Amphibian Immunology

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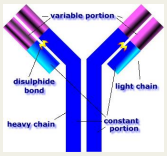
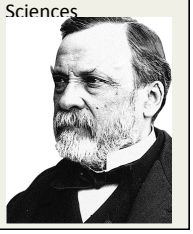


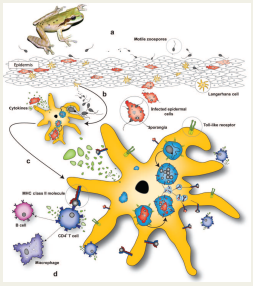


Today

- Part I Introduction to Immunology
- Part II Innate Immunity
- Part III Acquired Immunity
- Part IV Xenopus Specific Immunology

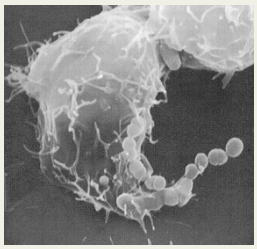
To Survive we need:

- Blood flow
- Oxygen
- Nutrients
- An immune system



Why Do We Need an Immune System?

- We are constantly challenged with:
  - Viruses
  - Bacteria
  - Fungi
  - Parasites
  - Toxins
  - Malignantly transformed cells



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Consequence of Impaired Immunity

- Animals that do not have functional immune systems
- Athymic (nude) mice
- Cats with advanced feline immunodeficiency virus (FIV)
- Humans with HIV
- Animals on high doses of immunosuppressive therapy
- May not survive a strong challenge from pathogenic organisms

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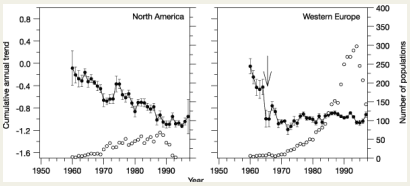
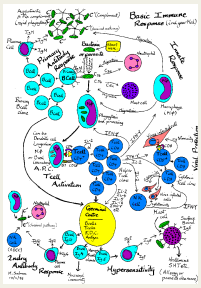
Amphibian Decline



Immunity is Provided by a Complex Network of Overlapping Systems



- Cytokines
- Chemokines
- Complement factors
- Antigen presenting cells
- Leukocytes
- Toll like receptors
- Antibodies

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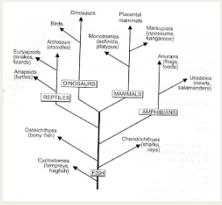
Which Animals Have Immune Systems?

- All vertebrate animals have some form of immune system



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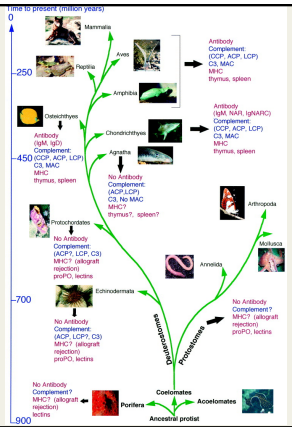
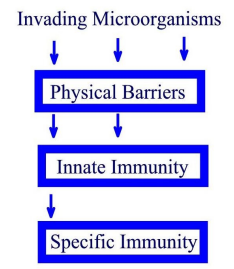


Immune System Organization

- The immune system is actually a combination of branches that work together



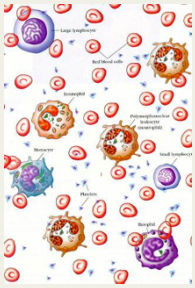
Innate Immunity vs Specific Immunity

- Innate immunity
  - Does not discriminate between different organisms or between self and non-self
  - Limited effectiveness but important first line of defense
- Adative (specific) immunity
  - Very effective against established infections
  - Relatively slow to respond
  - Immunological memory

INNATE IMMUNITY

Components of the Innate Immune system

- Mononuclear cells
  - Monocytes- blood
  - Macrophages- tissues
- Natural Killer (NK) cells
- Granulocytic cells
  - Neutrophils
  - Eosinophils
  - Basophils
- Mast cells
- Dendritic cells
- Complement
- Toll like receptors
- Antimicrobial peptides



