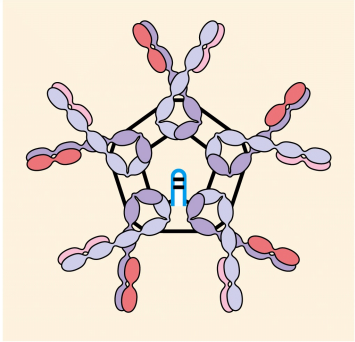
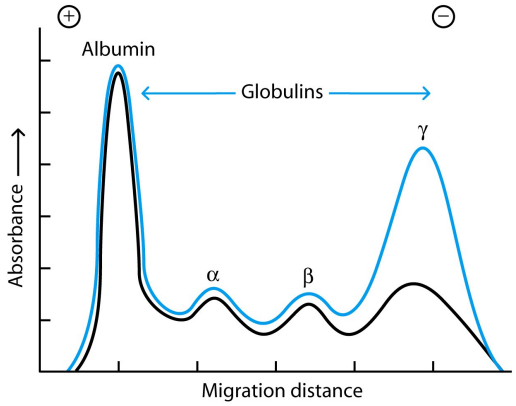
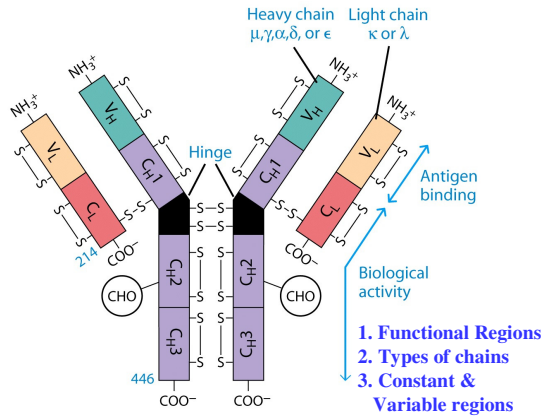
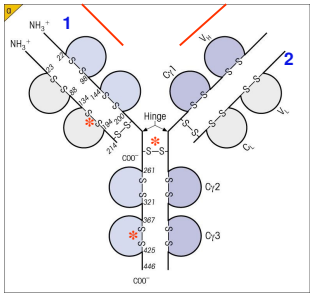
Chapter 4. Immunoglobulin Structure and Function



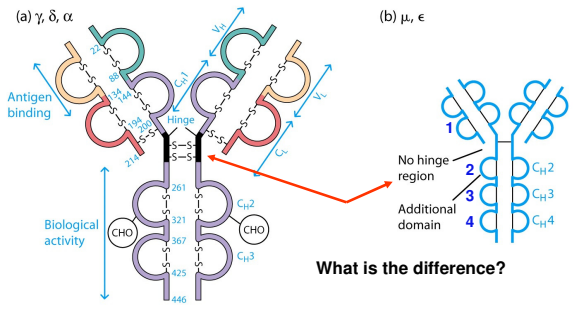






- Each heavy and light chain is made up of a number of domains (= Ig folding or Ig domains).
- Light chains consist of 2 domains (C and V).
- Heavy chains have 4-5 domains (depending on the class of antibody)
- Each domain is about 110 amino acids in length and contains an intrachain disulfide bond between two cysteines about 60 amino acids apart.

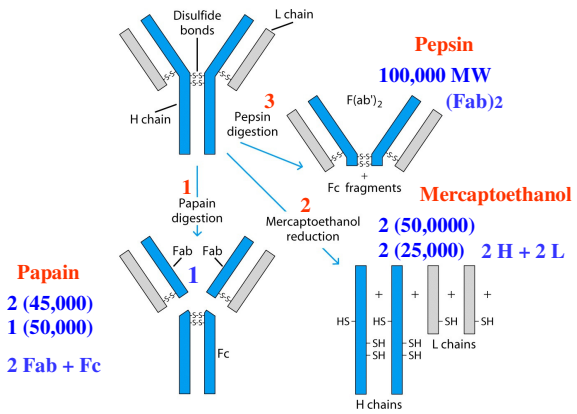
-150,000 molecular weight



- Constant (C) and Variable (V) regions

Basic Antibody Structure

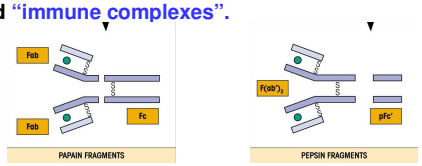
- Multiple myeloma = cancerous plasma cells
- Monomer = 150,000



RECAP:

- The Fc region plays NO role in antigen binding.

