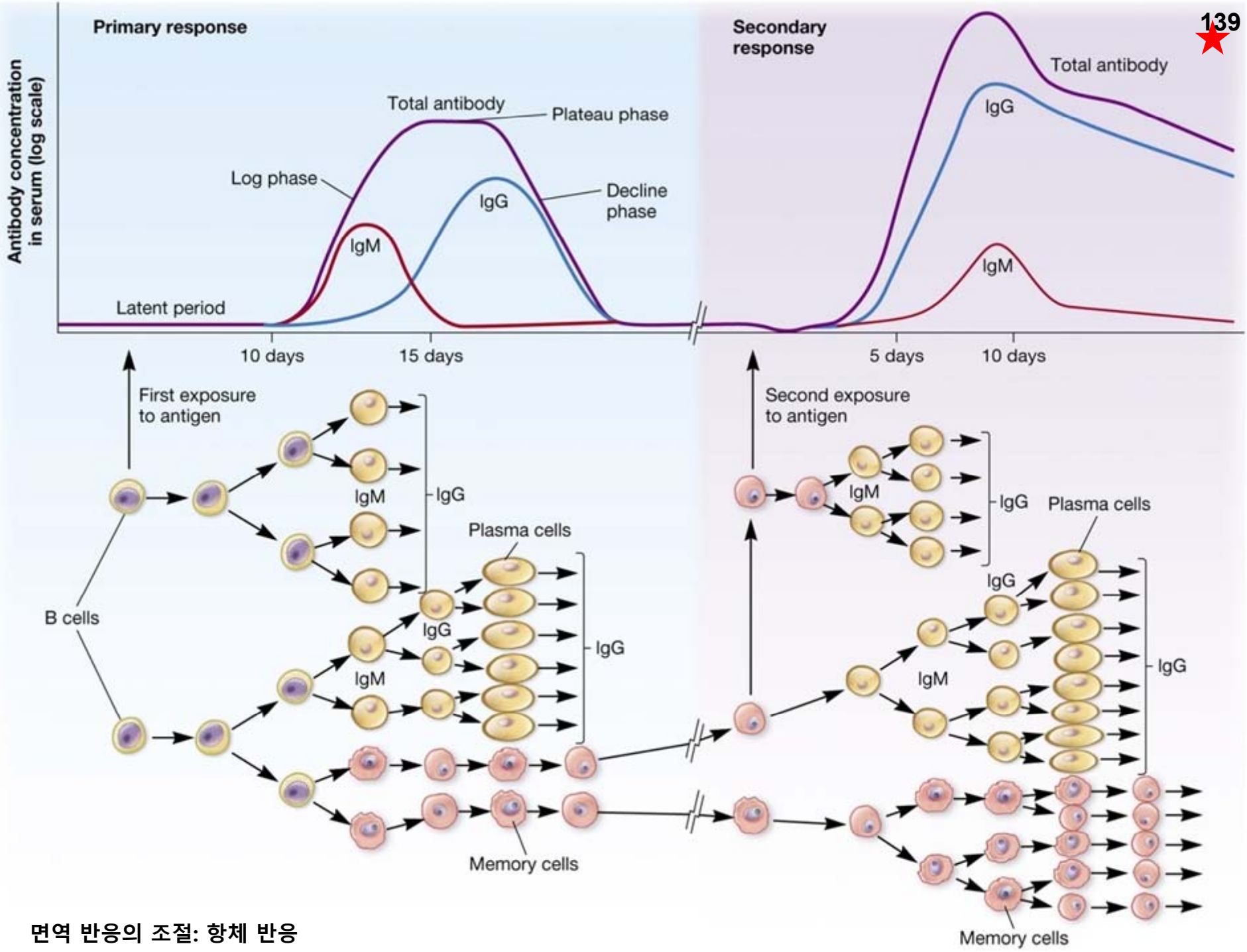

면역 반응의 조절: 항체 반응

Antibody Responses

✓ **Primary Ab response**

- several days to weeks lag or latent period after initial exposure to Ag
  - no antibody detectable in blood
- after B cell differentiation into plasma cells, antibody is secreted
- IgM appears first, followed by IgG

✓ **Secondary Ab response** (only to TD antigens)

- upon secondary exposure to the same Ag, B cells mount a heightened, **memory** response
- characterized as having a shorter lag, a more rapid log phase, longer persistence, a higher IgG titer and production of antibodies with a higher affinity for the antigen

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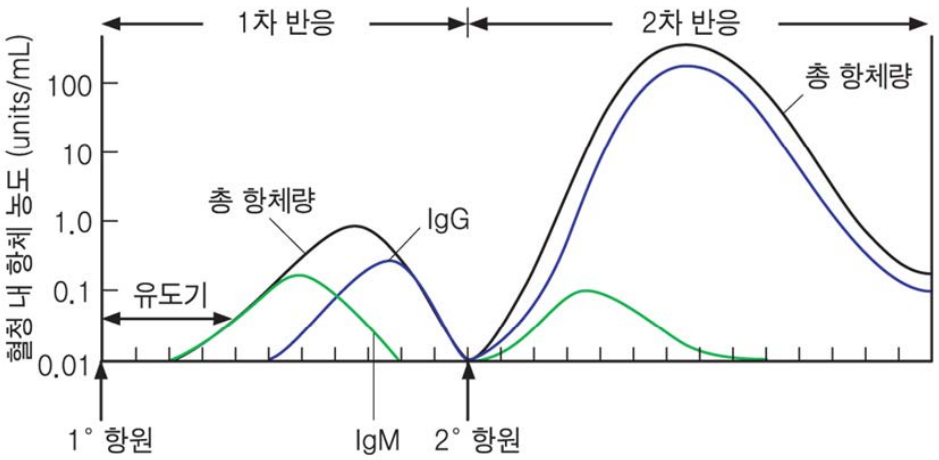
Antibody Responses



특 성	1차 반응	2차 반응
반응하는 B 세포의 종류	미감작 B 세포	기억 B 세포
항원 주입 후 반응이 나타나기 전까지 걸리는 지체 시간 (lag period)	4~7일	1~3일
정점에 이르는 시간	7~10일	3~5일
정점에 이를 때 항체의 양	항원에 따라 다름	1차 반응보다 100~1,000배 높음
항체의 종류	주로 IgM	주로 IgG
항원의 종류	흉선 의존 및 비의존	흉선 의존
항체의 친화력	낮음	높음

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Diversity of Antibodies

✓ **three mechanisms** contribute to generation of antibody diversity

- **combinatorial joining:** rearrangement of antibody gene segments
  genes are split or interrupted into many gene segments
- **splice site variability**: generation of different codons during the joining
  VJ joining can produce polypeptides with different amino acid sequences
- **somatic hypermutation**: exceptionally high mutation in V regions
  during an Ag challenge, V regions are susceptible to somatic mutation
  produce antibodies with different epitope recognition
  cf. affinity maturation

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Combinatorial Joining

- segments clustered separately on same chromosomes
  exons that code for constant regions
  exons that code for variable regions
- exons for constant region are joined (spliced together) to one segment of the variable region
- **RAG-1** & **RAG-2**: splicing enzymes
- occurs on heavy and light chains

Table 34.3	Theoretical Antibody Diversity Resulting from Combinatorial Joining of Germ-Line Genes[1]
λ light chains	V regions = 2 J regions = 3 Combinations = $2 \times 3 = 6$
κ light chains	V_κ regions = 250 − 350 J_κ regions = 4 Combinations = $250 \times 4 = 1{,}000$ $= 350 \times 4 = 1{,}400$
Heavy chains	V_H = 250 − 1,000 D = 10–30 $J_H = 4$ Combinations = $250 \times 10 \times 4 = 10{,}000$ $= 1{,}000 \times 30 \times 4 = 120{,}000$
Diversity of antibodies	κ-containing: $1{,}000 \times 10{,}000 = 10^7$ $1{,}400 \times 120{,}000 = 2 \times 10^8$ λ-containing: $6 \times 10{,}000 = 6 \times 10^4$ $6 \times 120{,}000 = 7 \times 10^5$

1 Approximate values.

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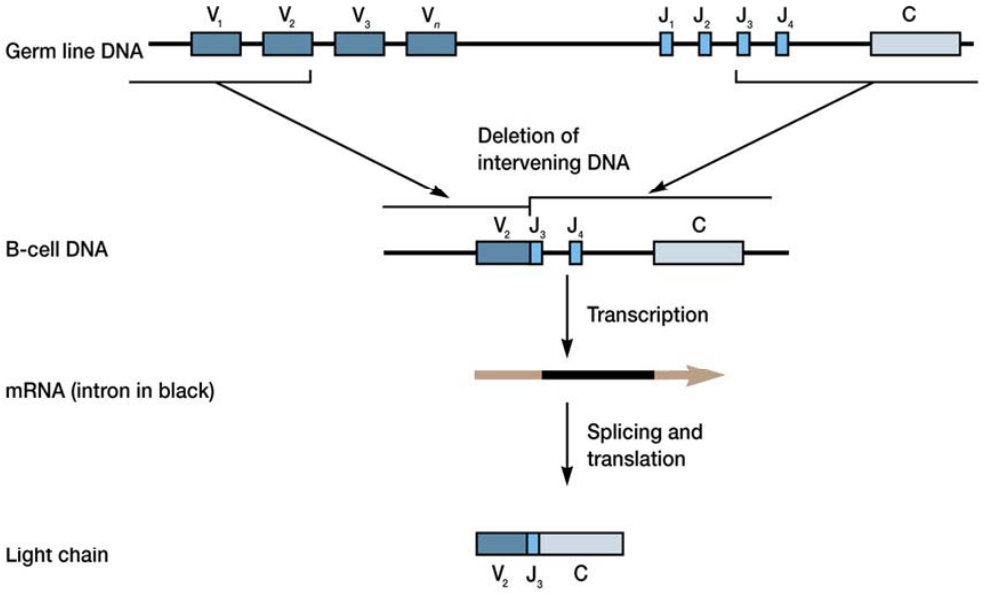
Light Chain

- germ line DNA for light chain contains multiple coding sequences, V and J (joining)
- in B cell development
  - one V is joined with one J region
  - many possible combinations formed
  - VJ joined with C (constant) exon



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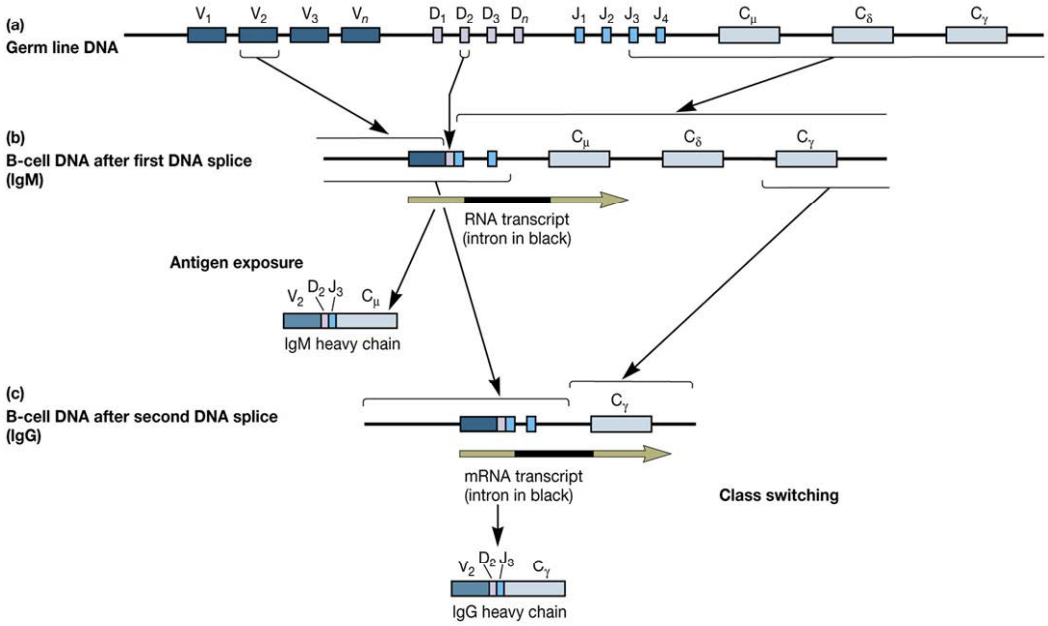
Heavy Chain

- V and J regions are joined to 3rd coding region called D (diversity) sequences
- VDJ joined to C region
- antibody class switch
  - initial C region results in IgM but changes as the immune response progresses and B cells proliferate



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Clonal Selection Theory

- body forms large, diverse B lymphocyte pool that can bind to large range of antigenic epitopes
- self-reactive cells are eliminated at an early stage of development (clonal deletion)
- encounter with antigen stimulates only those B cells that recognize and bind antigen
- stimulated B cells proliferate to produce B cell clone (all have same antigen specificity)
- B cell clone differentiates to form two cell populations
  - plasma cells and memory B cells

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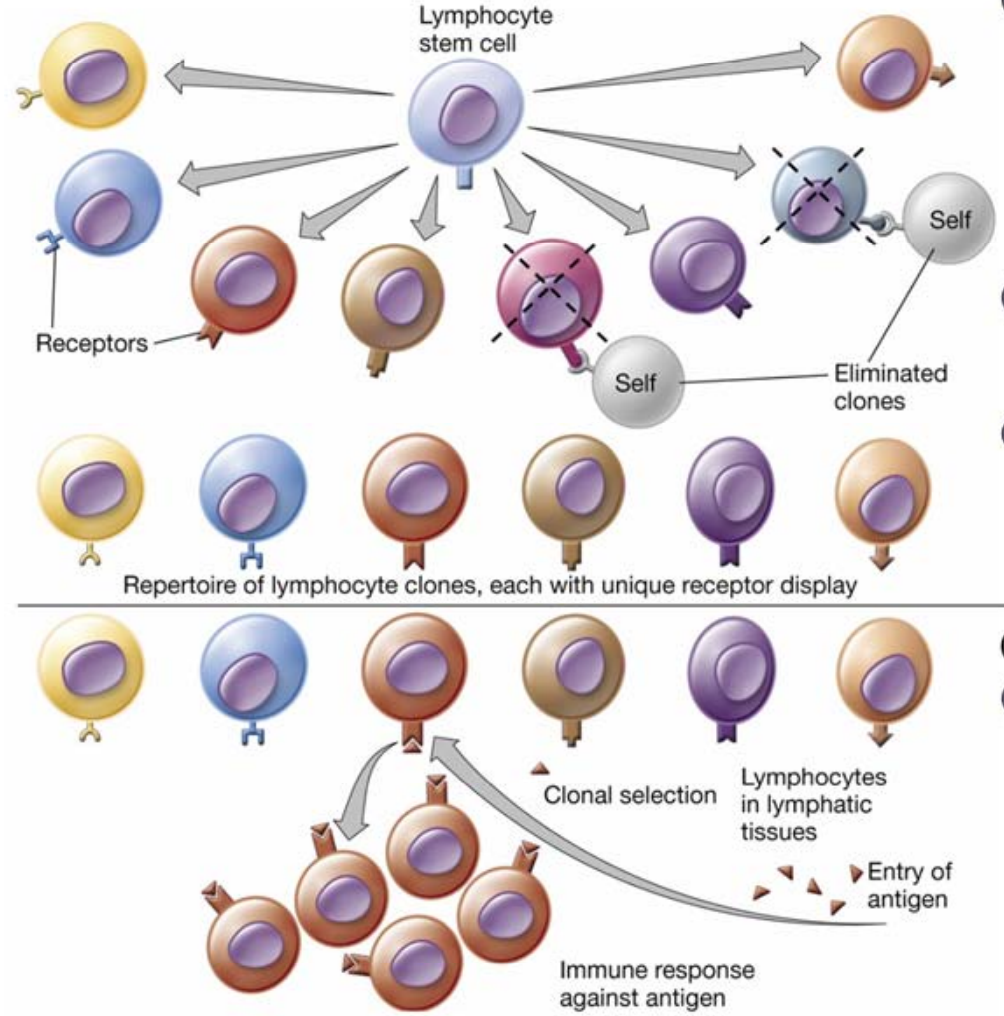
Clonal Selection Theory



(a) Antigen-Independent Period

1. During development of early lymphocytes from stem cells, a given stem cell undergoes rapid cell division to form numerous progeny.

   During this period of cell differentiation, random rearrangements of the genes that code for cell surface protein receptors occur. The result is a large array of genetically distinct cells, called clones, each clone bearing a different receptor that is specific to react with only a single type of foreign molecule or antigen.

2. At the same time, any lymphocyte clones that have a specificity for self molecules and could be harmful are eliminated from the pool of diversity. This is called immune tolerance.

3. The specificity for a single antigen molecule is programmed into the lymphocyte and is set for the life of a given clone. The end result is an enormous pool of mature but naïve lymphocytes that are ready to further differentiate under the influence of certain organs and immune stimuli.

(b) Antigen-Dependent Period

4. Lymphocytes come to populate the lymphatic organs, where they will finally encounter antigens. These antigens will become the stimulus for the lymphocytes' final activation and immune function. Entry of a specific antigen selects only the lymphocyte clone or clones that carry matching surface receptors. This will trigger an immune response, which varies according to the type of lymphocyte involved.

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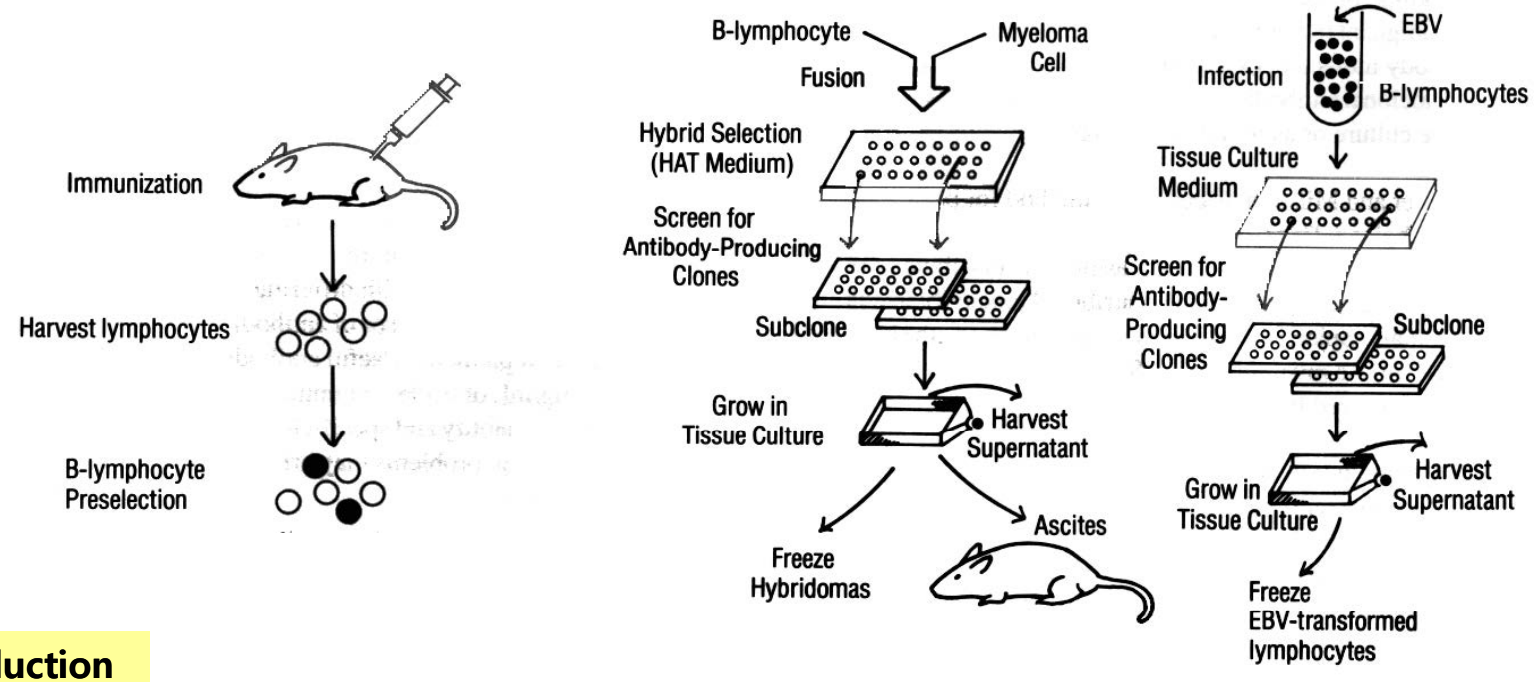
Monoclonal Antibody Technology