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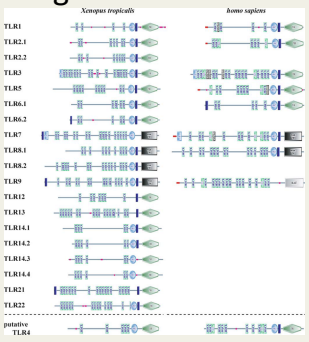
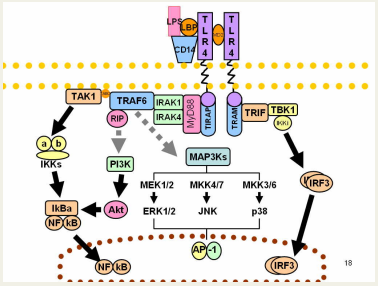
Innate Immunity: Terminology

• **Antimicrobial and serum proteins**
-potent, broad spectrum antibiotics
-enhance immunity
• **Pattern Recognition Receptors (Toll like receptors)**
-primitive part of the immune system
-found on host cell surface
-recognize pathogen-associated molecular patterns
• **Pathogen-associated Molecular Patterns (PAMPs)**
-molecules associated with groups of pathogens
-activate immune responses

Frogs have 20 TLRs!



TLR induce expression of IL-6, Interferon and GM-CSF in macrophages



Innate Immunity: Terminology

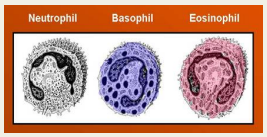
- **Antigen**: Binds to an immunoglobulin or T cell receptor
- **Immunogen:** Induces an immune response to something foreign or toxic to the cell or body

Requirements for Immunogenicity

- Foreignness – a rabbit immunized with its own serum albumin should not produce antibody
- High Molecular Weight -- These are general categories and that there are some exceptions. How could you make a small "non-immunogenic" molecule "immunogenic"?
- Chemical Complexity --

Granulocytic Cells
Polymorphonuclear Leukocytes

- Basophils
- Eosinophils
- Neutrophils
- **Act rapidly but are not capable of sustained effort**



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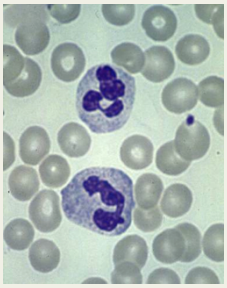
Neutrophils

- Most abundant leukocyte
- Mediate acute inflammatory response to bacterial infection
- Ingest foreign particles and destroy with a respiratory burst



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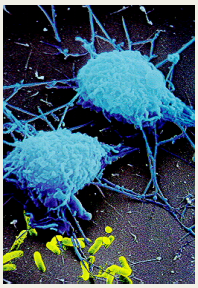
Innate Immunity: Terminology

Monocytes/ Macrophages: "Big-eater"

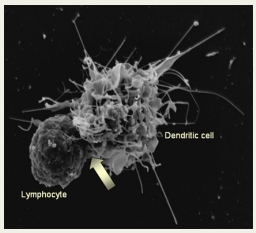
- role is to phagocytose
- stimulate lymphocytes
- attack foreign substances, infectious microbes, and cancer cells



Innate Immunity: Terminology

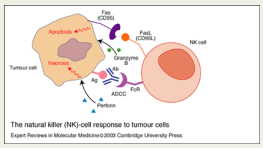
Dendritic Cells: Process antigen and present it to cell surface of T cells of the immune system

-Messengers between innate and adaptive



Natural Killer Cells

- Lymphocytes
- Contain granules filled with potent chemicals
- Do not need to recognize a specific antigen
- Target tumor cells and infectious organisms
- **ADCC= antibody dependent cell cytotoxicity**