- Papain breaks antigen molecules into 2 Fab fragments and an Fc fragment.
- Pepsin breaks antibody molecules into an $F(ab')_2$ fragment and a VERY SMALL pFc' fragment.
- Mercaptoethanol treatment results in 2 heavy and 2 light chains
- Complexes of antibodies cross-linked by antigen are called "immune complexes".



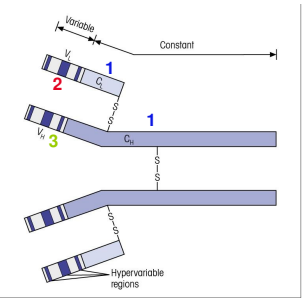


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Figure 3.3

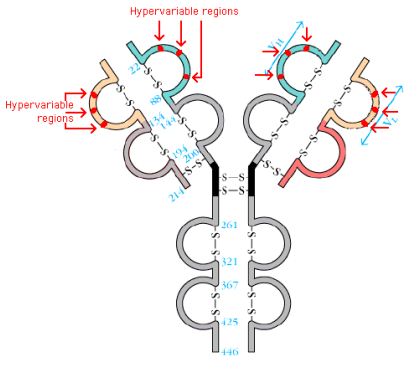
1. Constant region - amino acid sequence in the C-terminal regions of the H and L chains is the same.

2. Variable region - amino acid sequence in the N-terminal regions of the H and L chains is different. This region provides antibodies with unique specificity.

3. Hyper-variable regions are regions within the variable regions (greater specificities).

Summary

- Molecule consists of Constant and Variable regions for both Light and Heavy chains (CH, VH, CL, VL)
- Ig molecule made of domains
- Domains ~ 110 aa
- Each antigen-binding site is made up of the N-terminal domain of the heavy and the light chains
- IgM and IgE possess 4 CH domains (CH1-CH4) while IgG, IgA and IgD have 3 CH domains (CH1-CH3). Hinge region is missing.
- Hypervariable regions in the Variable regions of both H and L chains.



-Within the variable domains are three regions of extreme variability.

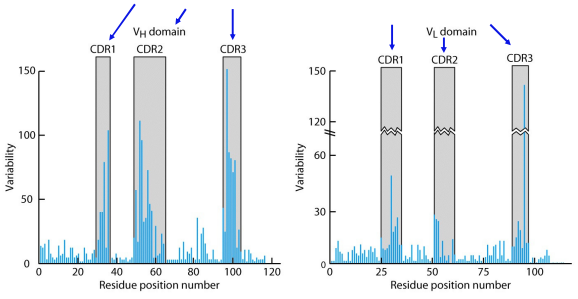
These are referred to as the hypervariable regions.

These regions of the variable domains actually contact the antigen.

They therefore make up the antigen-binding site.

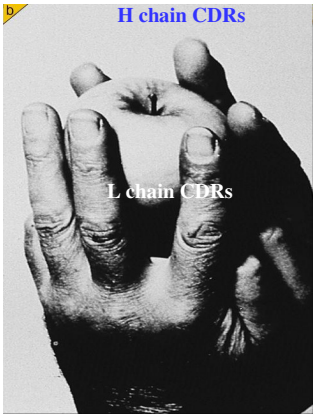
These regions are also called the complementarity-determining regions, or CDRs.

Complementarity-Determining Regions, or CDRs.



Heavy Chain

Light Chain



- A simulated antigen-binding site showing how the CDRs form points of contact with the antigen.

RECAP:

- Antibodies are comprised of repeating 110 aa units referred to as domains or Ig folds.
- The C-terminal domains are constant from antibody to antibody (within a class).
- The constant region domains are responsible for all functions of antibody other than antigen binding (opsonization, ADCC, complement activation) → Biological Function!
- The N-terminal domains are variable from antibody to antibody and are referred to as "variable domains".
- The variable domains contain 3 hypervariable regions - the CDRs.
- The CDRs of the V domains in both H and L chains make up the antigen-binding site.