- hybridomas
  - overcome some of limitations of antisera as a source of antibodies
  - used to produce monoclonal antibodies (mAb) recognize one epitope
- potential for numerous biomedical applications
  - most of current applications involve in vitro diagnostic testing and research



mAb production

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Action of Antibodies

- bind antigens with great specificity
  - can occur within animal body (in vivo)
    - essential for the protection of animal from viruses, microbes, and cancer cells
- antibody coats foreign invading material
  - marks it for recognition by components of the innate and adaptive immune systems
  - neutralization, opsonization, and immune complex formation

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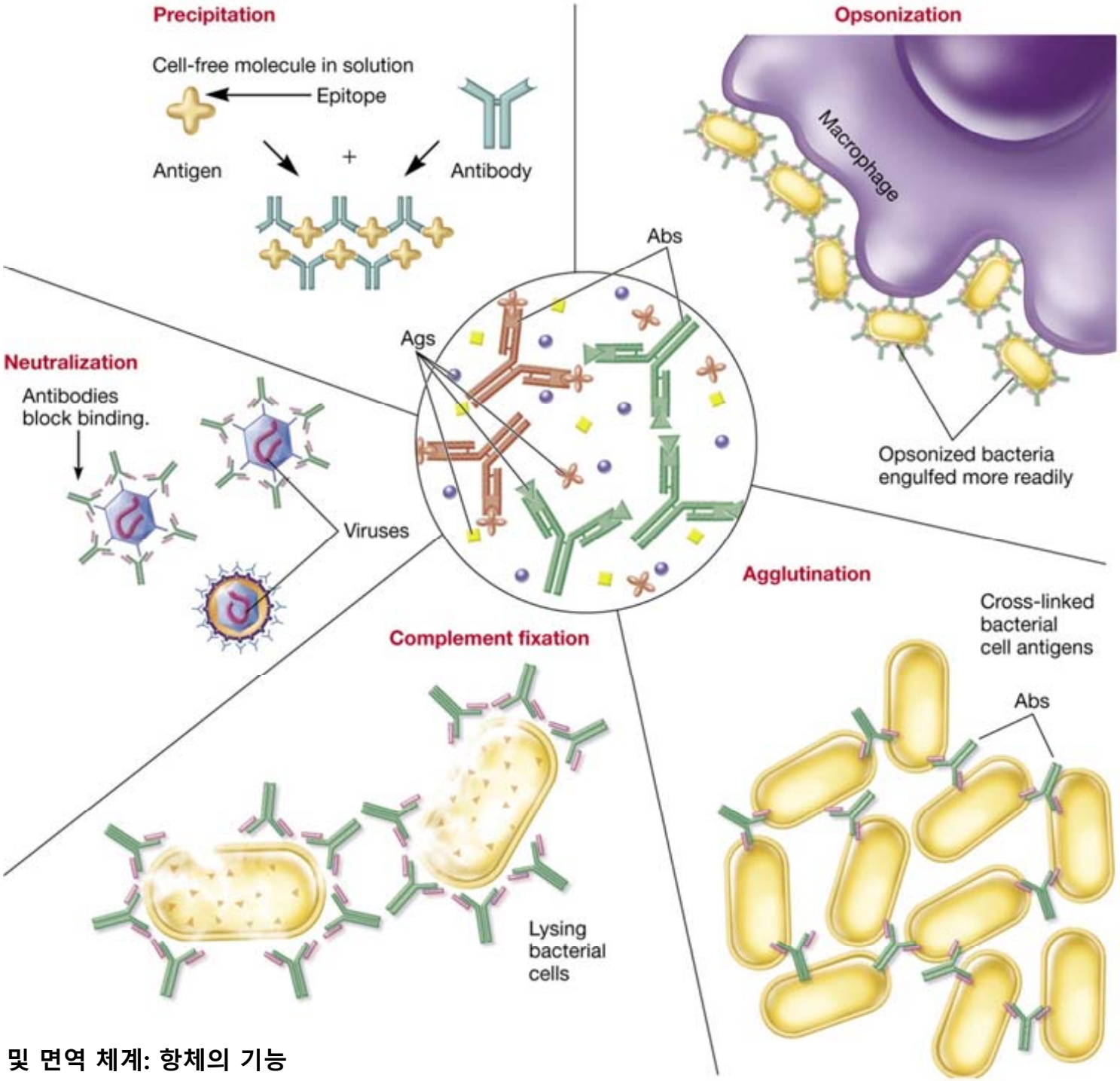

면역 반응 및 면역 체계: 항체의 기능

Immune Complex Formation

- antigens and antibodies can crosslink, producing immune complexes
- **agglutination** reaction occurs when cells or particles are cross-linked
  - the immune complex formed is more readily phagocytosed in vivo than are free antigens
  - caused by agglutinin antibodies
- **precipitation** (precipitin) reaction occurs when antigens are soluble molecules and the immune complex settles out of solution
  - caused by precipitin antibody
- antibody:antigen ratio is in equivalence zone when their concentration is optimal for formation of the immune complex

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Neutralization

✓ **Toxins**

- inactivation of toxins resulting from interaction between toxin and specific antitoxin antibodies
- complexing toxin with antibodies
  - can prevent the toxin from attaching to host cells
  - can prevent toxin from entering host cells
  - can result in ingestion by macrophates

✓ **Viruses**

- IgG, IgM, and IgA antibodies can bind to some extracellular viruses and inactivate them
- fixation of complement component C3b, from classical complement pathway, helps in the neutralization process
- viral infection is prevented because neutralization of viruses prevents them from binding and entering target cells

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Opsonization

- microorganisms or other foreign particles become coated with antibodies and/or complement
- opsonizing antibodies bind Fc receptors on macrophages and neutrophils, creating bridge between phagocyte and antigen

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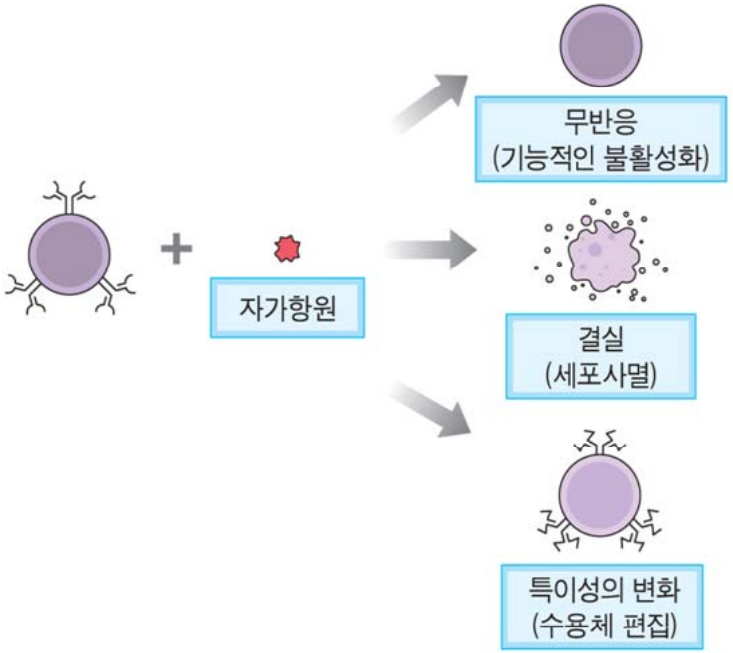
Immunological Tolerance

- 면역관용이란?
  한 번 노출되었던 항원에 다시 노출되었을 때 면역반응이 일어나지 않는 현상
- Tolerogen: 면역관용을 유발하는 항원 (모든 자가항원 + 일부 외래항원)
- Self-tolerance: 자가항원에 대한 면역관용
  - Central tolerance
  - Peripheral tolerance

- 관용을 촉진하는 요인
  많은 양의 항원
  항원의 숙주내 지속성
  정맥주사나 경구 투여
  <u>면역증진활성 부족</u>
  - **adjuvant** (항원보강제) activity
  - co-stimulation activity



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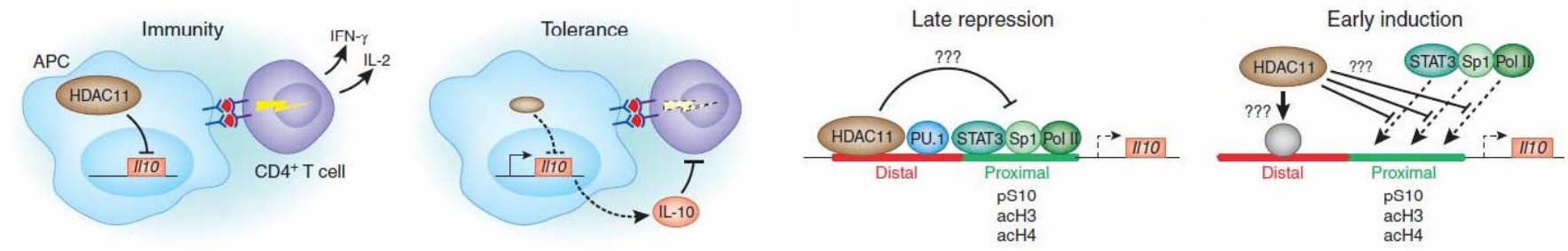
Immunological Tolerance

표 16-1 단백질 항원의 면역원성 관용원성을 결정하는 요인

요 인	면역반응을 자극하기 쉬운 요인	관용을 일으키기 쉬운 요인
양	각기 다른 항원에 따라 다양한 적정 농도	고농도
저항성	짧은 생존기간(면역반응에 의해 제거됨)	지속성
침입 부위	피하, 표피 아래, 생성기관에 없음	혈관 내, 구강, 생성기관 내 존재
항원보강제의 존재	항원보강제와 함께 하는 항원: 보조 T 세포를 자극함	항원보강제 없는 항원: 비면역 유발원 또는 면역관용원
항원제시세포의 특성	다량의 공동자극분자	소량의 공동자극분자 및 싸이토카인



IL-10 유전자 발현의 derepression과 면역관용 (HDAC11의 역할)

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Vaccine: Prevention from Infection

표 14-1 병원균 감염에 의한 전 세계 5세 미만의 영유아 사망자 수

원인균 및 질병	영유아 사망자 수 (단위: 천 명)
폐렴구균	841
홍역	530
헤모필루스 인플루엔자	945
로타바이러스	800
말라리아	700
인간면역결핍바이러스 (HIV)	500
백일해	285
파상풍	201
결핵	100

출처: 세계보건기구(WHO)에서 발표된 통계결과.

표 14-2 수동 및 능동면역의 획득

면역 형태	획득 방법
수동면역	모유
	면역글로빈
	인간화 단클론항체
	항독소
능동면역	자연감염
	백신접종:
	- 약독화균주
	- 불활성화 균주
	- 정제된 균주의 거대물질
	- 균주에서 분리된 항원:
	재조합법으로 발현된 단백질
	DNA 자체 또는 바이러스벡터에 삽입된 DNA
	다가복합체
	- 독소

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Vaccination Program in Korea

표 14-3 한국 표준 예방접종 일정표

	대상 전염병	예방접종 시기
국가필수예방접종*	결핵(BCG, 피내용)	생후 4주 이내
	B형 간염	임신부가 HBsAg 양성인 경우에는 출생 후 12시간 이내에 백신과 B형 간염인 면역글로불린(HBIG)을 동시에 주사하고, 음성인 경우에도 생후 2개월 이내에 첫 접종을 시작함. 이후의 접종 일정은 약품설명서에 기재된 접종방법 대로 실시함.
	디프테리아(D)/백일해(P)/파상풍(T); DPaT(= DPT)	DPaT 혼합백신은 총 5회(생후 2, 4, 6, 15~18개월, 4~6세) 접종하며, 11~12세에 백일해를 제외한 Td로 1차 접종
	소아마비(IPV)	모두 3회(생후 2, 4, 6개월) 접종하며, 3차 접종은 생후 18개월 이내에 접종도 가능
	홍역(M)/유행성이하선염(M)/풍진(R); MMR	홍역 유행 시, 생후 6개월에 홍역 단독 백신(또는 MMR)으로 조기접종하며, 이 경우 생후 12개월에 다시 MMR로 접종한다.
	수두	생후 12~15개월 이내
	일본뇌염	일본뇌염(사균백신) 기초접종은 3회(1~2주 간격으로 2회 접종한 다음 12개월 후 3차 접종), 만 6세와 만 12세에 각 1회 추가접종
	Td(파상풍/디프테리아)	만 11~12세
기타 예방접종†	장티푸스	추정위험군에서 선별접종하며, 접종대상자의 경우 경구용 생균백신은 격일로 3~4회(6세 이상), 주사용은 1회 근육주사(2세 이상)
	인플루엔자	인플루엔자 고위험군을 대상으로 우선접종을 권장(6개월~9세 미만 처음 접종할 경우 1개월 간격으로 2회 접종, 이후 매년 1회 접종)
	B형 헤모필루스(Hib), 뇌수막염	접종시기가 매우 다양함. (접종에 대한 견해가 상반됨)
	A형 간염	만 1~16세에 1회 기초접종, 기초접종 6~12개월 후 추가접종
	폐렴구균	생후 12~15개월 이내
	신증후군 출혈열	고위험군을 대상으로 선별접종하며, 접종대상자의 경우 1개월 간격으로 2회 접종 후 12개월 후 3차 접종

* 국가가 권장하는 예방접종 8종류(국가는 전염병 예방접종을 통해 예방접종대상 전염병과 예방접종의 실시기준 및 방법을 정하고 국민과 의료인들에게 이를 준수토록 하고 있음).
† 국가필수예방접종 이외 민간의료기관에서 접종 가능한 예방접종.

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일본뇌염: 생백신 vs 사백신 비교

- **국내 백신의 종류**

백신균주	백신 종류	제조(수입)사	국내허가	국내도입
Nakayama	쥐뇌조직배양 (사백신)	녹십자/보령	1971	1971
SA-14-14-2	BHK세포배양 (생백신)	글로박스	2002	2014.2.10
Beijing-1	Vero세포배양 (사백신)	녹십자/보령	2014	2015.3.1

- **접종 시기** (cf. 6~12 개월은 면역력 있음)

접종 차수	접종 시기	사백신	생백신
1차 기초접종	12~24개월	○	○
2차 기초접종	1차 접종 후 1~2주	○	
3차 기초접종	2차 접종 후 12 개월	○	○
4차 추가접종	만 6세	○	유행시 선택
5차 추가접종	만 12세	○	

백신의 종류 및 실제 (바이오의약품학에서 상세히 배움)

Types of Vaccines

- *Types of vaccines*
  - 전균 (**WCV. whole cell vaccine**): 약독화생백신, 불활화사백신
  - 정제항원 (**ACV. acellular vaccine**): 변독소 (toxoid), **서브유닛백신**, 접합백신
  - 기타 (임상시험 단계): 벡터백신 (=키메라백신) , 핵산 백신

- **(약독화)생백신**: 강력한 효과; 낮은 안전성 (잠재위험성, 냉장보관필수)
  : 천연두, 홍역, 유행성이하선염, 풍진, **소아마비 (Sabin)**, **결핵 (BCG)**, 수두 etc.
- **(불활화)사백신**: 비교적 높은 안전성, cost-effective; 추가 접종필요
  : 콜레라, A형간염, 인플루엔자, 페스트, **소아마비 (Salk)**
- **변독소, 서브유닛백신**: 높은 안전성; 적절한 효과
  : 디프테리아, 파상풍, 백일해, B형간염 (HBsAg), 폐렴구균
- **접합백신**: 다당체항원에 대한 백신. 단백질과의 접합
  : Hib 백신 (Hib capsule + 파상풍독소), 진균백신 (Phosphomannan + BSA)
- **벡터백신**: 항원유전자를 발현시켜 항원성을 높인 생백신
  : 약독화 *Salmonella* 벡터: 소화기관 **점막감염을** 통한 sIgA의 분비기능이 탁월

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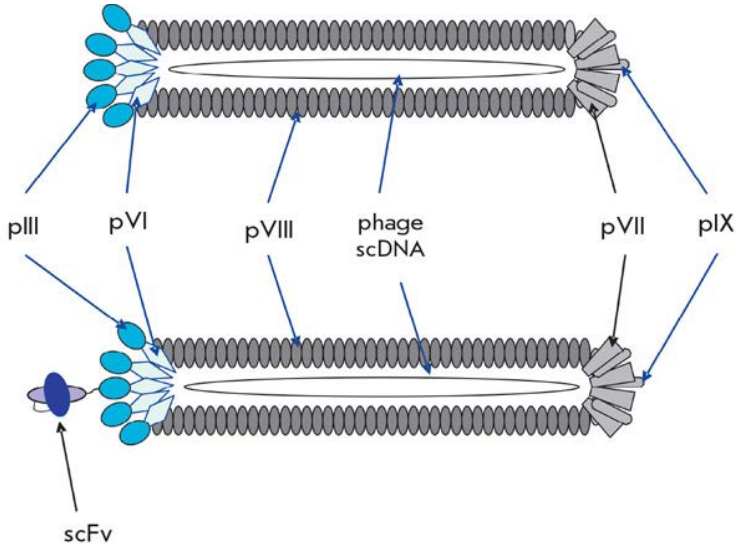
Nucleic Acid Vaccines

- DNA 백신이란?

  항원 유전자를 플라스미드에 삽입하여 제형화
  근육주사로 DNA를 주입하여 (gene gun 이용), 항원 발현을 유도
  접종경로에 따라 DNA 안정성 정도, 유전자 발현 정도 등이 달라질 수 있음

- DNA 백신의 장점

  항원 상태 완벽 재현 (세포 내에서 발현)
  약독화생백신 수준의 강력한 효과,
  but 높은 안정성, 냉장보관 불필요
  - 단백질항원: SARS DNA 백신 실험 성공
  - 탄수화물항원: peptide로 탄수화물 모방
    (phage-display-peptide library. PDPL)



- DNA 백신의 문제점? 모니터링?
- RNA 백신?

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Adjuvants

- **IFA and CFA by Jules T. Freund**

  표면활성제, 유동파라핀 등으로 항원을 유제화하여 동물의 생체 내에 주사하여 더 강한 면역반응을 유도함 (1947). *Mycobacterium* (usu. Mtb)의 사균을 추가하면 더 강한 면역반응이 유도됨.

