

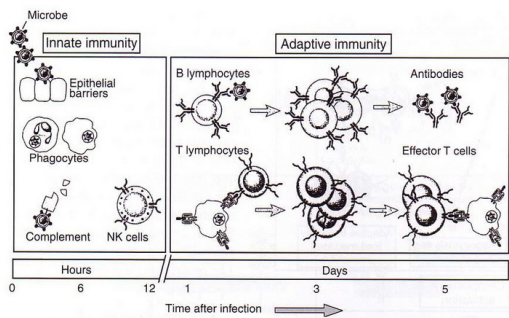
Immunology Review

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Outline

- Biology review
 - Innate Immunity
 - Acquired Immunity
 - Complement
 - Cytokines
- Immunology and Disease
- Therapeutics used in immune-mediated disease

Innate vs. adaptive immunity



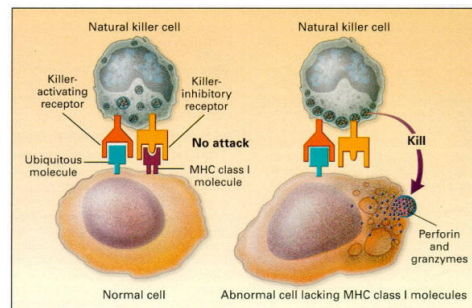
Innate Immunity

- Physical barriers
- Non-specific receptor-mediated endocytosis & pinocytosis
- Acute phase proteins
- Complement & cytokines
- NK cells
- Pattern-recognition receptors

Natural killer cells

- Virally-infected & transformed cells often lack class-I MHC receptors
- KIR (killer-cell inhibitory) bind class MHC class-I antigen and prevent NK-cell mediated death
- Death via lysis or apoptosis
- Also play a role in adaptive immunity

NK cell



Pattern-recognition receptors

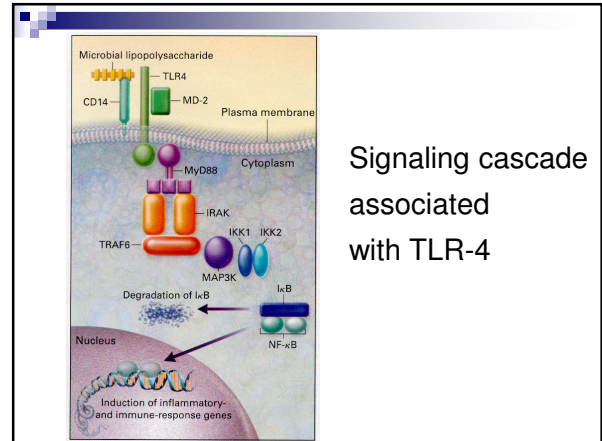
- Recognize pathogen-associated molecular patterns
 - Bacterial LPS, peptidoglycan, lipoteichoic acids, mannans, bacterial DNA, dsRNA, glucans
- Receptors found on macrophages, dendritic cells and B-lymphocytes

Pattern-recognition receptors

- 3 functionally different classes
 - Secreted
 - Mannan-binding lectin receptor
 - Endocytic
 - Macrophage mannose receptor
 - Signaling
 - Toll-like family receptors

Toll-like receptors

- >11 mammalian TLR have been identified
- Recognition of microbial products → activation of NF- κ B pathway
- Polymorphisms in human TLR-4 gene associated with an increased susceptibility to gram-negative infections



Adaptive immunity

- Humoral (B-cell) immunity
 - B-lymphocytes preprocessed in fetal liver and bone marrow
 - Antibodies against invading agent
- Cell-mediated (T-cell) immunity
 - T-lymphocytes preprocessed in the thymus
 - Formation of large numbers of activated T lymphocytes

Antigen Presenting Cells

- Macrophages:
 - Kupffer cells, alveolar, splenic, peritoneal, microglial
- Dendritic cells
- Interdigitating cells of the thymus
- B-cells
- Langerhaans cells in skin

Antigen Presenting Cells

- Functions:
 - Phagocytosis (with granulocytes)
 - Antigen presenting to T-lymphocytes (CD4)

Major Histocompatibility complex (MHC)

