

BI-311

General Microbiology

Handout #3

LD-50 (Lethal Dose 50%). Strain attenuation vaccines.

Disease - Arsenal of Virulence:

A - ENZYMES

- 1 - Hyaluronidase - Collagenase dissolve connective tissues
- 2 - Kinase (Streptokinase) dissolves fibrin clots
- 3 - Coagulase produced protective fibrin clots
- 4 - Hemolysins lysis of erythrocytes
- 5 - Leucocidins lysis of leucocytes

B - TOXINS

1 - Exotoxins:

Proteins - released into media - teledirected damage -

Diphtheria toxin *Corynebacterium diphtheriae* G+
Protein synthesis in Eukaryotes (& Archaea).
Pseudomembrane - DTP vaccine

Tetanus & Botulism toxin *Clostridium tetani*, *C.botulinum*
Proteins - nerve synapse two opposing neuron signals

Enterotoxin - Cholera *Vibrio cholerae* G- attaches to
ganglioside GM1 (complex glycolipid) -
ATP -> cAMP; Na - entry blocked, Cl & H₂O loss.

2 - Endotoxins:

Location: G- Lipopolysaccharide (LPS) layer: Lipid A
(N-acetylglucosamine-linked): *Escherichia*,
Salmonella, *Shigella*

Effects: fever - endogenous pyrogens (cell reaction)

Test: Amoebocytes of *Limulus polyphemus*

Host Defenses:

Non-specific, Specific

Non-specific:

Natural host resistance vs. susceptibility

Age - Stress - Diet

Anatomical defenses:

Skin layers, Sebaceous glands,

Ciliated epithels, mucus, Lysozyme

pH

Competition.

Compromised host.

Inflammation:

Symptoms: Local redness, swelling, pain, heat.

Pyrogenesis: Fever induction - endogenic pyrogenic proteins

Fever: Continuous, Remittent, Intermittent, Relapsing

Phagocytes:

Neutrophils - polymorphonuclear leucocytes (PMN)

Macrophages & Monocytes

Oxygen-dependent parasite destruction

ADHERENCE

tissue-specificity - host-specificity - receptor molecules
glycocalyx - fimbriae type I - *E. coli*, *Klebsiella*,
Salmonella, *Shigella*

INVASION

bacteremia
virulence : enzymes - hyaluronidase - collagenase -
streptokinase - coagulase - hemolysins - phospholipase -
leucocidins

EXOTOXINS produced extracellularly + exported

Diphtheria *Corynebacterium diphtheriae*
Tetanus *Clostridium tetani*, *C. botulinum* - binds nerve synapses
Enterotoxin *Vibrio cholerae* -
strong - nonpyrogenic - immunogenic (toxoids) - specific

ENDOTOXINS cell bound local effect

G-, Lipopolysaccharide-lipoprotein complexes LPS
Salmonella. - *Limulus* assay
weak - pyrogenic - poor immunogens - general

VIRULENCE ability of a parasite to cause disease

invasiveness vs. toxigenicity - *Streptococcus pneumoniae*
LD50 - attenuation

HOST DEFENSE

non-specific vs. specific (immune response)
Natural resistance and host specificity
age - infant botulism --- old age
anatomical defenses: Sebaceous glands - pH - beta lysins
compromised host - smoking, alcohol, drugs, lack of sleep
malnutrition, infections - AIDS

INFLAMMATION & FEVER

cytokines (leucocytes) - fibrin clot - isolation
pyrogens - (endogenous)
continuous - remittent - intermittent

Components of the immune system

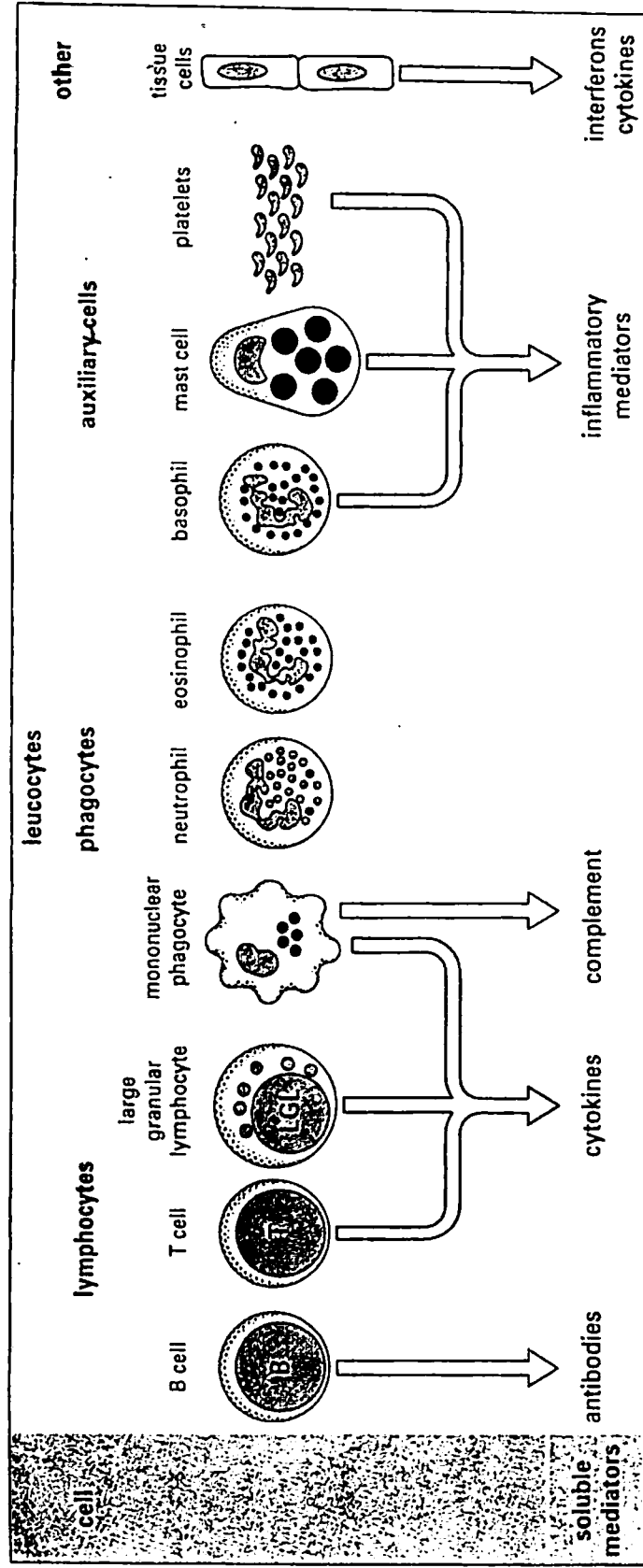
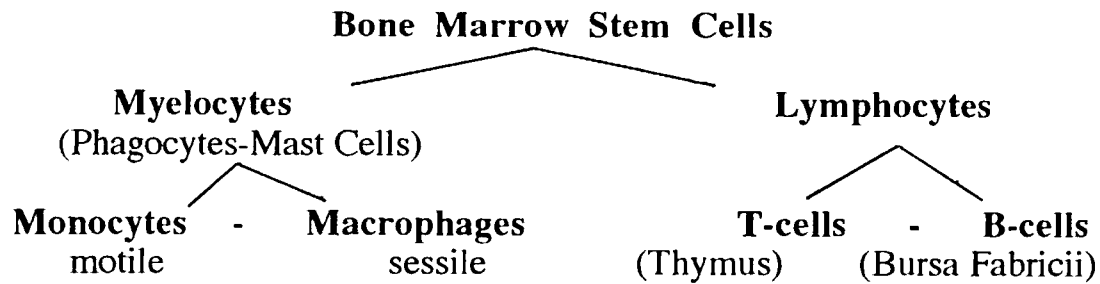