  - Freund's incomplete adjuvant (FIA or IFA): 유제화된 항원의 emulsion
  - Freund's complete adjuvant (FCA or CFA): IFA에 Mtb 추가

- An adjuvant is a pharmacological or immunological agent that modifies the effect of other agents, such as a drug or vaccine.
- It has few direct effects when given alone.
- Adjuvants are often included in vaccines (why?)

  usu. emulsification (제형) / PRR ligands (추가항원) or both

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Examples of Vaccine Adjuvants

Vaccine Adjuvants Products

Alum and Emulsions		
AddaVax™	Vaccine Adjuvant: Squalene-Oil-in-water	2 ml 5 x 2 ml
Alhydrogel 2%	Vaccine Adjuvant: Aluminium hydroxide gel	50 ml 250 ml
IFA	Vaccine Adjuvant: Incomplete Freund's adjuvant Water-in-oil	10 ml 6 x 10 ml
PRR Ligands		
Flagellin FliC VacciGrade™	TLR5 agonist - Recombinant flagellin from *S. typhimurium*	50 µg
Gardiquimod VacciGrade™	TLR7 agonist - Imidazoquinoline compound	5 mg
Imiquimod VacciGrade™	TLR7 agonist - Imidazoquinoline compound	5 mg
MPLA VacciGrade™	TLR4 agonist - Monophosphoryl Lipid A	1 mg
N-Glycolyl-MDP VacciGrade™	NOD2 agonist - N-glycolyted muramyldipeptide	5 mg
ODN 1826 VacciGrade™	TLR9 agonist - CpG ODN, type B (murine)	1 mg
ODN 2006 Vaccigrade	TLR9 agonist - CpG ODN, type B (human)	1 mg
Poly(I:C) VacciGrade™	TLR3 agonist - Polyinosine-polycytidylic acid	10 mg
R848 VacciGrade™	TLR7/8 agonist - Imidazoquinoline compound	5 mg

http://www.invivogen.com/vaccine-adjuvants

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Immune Disorders

- hypersensitivities (과민반응)
- autoimmune diseases (자가면역 질환)
- transplantation (tissue) rejection (이식 거부 반응)
- Immunodeficiencies (면역결핍증)

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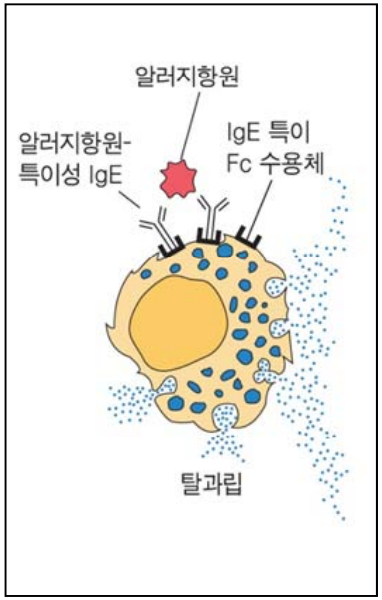
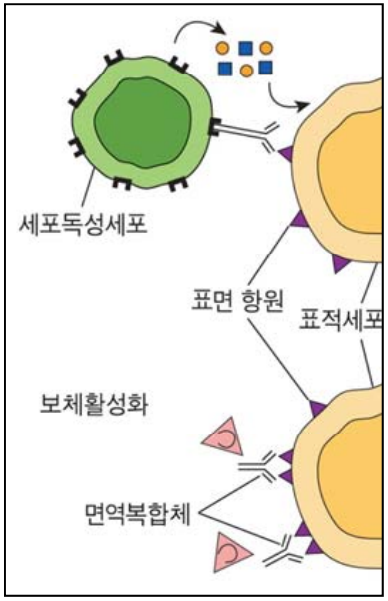
Hypersensitivities

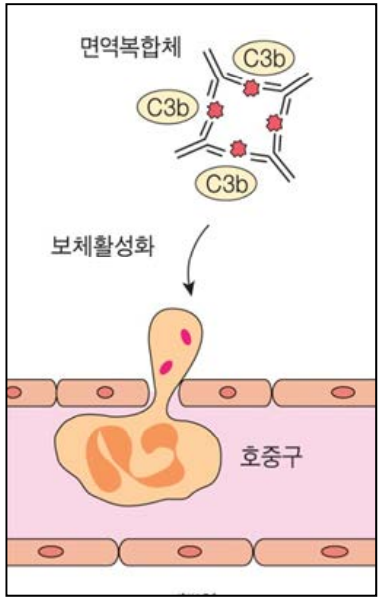
- exaggerated immune response upon second or subsequent contact with antigen
- causes tissue damage
- reactions classified as **immediate** or **delayed**
- Gell-Coombs classification four different types: I, II, III, and IV



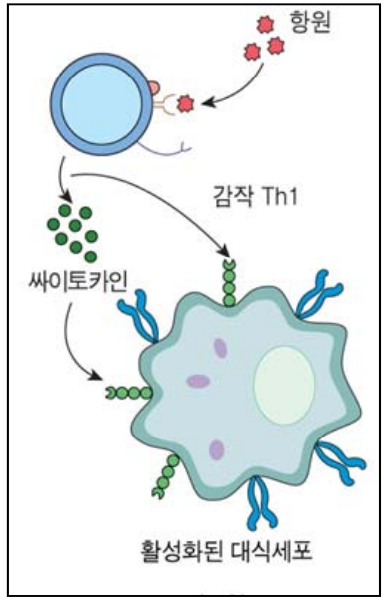
IgE-매개
즉시 과민반응



IgG/IgM-매개
세포독성 과민반응



면역복합체-매개
과민반응



지연형 과민반응

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Type I Hypersensitivity

- allergy
  - one kind of Type I hypersensitivity
- allergen
  - antigen that causes allergic reaction
- occurs immediately following second contact with allergen
- involves production and action of **IgE** (sometimes called reagin) and **mast cells**, and sometimes basophils or eosinophils

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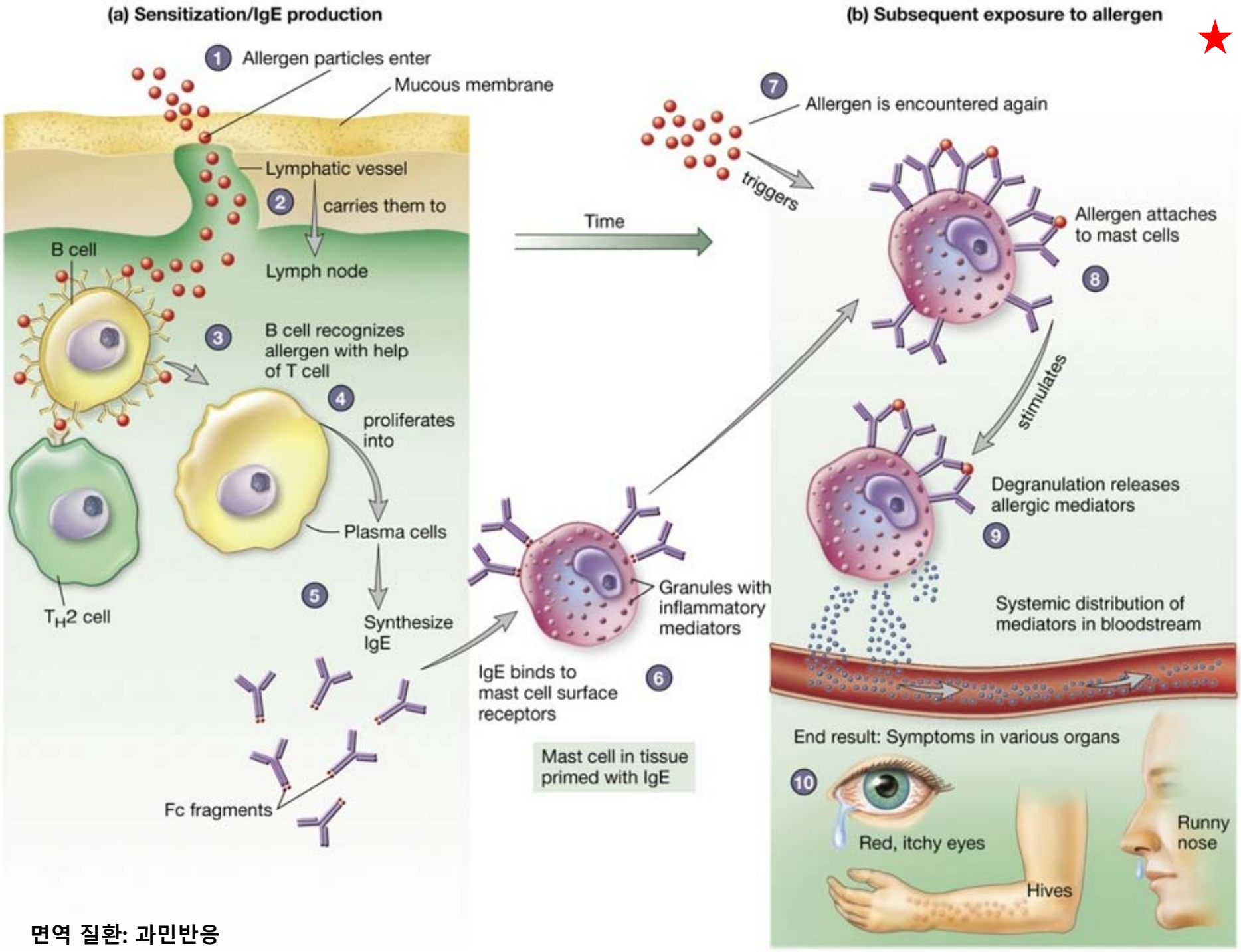
(a) Sensitization/IgE production
(b) Subsequent exposure to allergen
1 Allergen particles enter
Mucous membrane
Lymphatic vessel
2 carries them to
Lymph node
B cell
3 B cell recognizes allergen with help of T cell
4 proliferates into
Plasma cells
5 Synthesize IgE
T_H2 cell
Fc fragments
IgE binds to mast cell surface receptors
6
Granules with inflammatory mediators
Mast cell in tissue primed with IgE
Time
7 Allergen is encountered again
triggers
Allergen attaches to mast cells
8
stimulates
Degranulation releases allergic mediators
9
Systemic distribution of mediators in bloodstream
End result: Symptoms in various organs
10
Red, itchy eyes
Runny nose
Hives

면역 질환: 과민반응

Anaphylaxis

phylaxis:

aphylaxis:

prophylaxis:

anaphylaxis?

- release of physiological mediators in response to allergen cause
  - smooth muscle contraction
  - vasodilation
  - increased vascular permeability
  - mucous secretion
- can be systemic or localized

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Systemic Anaphylaxis

- results from **massive release of mast cell mediators** in a short time
- usually results in **respiratory impairment**, **decreased blood pressure**, and **circulatory shock**
- can cause death within a few minutes

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Localized Anaphylaxis

- an atopic ("out of place") reaction
  - symptoms depend on route by which allergen enters body
- hay fever
  - upper respiratory tract
- bronchial **asthma**
  - lower respiratory tract
- hives
  - skin
  - common with true food allergies

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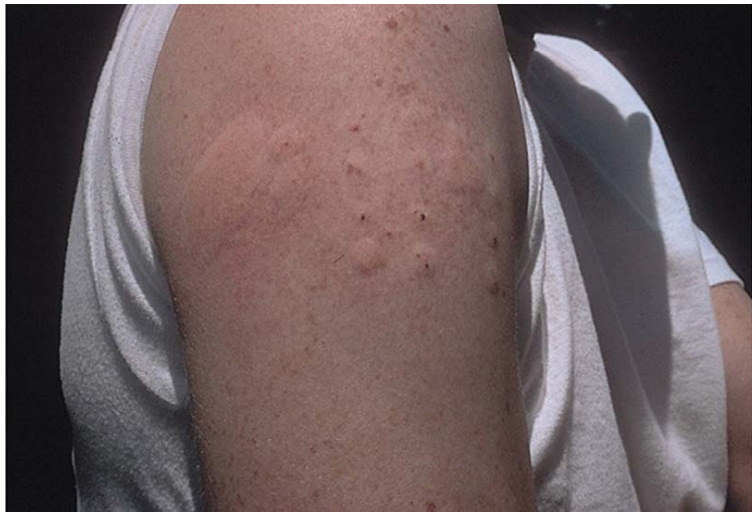
Diagnosis and Treatment

- diagnosed by skin tests
  - inoculation of small amounts of allergens into skin
  - rapid inflammatory reaction characterized by redness, swelling, and itching



In vivo skin testing

- desensitization
  - controlled exposure to allergen
  - stimulates IgG production
    - intercept and neutralize allergen before it binds to IgE-bound mast cells

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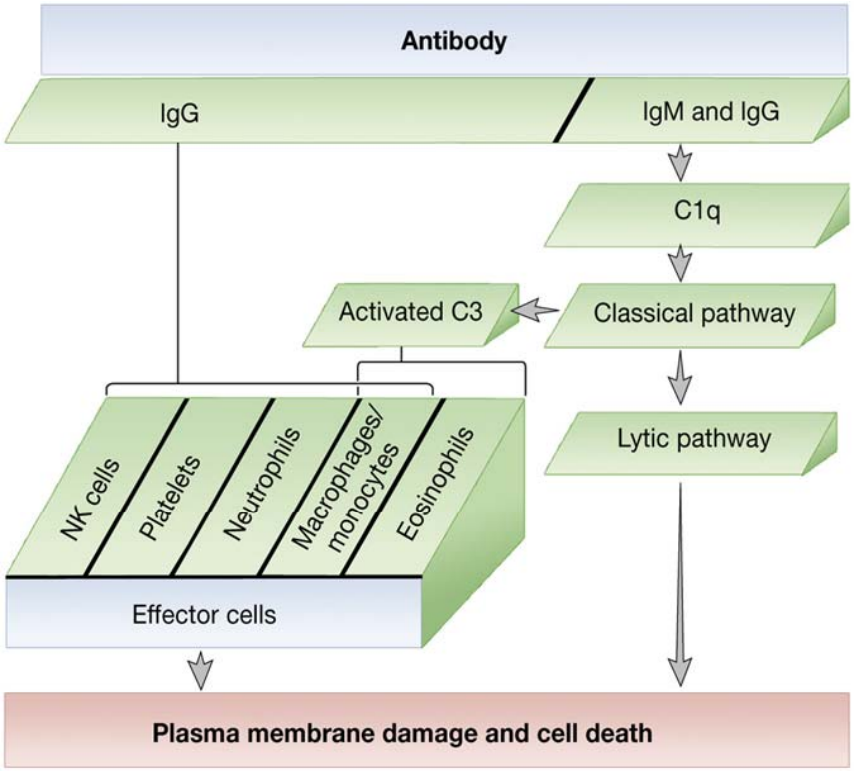
Type II Hypersensitivity

- cytolytic or cytotoxic reaction
- involves **IgG** and **IgM** antibodies
  - directed against cell surface or tissue antigens
  - stimulate complement pathway and effector cells



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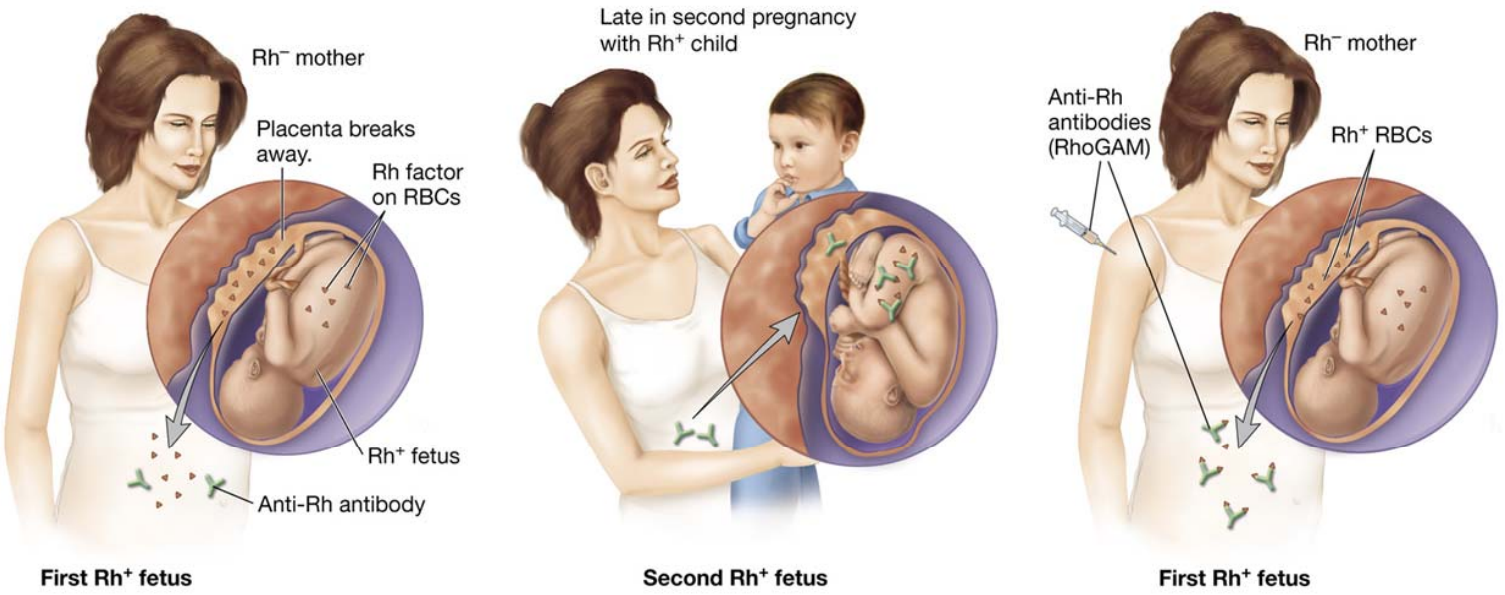
Type II Hypersensitivity: Examples

- **blood transfusion** reaction in which donated blood cells are attacked by recipient's antibodies
- **erythroblastosis fetalis**
  - mother can be passively immunized with anti-Rh factor antibodies or RhoGam to control this disease which is potentially fatal for newborn



Rh Incompatibility and RBC lysis

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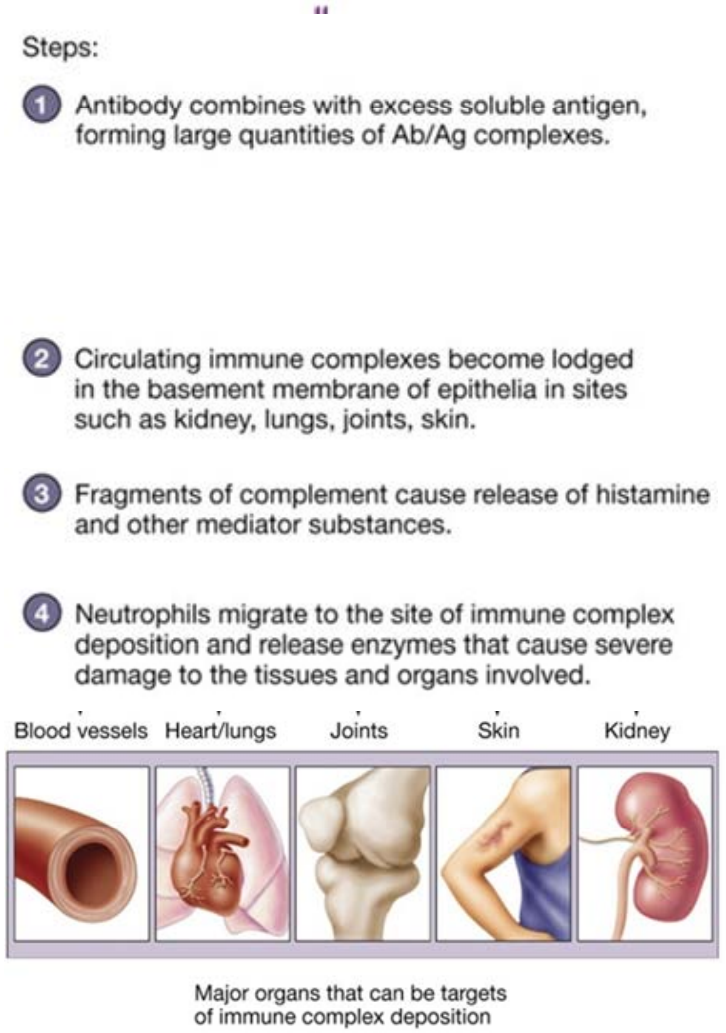
Type III Hypersensitivity

- involves formation of **immune complexes**
  - usually removed by monocytes and macrophages
  - if accumulate, leads to hypersensitivity reaction
    - resulting inflammation causes tissue damage

- examples
  - vasculitis (혈관염)
  - glomerulonephritis (사구체신염)
  - arthritis (관절염)
  - systemic lupus erythematosis (SLE)



Major organs that can be targets of immune complex deposition

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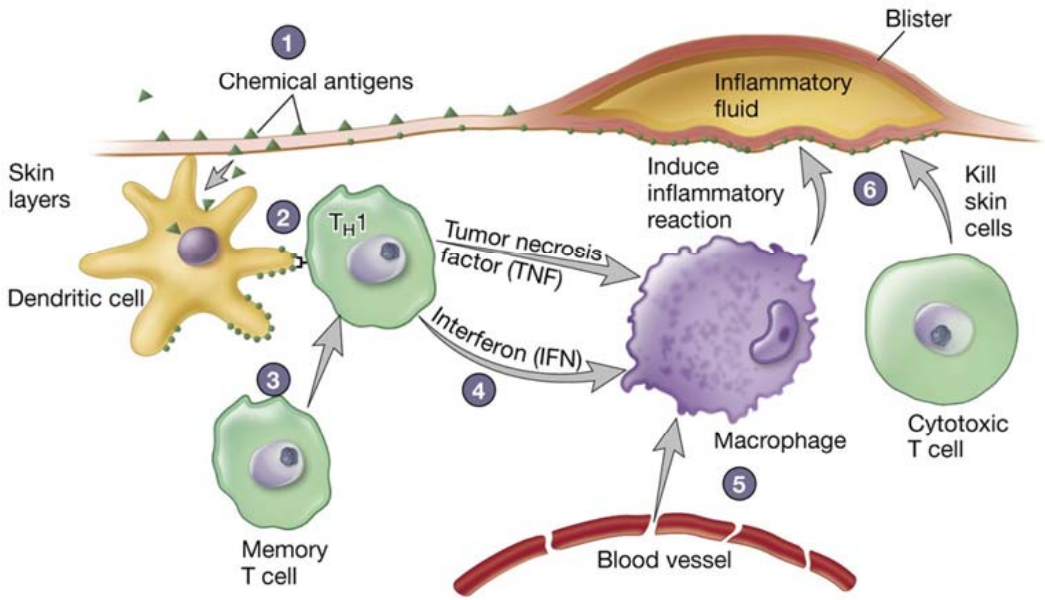
Type IV Hypersensitivity

- involves delayed, **cell-mediated immune** reactions
- important factor is time required for T cells to reach and accumulate near antigens
- **Th** and **CTL** cells can elicit type IV (i.e. **DTH**) reactions
- examples
  - tuberculin hypersensitivity
  - some autoimmune diseases
  - transplantation rejection
  - cancer cell killing
  - **allergic contact dermatitis**



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Autoimmune Diseases

- autoimmunity
  - presence of serum antibodies that react with self antigens (autoantibodies)
  - often benign
  - natural consequence of aging
  - reversibly induced by numerous stimuli (e.g., infectious organisms, drugs)
- autoimmune disease
  - results from activation of self-reactive T and B cells
  - leads to tissue damage
  - e.g. rheumatoid arthritis, insulin-dependent diabetes mellitus
- facts that influence the development of autoimmune disease
  - infection
  - genetic
  - viral
  - hormones
  - influence of stress and neurochemicals on the immune response

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Autoimmune Diseases

Table 34.4 Some Autoimmune Diseases in Humans

Disease	Autoantigen	Pathophysiology
Acute rheumatic fever	Streptococcal cell wall antigens mimic self antigens and induce antibodies that cross-react with antigens on cardiomyocytes and other cells.	Type II hypersensitivity leads to myocarditis, heart valve scarring, and arthritis.
Autoimmune hemolytic anemia	Rh blood group antigen induces antibody to Rh antigen on red blood cells.	Type II hypersensitivity leads to anemia when red blood cells are destroyed by complement and phagocytosis.
Goodpasture's syndrome	Damage to kidney basement membrane exposes cryptic collagen protein, inducing anti-collagen antibody.	Type II hypersensitivity results in glomerulonephritis and pulmonary hemorrhage.
Graves' disease	Antibody to thyroid-stimulating hormone (TSH) receptor mimics TSH.	Overstimulation of TSH receptor leads to hyperthyroidism.
Multiple sclerosis	Antibody and activated T cells to several nervous system antigens	Types II and IV hypersensitivities alter nerve communications, leading to numbness, weakness, spasm, and loss of motor and cognitive function.
Myasthenia gravis	Antibody to acetycholine receptor in skeletal muscle	Antibody blockade of neurotransmitter receptors results in progressive muscular weakness.
Rheumatoid arthritis	IgG antibody (to synovial joint cartilage antigen) recognized as foreign	Type III hypersensitivity from immune complexes of antibodies to antibodies results in joint inflammation and destruction.
Systemic lupus erythematosus	Antibodies to various cellular (DNA, nucleoprotein, cardiolipin) and blood clotting components	Type III hypersensitivity results in immune complex-induced arthritis, glomerulonephritis, vasculitis, and rash.
Insulin-dependent diabetes mellitus	Antibody and activated T cells to pancreatic beta cell antigens	Types II and IV hypersensitivities destroy beta cells, resulting in insulin deficiency.