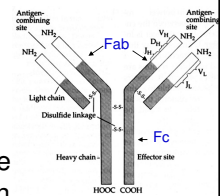
- MHC-I
 - Expressed on most nucleated cells in body
 - Present peptides to CD8 T-lymphocytes
 - Peptides generated in cytoplasm
- MHC-II
 - Constitutive on B-cells, dendritic cells & thymic epithelial cells; induced expression on macrophages, endothelial cells
 - Present peptides to CD4 T-lymphocytes
 - Peptides generated in lysosomes

B-lymphocytes

- Lymphoid macrophages present antigen to adjacent B-cells →
 - Lymphoblasts → plasma cells
 - ↘ memory cells
 - Memory cells
 - More rapid, potent and longer-lasting response in future
- Activation modulated by coreceptors

Immunoglobulin

- Gamma globulins
 - ~20% of plasma proteins
- Classes of Ig differ by virtue of Fc region on heavy chain
- Variable & constant portions
- Five classes that differ in structure of heavy chains



Functions of antibodies

- Direct action on pathogens
 - Agglutination
 - Precipitation
 - Neutralization
 - Covers toxic sites of antigenic agent
 - Lysis
- Activates complement
 - Amplifying effects

Immunoglobulin

- 5 classes: IgM, IgG, IgA, IgD, IgE
- IgG
 - ~75% of Ab
 - Longest 1/2 life
 - Opsonizing antibody
 - Neutralizes specific toxins (tetanus, botulisms) & viruses
 - Immobilizes various motile bacteria (clumping)

Immunoglobulin

- IgM
 - Major immunoglobulin in primary immune response
 - Efficient at complement activation, opsonization, virus neutralization, agglutination
 - Part of B cell receptor with IgD
- IgA
 - Prevents adherence of bacteria/viruses to epithelial surfaces
- IgE
 - Immunity for parasitic worms
 - Type I hypersensitivity reaction

Functions of Antibodies

Figure 2.28

Function	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	-	++	++	++	++	++	-
Opsonization	-	-	+++	*	++	+	+	-
Sensitization for killing by NK cells	-	-	++	-	++	-	-	-
Sensitization of mast cells	-	-	+	-	+	-	-	+++
Activation of complement system	+++	-	++	+	+++	-	+	-

T-lymphocytes

- Types:
 - Helper T cells (CD4)
 - Cytotoxic T cells (CD8)
 - Suppressor T cells
- TCR expressed on cell surface in association transmembrane molecule (CD4/CD8)

T-lymphocytes

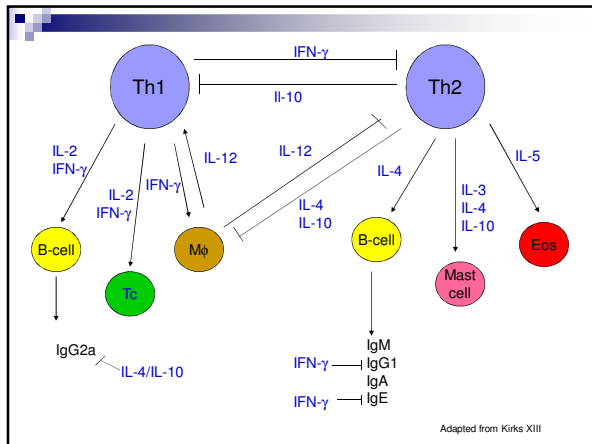
- Require 2 signals to become activated
 - Complex of a peptide-MHC molecule
 - Costimulatory signal on APC
 - Absence → inactivation or PCD of T cell
- APC
 - Dendritic cells: major APC in CD4 T-cell responses
 - B-cells: major APC in primed (memory) T-cell responses

Helper T-lymphocytes (Th)

- Functions:
 - Stimulation of growth/proliferation of cytotoxic & suppressor T cells
 - Stimulation of B cell growth & differentiation
 - Feedback stimulatory effect on helper T-cells
 - Activation of the macrophage system

Helper T-lymphocytes

- T-cell differentiation partially determined by presence of cytokines synthesized by innate immune response
- IL-12, IFN- γ , and IL-18 → Th1 (CMI)
 - Viruses, bacteria
- IL-4 → Th2 (humoral)
 - Allergens, parasites