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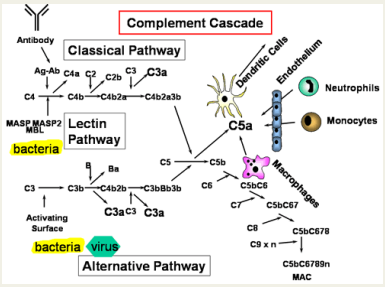
Complement



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Barriers

- Anatomic barriers
  - Skin
  - Mucous membranes
- Physiologic barriers
  - Temperature
  - Low pH
  - Chemicals
  - Tears

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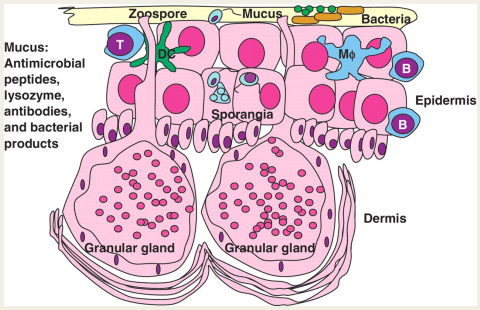
Schematic diagram of immune defenses against Batrachochytrium dendrobatidis in the skin.

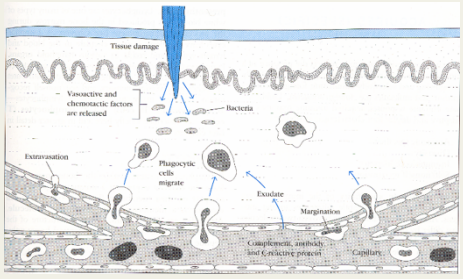


Louise A. Rollins-Smith et al. Integr. Comp. Biol. 2011;icb.icr095

Integrative and Comparative Biology

Barriers Can be Breached

Tissue damage induces an inflammatory response



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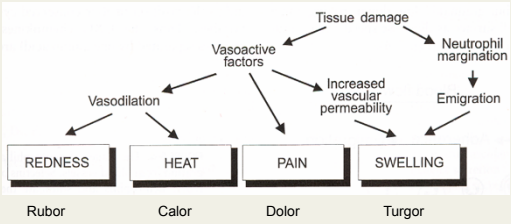
Inflammation Characterized by:



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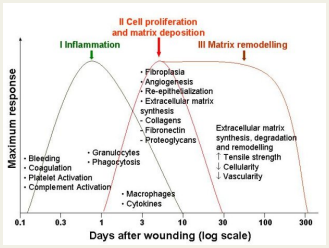
First Responders

- Complement
- Neutrophils
- Peptides



Adaptive Immunity

- Only cells of the acquired branch of the immune system can be educated
- Antigenic specificity
- Immunological memory
- Self/ non-self recognition

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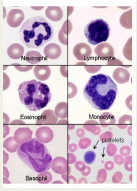
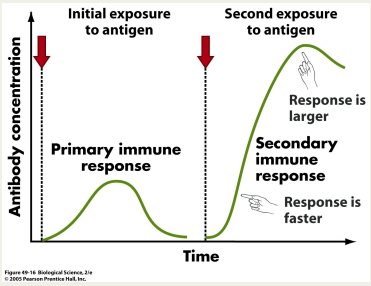
Leukocyte

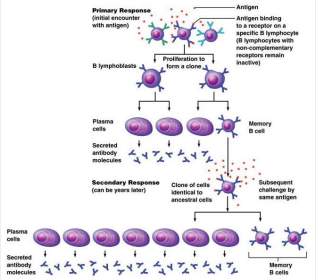
- Definition- a colorless cell that circulates in the blood and body fluids and is involved in counteracting foreign substances and disease; a white (blood) cell.



