Veterinary Immunology: An Introduction, 8th Edition
Chapter 14 Antibodies: Soluble Antigen Receptors

KEY POINTS:

1. There are five classes of immunoglobulins in mammals (IgG, IgM, IgA, IgE, IgD). All start as B cell antigen receptors shed into body fluids.
2. IgG is predominant in serum and is responsible for systemic defense.
3. IgM is very large produced mainly during primary immune response.
4. IgA is produced on body surfaces and is involved in defense of intestinal and respiratory tracts. It is predominant in secretions such as saliva, milk and intestinal fluid.
5. IgE is found in small quantities in serum and is involved in defense against parasites and allergies.
6. IgD is on surface of immature lymphocytes and has unknown function.

Introduction

- Serum concentrations: IgG > IgM > IgA > IgD > IgE
- Electrophoresis separates serum into 4 fractions: albumin and alpha/beta/gamma globulins.
    - Most immunoglobulins are in gamma globulins.
    - IgM migrates among beta globulins.

Immunoglobulin G

- made and secreted by plasma cells spleen lymph nodes, bone marrow
- predominant in blood
- smallest of immunoglobulin molecules > can escape from blood vessels
- binding to bacterial surfaces can cause agglutination and opsonization
- can activate classical complement pathway only when sufficient molecules have accumulated in a correct configuration on the antigenic surface

Immunoglobulin M

- made by plasma cells in the spleen, lymph nodes and bone marrow
- on B cell surface is a monomer, when secreted has 5 units
- predominant immunoglobulin made in primary immune response
- more efficient than IgG at complement activation, opsonization, neutralization of viruses and agglutination
- very large so rarely enter tissue fluids even at sites of acute inflammation

Immunoglobulin A

- secreted by plasma cells under body surfaces
- made in walls of intestine, respiratory tract, urinary system, skin and mammary gland
- secreted as a dimer
- IgA produced in body surfaces pass through epithelial cells into external secretions
    - transport through intestinal epithelial cells bound to polymeric Ig receptor or secretory component. Secretory component binds IgA dimers to make SIgA to protect IgA from digestion by intestinal proteases.

Immunoglobulin E

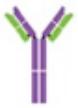
- made by plasma cells located beneath body surfaces
- very low concentrations in serum so has do do more than bind and coat antigens to have effect
- triggers acute inflammation (signal transducing molecule)
- Bind to FceRI receptors on mast cells and basophils
    - antigen binds
    - triggers rapid release of inflammatory molecules from mast cells
    - enhance local defenses
- type I hypersensitivity reactions
- immunity to parasitic worms
- shortest half life of all immunoglobulins
- destroyed by mild heat

Immunoglobulin D

- not yet detected in rabbits or cats or chickens
- BCR mainly found attached, very little secreted into blood
- evolutionarily labile, many variations in structure

Table 14-1 Major Immunoglobulin Classes in the Domestic Mammals

	Immunoglobulin Class				
Property	**IgM**	**IgG**	**IgA**	**IgE**	**IgD**
Molecular weight	900,000	180,000	360,000	200,000	180,000
Subunits	5	1	2	1	1
Heavy chain	μ	γ	α	ε	δ
Largely synthesized in:	Spleen and lymph nodes	Spleen and lymph nodes	Intestinal and respiratory tracts	Intestinal and respiratory tracts	Spleen and lymph nodes

					
	IgM	IgG	IgA	IgE	IgD
Heavy Chain	μ (mu)	γ (gamma)	α (alpha)	ε (epsilon)	δ (delta)
MW (Da)	900k	150k	385k	200k	180k
% of total antibody in serum	6%	80%	13%	0.002%	1%
Fixes complement	Yes	Yes	No	No	No
Function	Primary response, fixes complement. Monomer serves as B-cell receptor	Main blood antibody, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva	Antibody of allergy and anti-parasitic activity	B cell Receptor

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3D Structure of Immunoglobulins

- Ig peptide chains fold in complex manner
- IgG 3 globular regions (2 Fab and one Fc region)
- each globular regions made of paired domains
- in the Fab globular region there is groove located between two variable domains VH and VL
    - groove is lined by amino acids of complementary determining regions (CDRs) so surface of groove (antigen binding site) has highly variable shape
    - CDRs from light and heavy chains, heavy chains contribute more to binding of antigen
    - CDRs on each of Fab region are identical so each molecule has 2 identical antigen binding sites
    - hinge region in the middle of the heavy chain makes IgG more flexible > crosslink two antigens at the same time > clump together bacteria > agglutination