Antibody-Mediated Effector Functions

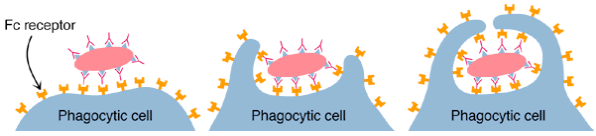
- Binding to Antigen
- OPSONIZATION: FcR in Macrophages and neutrophils
- COMPLEMENT ACTIVATION: IgG and IgM
- ADCC – NK cells trough FcR
- CROSSING EPITHELIAL LAYERS – IgA (but also IgM)
- CROSSING PLACENTA- IgG

Fcγ receptors enhance phagocytosis of foreign cells/particles coated with IgG

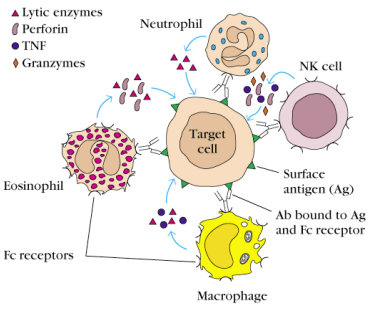
Antibody made in response to foreign cells (cells/viral particles/bacteria etc) will bind to those cells.

Macrophages (and neutrophils) possess **receptors for the Fc** region of IgG.

Binding of macrophage Fc receptors to antibody bound to cells/particles facilitates and increases phagocytosis of cells/particles.



ADCC - Antibody-dependent cellular cytotoxicity - mediated by IgG

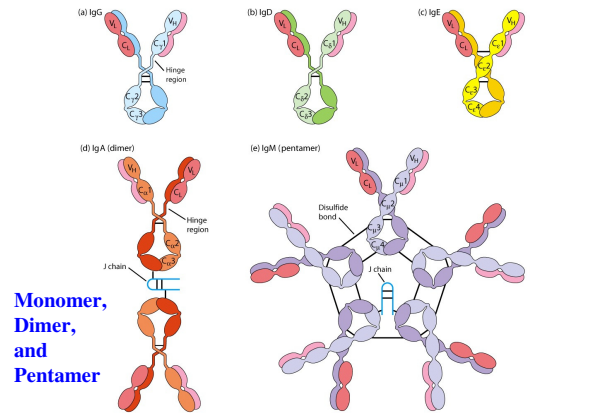


Kuby Figure 14-12

Antibody made in response to foreign cells (cells/viral particles/bacteria etc) will bind to those cells.

Cells of the innate immune system (neutrophils, eosinophils, macrophages, NK cells) possess receptors for the **Fc region** of IgG.

These cells bind to antibody on the surface of foreign cells and release lytic compounds → lysis.



Monomer, Dimer, and Pentamer

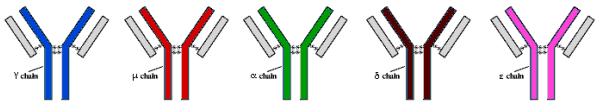
Structural Variants of the Basic Immunoglobulin Molecule

Different heavy chains can be used

There are five major types of heavy chain --> five major classes (isotypes) of antibody

- gamma --> IgG (in humans 4 subclasses: IgG1, IgG2, IgG3, IgG4)
- mu --> IgM
- alpha --> IgA (in humans, 2 subclasses: IgA1, IgA2)
- delta --> IgD
- epsilon --> IgE

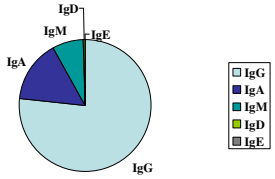
The function of antibody varies depending on which heavy chain is used.



IgG IgM IgA IgD IgE

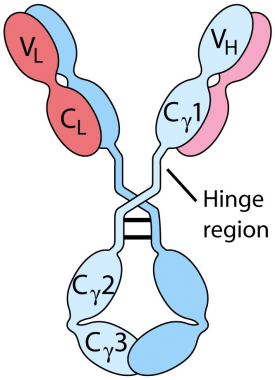
Relative abundance in normal serum:

IgG	8 - 16 mg/ml
IgA	1.4 - 4 mg/ml
IgM	0.5 - 2 mg/ml
IgD	0.003 - 0.04 mg/ml
IgE	17 - 450 ng/ml (<0.0005 mg/ml)