Fig. 1.4 The principal components of the immune system are shown, indicating which cells produce which soluble mediators. Complement is made primarily by the liver, although there is some synthesis by mononuclear phagocytes. Note that each cell produces and secretes only a particular set of cytokines or inflammatory mediators.

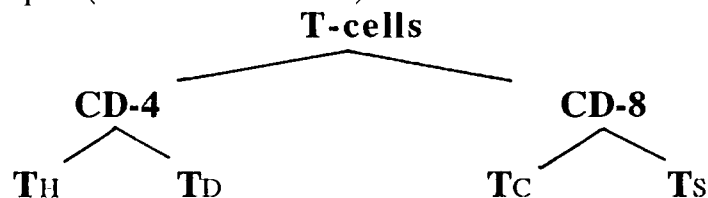


Phagocytes: Monocytes, Macrophages, Polymorphonuclear Leucocytes

Lysosomes - inclusion (digestive vacuoles with enzymes and bactericides)
 Enzymes: Lysozyme - Protease - Phosphatase - Lipase - Nuclease
 Bactericides: H-peroxide, superoxide, hydroxyl radical, singlet oxygen
 Aerobic/Anaerobic Switch: Upon phagocytosis - Lactate production,
 pH-drop
 APC - system: Antibody Presenting Cells.

Lymphocytes: T-cells, B-cells & NK-cells

T-cells: Equipped with TCRs: T-Cell-Receptors for MHC-presented epitopes (AG-determinants)



TH - Detect the MHC-class 2 w. Antigens on APC-Lukocytes
 - Produce lymphokines to activate B-cells

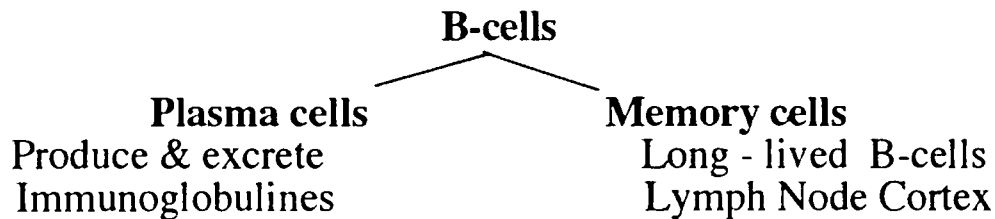
TD - Delayed hypersensitivity cells - activate phagocytes involved in allergy reactions

TC - Killer cells - Detect the MHC-class 1 w Antigens
 - Kill infected regular APC-cells: Perforins

Ts - Regulate the Immune response. Suppressing B-cells

NK-cells: Natural Killer cells, fixed number - no memory
Kill with Perforin - Attack cells with improper ID. Altered
MHC-class 1-by viruses or oncogens.

B-cells: Equipped with specific surface A-bodies; Produce and
release Abs



Means of Communication

1. - Abs (Antibodies) or Immunoglobulins

Antigen-Antibody reaction is specific

Immunogens - substances with parts that cause immune response:

Haptens = antigenic determinants or epitopes (sugars, AA, A, bases)

Antigen Receptor Molecules = Immunoglobulines,

proteins that combine with Haptens.- In serum (Antiserum), milk,
body fluids - Amino terminal variable, carboxy terminal constant.