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Autoimmune Diseases

✓ **Tissue-specific** autoimmunity
- Autoimmune hemolytic anemia
- Hashimoto's thyroiditis
- Type I DM
- Goodpasture's syndrome

- Grave's disease
- Myasthenia gravis

✓ **Systemic** autoimmunity
- Systemic lupus erythematosus
- Rheuamtoid athritis
- Multiple sclerosis
- Ankylosing spondylitis
- Sjögren syndrome

표 17-1 사람에서 대표적으로 나타나는 자가면역질환

질 환	자가항원	주요 면역반응
조직 특이적 자가면역질환		
자가면역용혈성 빈혈	적혈구 막 단백질	자가항체
굿패스쳐증후군 (Goodpasture's syndrome)	신장, 폐 기저막	자가항체
그레이브스병 (Grave's disease)	TSH 수용체	자극성 자가항체
하시모토 갑상선염 (Hashimoto's thyroiditis)	갑상선 단백질	T_H1, 자가항체
인슐린 의존성 당뇨	췌장 β 세포	T_H1, 자가항체
중증근무력증 (Myasthenia gravis)	아세틸콜린 수용체	저해성 자가항체
전신성 자가면역질환		
강직성 척추염 (ankylosing spondylitis)	척추	면역복합체
다발성 경화증	뇌 신경세포	T_H1, T_C, 자가항체
류마티스성 관절염	관절조직, IgG	자가항체, 면역복합체
Sjögren's syndrome	침샘, 간, 신장, 갑상선	자가항체
전신홍반루푸스 (SLE)	DNA, 핵단백질, RBC, 혈소판	자가항체, 면역복합체

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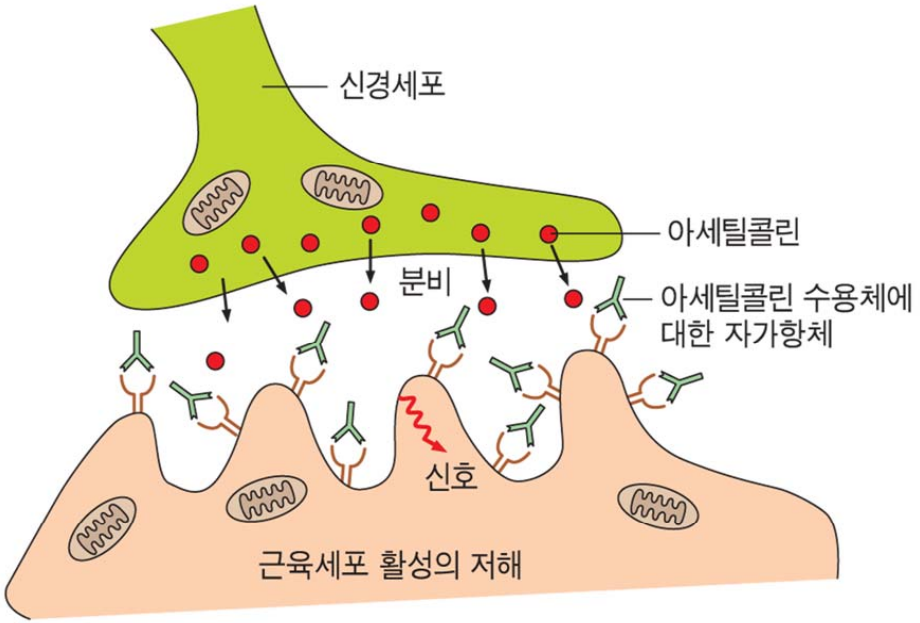
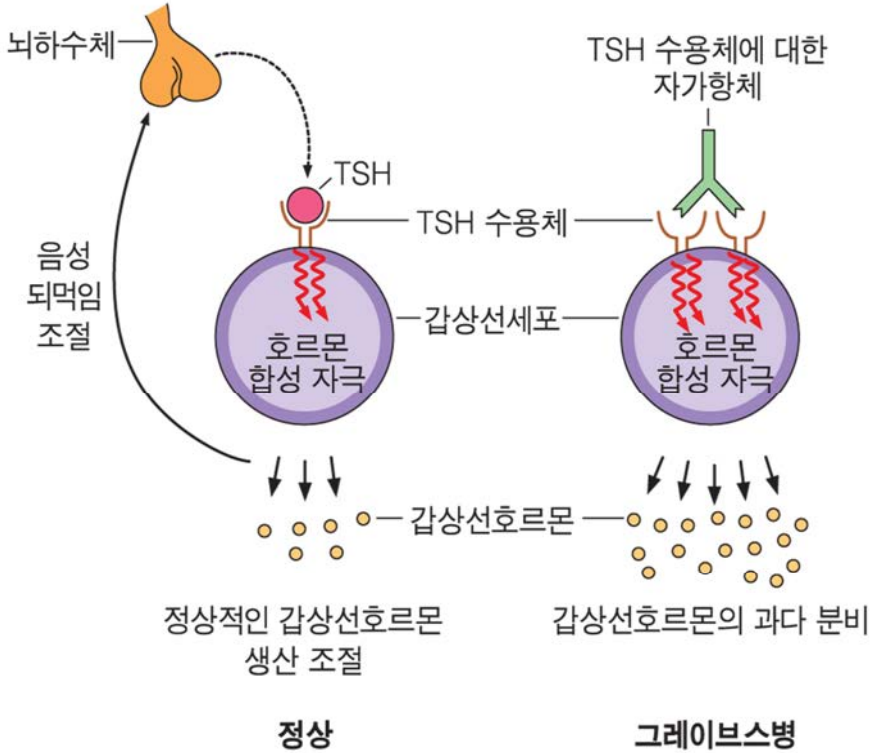
Autoimmune Diseases





중증근무력증 (Myasthenia gravis), 그레이브스병 (Grave's disease)

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Mechanisms of Autoimmune Diseases

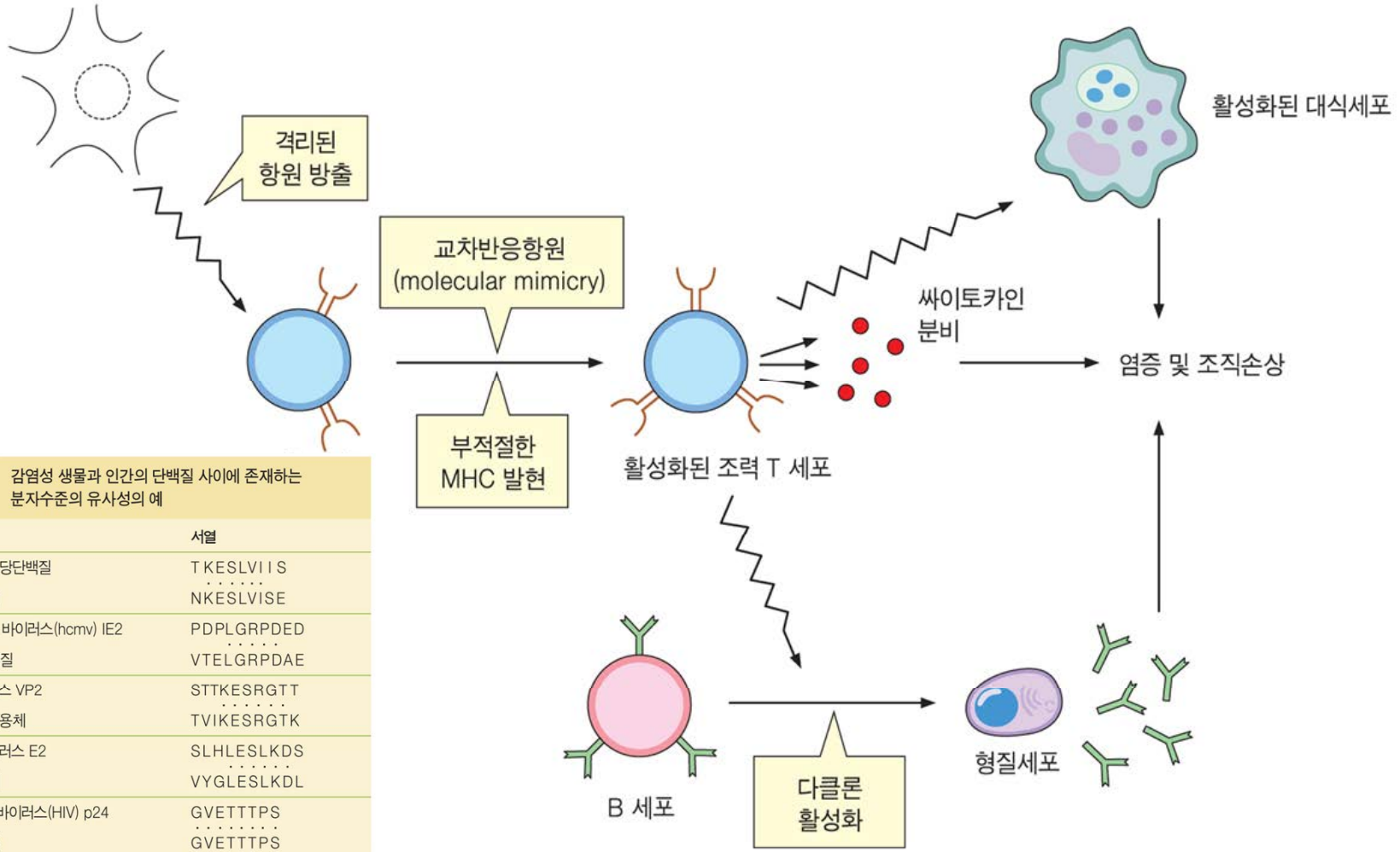

표 17-2 감염성 생물과 인간의 단백질 사이에 존재하는 분자수준의 유사성의 예

단백질	서열
Rabies virus 당단백질	TKESLVIIS
인슐린 수용체	NKESLVISE
인간 거대세포 바이러스(hcmv) IE2	PDPLGRPDED
HLA-DR 단백질	VTELGRPDAE
폴리오 바이러스 VP2	STTKESRGTT
아세틸콜린 수용체	TVIKESRGTK
인유두종 바이러스 E2	SLHLESLKDS
인슐린 수용체	VYGLESLKDL
인간 면역결핍바이러스(HIV) p24	GVETTTPS
인간 IgG 중쇄	GVETTTPS
홍역 바이러스 P3	EISFKLGQE
Myelin basic protein	EISFKLGQE

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Treatment of Autoimmune Diseases

✓ **Immunosuppressive drugs**

- Corticosteroid, azathioprine, cyclophosphamide etc
- Cyclosporin A, FK-506

✓ **Anti-inflammatory drugs**

- TNF-α antagonists: **Enbrel**, **Remicade**, **Humira**
- IL-1 antagonists: **Kineret**

✓ **B cell antagonists**

- **Rituxan**: mAb against CD20

cf. T cell antagonists, 면역관용 유도?

표 18-1 임상에 사용되는 면역억제 방법

면역억제제	작용기작
사이클로스포린 및 FK-506	NFAT 전사요소의 활성화를 억제하여 T 세포의 싸이토카인 생산을 차단
아자티오프린	임파구 전구세포의 증식을 차단
미코페놀레이트 모페틸	임파구의 구아닌 뉴클레오티드 합성을 억제하여 임파구 증식을 차단
라파미신	IL-2 신호 전달을 억제하여 임파구 증식을 차단
코르티코스테로이드	대식세포의 싸이토카인 분비를 억제하여 염증을 감소
항-CD3 단클론항체	CD3에 결합하여 T 세포를 제거하고 탐식작용 혹은 보체-매개 세포용해를 촉진
항-IL-2 수용체 (CD25) 항체	IL-2 결합을 차단하여 T 세포 증식을 억제하고 CD25을 발현하는 활성화된 T 세포를 제거
CTLA-4-Ig	B7 공동자극분자가 T 세포의 CD28에 결합하는 것을 차단하여 T 세포 활성화를 억제; 임상시험 중
항-CD40 리간드	T 세포의 CD40 리간드가 CD40에 결합하는 것을 차단하여 대식세포와 혈관상피의 활성화를 억제; 임상시험 중

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Immunosuppressive Drugs

- **Immunosuppressant**

  Corticosteroids: Prednisone, **Prednisolone**, Methylprednisolone
  Calcineurin inhibitors: **Ciclosporin**, **Tacrolimus**
  mTOR inhibitors: Siorlimus, Everolimus
  Antimetabolites: **Azathioprine**, **Mycophenolate** (**MMF**, sodium salt)
  Anti-lymphocyte polyclonal antibodies: anti-thymocyte globulin (= **ATG**)
  Anti-CD3: **Muromonab-CD3** (= orthoclone, **OKT3**)
  Anti-CD25 (IL-2R): **Basiliximab**, **Daclizumab**
  Anti-CD52: Alemtuzumab
  Anti-CD20: Rituximab

- **면역 신호 전달 및 면역억제제의 실제 (immunosuppressive regimens)**

  T 세포의 분화/증식/활성화와 cytokine들: 3-signal model
  : Th0 (IL-2), Th1 (INF-γ), Th2 (IL-4)
  활성과 부작용 및 개선점?

History of Immunosuppression

- First successful renal transplant (1954)
  between identical twins without immunosuppression

- First successful allograft (1959)
  between non-identical twins using sublethal total body irradiation

- First successful unrelated allograft (1962)
  using **azathioprine**
  >1 yr survival

- Reversal of rejection with steroids (1963)

- *For modern biological therapy?*

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Immunosuppressive Drugs

Challenges for post-transplant recipients

- To provide adequate immunosuppression
- Minimize adverse effects
- Treat adverse effects and chronic, drug-related complications

Drugs are good for preventing acute rejections, but not chronic, Ab-mediated rejection

- Recent improvement in short-term outcomes
- Less improvement in long-term outcomes

Combination therapy using multiple drugs

- To create synergy between drugs targeting different stages
- Minimize the dose of each drug

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Immunosuppressants

SELECTIVE INHIBITORS OF CYTOKINE PRODUCTION AND FUNCTION

Cyclosporine NEORAL, SANDIMMUNE
Everolimus ZORTRESS
Sirolimus RAPAMUNE
Tacrolimus PROGRAF

IMMUNOSUPPRESSIVE ANTIMETABOLITES

Azathioprine IMURAN
Mycophenolate mofetil CELLCEPT
Mycophenolate sodium MYFORTIC

ANTIBODIES

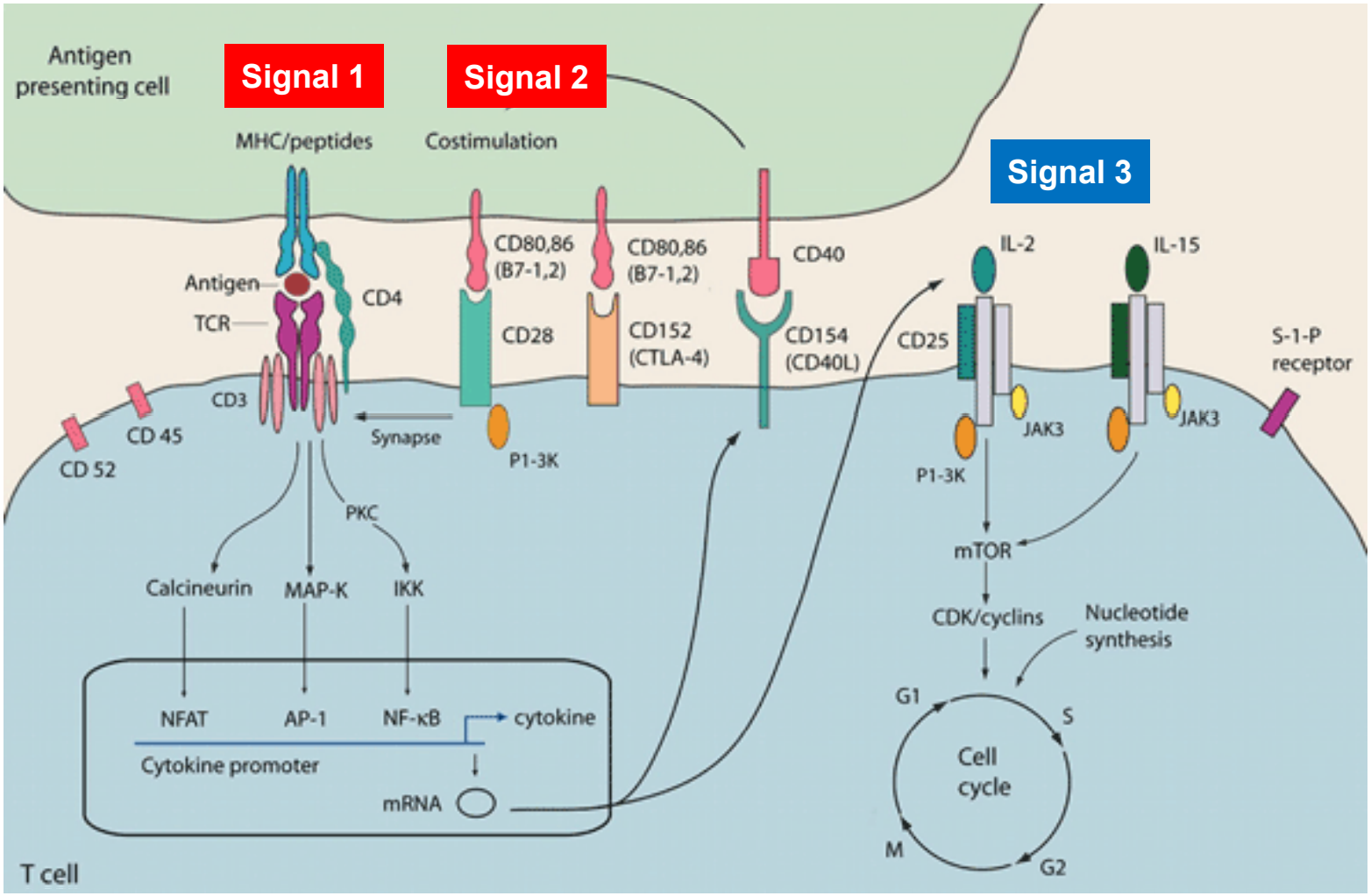
Alemtuzumab CAMPATH
Antithymocyte globulins ATGAM, THYMOGLOBULIN
Basiliximab SIMULECT
Daclizumab ZENAPAX
Muromonab-CD3 ORTHOCLONE OKT3

ADRENOCORTICOIDS

Methylprednisolone MEDROL
Prednisolone ORAPRED, PRELONE
Prednisone DELTASONE

Fig. 40.1 책에서 다루는 면역억제제

Steps in T cell activation



T세포의 활성화 단계: 3-signal model

Induction Theory: Antibodies

- The most ***intense*** therapy, when alloimmune response is greatest
- "Induce" tolerance
- Provide *background immunosuppression* during immediate post-op period, while renal function stabilizes
- Use antibodies against cell surface receptors
  - ATG (eATG/ATGAM, rATG/Thymoglobulin)
  - Basiliximab (CD25), Daclizumab (CD25), **Alemtuzumab (CD52)**

  cf. These are also used for leukemia
- Antibody-mediated signal blockage leads to *hyper-activation* of immune signalings, eventually resulting in immune tolerance

cf. Antibodies from mouse contain Muro in their names.