(a) IgG

- Most abundant in secondary responses
- Crosses placenta (FcRn)
- Complement activation
- Binds to FcR in phagocytes



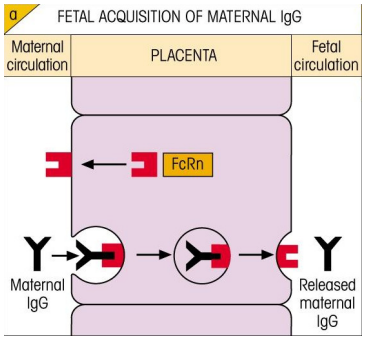


Figure 3.15a

Crosses placenta
Complement Activator
Fc binding

IgG1 IgG2

Disulfide bond

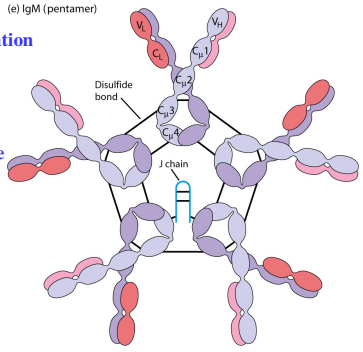
Crosses placenta
Complement Activator
Fc binding

IgG3

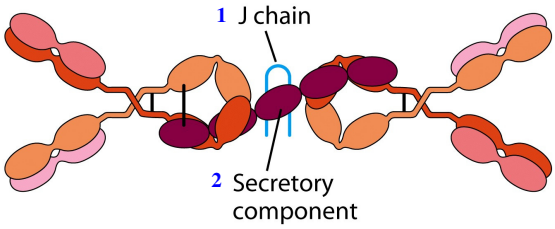
Crosses placenta
Complement Activator

IgG4

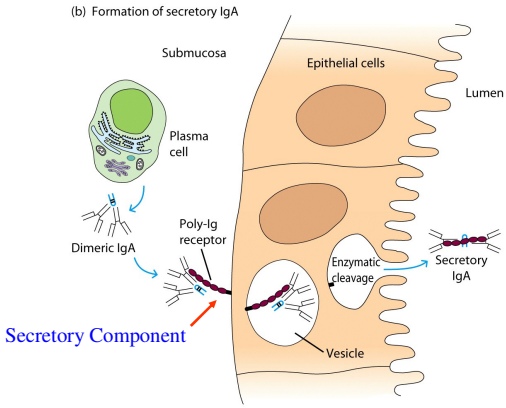
- Best Complement activation
- First Ab produced in neonate
- First antibody produced after challenge
- Mucosal transport (to some degree)
- Monomer on B cells
- J chain: polymeric



(a) Structure of secretory IgA



- Dimer in mucosal secretions
- Mucosal transport
- Monomer in circulation
- J chain (polymeric) and Secretory components



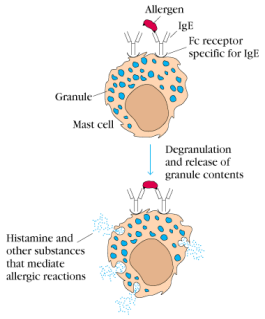
Role of IgE in allergic reactions

IgE antibodies mediate the immediate-hypersensitivity (allergic) reactions that are responsible for symptoms of hay fever, asthma, hives and anaphylactic shock.

IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells.

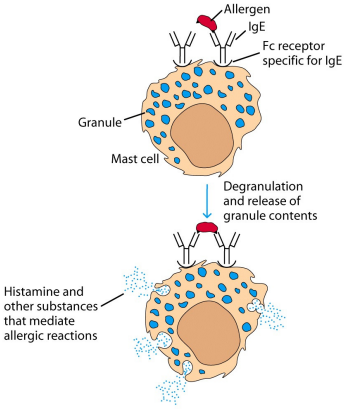
Cross-linkage of receptor-bound IgE molecules by antigen (allergen) induces degranulation of basophils and mast cells.

A variety of pharmacologically active mediators present in the granules are released, giving rise to allergic manifestations



IgD

- Role unknown
- Present on the surface of MATURE B cells → Marker!!