Y-shaped molecules w. 2 light + 2 heavy chains - Light chains:

1 constant (lambda or kappa) +1 variable region - Heavy chains:

3 constant + 1 variable

IgG - monomer - serum circulating IgG 1-4

IgA - dimer - Body fluids produced by mucosa - most numerous

IgM - pentamer - 1st circulating - strong complement binder

IgD - monomer - small amounts - unknown function

IgE - monomer - small amounts - Allergy related

2. - TCRs --- T-cell receptors - 2 S-S linked peptides - NH-

Variable domain - transmembran rooted - recognize only presented

Antigens: MHC+A-gen

3. - MHC --- Major Histocompatibility Complex Proteins

Binding semi-specific by motifs

Class I --- surface of all nucleated cells

alpha chain 45K glycoprotein in CM, encoded in MHC region
beta 12K not. Ag-binding at ER moves to CM - Labeled target cells for Tc.

Class II -- surface of B-cells, Macrophages & other APCs.

both chains alpha 33K & Beta 28K are transmembrane in CM
individual variability. - Ag-binding in Lysosomes moves: CM - Labeled target for TH-cells

4. - Cytokines - Small soluble proteins that regulate cell functions

Lymphokines - Cytokines produced by lymphocytes

4.1 **Interleukins** - Regulate interaction between leukocytes

IL1- Macrophages to IL1-receptor of TH-cells

IL2 - TH-cells to Tc-cell proliferation

IL4 + IL2 + IL6 - TH-cells to B-cell proliferation

4.2 **IFN-Interferons** (alpha, gama) nonspecific: viruses

4.3 **TNF**- Tumor Necrosis Factor (alpha, beta) by T-cells

5. - Tolerance and Clonal Selection

Positive & Negative selection of T-cels in Thymus

Clonal selection of B-cells

6. - The Complement System - Cascading Enzyme Sequence

Receptors for **IgM** and **IgG** Immunoglobulines upon

Ag-Ab contact + reconfiguration of the -COO tail

11 proteins C1-11

Phases: 1) initiation; 2) recognition by C1; 3) C4 -2; 4)C3;

Attraction of Phagocytes

5) C5 - 6 - 7; 6) C8 - 9 ring and puncture -- leak

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The central role of T_H cells in cell-mediated immunity

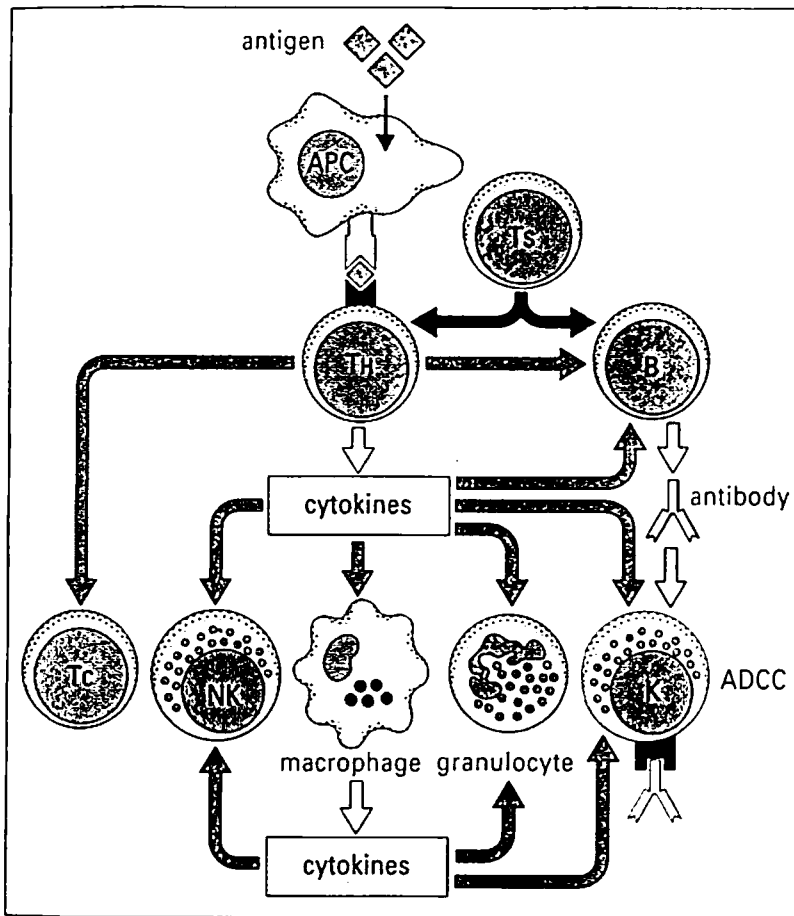


Fig. 9.3 Antigen-presenting cells (APCs) present processed antigen to helper T cells (T_H), which are central to the development of immune responses. These cells recognize particular epitopes and thus select those as targets. They then select and activate the appropriate effector mechanisms. They can help B cells to make antibody and activate or suppress the actions of a variety of other effector cells. These are described in greater detail later, and include cytotoxic T cells (T_C), natural killer (NK) cells, macrophages, granulocytes and antibody-dependent cytotoxic (K) cells. Many of these effects are mediated by lymphokines, but cytokines from other cells, particularly macrophages, are also important. Both T and B cells may in turn be influenced by 'suppressor' (T_S) or regulatory cells.

Immune Disease

1. Immediate Hypersensitivity – Type I. = Allergy

Mechanism: Memory through IgE attached to:

- (a) Mast Cells (non-motile connective tissue cells) and
- (b) Basophiles (motile leukocytes in the blood) --> release of
- (c) Histamines + Serotonine (modified amino acids)

Symptoms: minor – skin reaction, itch, watery eyes, sneezing
major – *asthma*, *anaphylactic shock*

Allergens (allergy antigens): pollen, hay fever, insect bites ...

Antidot: Antihistamines, Adrenalin.