Humanized/chimeric antibodies contain ZU/XI in their names

→ less antigenicity, longer half-life

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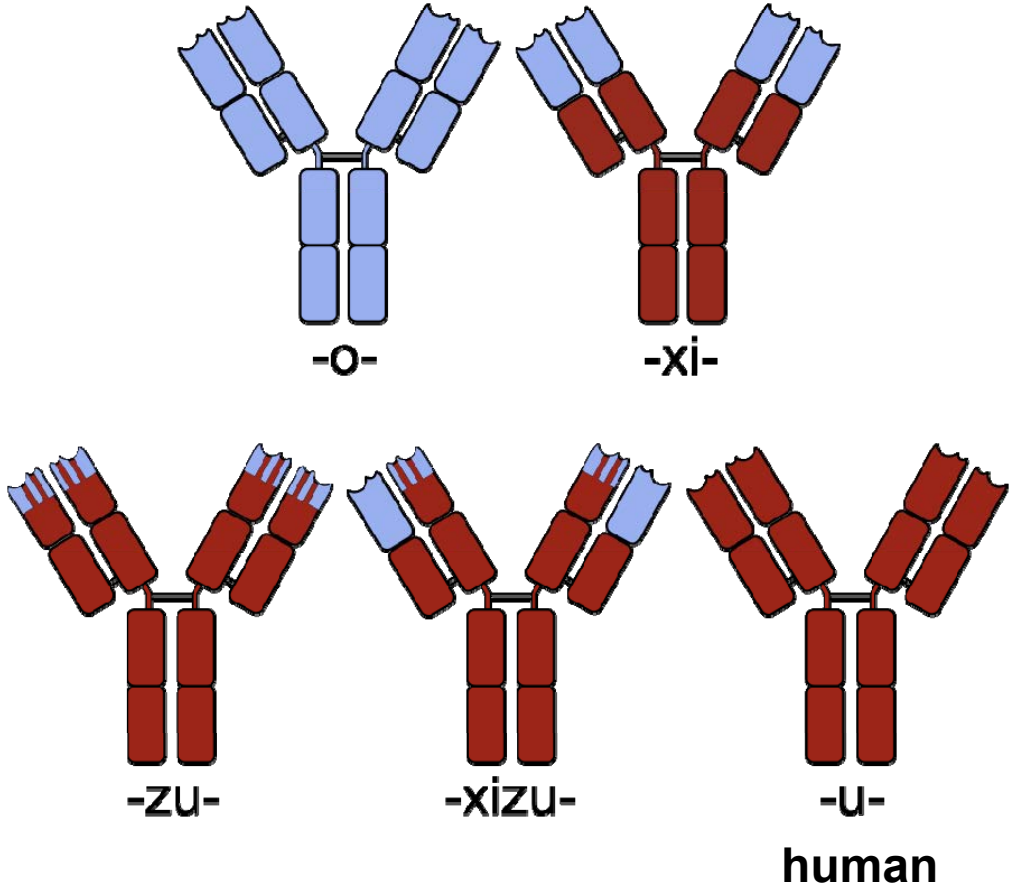
Humanized/Chimeric Antibodies

- Basiliximab (anti-CD25)
- Daclizumab (anti-CD25)
- Alemtuzumab (anti-CD52)

- Infliximab (anti-TNF-α)
- Omalizumab (anti-IgE)



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Humanized/Chimeric Antibodies

	Absolute difference (95% CI)	Half-life	Ref.
Daclizumab (90% human)	-16% (-24% to –8%)	30 days	NEJM. 1998 Transpl.1999
Basiliximab (75% human)	-14% (-20% to –9%)	7 days	Lancet. 1997 Transpl.1999 Transpl. 2001

Biopsy-confirmed acute rejection

Humanized/Chimeric Antibodies

Advantages

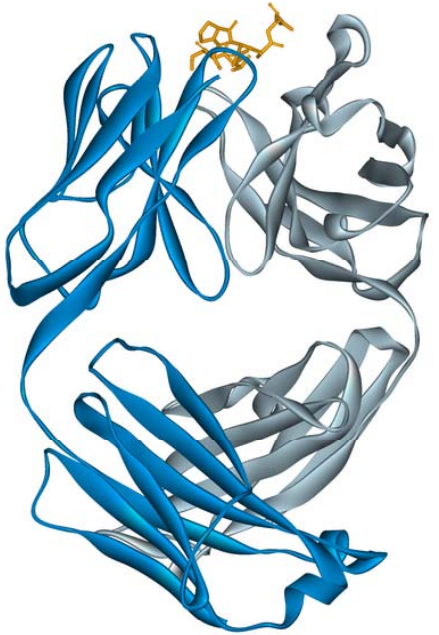
- High efficacy
  - reduce renal rejection rates by approx 30% in classic triple therapy
- Short courses
  - IV, in hospital only
  - Compliance guaranteed
- 'Few' adverse effects
  - Severe re-exposure hypersensitivity observed

Disadvantages

- Cost
  - But cost-effective to add basiliximab to ciclosporin regimens (NICE TA 99)
  - Same report states that should not be added to tacrolimus regimens.
- Discomfort
  - Daclizumab is a 5 dose, fortnightly regimen & requires infusion

Alemtuzumab

- **Campath**, **MabCampath** or **Campath-1H**
- A new mAb binding to CD52 (not CD25), present on the mature lymphocytes, but not on the stem cells.
- used for chronic lymphocytic leukemia (CLL), cutaneous T-cell lymphoma (CTCL) and T-cell lymphoma.
- Also used for transplantation (BM, kideny etc)
- Also used for treatment of multiple sclerosis.
- A complication of therapy with alemtuzumab is that it significantly increases the risk foropportunistic infections, in particular, reactivation of cytomegalovirus (CMV).



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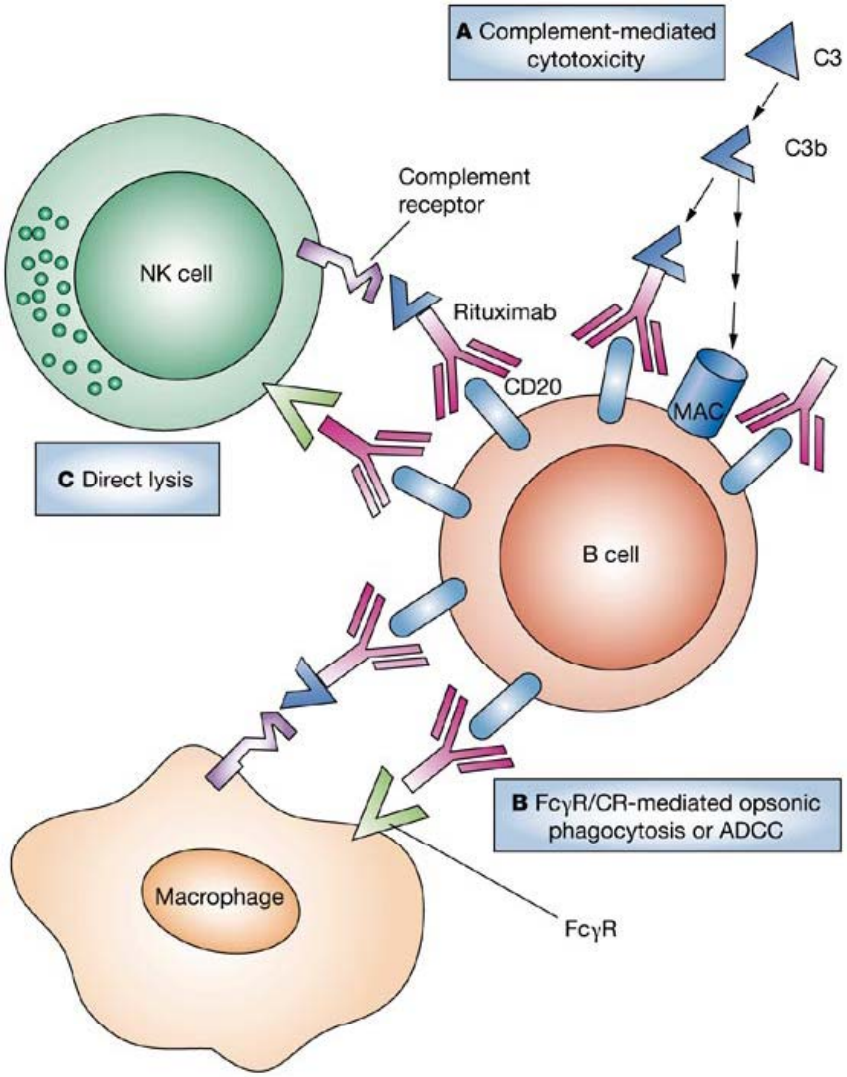
Rituximab

- **Rituxan** and **MabThera**
- A new mAb against CD20
- CD20 is primarily found on B cells (a new horizon!)
- Used to treat diseases characterized by excessive numbers of B cells, overactive B cells, or dysfunctional B cells.

cf. V geting CD20 have d.



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Prednisone

- A synthetic corticosteroid (anti-inflammation)
- used to treat certain inflammatory diseases (such as moderate allergic reactions) and (at higher doses) some types of cancer.
- Highly effective to prevent rejection, but with significant (long-term) adverse effects.
- used for many different indications including: asthma, COPD, CIDP, rheumatic disorders, allergic disorders, ulcerative colitis and Crohn's disease, adrenocortical insufficiency, hypercalcemia due to cancer, thyroiditis, laryngitis, multiple sclerosis, poison oak exposure, and as part of a drug regimen to prevent rejection post organ transplant.

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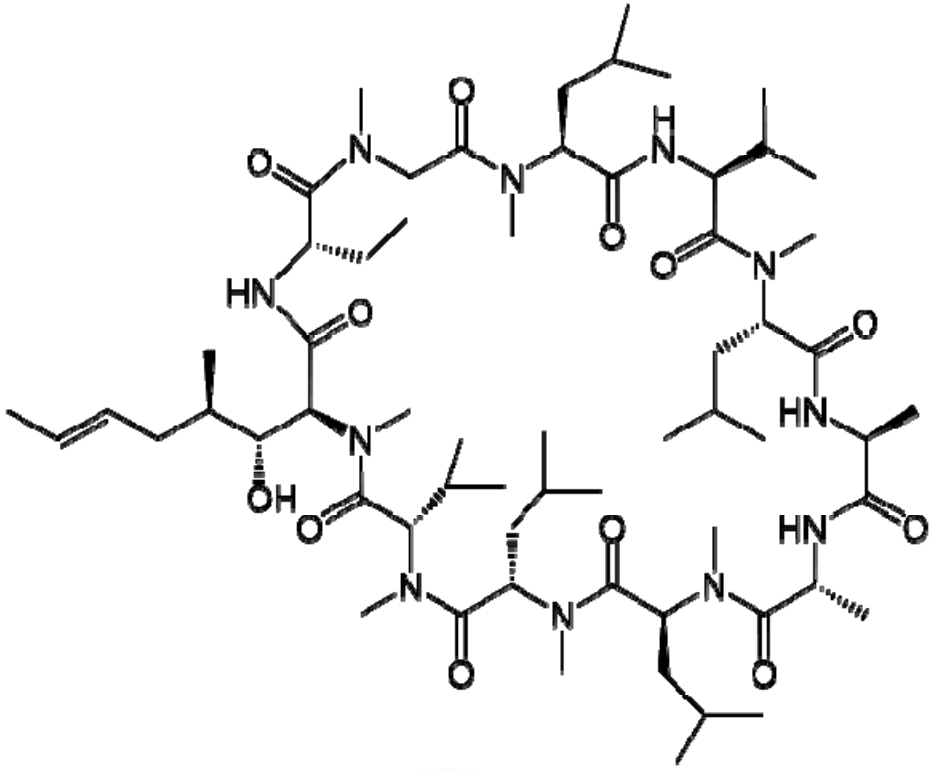
Cyclosporine (CsA)

- A peptide from fungus *Tolypocladium inflatum* (= *Beauveria nivea)*
- Binding to cyclophilin
- Widely used in organ transplantation to prevent rejection.
- Multiple formulations: Oil-based (variable absorption), Microemulsion (preferred)



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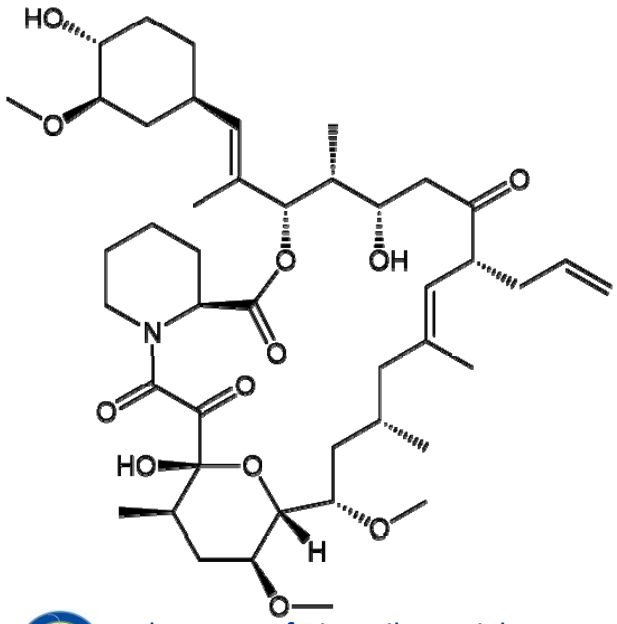
Tacrolimus (FK-506)

- A 23-membered macrolide lactone discovered in 1984 from the fermentation broth of *Streptomyces tsukubaensis*.
- Binding to FKBP-12 (= immunophilin)
- Mainly used after allogenic organ transplant to lower the risk of organ rejection.
- Also used in a topical preparation in the treatment of atopic dermatitis (eczema), severe refractory uveitis after bone marrow transplants, exacerbations of minimal change disease, and the skin condition vitiligo.



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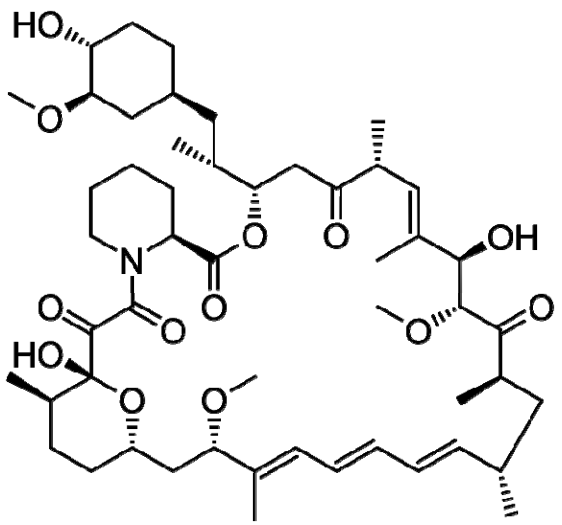
Sirolimus (Rapamycin)

- **Rapamune** by Pfizer (formerly by Wyeth)
- A macrolide discovered from *Streptomyces hygroscopicus* (Rapa Nui. Easter Isalnd)
- Originally developed as an antifungal agent.
- However, this use was abandoned due to potent immunosuppressive and antiproliferative properties.
- Prolong the life of experimental mice and useful in the treatment of certain cancers.
- It binds to FKBP, but inhibits FRAP1 (**mTOR**, mammalian target of rapamycin)



cf. mTOR is a Ser/Thr kinase signaling in cell proliferation

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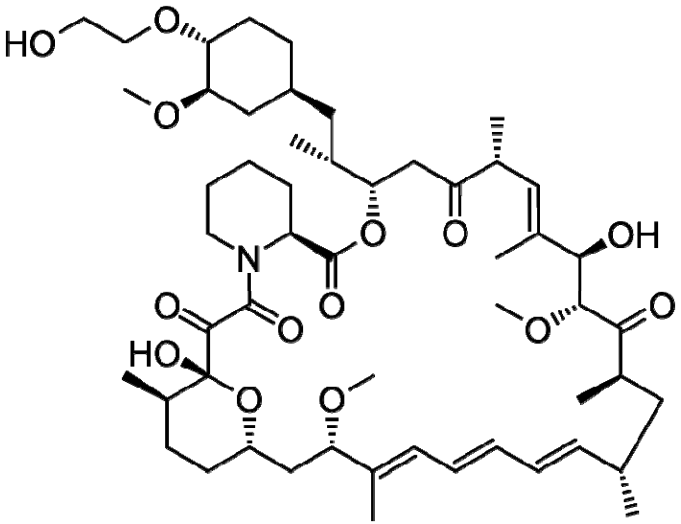
Everolimus

- Everolimus (RAD-001) is the 40-*O*-(2-hydroxyethyl) derivative of sirolimus and works similarly to sirolimus.
- Currently used as an immunosuppressant in organ transplants and treatment of renal cell cancer and other tumors.



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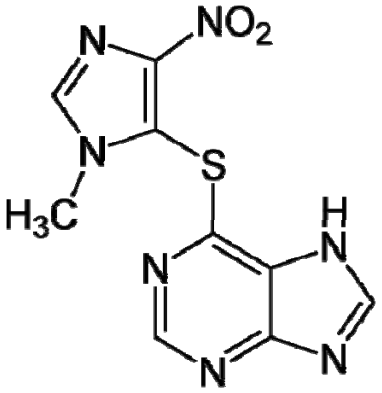
Azathioprine

- A purine analogue first used to prevent transplant rejection and used for more than 50 years. (not widely used any more)
- Synthesized originally as a cancer drug and a pro-drug for mercaptopurine in 1957
- Mercaptopurine, inhibiting an enzyme that is required for DNA synthesis. Thus it most strongly affects proliferating cells, such as the T cells and B cells of the immune system.
- The main adverse effect is bone marrow suppression, which can be life-threatening, especially in people with a genetic deficiency of the enzyme thiopurine S-methyltransferase.



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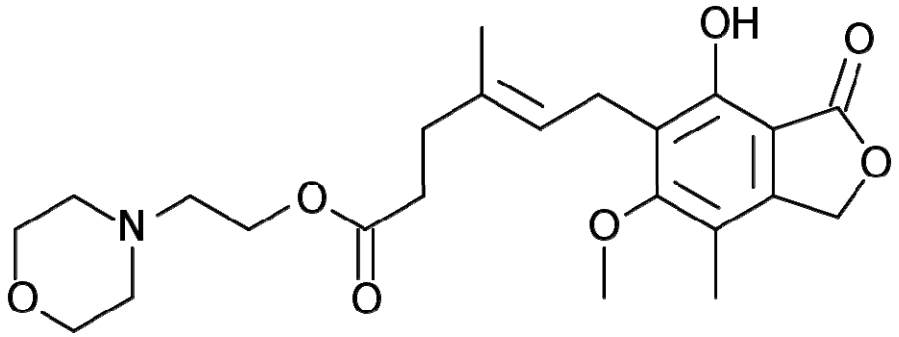
Mycophenolate mofetil (MMF)

- A pro-drug (morpholinoethyl ester) of mycophenolic acid, used extensively in transplant medicine (preferred over AZA).
- It is a reversible inhibitor of IMP DH in purine biosynthesis.
- Other cells (other than T and B cells) are able to recover purines via a separate scavenger pathway and are thus able to escape the effect.
- Also used in the treatment of autoimmune diseases
- Popular as a less toxic alternative



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Standard Immunosuppressive Regimens

- **Calcineurin Inhibitor**
  - Cyclosporine (CsA)
  - Tacrolimus

- **Anti-metabolites/mTOR inhibitors**
  - Azathioprine
  - Mycophenolate mofetil (MMF)
  - Sirolimus/Everolimus

- **Steroids**
  - Prednisone

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Standard Combination Regimens

- Tacrolimus + Steroid + Mycophenolate (49%)
- Cyclosporine + Steroid + Mycophenolate (28.5%)

- Tacrolimus + Mycophenolate(3.8%)
- Tacrolimus + Steroid (1.9%)
- Steroid + Mycophenolate (0.9%)
- Tacrolimus (0.6%)

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Standard Combination Regimens

- Immunosuppressive regimens have improved greatly since beginning of transplantation
- **3-drug regimens** with *tapering of steroids* are standard of care
- Current challenges are providing *adequate immunosuppression* and *minimizing complications of drugs*

- Current efforts are focused on further minimization of immunosuppression and use of alternative regimens
- While much of transplant and immunosuppression are protocol-driven, regimens should be ***individualized***
- Predicting those who are more likely to have rejection can be difficult

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Transplantation and Treatment

Organ transplant	Immunosuppressants
Kidney Heart	Cyclosporine, Azathioprine, Prednisone, ALG, Tacrolimus.
Liver	Cyclosporine, Prednisone, Azathioprine, Tacrolimus.
Bone marrouw	Cyclosporine, Cyclophosphamide, Prednisone, Methotrexate, ALG, total body radiation.