Cytotoxic T-lymphocyte

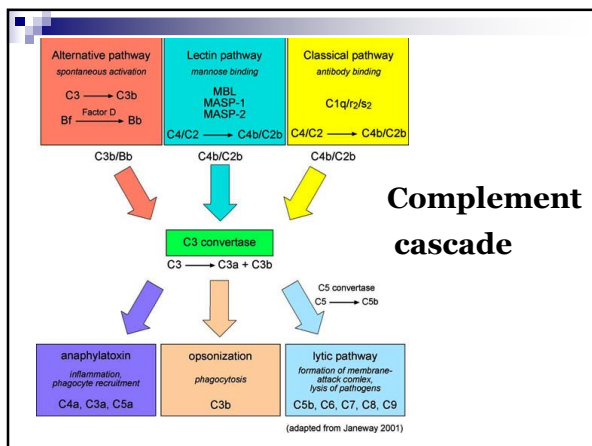
- CD8 T-cell
 - TCR recognizes peptide associated with MHC class I molecule
- Must be activated before killing target cell
- Mechanisms of killing
 - Perforin/granzymes (similar to MAC complex)
 - Fas/FasL → caspase cascade

Complement

- System of more than 30 proteins
 - Synthesized in the liver
 - Primarily constitutive
 - IL-6, TNF- α , IFN- γ increase complement production during active infections

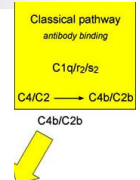
Complement- major activities

- Opsonization and phagocytosis
- Lysis
- Agglutination
- Neutralization of viruses
- Chemotaxis
- Activation of mast cells and basophils
- Disposing of immune complexes and the products of inflammatory injury



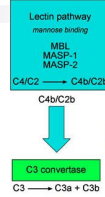
Classical Pathway

- Activated by antigen/antibody complex
 - IgM or IgG
- C4b2b- classical C3 convertase
 - C3b-opsonin
 - C3a-anaphylatoxin
- Can also neutralize viruses via C1



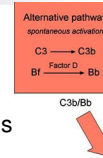
Lectin pathway

- Mannose-Binding Lectin (MBL) cascade
- MBL made in liver in response to macrophage cytokines
 - Pattern recognition (endocytic)
- MBL + serum proteases function like C1
- Converges with classical pathway



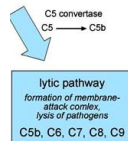
Alternative pathway

- Can be activated by foreign pathogens in absence of antibody
- Factor B binds C3b & Factor D cleaves → C3bBb (alternative pathway C3 convertase)
- Activated by foreign pathogens, IgA complexes, autoantibodies
- Efficient at opsonization



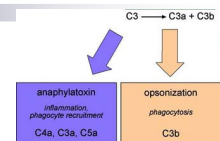
Terminal pathway

- C5a & C5b
 - C5b interacts with C6, C7, C8, C9 → C5b-C9
 - Membrane attack complex (MAC)
 - Pore formation in cell membranes → lysis
- Gram-negative & viruses most susceptible
 - Peptidoglycan layer on gram-positive cells



Anaphylatoxins

- C3a, C4a, C5a
- Binds to receptors on various cell types
 - Smooth muscle contraction
 - Increase vascular permeability
 - Activate mast cells
- C3a KO: increased susceptibility to LPS-induced shock
- C5a KO: attenuated response



Cytokines

- Soluble polypeptides that modulate many biological responses
- Differ from classic endocrine hormones
 - Produced by many cell types
 - Produced primarily *de novo* in response to exogenous stimuli
 - Commonly have autocrine and paracrine effects

Cytokines

- Interferons
- Chemokines (IL-8)
- Members of TNF family
- Interleukins
 - EGF family (EGF, TGF- α)
 - β -trefoil family (FGF)
 - Cysteine knots (TGF- β , VEGF, PDGF)
 - Colony-stimulating factors (GM-CSF)

Interleukins

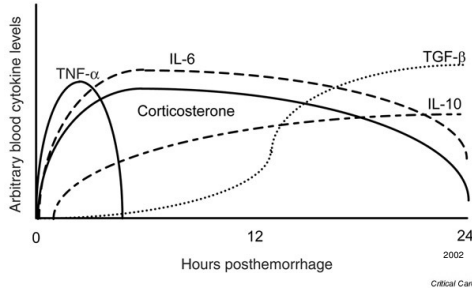
- Proteins secreted by activated cells of the immune system
- Form a complex network of signaling molecules in response to immunologic stimulation
- Activated monocytes and lymphocytes
- Biologically active at low concentrations
- Redundant, synergistic, antagonistic