Memory



Basis for Immunological Memory

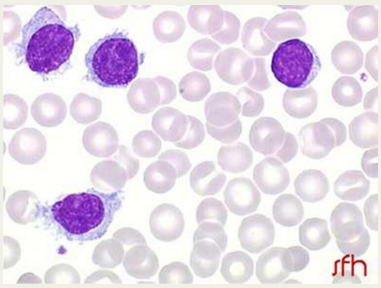


Adaptive Immunity: Terminology

Lymphocytes

- T cells (mature in Thymus)
  - -CD4 helper T cells
    - -Th1, promote macrophage activation and cytotoxic T cell proliferation
    - -Th2, evoke strong antibody response
  - -CD8 cytotoxic T cells kills infected/dysfunctional cells
- B cells (produced in the bone marrow)

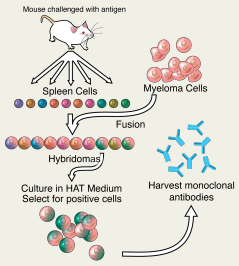
Lymphocytes

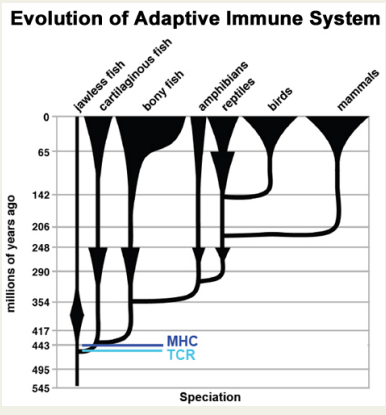


CD

- CD stands for cluster of differentiation, which indicates a defined subset of cellular surface receptors (epitopes) that identify cell type and stage of differentiation, and which are recognized by antibodies.

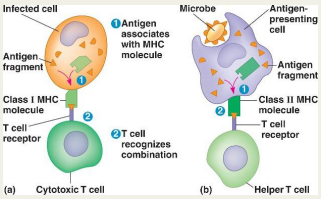
Monoclonal Antibodies





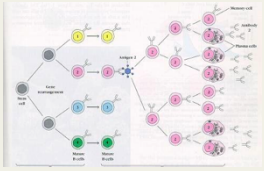
Major Histocompatibility Complex (MHC) Proteins

- MHC I- Expressed on all nucleated cells, present antigen to CTL
- MHC II- Expressed on antigen presenting cells, present antigen to helper T cells



B Cells

- The only antibody producing cells
- Also function as antigen presenting cells
- B cell receptor is membrane bound immunoglobulin



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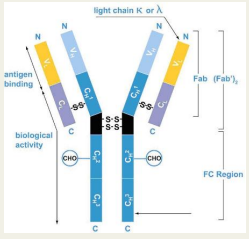
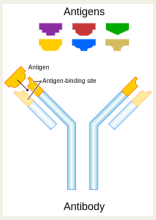
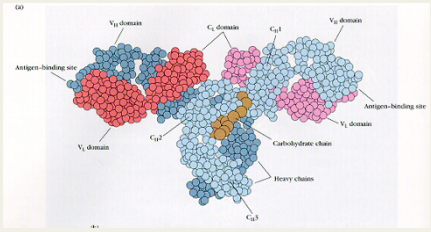




Immunoglobulins are globular molecules



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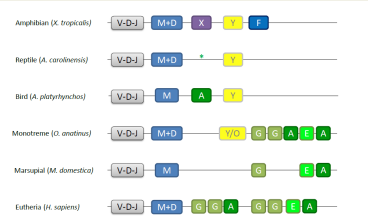
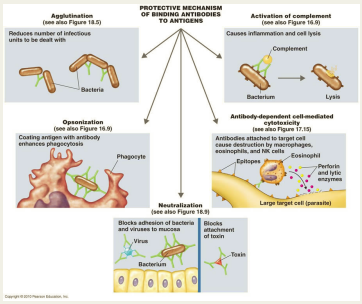
Ig Class Switching



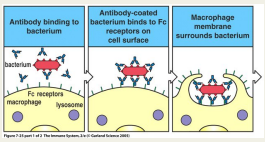
Supplemental Figure 4. Genomic synteny of IgX is consistent with its giving rise to IgA. Genomic assemblies and published genomic studies of the IgH locus were used to compare the position of the constant genes of IgX of amphibians with IgA of birds and mammals. The asterisk denotes that IgA is present in some reptiles such as the leopard gecko though genomic data is lacking.