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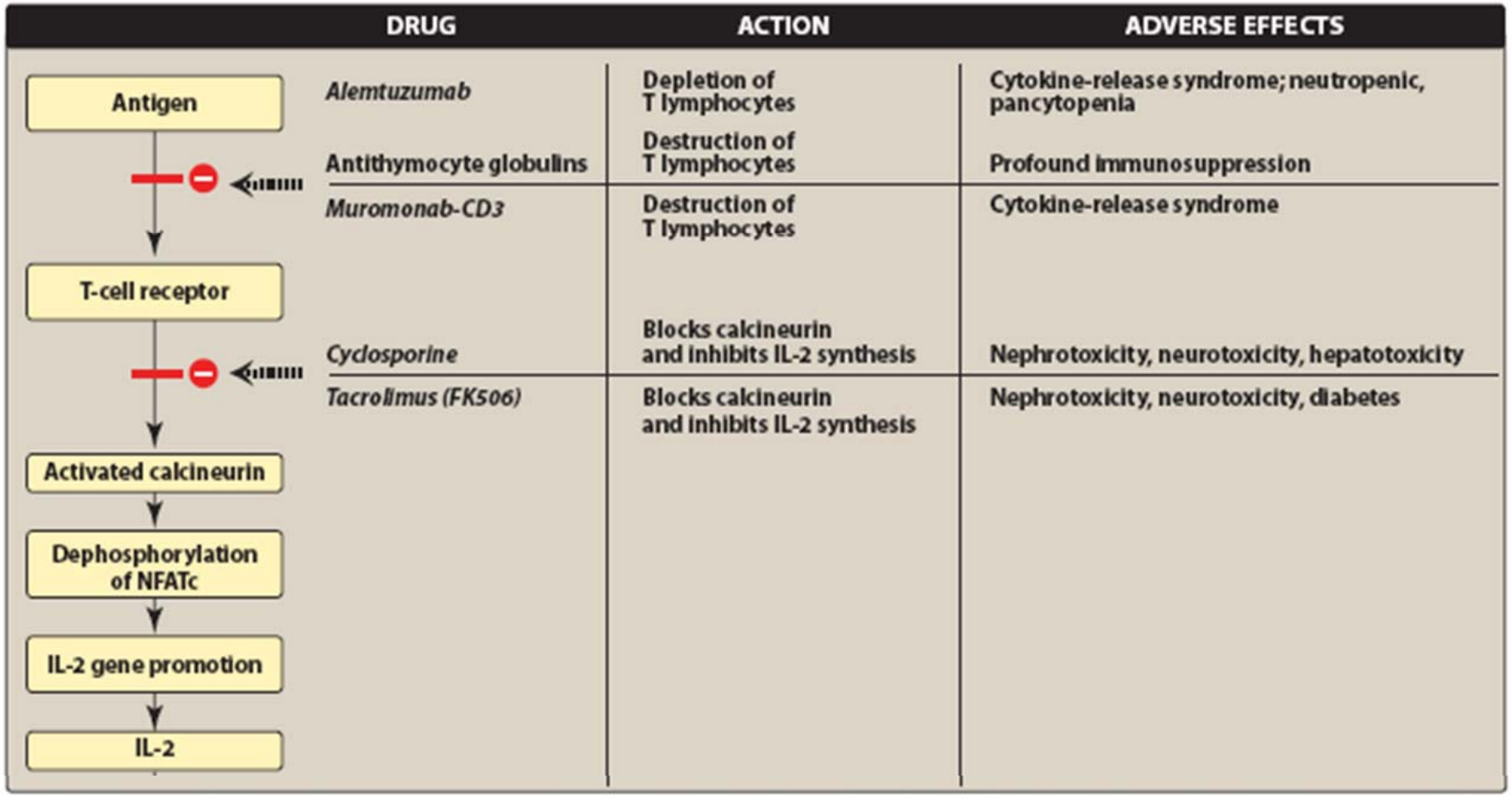
Suppression Steps: Roundup



DRUG	ACTION	ADVERSE EFFECTS
Alemtuzumab	Depletion of T lymphocytes	Cytokine-release syndrome; neutropenic, pancytopenia
Antithymocyte globulins	Destruction of T lymphocytes	Profound immunosuppression
Muromonab-CD3	Destruction of T lymphocytes	Cytokine-release syndrome
Cyclosporine	Blocks calcineurin and inhibits IL-2 synthesis	Nephrotoxicity, neurotoxicity, hepatotoxicity
Tacrolimus (FK506)	Blocks calcineurin and inhibits IL-2 synthesis	Nephrotoxicity, neurotoxicity, diabetes

Fig. 40.8 주요 면역억제제의 작용

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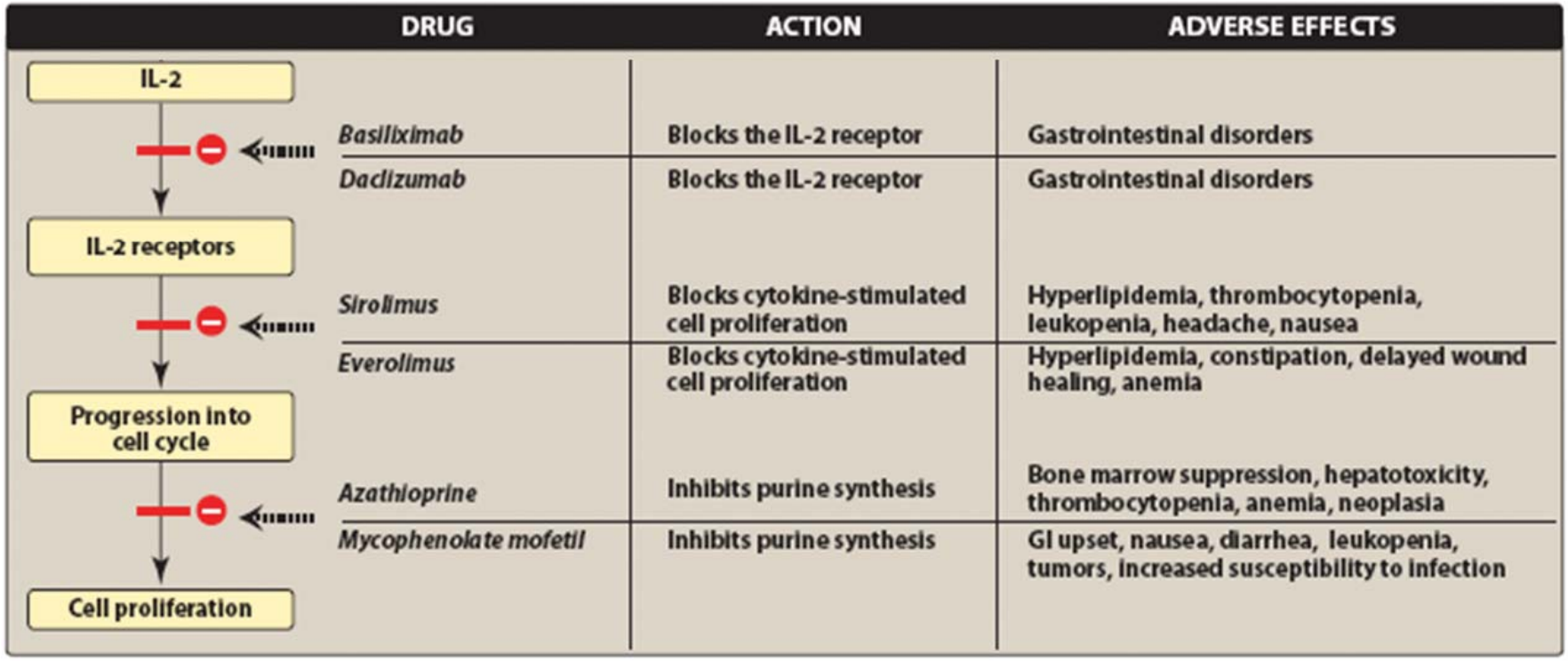
Suppression Steps: Roundup



DRUG	ACTION	ADVERSE EFFECTS
Basiliximab	Blocks the IL-2 receptor	Gastrointestinal disorders
Daclizumab	Blocks the IL-2 receptor	Gastrointestinal disorders
Sirolimus	Blocks cytokine-stimulated cell proliferation	Hyperlipidemia, thrombocytopenia, leukopenia, headache, nausea
Everolimus	Blocks cytokine-stimulated cell proliferation	Hyperlipidemia, constipation, delayed wound healing, anemia
Azathioprine	Inhibits purine synthesis	Bone marrow suppression, hepatotoxicity, thrombocytopenia, anemia, neoplasia
Mycophenolate mofetil	Inhibits purine synthesis	GI upset, nausea, diarrhea, leukopenia, tumors, increased susceptibility to infection

Fig. 40.8 주요 면역억제제의 작용

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Transplantation (Tissue) Rejection

- types of transplants
  - allograft: transplants between genetically different individuals within a species
  - xenograft: donor and recipient are different species
- two mechanisms can occur
  - foreign MHC molecules on the transplanted tissue (graft) are recognized by host helper T cells when aid cytotoxic T cells in destroying graft
  - T-helper cells react to graft by releasing cytokines which stimulate destruction of graft by macrophages

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Tissue Transplantation

- to be successful, the ABO blood group and the MHC molecules of the donor and recipient must be as closely matched as possible
  - after matching blood types of donor and recipient, the identification of Human Leukocycte Antigens (HLA) is done
  - family members are the first choice for a close match because HLA genes are usually inherited as a complete set from parents

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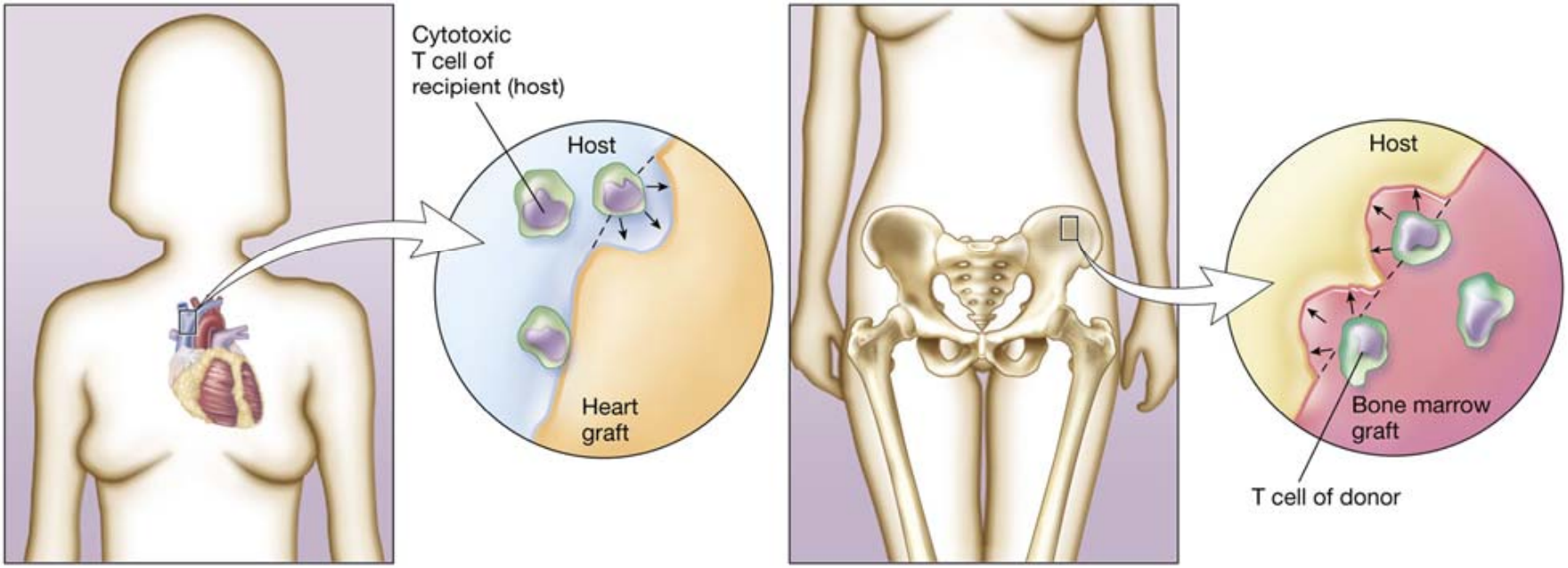
Graft-Versus-Host Disease

- can occur in organ transplant recipients
- immunocompetent cells in donor tissue reject host
- e.g., in bone marrow transplants
  - disease prevented by treating donor with immunosuppressive drugs to deplete marrow of mature T cells



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Immunodeficiencies

- failure to recognize and/or respond properly to foreign antigens
- primary (congenital) immunodeficiencies (1차 면역결핍)
  - result from genetic disorder
- acquired immunodeficiencies (2차 면역결핍)
  - result from infection by immunosuppressive microbes (e.g., HIV)

Table 34.5 Some Congenital Immune Deficiencies in Humans

Condition	Symptoms	Cause
Chronic granulomatous disease	Defective monocytes and neutrophils leading to recurrent bacterial and fungal infections	Failure to produce reactive oxygen intermediates due to defective NADPH oxidase
X-linked agammaglobulinemia	Plasma cell or B-cell deficiency and inability to produce adequate specific antibodies	Defective B-cell differentiation due to loss of tyrosine kinase
DiGeorge syndrome	T-cell deficiency and very poor cell-mediated immunity	Lack of thymus or a poorly developed thymus
Severe combined immunodeficiency disease (SCID)	Both antibody production and cell-mediated immunity impaired due to a great reduction of B- and T-cell levels	Various mechanisms (e.g., defective B- and T-cell maturation because of X-linked gene mutation; absence of adenosine deaminase in lymphocytes)

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HIV Drugs

- Nucleoside analogues (RT inhibition. NRTI)
  Zidovudine (AZT) / **Dideoxyinosine** (Didanosine, ddI), **Zalcitabine** (ddC), **Lamivudine**, Stavudine, **Emtricitabine**, Apricitabine, Elvucitabine, Stampidine / **Amdoxovir** / Abacavir

표 20-4 임상적으로 사용되는 항 HIV-1 약제

Entry/Fusion 저해제		Enfuvirtide (gp41), Maraviroc (CCR5), Ibalizumab (CD4)
역전사 저해제	Nucleoside 또는 Nucleotide (NRTI)	Abacavir (ABC), Emtricitabine (FTC), Lamivudine (3TC), Didanosine (ddI), Zidovudine (AZT), Stavudine (d4T), Zalcitabine (ddC), Tenofovir (TDF), Combivir (3TC + AZT), Trizivir (3TC + AZT + ABC), Truvada (TDF + FTC)
	Non-nucleoside (NNRTI)	Efavirenz, Nevirapine, Delavirdine (1st gen.) Etravirine (2nd gen.)
단백질분해효소(protease) 저해제		Atazanavir, Fosamprenavir, Lopinavir, Darunavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir, Amprenavir, Indinavir
인테그라아제 저해제		Raltegravir

Integrase inhibitors
MK-0518 (**Raltegravir**), **Elvitegravir**

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Immunotherapy

- Therapeutic Vaccines (치료용 백신)
- Therapeutic Monoclonal Antibodies (치료용 단클론항체)
- Cytokine Therapy (치료용 싸이토카인)
- Immune Gene and Cell Therapy (면역 유전자 및 세포 치료제)
- Immnosuppressive Drugs (면역 억제제)

표 18-1 임상에 사용되는 면역억제 방법

면역억제제	작용기작
사이클로스포린 및 FK-506	NFAT 전사요소의 활성화를 억제하여 T 세포의 싸이토카인 생산을 차단
아자티오프린	임파구 전구세포의 증식을 차단
미코페놀레이트 모페틸	임파구의 구아닌 뉴클레오티드 합성을 억제하여 임파구 증식을 차단
라파미신	IL-2 신호 전달을 억제하여 임파구 증식을 차단
코르티코스테로이드	대식세포의 싸이토카인 분비를 억제하여 염증을 감소
항-CD3 단클론항체	CD3에 결합하여 T 세포를 제거하고 탐식작용 혹은 보체-매개 세포용해를 촉진
항-IL-2 수용체 (CD25) 항체	IL-2 결합을 차단하여 T 세포 증식을 억제하고 CD25을 발현하는 활성화된 T 세포를 제거
CTLA-4-Ig	B7 공동자극분자가 T 세포의 CD28에 결합하는 것을 차단하여 T 세포 활성화를 억제; 임상시험 중
항-CD40 리간드	T 세포의 CD40 리간드가 CD40에 결합하는 것을 차단하여 대식세포와 혈관상피의 활성화를 억제; 임상시험 중

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Therapeutic Antibodies

출시	종류	개발자/개발업체	상품명	타겟	적응증
1986	마우스	J&J	Orthoclone® OKT3	CD3	장기이식 거부반응 완화
1994	키메릭	Centocor/Eli Lilly	Reopro®	GPIIb/IIIa	PCI
1997	키메릭	IDEC/Genentech/Roche	Rituxan®	CD20	비호치킨성 림프종
	인간화	Protein Design Labs/Hoffmann-La Roche	Zenapax®	CD25	장기이식 거부반응 완화
1998	인간화	Genentech	Herceptin®	EGFR2/HER	악성 유방암
		Medimmune	Synagis®	RSV	RSV감염 예방
	키메릭	Centocor/Schering-Plough	Remicade®	TNF-α	류마티스, Crohn병
	키메릭	Novartis	Simulect®	IL2R	장기이식 거부반응 완화
2000	인간화	Celltech/Wyeth	Mylotarg®	CD33	급성 골수 백혈병(AML)
2001	인간화	Millennuium/Schering AG	Campath®	CD52	급성 임파구성 백혈병(ALL)
2002	방사면역 접합체	IDEC	Zevalin®	CD20	비호치킨성 림프종
	인간(Phage Display)	Abbott Lab.	Humira®	TNF-α	류마티스
	인간화	Croxia& GlaxoSmithKline	Raptiva®	CD11a	만성 중증 건선 완화
2003	방사면역 접합체	Corixa/GSK	Bexxar®	CD20	비호치킨성 림프종
	인간화	Genentech & Novartis	Xolair®	IgE	중증 지속성 천식 완화
2004	키메릭	Imclone & Bristol-Myers Squibb	Erbituxv	EGFR	결장직장암
	인간화	Genentech	Avastin®	VEGF	결장직장암
	인간화	Biogen-IDEC/Elan	Tysabri®	α4 integrin	다발성 경화증
2005	인간화	YM Biosciences	Theracim®	EGFR	Glioma, Head&Neck, Lung, Pancrea cancers
	인간화	Roche/Chugai	Actemra®	IL-6R	관절염, benign B tumor
2006	인간화	Genentech	Lucentis®	VEGF	습성 황반변성
	인간(IgG2)	Amgen	Vectibix®	EGFR	대장암
2007	인간화 (IgG2-IgG4 hybrid)	Alexion Pharmaceuticals	SOLIRIS®	C5	Dermatomyositis, 신장염, 관절염, 등
2008	인간화 (Pegylated IgG4-Fab)	Celltech, UCB	Cimzia®	TNF-α	Crohn's disease, 관절염
2009	인간	Centocor/Orthobiotech	Simponi®	TNF-α	Psoriatic arthritis, 관절염
	인간	Norvatis\	ILARIS®	IL-1β	관절염 등
	인간	Centocor	Stelara®	IL-12, IL-23	건선
	인간	Genmab	Arzerra®	CD-20	CLL, NHL, RA, MS
	마우스-rat	Fresenius Biotech Trion Pharma	Removab®	CD3, EpCAM	malignant ascites
2010	인간	Amgen	Prolia®	RANKL	골다공증
	인간	HGS	Raxibacumab	anthrax toxin	Anthrax toxin

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Therapeutic Antibodies

중요	의약품명	상품명	타겟 항원	적응증
★★	Adalimumab	**Humira**	TNF-α	**관절염, 척추염, 크론병**
★★	Infliximab	**Remicade**	TNF-α	**관절염, 척추염, 크론병**
★★	Rituximab	**Rituxan**	CD20	**비호지킨성 림프종**
★★	Bevacizumab	**Avastin**	VEGF-A	**전이성 대장암/직장암, 폐암**
★★	Ranibizumab	**Lucentis**	VEGF-A (Bevacizumab의 Fab)	**습성 노인황반변성 (AMD)**
★★	Trastuzumab	**Herceptin**	HER-2/Errb2 (EGFR)	**전이성 유방암**
★☆	Cetuximab	**Erbitux**	EGFR	**전이성 직결장암, 편평세포암**
★☆	Natalizumab	**Tysabri**	α4 인테그린	**다발성경화증 (MS)**
★☆	Omalizumab	**Xolair**	IgE	**알러지**
★	Palivizumab	**Synagis**	RSV의 F단백질	**RSV 감염**
★	Basiliximab	**Simulect**	CD25 (IL-2R)	**면역억제**
★	Daclizumab	**Zenapax**	CD25 (IL-2R)	**면역억제**
★	Alemtuzumab	**CamPath**	CD52	**면역억제**
★	Muromonab-CD3	**Orthoclone OKT3**	CD3	**면역억제**