Cytokines & Innate Immune Response

- IL-1
- IL-6
- TNF- α

- IFN- α
- IFN- β

Cytokine levels post trauma



Cytokines

- | | |
|--|---------------------------------------|
| ■ Pro-inflammatory | ■ Anti-inflammatory |
| <input type="checkbox"/> IL-1 β | <input type="checkbox"/> IL-10 |
| <input type="checkbox"/> TNF- α | <input type="checkbox"/> IL-4 |
| <input type="checkbox"/> IL-8 | <input type="checkbox"/> TGF- β |
| <input type="checkbox"/> IL-12 | <input type="checkbox"/> IL-1Ra |
| <input type="checkbox"/> IL-6 | <input type="checkbox"/> IL-6 |

Immunology & Disease

- Hypersensitivity Reactions
- C5a & Sepsis
- Cytokines and heart disease
- Immune-mediated disease

Hypersensitivity Reactions

- Type I: Immediate hypersensitivity
- Type II: Cytolytic/Cytotoxic
- Type III: Immune Complex
- Type IV: Cell-mediated immunity

Type I: Immediate hypersensitivity/ anaphylaxis

- IgE mediated
- Primary mediators released from mast cells & basophils
 - Histamine, AA metabolites, cytokines
- Secondary mediators
 - Activated complement, coagulation and fibrinolytic systems
- Late phase reaction
 - 6-12 hours post immediate reaction

Anaphylaxis

- Effects of primary mediators
 - Increase in vascular permeability
 - Systemic arteriolar vasodilation
 - Bronchial smooth muscle contraction
 - Enhanced mucus secretion
 - Increased gastric acid secretion
- Effects of secondary mediators
 - Tissue injury
 - Intravascular coagulation
 - Free radical production

Anaphylaxis- Treatment

- Epinephrine
 - β -adrenergic effects
 - α -adrenergic effects
- IV fluids
- Corticosteroids

Type II: Cytolytic/Cytotoxic

- Ab's formed against target antigens
 - Complement-mediated cytotoxic reactions
 - Antibody-dependent cytotoxic reactions
 - Antibody-mediated cellular dysfunction
- Examples: Transfusion reactions, Myasthenia Gravis, ITP, IMHA

Type III: Immune Complex

- Formation of immune complexes
 - Exogenous antigens (bacteria/virus)
 - Endogenous antigens (DNA)
- Activate complement cascade
 - C3a, C5a
- Favored sites of complex deposition: kidneys, joints, skin, heart, small vessels
- Examples: SLE, GN

Type IV- Cell-mediated immunity

- Delayed-type hypersensitivity
- T-cell mediated (Th1)
- Allograft, intracellular parasites (viruses, mycobacteria, fungi), soluble proteins
- Appear 24-72 hours later
- Granulomas, erythema, induration

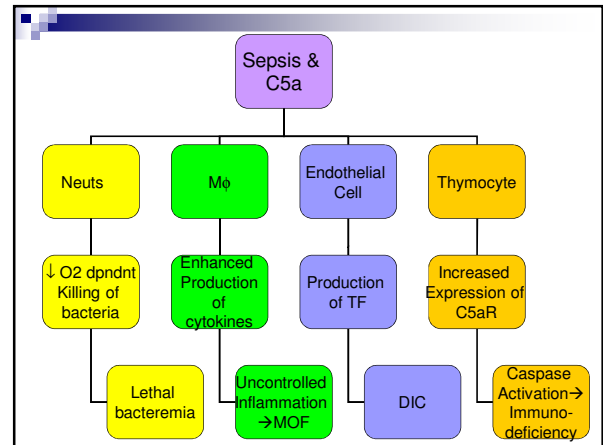
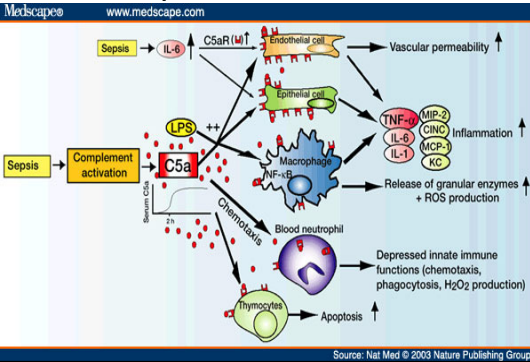
C3a & C5a