2. Autoimmunity -- Specific – Type II

Mechanism: Th-1 cells recognize “self” as “foreign” antigens

Symptoms: *Hashimoto disease* attack on thyroid
Juvenile diabetes attack on Langerhans islets in pancreas

-- Systemic – Type III

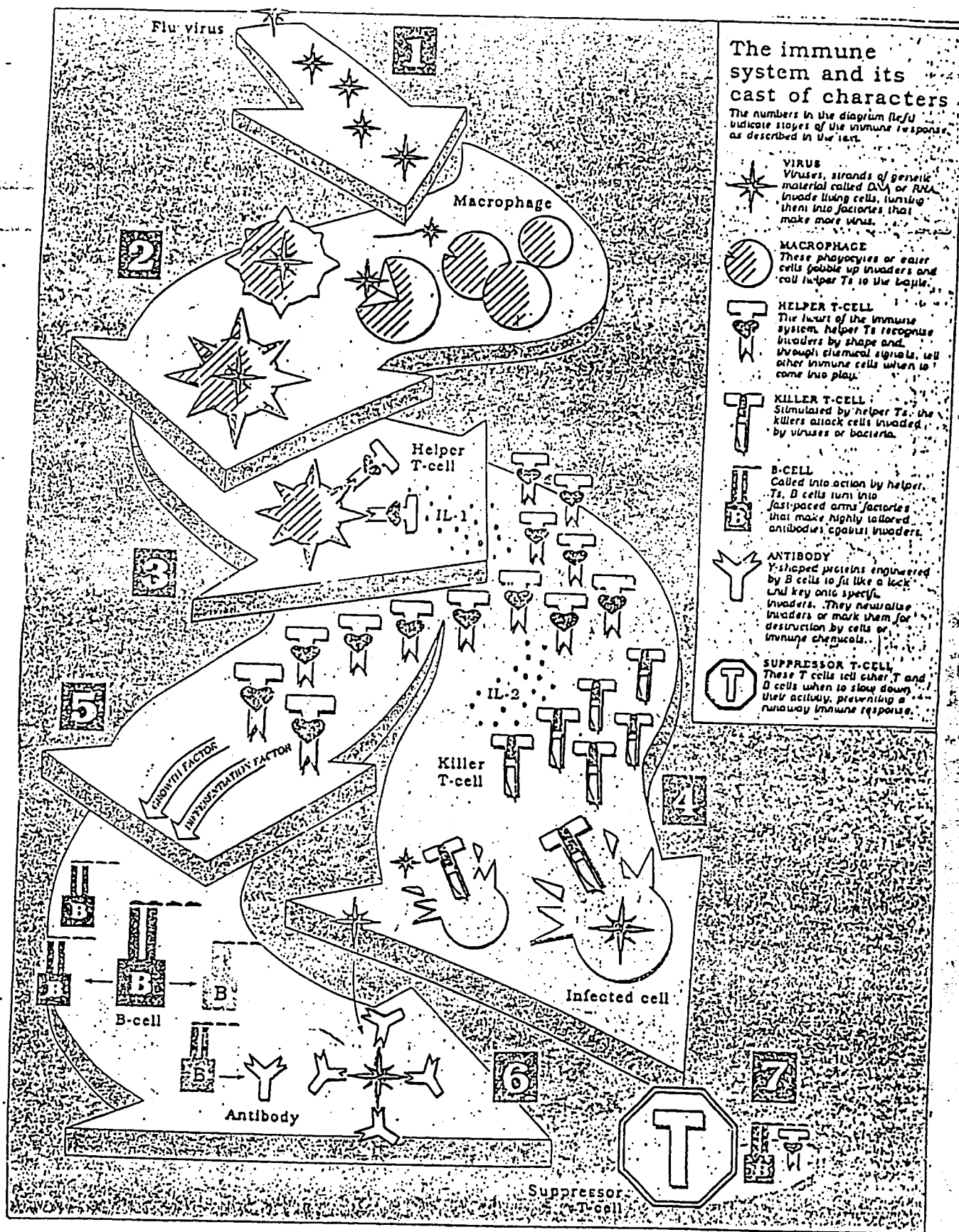
Symptoms: *Systemic Lupus* attack on different organs incl. nucleic acids

Rheumatoid Arthritis attack on cartilage

3. Delayed Hypersensitivity – Type IV

Mechanism: Th-1 cells inflammatory response

Symptoms: Contact dermatitis, e.g. poison ivy, jewelry, cosmetica, dust mites, animals, tuberculin test



Strategies of Pathogens.

1. Direct assault

Streptococcus streptolysin O - Lysis of leukocytes
Vaccinia protein inhibiting C5 of the complement
Yersinia pestis CM protein coating

2. Camouflage and Mimicry (stealth bomber strategy)

E. coli - covered with sialic acid - not safe against Antibodies.
Rabies virus - attaches to acetylcholine neurotransmitter site
Vaccinia virus - on epidermal growth factor receptor site
Rhino virus - binds to ICAM receptor on mucosa; viral genes encoding for hormones - 'fattening the cow'.
HIV - CD-4 receptor binding - avoidance of Ab-s by narrow fit

3. Hiding, escaping surveillance

Chlamydia, *Rickettsia*, *Shigella*, *Mycobacterium tuberculosis*,
Plasmodium vivax - hide inside the **endosome**. Prevent pH-change and fusion with lysosomes.