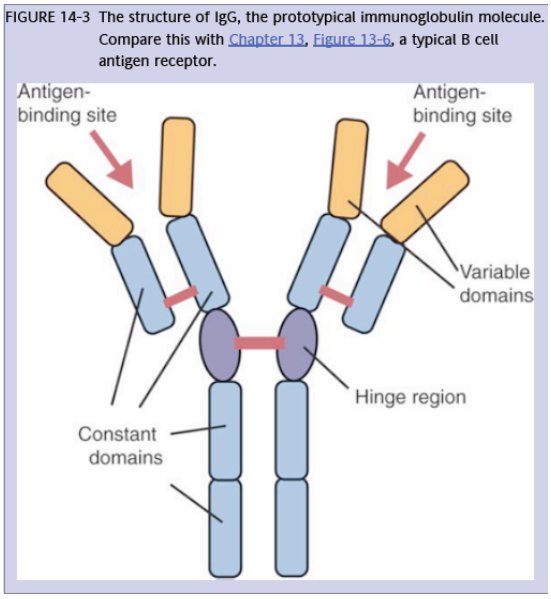

FIGURE 14-3 The structure of IgG, the prototypical immunoglobulin molecule. Compare this with Chapter 13, Figure 13-6, a typical B cell antigen receptor.

Immunoglobulin variants

1. Subclasses
    a. all are made of two heavy chains and two light chains
        i. structurally different heavy chains lead to subclasses (ie multiple IgG or IgA subclasses in a species)
        ii. result of duplication and mutation
        iii. variations among species are probably not of major biological significance

2. Allotypes
    a. inherited variations in immunoglobulin amino acid sequences (between members of the same species)
3. Idiotypes
    a. variations (idiotopes) in the amino acid sequences within the variable domains on light and heavy chains
    b. collection of idiotopes on an immunoglobulin = its idiotype
    c. some idiotopes are in antigen binding site and some on non antigen binding sites of V domain.

Production of Immunoglobulin Heavy Chains

- 2 different genes code for each immunoglobulin heavy chain
    - one gene for variable domain (antigen binding site)
    - one gene codes for constant domains
        - gene consists of several exons (expressed sequenced)
            - each exon codes for a constant domain, one codes for hinge region
        - all the heavy chain constant regions are located on one chromosome (in order M - D - G - E - A from 5' to 3'.)

Soluble Antigen Receptors

1. B cells undergo 2 different DNA recombination events
    a. V(D)J recombination: creates antigen binding site of B cells as they develop within the bone marrow in the absence of antigens
    b. Class Switch Recombination: when antigens activate B cells, change in class of antibody produced by a B cell
        i. does not affect antigen binding specificity
        ii. results in production of a different heavy chain constant region
2. Class Switch Recombination
    a. immunoglobulin classes change during antibody response, due to change in way heavy chain genes are constructed and used
        i. no change in antigen binding ability
    b. immunoglobulin classes are synthesized in a standard sequence
        i. B cell first uses IGHM genes to make IgM BCRs (remaining genes 3' to IGHM are ignored.
        ii. B cell then transcribes the IGHD genes and then expresses both IgM and IgD
        iii. eventually the B cell switches to using IGHG, IGHA or IGHE and commits to making BCRs and immunoglobulins of one of the other major classes (IgG, IgA or IgE)
        iv. unwanted, unused IGH genes are excised as a DNA circle and lost from the cell
        v. required IGH gene is spliced directly into the IGHV
            1. ie for IgM: IGHV gene spliced directly to IGHM genes

2. ie for IgA: genes coding for Cm to Ce inclusive are deleted and IGHV spliced to IGHA genes
    a. looping out deletion via enzyme recombinase

vi. two signals needed to initiate class switching in a B cell
1. activation signal: cross linking between CD40 (B cell) and CD154 (helper T cell)
2. specific class switch determination by cytokines (IL-4, TGF-beta, interferon-gamma, esp)
    a. signals from CD40 and antigen activate recombinase in the B cell while cytokines (by activating specific promoter regions) target the recombinase to a specific immunoglobulin gene.

3. BCRs and Soluble Immunoglobulins
a. Immunoglobulins can exist as either BCRs or secreted antibodies depending on inclusion of a hydrophobic transmembrane C terminal domain
b. switch between the two forms depends on the differential splicing of exons

4. Immunoglobulins of Domestic Mammals
a. all mammals posses genes for and express 4 or 5 major immunoglobulin classes (IgG, IgM, IgA, IgE, IgD)
b. dogs and cats
    i. dogs have 4 IGHG genes and therefore 4 IgG subclasses (IgG1, IgG2, IgG3, IgG4 in order of abundance), two IgE (IgE1 and IgE2) subclasses, and 4 allelic variants of IGHA gene
        1. an IgM allotype has been described in the dog
    ii. cats have 3-4 IGHG genes, one IgM subclass, two IgA subclasses and 2 IgE subclasses