- Anaphylatoxins
- Acute inflammatory response
 - Vasodilation, locally increased blood flow, contraction of smooth mm, edema, heat
- C3a, C5a, C5b-C9 in plasma implies loss of control

C5a

- Enhances innate immune functions of phagocytic cells
 - Generation of superoxide anion
 - Granule enzyme release
 - Confers neutrophil resistance to apoptosis
 - Helps initiate rolling of neutrophils on EC
- Endothelial cells
 - Increases endothelial cell production of IL-8 & IL-6
 - Induces TF expression in EC & monocytes

C5a & Sepsis



Interventions

- Rodents (not humans)
 - TNF specific Ab
 - LPS specific Ab
 - C1 inhibitor
 - IL-1 R antagonist
 - PAF antagonists
 - Eicosanoid inhibitors
 - Antioxidants
 - NO inhibitors
 - Thrombin III specific Ab
- Promise (not used in humans)
 - C5a specific Ab
 - C5a R antagonists
 - C5a R specific ab
 - High mobility group B1 protein specific antibody
 - Migration inhibitory factor (MIF) specific Ab
 - IL-6 specific Ab

Heart disease

- TNF- α
 - Negative inotropic effect
 - Cachexia, ventricular dysfunction, myocarditis, CHF
 - Elevated in cats with CHF? (10/26 cats)
 - Meurs et al, 2002, AJVR

Heart disease

- IL-6
 - Elevated in humans with chronic heart failure
 - Levels may be related to severity
- NF- κ B?, IL-1 β ?, IFN- γ ?
- Redundancy in immune system may preclude direct cytokine blocking

Autoimmune disease

- Genetic link?
- Environment?
- Infectious agents

Autoimmune disease

- Infectious agents
 - Polyclonal B cell activation
 - Monocytic ehrlichiosis, leishmaniasis
 - Superantigens
 - "Bystander destruction"
 - Viruses: epitope spreading, T-lymphocyte infection
 - Molecular mimicry

Molecular mimicry

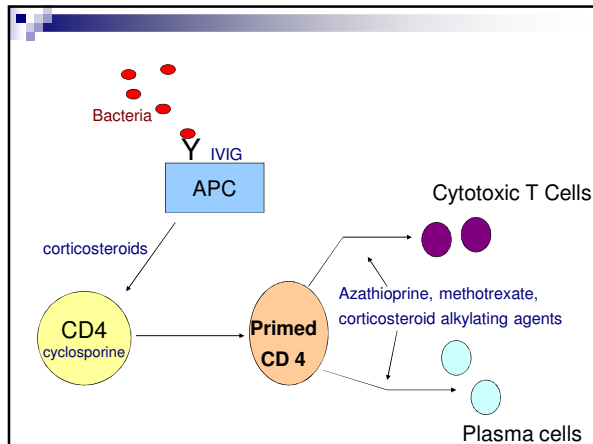
- Epitopes on microbial agents stimulate the production of antibodies or the proliferation of T cells that react with self-antigens
- Guillain-Barre (Campylobacter), IDDM (viral), Myasthenia gravis (Herpes) in humans
- Animals: Borrelia

Canine Diabetes Mellitus

- Multi-factorial
 - Breed predisposition
 - Hormonal antagonism
 - Secondary to chronic pancreatitis and islet destruction
 - Auto-immune

Immunosuppressive Agents

- Inhibit the proliferation & expansion of immune cells
 - Myelotoxic agents
- Prevent activation of the immune system
 - Corticosteroids, Calcineurin inhibitors
- Inhibitors of cytokine and growth factor action
- Interfere with antibody/antigen presentation
 - IVIG, Danazol



Myelotoxic Agents

- **Cyclophosphamide**
 - Alkylation of DNA during S phase
 - Suppresses T-cell activity and Ab production
- **Azathioprine**
 - Purine analogue metabolized to ribonucleotide monophosphates
 - Humoral > cell-mediated
- **Methotrexate**
 - Competitively inhibits folic acid reductase
 - Anti-neoplastic agent