4. Disabling Antigen presentation

Attachment of a protein on the carboxy tail of MHC, anchoring and preventing passage to the CM.
Adenovirus - pneumonia, dysentery

5. Decoy

Usind delay in B-cell response of 2 weeks by altering epitope.
VSG - variable Surface Glycoprotein, 1000 VSG genes in
Trypanosoma gambiense.
Error prone genes: Replication - Transcription - Translation
Neisseria gonorrhoeae

6. Endosymbiosis

Chlorella & *Hydra*

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B-CELL (ANTIBODY) ATTACK

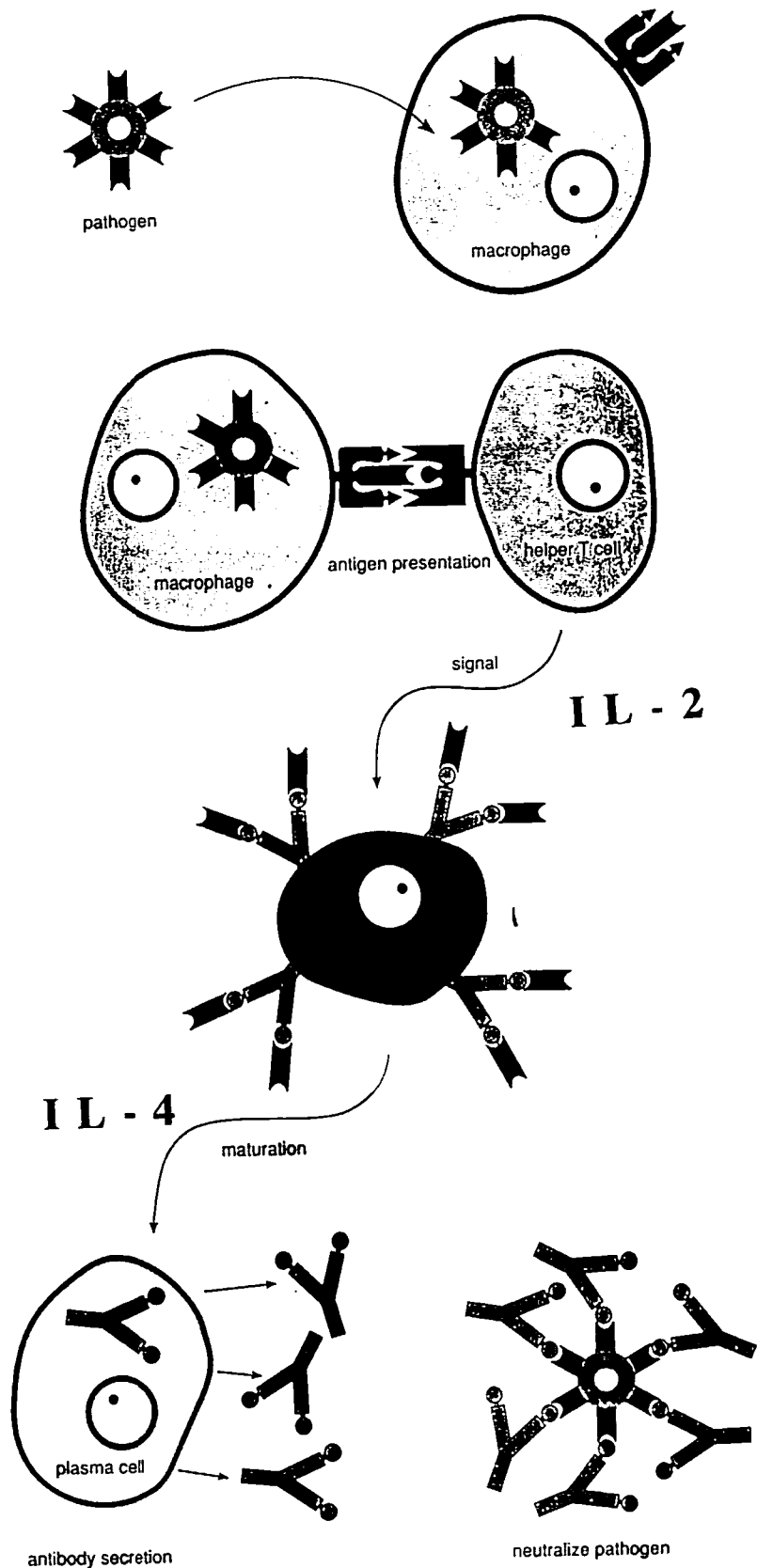


Figure 3. Humoral immune response involves the formation of specific antibodies (orange) that bind to a foreign antigen (green), eventually leading to the destruction of the pathogen. After a host organism is infected, an immune-system cell called a macrophage will attach the foreign antigen to a class II MHC molecule (brown), which the macrophage displays on its surface. A helper T cell, bearing a T-cell receptor molecule, recognizes the foreign antigen attached to the class II MHC molecule and signals another immune-system cell, a B cell, to differentiate and proliferate. The progeny of the selected B cell produces antibodies that specifically bind to the foreign antigen.