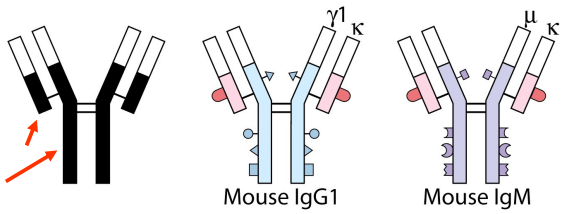
SUMMARY

- IgA and IgM are secreted across epithelial surfaces
- IgG, IgD and IgE can be found only within the body - in serum or lymph.
- IgA and IgM are also found in serum and lymph BUT IN ADDITION can also be found in secretions such as mucous secretions, saliva and tears.
- The IgA and IgM found in external secretions differs from that found in serum by the presence of an additional component referred to as the "secretory component".
- This component is acquired as the IgA or IgM is transported across the epithelial cell barrier.

Antigenic Determinants on Immunoglobulins

- Abs are glycoproteins and themselves very immunogenic
- Epitopes on immunoglobulins are divided into:
  - ISOTYPIC
  - ALLOTYPIC
  - IDIOTYPIC

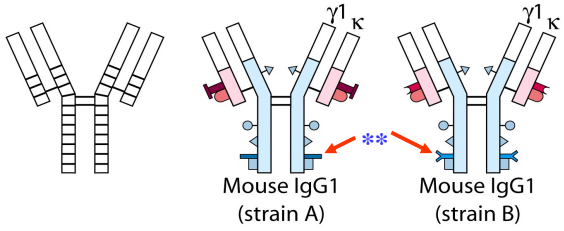
(a) Isotypic determinants



Constant region determinants that define each antibody class and subclass

The function of antibody varies depending on which heavy chain is used.

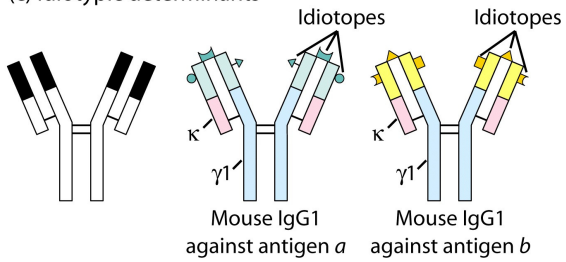
(b) Allotypic determinants



Allelic variation (Allotypes): IgG of a particular class may be slightly different between individuals (e.g. variation in the IgG amino acid sequence)

Note: This type of variation has no effect on antibody function.

(c) Idiotypic determinants



Generated by variation in amino acid sequence in the V_H and V_L. Most exactly, in the CDRs in the V regions

Variation in the antigen binding site (Idiotypes)

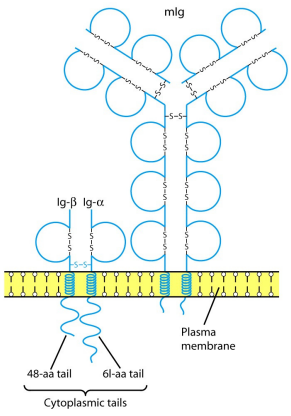
Remember: Idiotype = Ag binding site

RECAP - Sequence variation in antibodies:

1. **Different light changes - no significant functional effect**
2. **Different heavy chains - very significant functional effect - isotypic variation**
3. **Allelic variation between individuals - no large functional effect - allotypic variation**
4. **Variation in the antigen-binding site - idiotypic variation**

B Cell Receptor (BCR):

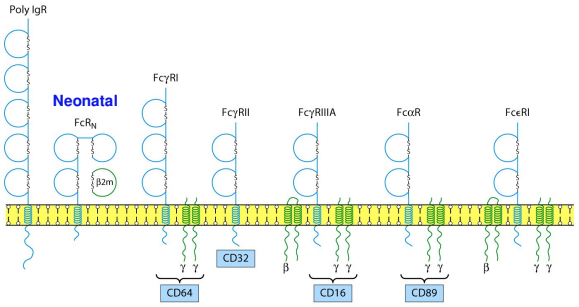
- **Short cytoplasmic tail (3-28 aa) ….signaling?**
- **Signaling through a homodimer, Ig-α and Ig-β**
- **Ig molecule + Ig-α/Ig-β is the BCR**
- **The homodimer molecule is member of the Ig superfamily group**