Glucocorticoids

- Stabilizes endothelial cell membranes
- Inhibit secretion of proteolytic enzymes
- Inhibits the release of AA from membrane phospholipids
- Redistributes monocytes and lymphocytes from the peripheral circulation to lymphatics & BM
- Reduce T-cell activation
- Suppress cytokine activity & alter M ϕ function

Calcineurin inhibitors

- Cyclosporine
 - Impedance of calcium-dependent signal transduction
 - Blocks transcription of genes regulated by NF-AT \rightarrow inhibition of early T-cell activation and cytokine synthesis.
 - Lymphocyte specific

Calcineurin inhibitors

- Tacrolimus
 - Similar MOA to cyclosporine
 - 50-100x more potent inhibitor of lymphocyte activation than cyclosporine *in vivo*
 - Greater toxicity

Inhibitors of Cytokine & Growth Factor Action

- Sirolimus (rapamycin)
 - Macrocyclic Ab
 - Kinase inhibitor
 - Blocks T-cell activation
 - Blocks stimulation of B-cell proliferation by LPS
 - Inhibits the proliferation of fibroblasts, endothelial cells, hepatocytes, smooth muscle cells

Inhibitors of Cytokine & Growth Factor Action

- Mycophenolate Mofetil
 - Selective inhibitor of T & B cell proliferation
 - Toxic to animals at therapeutic dosages
- Leflunomide & analogues
 - Inhibits de novo pathway of pyrimidine synthesis
 - Inhibitor of tyrosine kinases

Intravenous immunoglobulin

- Polyspecific human IgG
- Blockage of Fc receptors on M ϕ → ↓ Fc-mediated phagocytosis of IgG-coated cells
- Interfere with complement action
- Suppress antibody production

Danazol

- Synthetic androgen
- Mechanism uncertain
- May reduce the binding of IgG and complement to red blood cells & platelets

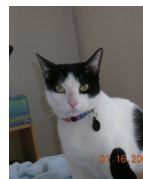
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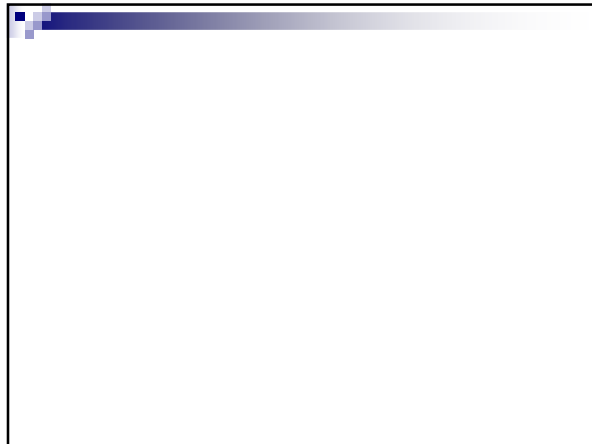
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Questions



The Huey Longs





Acute phase proteins

- C-reactive protein
- Serum amyloid A protein
- Proteinase inhibitors
- Coagulation proteins

Requirements for immunogenicity

- Foreignness
- High molecular weight(>6000 Daltons)
- Chemical complexity
- Degradability
- B/T cells

- In acquired myasthenia, antibodies, usually immunoglobulin G (IgG), are generated against acetylcholine receptors. These autoantibodies block neuromuscular transmission either by directly interfering with the actions of acetylcholine on receptors, accelerating the normal turnover rate of receptors, or activation of complement-mediated lysis of the postsynaptic membrane.¹ Acquired MG is the most common form of the disease. An increased risk has been identified in Akitas, various terriers, German shorthaired pointers, and Chihuahuas, whereas rottweilers, Doberman pinschers, dalmatians, and Jack Russell terriers have lower relative risks compared with mixed-breed dogs. Despite these statistics, German shepherds, and Labrador and golden retrievers are the breeds most commonly diagnosed with the disease.¹ Abyssinians and Somalis appear over-represented in cats.³⁴ Two age groups appear most susceptible in dogs (2 to 3 years of age; >9 years of age). Cats can be affected at any age.

Activation of T-lymphocytes

■ APC	T-lymphocyte
MHC II	TCR + co-R
CD40	CD154
B7	CD28, CTLA-4
CD58	CD2
ICAM-1	LFA-2