Antibody Protective Mechanisms



Opsonization

- Opsonins include IgG and complement C3
- Opsonins improve phagocytosis efficiency
- Opsonins are produced by innate and specific systems



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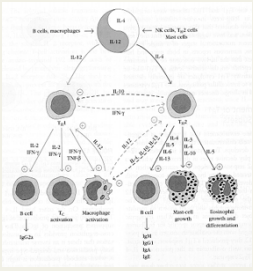
T Helper Cells

- T cells are the gatekeepers of the immune response



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T helper cells are subdivided into Th1 and Th2 cells based upon their cytokine profiles

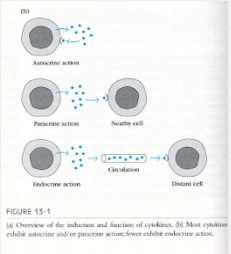

FIGURE 15-1
(a) Overview of the induction and function of cytokines. (b) Most cytokines exhibit autocrine and/or paracrine action; fewer exhibit endocrine action.

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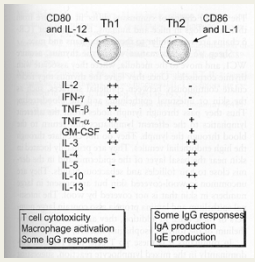
The Roles of T helper Cells

- **Th1**
  - Help IgG2a production
  - Activate macrophages
  - Delayed type hypersensitivity
  - Cytotoxic T cell activation



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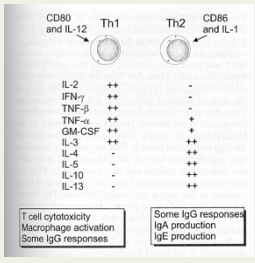
The Roles of T helper Cells

- **Th2**
  - Help for total Ab production
  - Help for IgE production
  - Stimulates eosinophil and mast cell production



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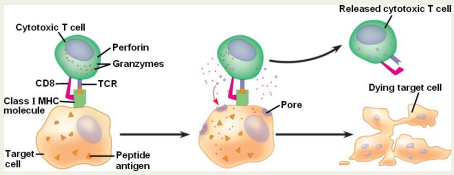
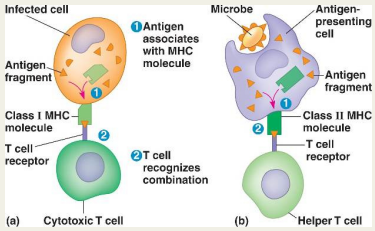
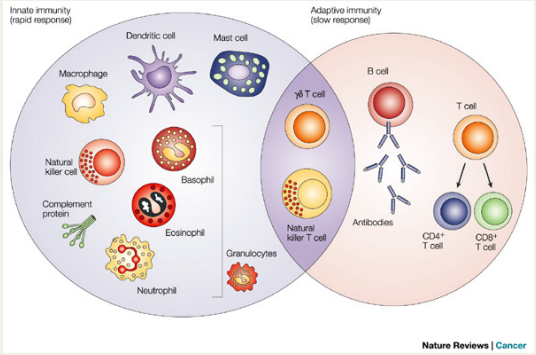
Cytotoxic T lymphocytes (CTL)