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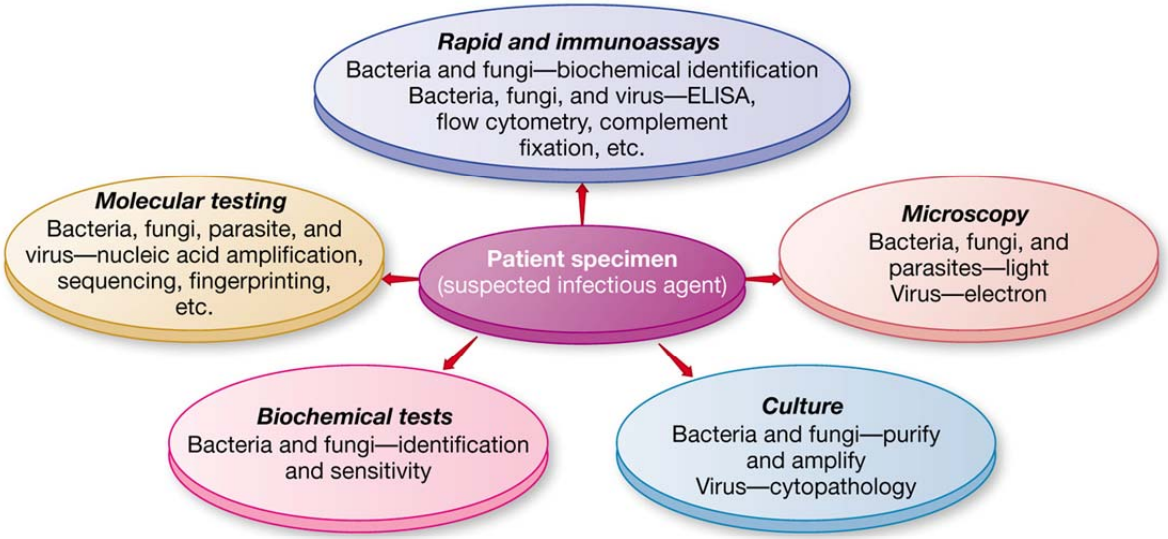
Clinical Microbiology Laboratory

- clinical microbiologist
  - the major function is to isolate and identify microbes from clinical specimens *rapidly*
- clinical specimen
  - portion or quantity of human material that is tested, examined, or studied to determine the presence or absence of specific microbes



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Working with Specimens

- safety concerns
  - Standard Microbiological Practices have been established by the Centers for Disease Control and Prevention (CDC)
- specimen should:
  - represent diseased area and other appropriate sites
  - be large enough for carrying out a variety of diagnostic tests
  - be collected in a manner that avoids contamination
  - be forwarded promptly to clinical lab
  - be obtained prior to administration of antimicrobial agents, if possible

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Standard Microbiological Practices

- are minimum guidelines that should be supplemented with other precautions based on exposure risks and lab biosafety level regulations
- goal is to protect workers from contact with agents by their taking precautions and by their working in a safe laboratory environment

- e.g. workers can limit their contact with microbes by not eating or smoking in lab and by preventing injuries caused by sharp objects
- e.g. coverings such as lab coats and bandages should be used
- e.g. workers should know how to use emergency eye wash and shower stations
- e.g. work space should be disinfected
- e.g. hands should be washed thoroughly after any exposure and before leaving lab

Biosafety Levels and Identification

- recommended guidelines for additional precautions reflect the laboratory's biosafety level (BSL)
  - BSL 1 – not known to cause disease in healthy adults
  - BSL 2 – associated with human disease
  - BSL 3 – disease may have serious or lethal consequences
  - BSL 4 – agent poses high risk of life-threatening disease

- preliminary or definitive identification of microbe based on numerous types of diagnostic procedures
  - microscopy
  - growth and biochemical characteristics
  - immunologic tests
  - bacteriophage typing
  - molecular methods

Molecular Genetic Methods

- accurate, routine methods used in clinical microbiology labs
- comparison of proteins
- nucleic acid-based detection methods
- molecular methods widely used
  - nucleic acid probes; DNA hybridization
  - **PCR** and **real time PCR (RTi-PCR)**
  - **ribotyping** (16S rRNA analysis)
  - multilocus sequence typing (MLST)
  - genomic fingerprinting
  - plasmid fingerprinting

표 5-7	바이오센서: 생물의학, 산업, 환경에서의 잠재적 응용방법
의학진단과 생물의학 조사	
농업, 원예, 수의학적 분석	
수질의 오염물, 미생물 오염의 검출	
발효분석과 조절	
공업 기체와 액체의 관찰	
광업에서 독성 기체의 측량	
냄새, 에센스, 페로몬의 직접적인 측정	

cf. **Biosensors**: biological detection systems

microfluidic antigen sensors, real time PCR, highly sensitive spectroscopy systems, liquid crystal amplification of microbial immune complexes

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Immunological Techniques

- detection of **antigens** or **antibodies** in specimens
  - especially useful when cultural methods are unavailable or impractical or antimicrobial therapy has been started
- use of immunological systems has many advantages
  - easy to use
  - give relatively rapid reaction endpoints
  - are sensitive and specific

- number, sensitivity, and specificity of **immunological techniques** are increased due to better understanding of immunology

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Immunological Methods

- Surface plasmon resonance (SPR)

- Agglutination (응집반응)
- Precipitation (침강반응): 침강이 되려면?
- Immunoelectrophoresis (면역전기영동법): 침강반응을 이용
- Complement fixation: *very sensitive*
- Immunoblotting (= Western blotting)
- Enzyme-linked immunosorbant assay (ELISA)
- Radioactive Immunoassay (RIA)
- Immnofluorescence/Fluorescence-Activated Cell Sorter (FACS)
- Biochips

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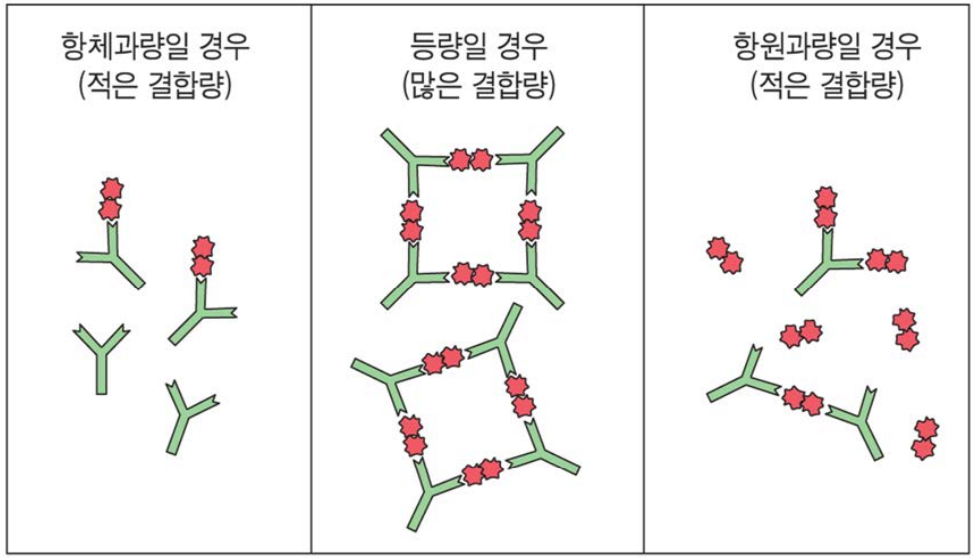
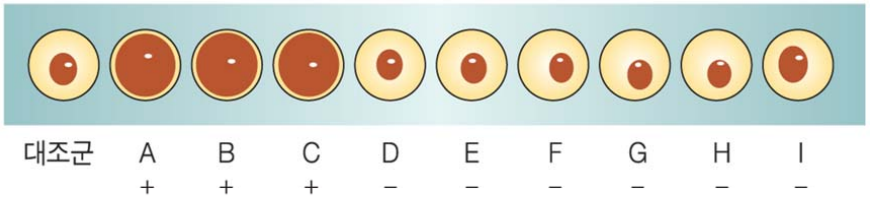
Agglutination

- 응집반응이 일어나려면?
  항원/항체 모두 multivalent
  적정비율로 존재

- 응집의 측정
  (원심분리)
  가시적 관찰





항원/항체를 이용한 응집반응

- 응집 억제 시험?

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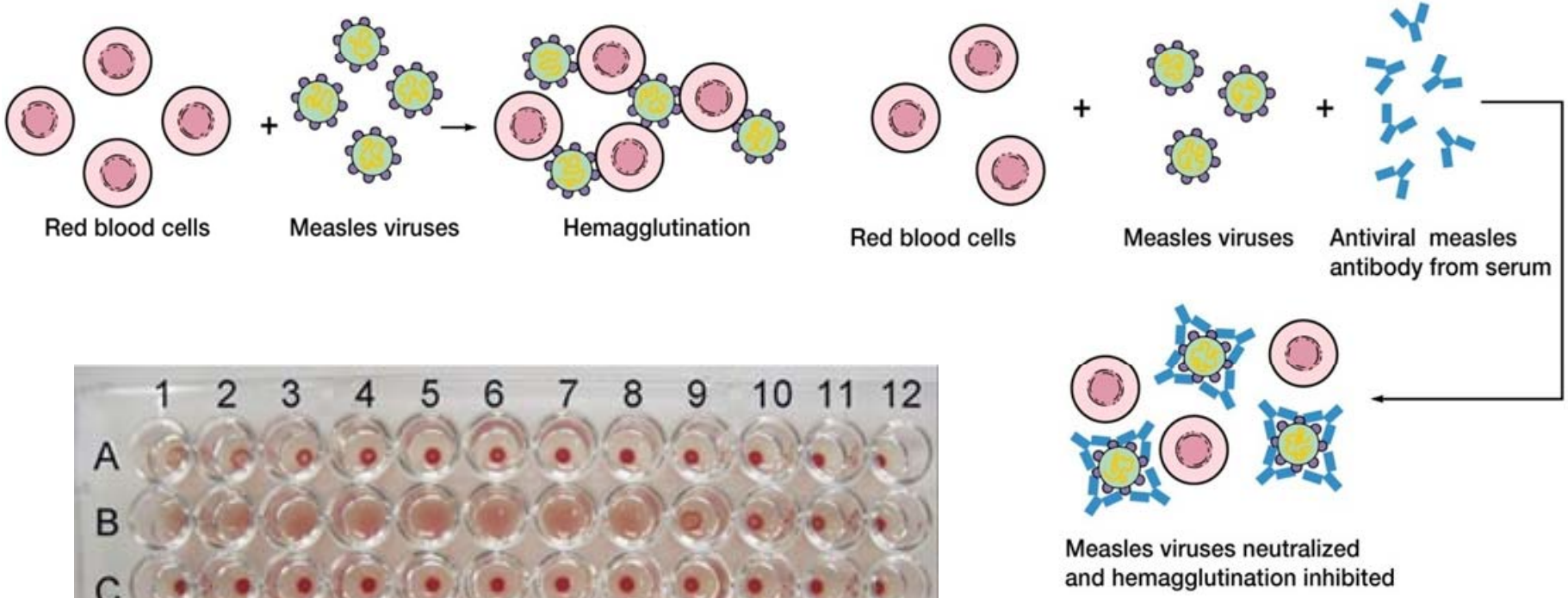
Agglutination



응집억제

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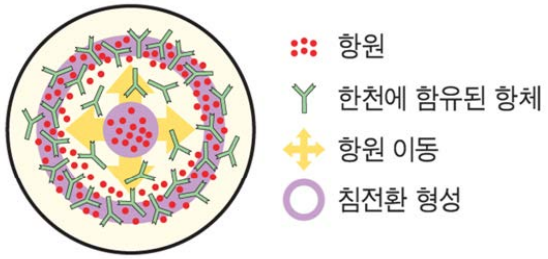
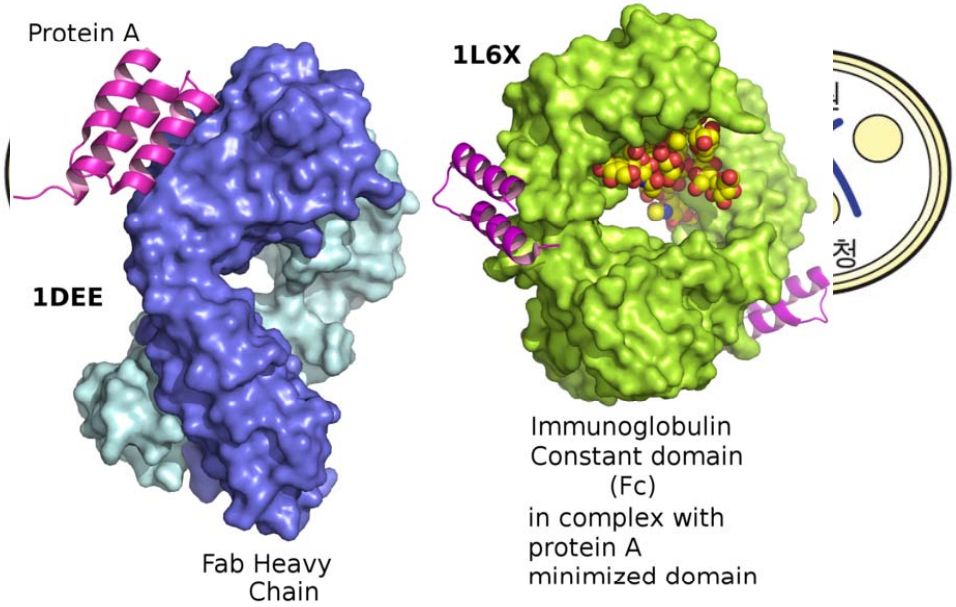
Precipitation

- 침강의 측정
  면역확산법 (immunodiffusion)
  면역전기영동법 (immunoelectrophoresis)
  면역침전 (**immunoprecipitation**): 원심분리, Tagging (bead, magnet)
  cf. *Staphylococcus* A protein (Spa): affinity to IgG (Fc)



Radial immunodiffusion
(*Mancini*)



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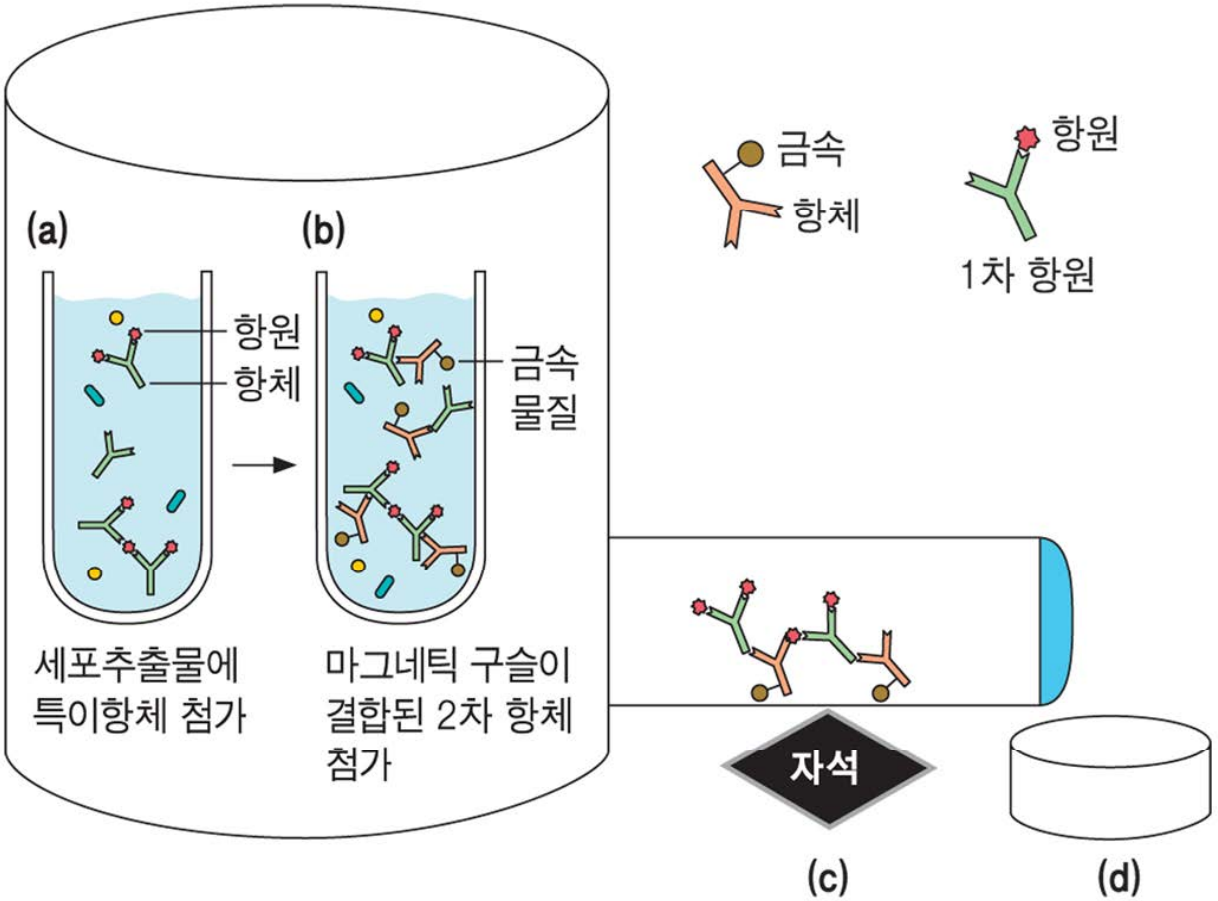
Precipitation



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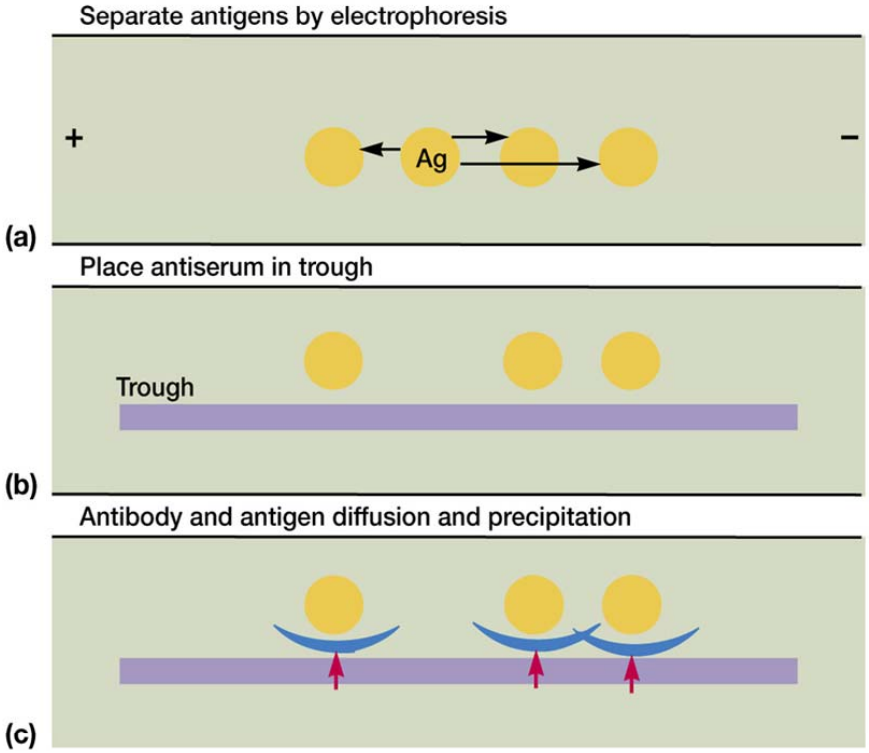
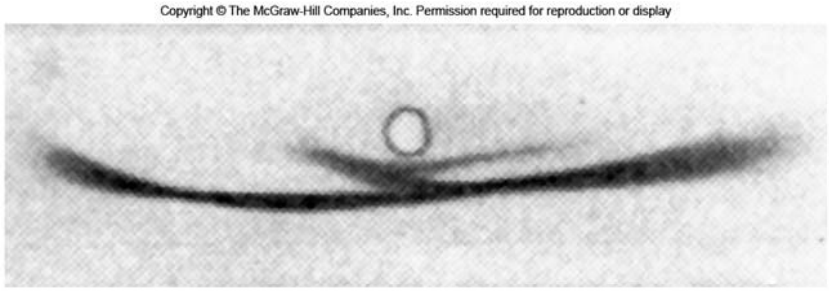
Immunoelectrophoresis