T-CELL (CYTOTOXIC) ATTACK

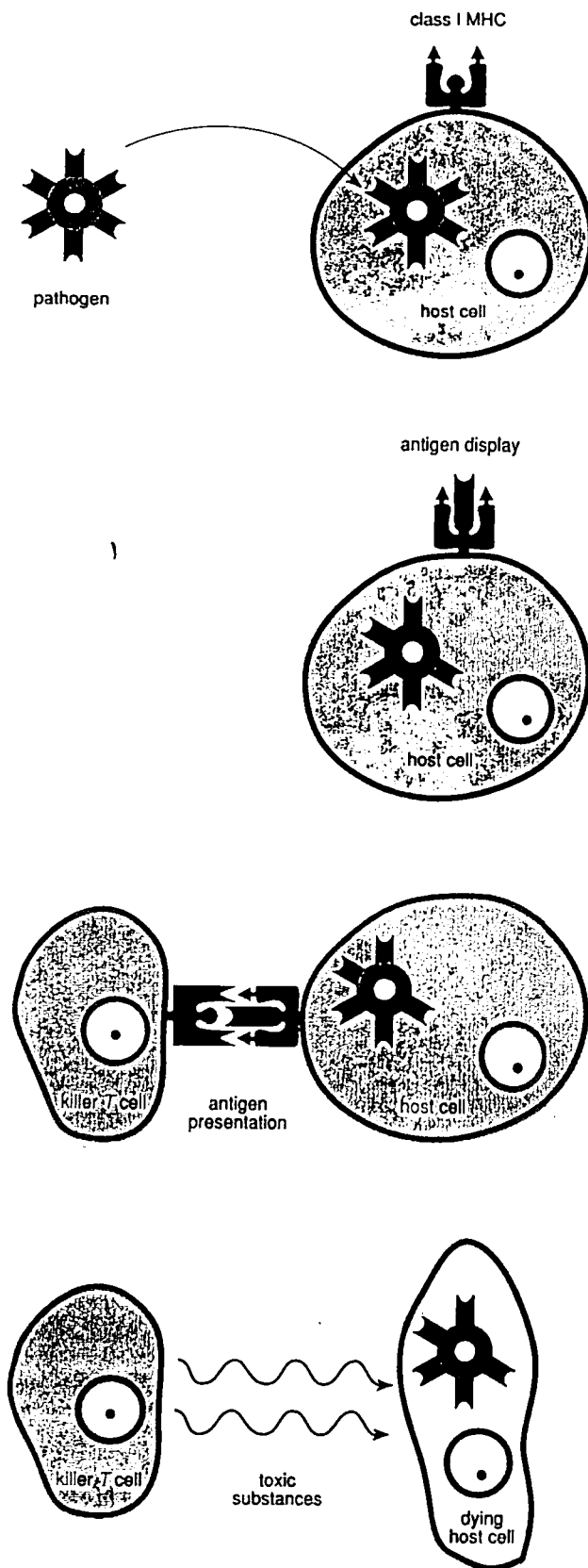


Figure 2. Cell-mediated immunity provides one of the body's defense mechanisms against an invading pathogen. When a virus infects a host cell, a class I molecule (dark brown) of the major histocompatibility complex (MHC) displays a fragment of the pathogen, called an antigen (green) on the infected cell's surface. The displayed antigen acts as a flag that identifies the cell as pathological. The complex formed by a class I MHC molecule and a foreign antigen is recognized by a killer T cell, whose T-cell receptor protein (light brown) binds to the complex. The killer T cell destroys the infected cell by releasing toxic substances.

COMPLEMENT ATTACKS

ANTIBODY-MEDIATED

ALTERNATIVE (DIRECT)

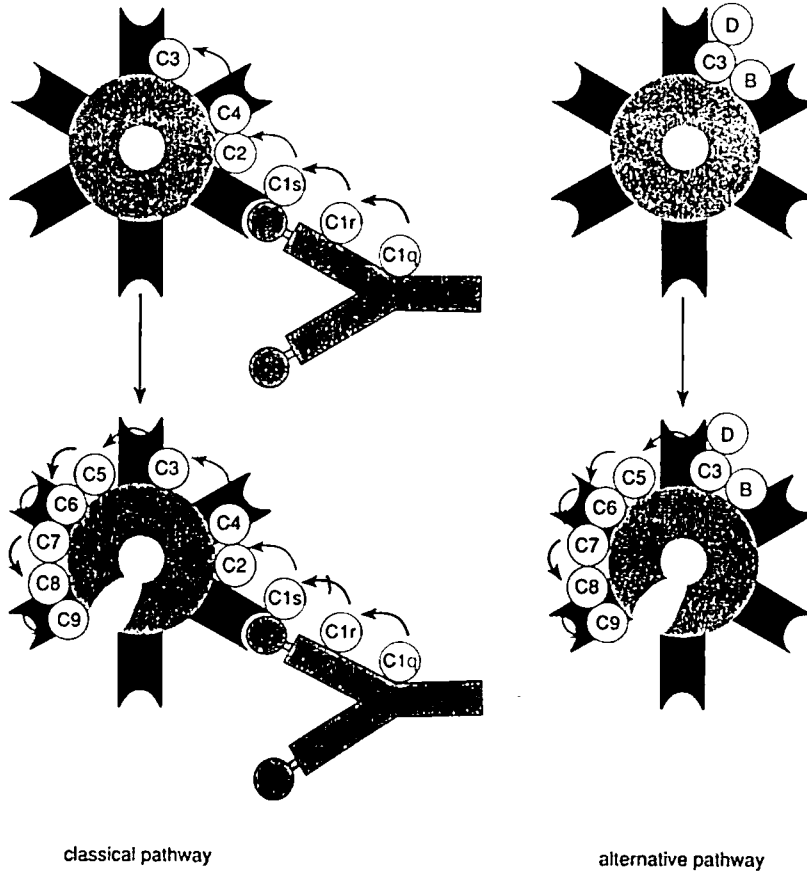


Figure 4. Complement system is a coordinated set of proteins that act—either alone or in concert with a specific antibody—to destroy a foreign cell or a host cell infected by a pathogen. The primary, or classical, complement pathway is a cascade of 11 enzymes—designated C1q, C1r, C1s and C2 to C9—triggered by an antigen-antibody complex to bring about the destruction of the cell. The final five proteins in the chain (C5 through C9) kill the cell by opening holes in its membrane. The alternative complement pathway is a shorter cascade triggered without the participation of an antibody molecule. The C3 protein, abetted by molecules designated B and D, reacts directly with a foreign antigen to initiate the lethal C5-to-C9 cascade.