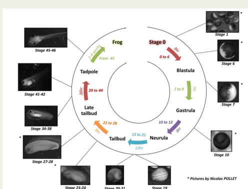


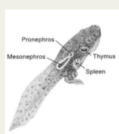




Amphibian Immunity

Juvenile Immunity





Summary of the Main Developmental Steps of the *Xenopus* Immune System

Devel. stages (days)	Liver	Thymus	Spleen	GALT
40 (d3)		Thymic epithelium buds from 2nd visceral pouch	Absent	Few scattered CD3ε Expressing leukocytes
46 (d4)	Lymphopoiesis in peripheral layer, Ig (μ)and sterile TCRβ RAG, AID	Epithelium, no precursors	Spleen anlage mesenchymal thickening in the mesogastrium	No B cells
47 (d4–5)	Lymphopoiesis, and B cell development in absence of Ag	Colonization by Lymphopoietic precursors from post-VBI (~100 cells) RC47^{+}	Blood cells (No Lymphopoiesis)	
48 (d6–7)		Cortex-meddula, full TCRβ mRNA, CD3ε CD8^{+} Thym., class II^{+} epithelial cells		
49 (d10–13)	IgL rearrangements	First CTX^{+} thymocyte, more CD8^{+}	Spleen B cells (~200) and 1st detect Ab responses	
50 (d15)		Ongoing thymocyte differentiation (3×10^{4} cells)		
56 (d38)		Ongoing thymocyte differentiation (9×10^{5} cells)	Detectable T cell responses	
58 (d44)		Max. size of the thymus ($1–2\times10^{6}$ cells)	Max. larval T cell response (1×10^{6} cells)	
Adult (>d60)	Adult-type leukocytes	Thymus move near tympanum New adult–type thymocyte differentiation	Adult T cell responses ($1–2\times10^{7}$ cells)	
Adult (> 1 yr)		Thymus progressively filled by fat tissues	($1–2\times10^{7}$ cells)	Many IgM^{+} and IgX^{+} B cells, as well as T cells (CD8^{+} and CD8-)

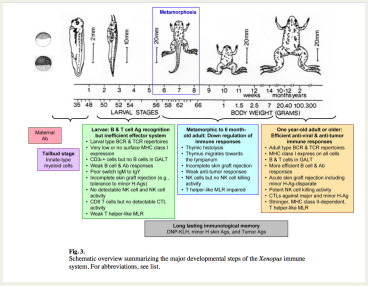


Fig. 3.
Schematic overview summarizing the major developmental steps of the *Xenopus* immune system. For abbreviations, see list.

Larval Immunology

- Competent Innate Immune System
- Weak Adaptive Immune System
  - -Small amount of T cells and B cells in larvae
  - -NK cells only right before metamorphosis

What is going on during Metamorphosis?

- Tadpoles are free-living so must be protected against potential pathogens
- During metamorphosis, they acquire adult specific molecules
- Must show tolerance to new self molecules
- T cell function becomes impaired
- Express different antibody repertoire
- Lessening of skin graft tolerance
- The appearance of MHC class I antigens
- Persistence of immunological memory

Adult Immunology

- Immune system becomes complete 2-3 weeks after metamorphosis
- Strong Innate and adaptive immune responses
  - -Antimicrobial peptides
  - -NK cells
  - -Complement
- Adaptive Immune system similar to other mammals
  - -Except B cells differentiate mostly in the liver and spleen (instead of bone marrow)

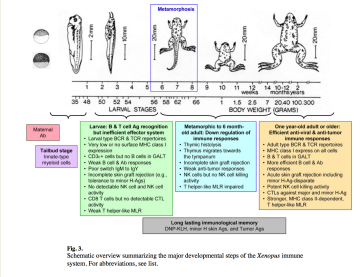


Fig. 3.
Schematic overview summarizing the major developmental steps of the *Xenopus* immune system. For abbreviations, see list.

Temperature and Immune Status

- Helminth clearance is slower at 15°C that 25°C
- Skin grafts are rejected faster at 27°C than at 21°C
- These and other data suggest selective inhibitory effect of low temperature on T-cell function

Other modulators of immune response

- Temperature
- UV
- pH
- Predators
- Man-made modulators
- Psychosocial factors
- Xenobiotics

Increases in Susceptibility not simply due to poor immunity

- Exposure to new, highly virulent pathogens
- New pathogens are immunosuppressive
- Pathogen "bloom"
- Stress response
- Environmental contaminants

Immunology tests that can be performed without species specific reagents

- Serum virus neutralization
- Serum protein electrophoresis
- Competitive ELISA (maybe)
- Hemagglutination inhibition
- Lymphocyte proliferation
- Apoptosis
- Cytotoxic T cell killing assays