- antigens first separated by electrophoresis according to charge
- antigens visualized by precipitation reaction
- has greater resolution than immunodiffusion assays





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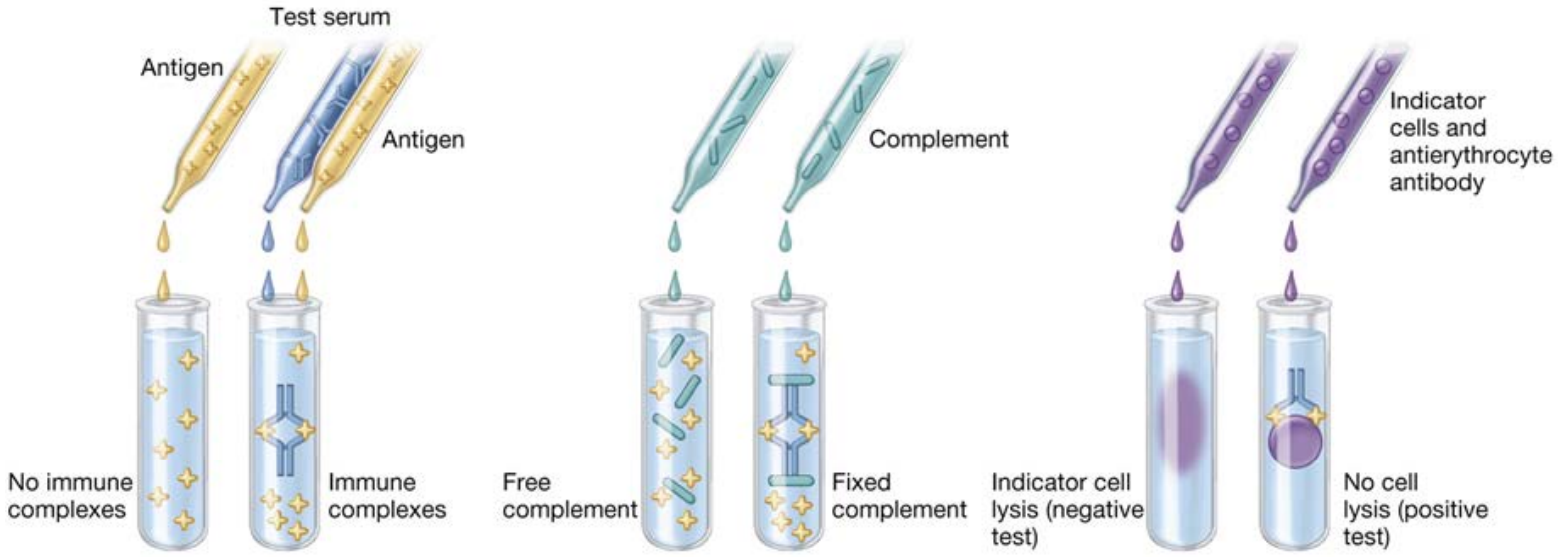
Complement fixation

- Immune complex 측정의 한 방법
  binding of complement to an Ag-Ab complex
  basis of diagnostic tests of Ab presence in the patient's serum
  very sensitive, measure extremely small amounts of antibody



Ag + Test serum Complement added Indicator cells

: complement consumed?

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Immunoblotting (Western Blotting)

- procedure
  - proteins separated by electrophoresis
  - proteins transferred to nitrocellulose sheets
  - protein bands visualized with enzyme-tagged antibodies
- sample uses
  - distinguish microbes
  - diagnostic tests
  - determine prognosis for infectious disease

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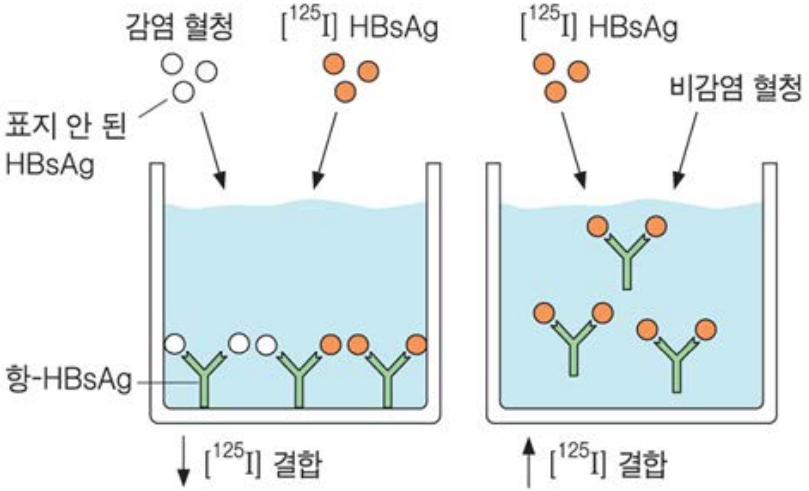
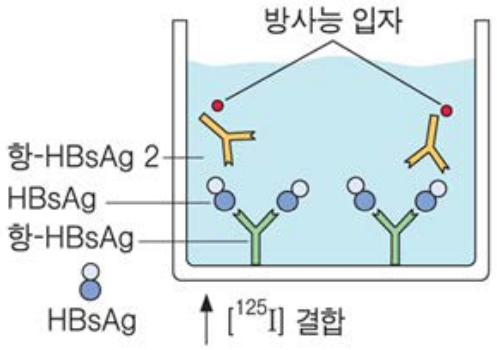
Radioimmunoassay (RIA)

- purified antigen labeled with radioisotope competes with unlabeled standard for antibody binding
- amount of radioactivity associated with antibody is measured





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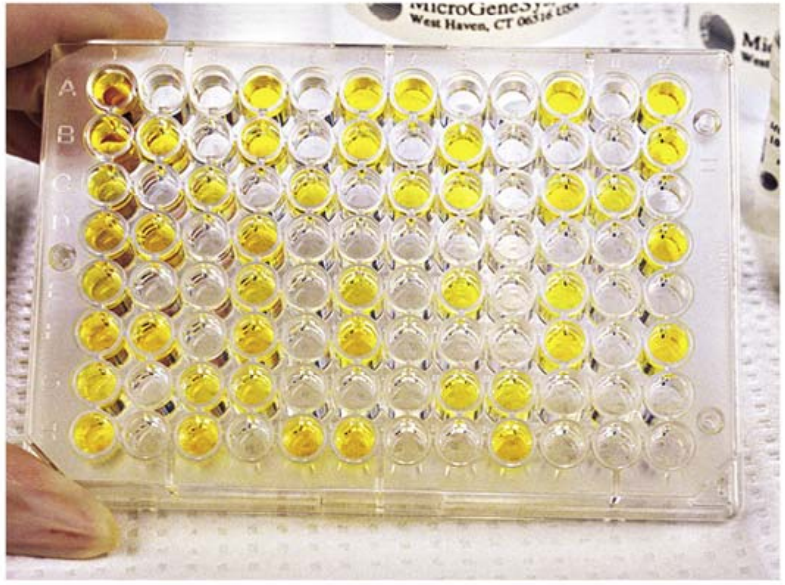
Enzyme-Linked Immunosorbent Assay (ELISA)

- one of the most widely used **serological** tests
  - direct test can be used to detect antigens in a sample
  - indirect test can be used to detect antibodies in a sample
- reaction visualized by addition of **chromogen**

Microtiter ELISA plate with 96 tests for HIV antibodies.
Colored wells indicate a positive reaction.



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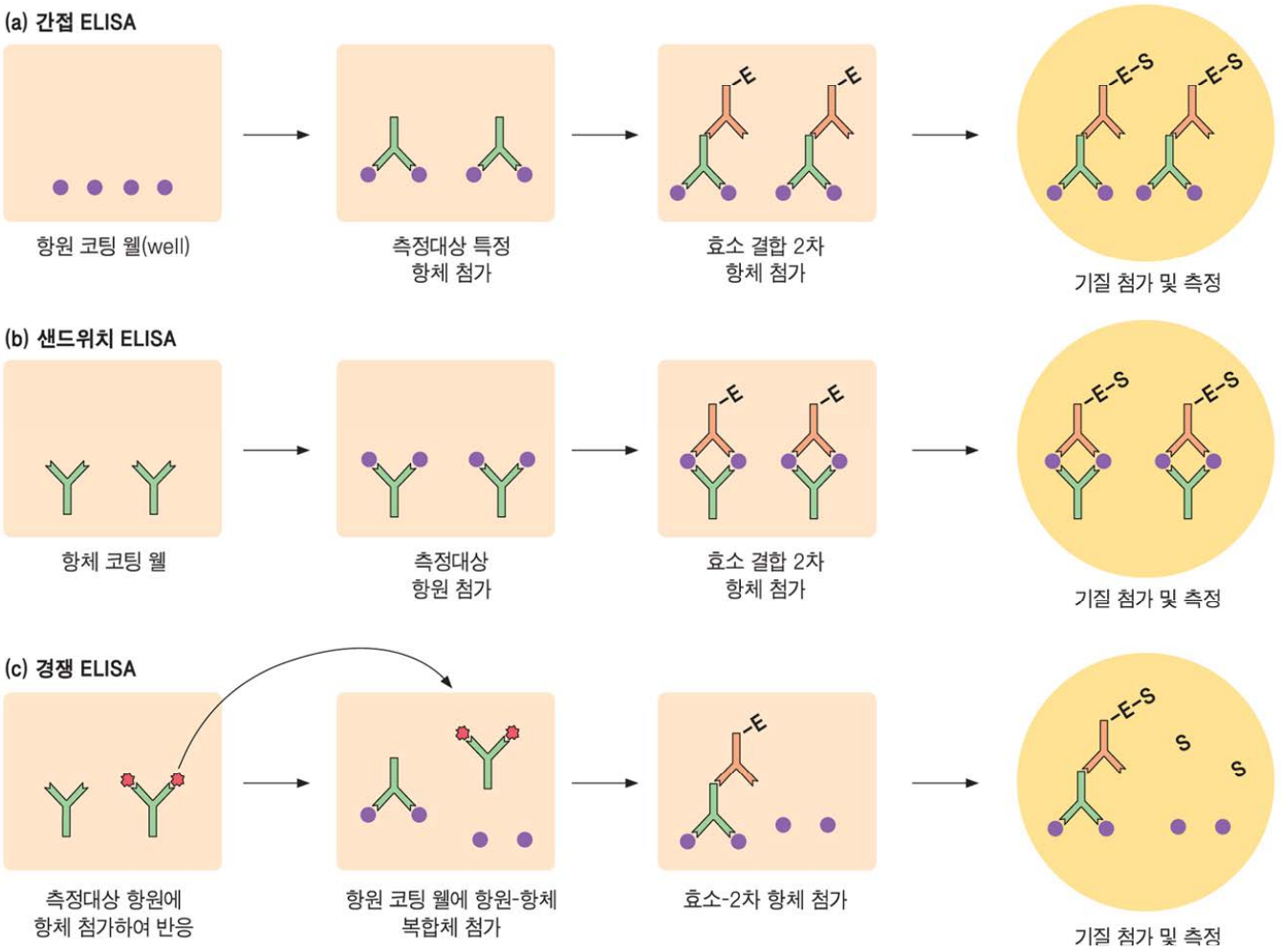
Types of ELISA



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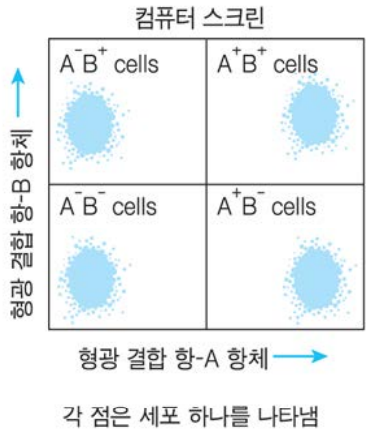
Flow Cytometry

- **flow cytometry**
  - detects organisms in clinical samples
  - detection based on cytometric parameters or by use of fluorochromes
    - fluorochromes often bound to antibodies or oligonucleotides
- **flow cytometer**
  - forces suspension of cells through laser beam and measures amount of light scattering of fluorescence
  - can detect heterogeneous microbial populations with different responses to antimicrobial treatments



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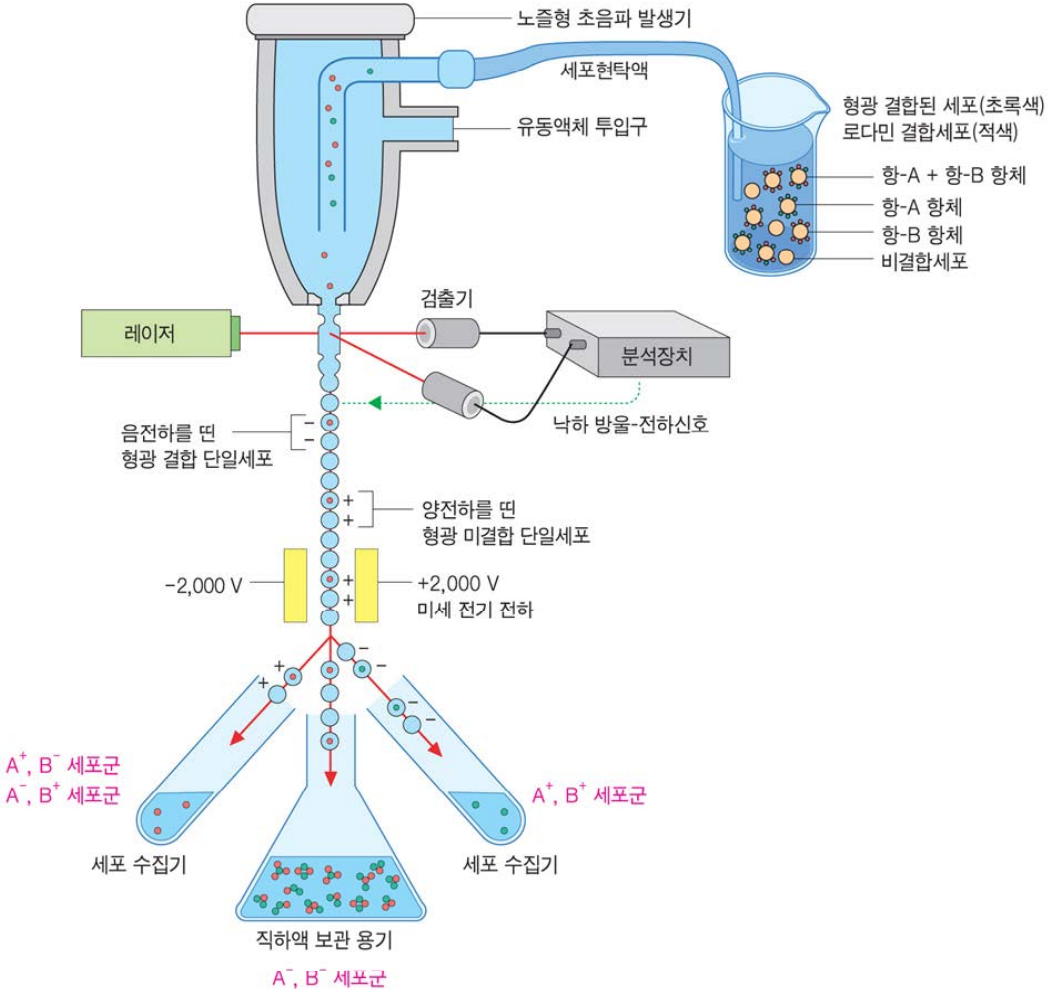
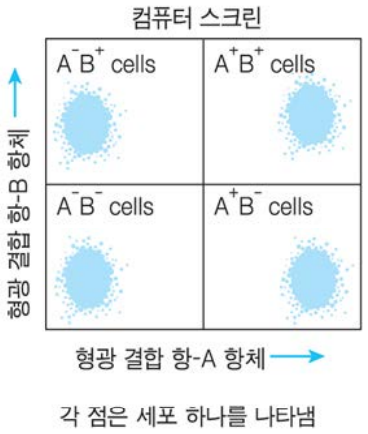
Flow Cytometry





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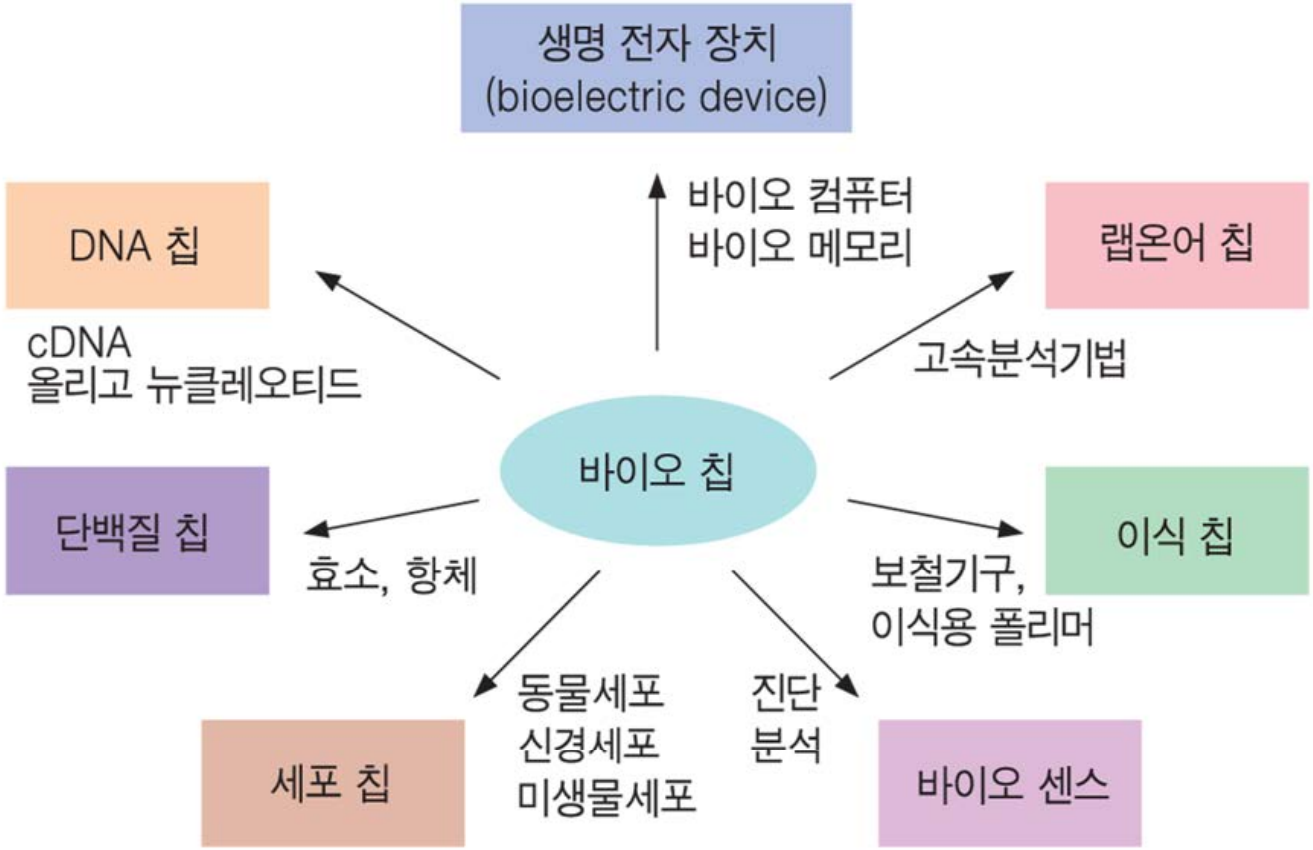
Biochips



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면역학적 방법들의 감도 비교

측정법	민감도* (μg 항체/mL)
액체 내 침강반응	20~200
겔 내 침강반응	
만시니법(방사면역확산법)	10~50
오흐테로니법(이중면역확산법)	20~200
면역 전기영동법	20~200
로켓 전기영동법	2
응집반응	
직접 응집반응	0.3
수동 응집반응	0.006~0.06
응집 억제반응	0.006~0.06
방사면역측정법(RIA)	0.0006~0.006
효소면역측정법(ELISA)	~0.0001~0.01
화학발광 ELISA**	~0.00001~0.01
면역형광법	1.0
면역입자분석법	0.005~0.001
유세포측정법	0.006~0.06
Immunochip법	~0.0001~0.01

* 민감도는 사용하는 항체의 친화력과 항원결정기 밀도 및 항원 표면의 분포에 의해 좌우된다.
** 화학발광에 기반을 둔 ELISA는 RIA에 유사한 민감도를 나타낸다.

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