DECOY

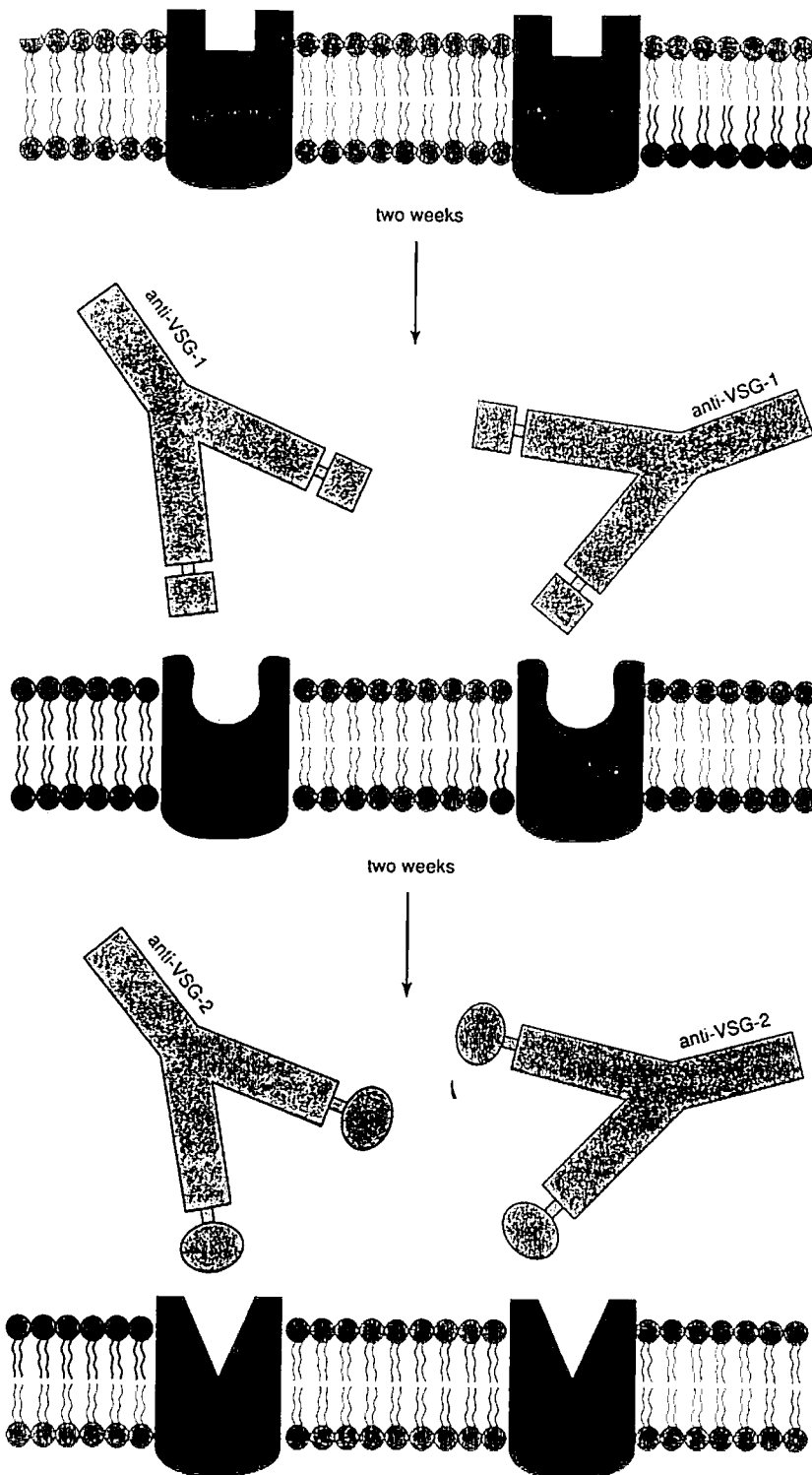


Figure 11. Decoy antigens distract the immune system by changing their structure more frequently than the body can produce the appropriate antibodies. The trypanosome that causes African sleeping sickness is able to change the structure of a surface membrane protein, called the variable surface glycoprotein (VSG), in less than two weeks—the time required for immune cells to produce new antibodies. The trypanosome is able to make more than a thousand different versions of the VSG antigen, leading to a chronic infection.

Microbiological diagnostics

Culturing: Selective medium
Differential medium

Stains:

EMB	-	Eosine-MethyleneBlue
TSI	-	Three Sugar Iron
		(glucose, lactose, sucrose, ferrous iron)

Immunodiagnosics:

- 1. Antibody titer**
- 2. Immunoelectron microscopy**
- 3. Immunofluorescence**
Rhodamine B, Isothiocyanate
- 4. Elisa - Enzyme-Linked ImmunoSorbent Assay**
- 5. RIA - Radio-Immuno-Assay** ¹²⁵
J
- 6. Immunoblot**
 - 1. Virus inactivation protein dissolution**
(Sodium Dodecyl Sulfate - SDS)
 - 2. Polyarcrylamide gel Electrophoresis**
 - 3. Nitrocellulose transfer**
 - 4. Sample serum (unknown)**
 - 5. Antihuman IgG + Peroxidase - brown band indicates +**

Direct:

Cholera toxin
E. Coli toxin
Staphylococcus
enterotoxin

Indirect:

**Viruses: HIV, Hepatitis
Measles, Mumps**

MIMICRY

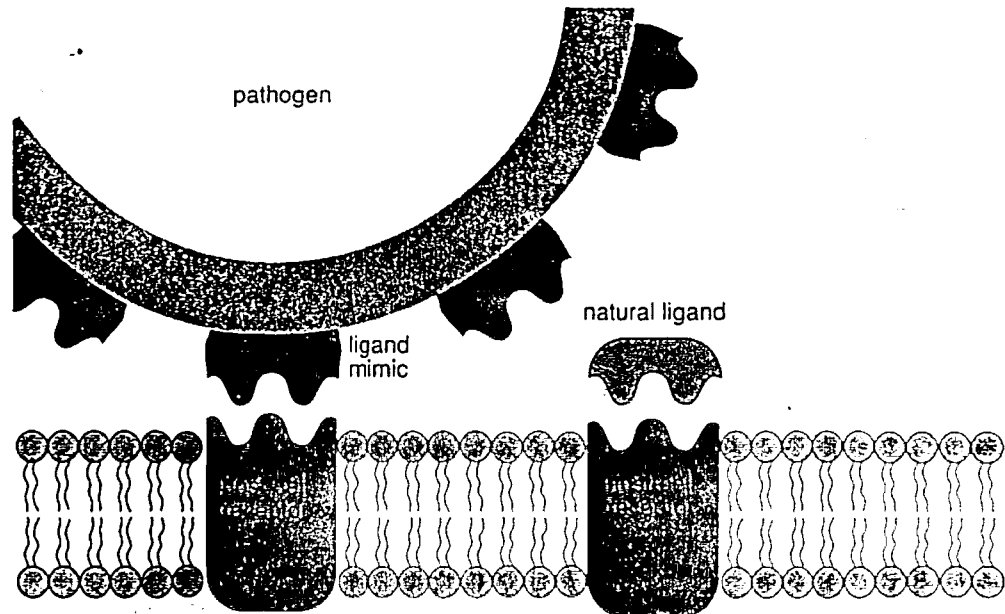


Figure 12. Mimicry of the molecule that normally binds to a host-cell receptor—the natural ligand—allows a pathogen to enter the cell. Ligand mimicry is a common strategy of pathogens. In some cases the receptor used by a particular virus has been identified: the rabies virus binds to the receptor for acetylcholine (the neurotransmitter of the nerve-muscle junction); and the vaccinia virus, which causes cowpox, binds to the receptor for epidermal growth factor (which stimulates cells to divide).

COMMUNICATION INTERRUPTION

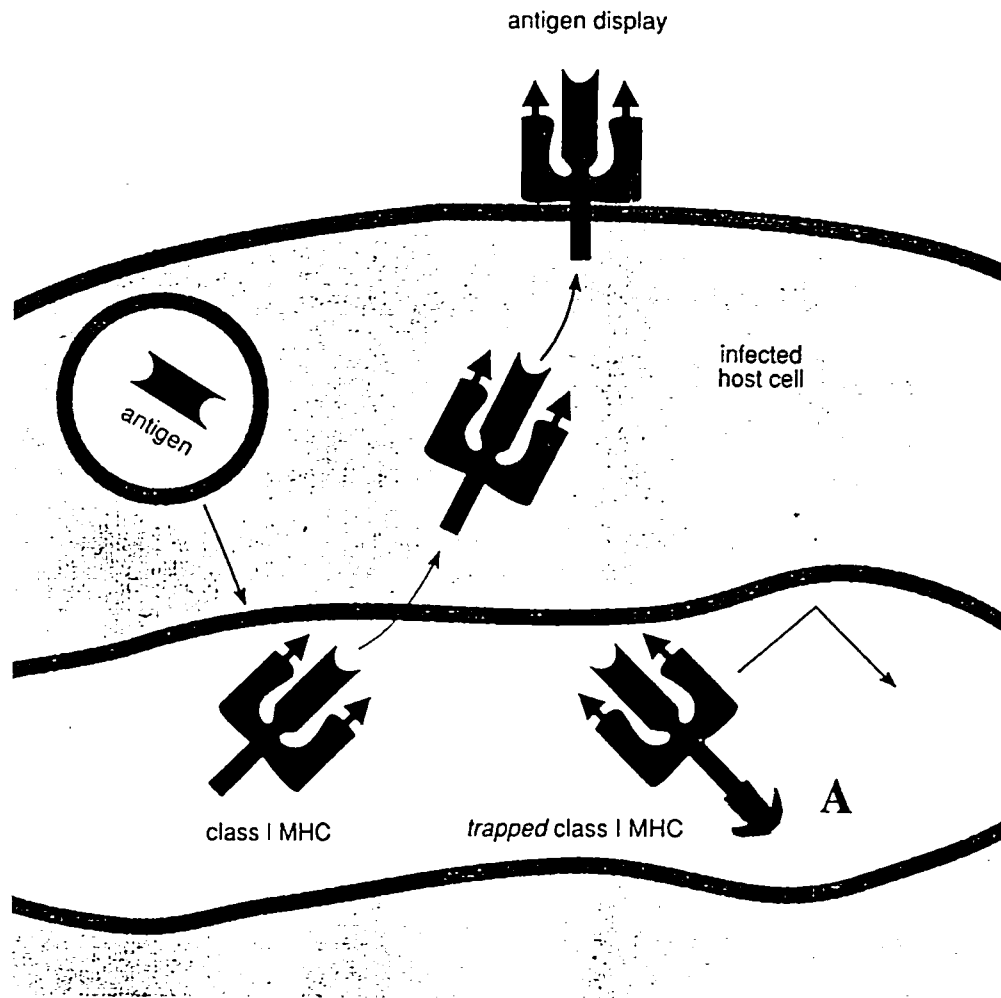


Figure 10. Disabling an infected cell's pathogen-alert system enables some viruses to hide from killer T cells, which would otherwise destroy the cell and its contents. A host cell normally advertises an infection by displaying a viral antigen (*green*) complexed with a class I MHC molecule (*brown*) on its membrane surface. The adenovirus (a cause of some respiratory infections and dysentery) subverts this mechanism by adding an extra piece of protein (*red*) to the class I MHC molecule that prevents the antigen-MHC complex from moving to the cell surface. As a result, killer T cells do not recognize that the cell is infected, thus allowing the virus to reproduce.