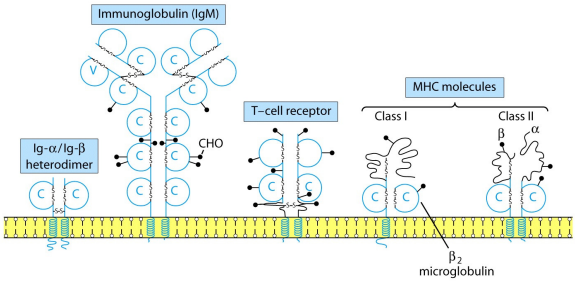
Ig Superfamily

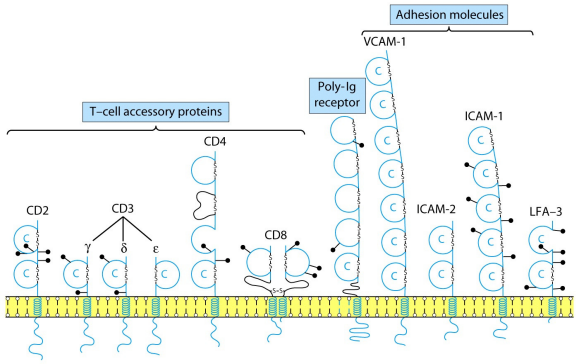
- Divergence from a common gene ancestor coding for 110 aa.
- A member MUST have a "typical" Ig domain or fold→ 110 aa with an intra chain disulfide bond 50-70 aa apart.
- Most members do not bind Ag!! Then, they must facilitate interaction with surface proteins
- You must know members with roles in: a) immune function, b) Receptor/Signal transduction, and c) Adhesion

Receptors



Immune Function





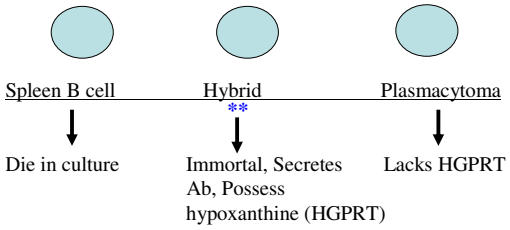
Monoclonal Antibodies

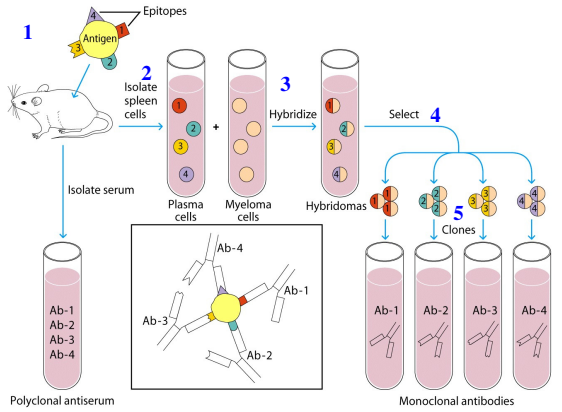
- Kohler & Milstein 1975
- Fusion of normal, activated B cell and plasmacytoma (cancerous plasma cell)
- Hybrid: immortal, secrete Ab, hypoxanthine

Plasmacytoma VS B cell

- Plasmacytoma:
  - Cancerous plasma cell (Immortal)
  - Does not secrete Abs
  - Lacks HGPRT
- Normal spleen B cell
  - Limited life span
  - Secretes Abs
  - Possess HGPRT

RESULTS:





Applications?

- Diagnosis
- Research
- Treatment
- Affinity VS Avidity

  Affinity (polyclonal Ab) = high because of multiple epitopes

  Avidity (monoclonal Ab) = low affinity but high avidity because of strong epitope-Ab interaction

The End

IgG - Most abundant Ig of internal body fluids (serum, extracellular fluids) - combats microorganisms and toxins within the body tissues.

IgA - Most abundant Ig in mucous secretions - protects external surfaces of the body

IgM - The first class of antibody produced during an immune response. Present both in internal body fluids and in secretions.

IgD - Functions not well defined. Found mostly on the B cell plasma membrane

IgE - Increases during parasitic infections. Causes symptoms of allergy.

	IgG	IgA	IgM	IgD	IgE
Complement fixation by classical pathway	++	-	+++	-	-
Ability to cross the placenta	++	-	-	-	-
Binds to mast cells and basophils	-	-	-	-	+++
Binds to macrophages and polymorphs	+++	+	-	-	+