

The Microbial Challenge and the Mammalian Response –
(The Biological Basis of Antibody Production)

- I. Properties of Antibodies
- II. The Microbial Challenge
- III. The Mammalian Answer

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Two Major Objectives

- ◆ Relate immunochemical properties of antibodies to their biological function
- ◆ Rekindle a sense of awe concerning the workings of the immune system.

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Lesser Objectives

- ◆ Define antibody “diversity”, “specificity” and “avidity”.
- ◆ List two survival advantages of microorganisms over mammals.

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Lesser Objectives

- ◆ List two survival advantages of mammals over microorganisms.
- ◆ Identify the mechanisms by which the mammalian specific adaptive immune system attempts to respond to all possible pathogenic microorganisms.



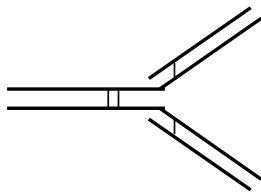
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I. Properties of Antibodies



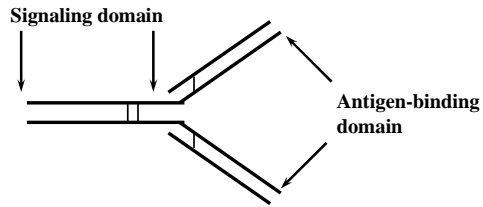
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Antibody monomers are composed of four polypeptide chains connected by disulfide bonds.



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Antibody molecules have two functional domains.



The signaling domain exists within the crystallizable fragment (F_c).

The antigen-binding domain exists within the antigen-binding fragment (F_{ab}).

The domains have different functions.

- ◆ The signaling domain communicates the antigen-binding event to appropriate host cells, and evokes such events as complement fixation, phagocytosis, and placental transport (depending on the antibody isotype).
- ◆ The antigen-binding domain (our focus) recognizes and binds to foreign molecules.

Antigen-binding domains confer three remarkable properties on antibodies.

- ◆ Diversity
- ◆ Specificity
- ◆ Avidity

Among all known molecules, only T cell antigen receptors (TCRs) so powerfully combine these three properties.

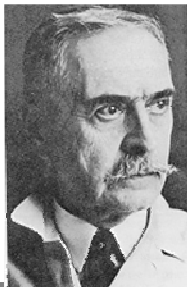
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Antibody Diversity

- ◆ Refers to the seemingly infinite variety of molecular structures against which specific antibodies can be generated.

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Antibody Diversity



Karl Landsteiner's research demonstrated that antibodies could be generated against hundreds of diverse artificial compounds, given the appropriate conditions.

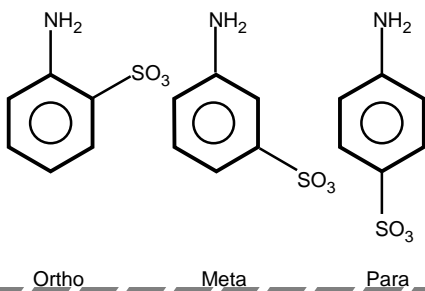
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Antibody Specificity

- ◆ Describes the measurable ability of chosen antibodies to distinguish between antigens with subtle structural dissimilarities.
- ◆ Consider Landsteiner's work again.

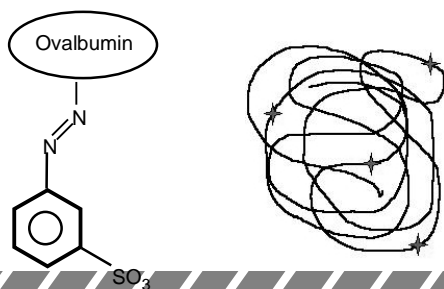
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Aminobenzene Sulphonate, an Artificial Hapten



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Carrier-Protein-Bound Hapten



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Antibody Avidity

- ◆ Describes the strength of antibody binding to antigen.
- ◆ Is indicated by the very low antigen concentration at which half of the antibody molecules present are bound

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Why are antibodies so remarkably diverse, specific and avid?

The *biological basis* of antibody production explains the existence of these molecular properties.

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II: The Microbial Challenge

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Consider Examples of Terms We Use to Describe Infections and Our Responses to Them:

- ◆ Microbial invaders
- ◆ *Dread enemy* of mankind
- ◆ An attack of the flu
- ◆ Armamentarium of antibiotics
- ◆ Minimum bactericidal concentration
- ◆ Immune surveillance



Further Examples of the Terms We Use to Describe Infections and Our Responses to Them:

- ◆ Natural killer cells
- ◆ Membrane attack complex
- ◆ Fighting off the flu
- ◆ Antibody
- ◆ Antibiotic
- ◆ Magic bullet
- ◆ Silver bullet




These terms use the vigorous metaphor of war to describe our interaction with the microbial world.

- ◆ Our very survival depends on warding off or destroying many microorganisms that surround and seek to colonize us.
- ◆ In the simplest possible terms, "It is us against them!"




Our Enemies ...


Fungi

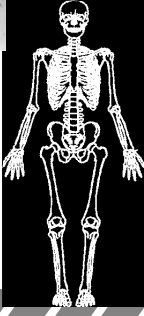


Protozoans

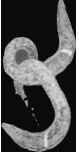


Bacteria






Helminths



Viruses



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Survival advantages of microbes versus humans:

- ◆ Numbers
- ◆ Diversity
- ◆ Generation times

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Which are more numerous?

- ◆ Individual E. coli bacteria on a Petri dish with over 100 colonies?
- ◆ Individual humans on earth?
- ◆ Microorganisms profoundly outnumber mammals.

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There are hundreds of species of pathogenic and opportunistic microorganisms.

- ◆ Viruses
- ◆ Bacteria
- ◆ Fungi
- ◆ Protozoans
- ◆ Helminths

◆ Humans are a single species.

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With swift generation times, microorganisms can rapidly spread mutations across their population.

- ◆ M. tuberculosis (slow-growing bacterium):
doubling time ~ 20 hours
- ◆ E. coli (rapidly growing bacterium)
doubling ~ 20 minutes
- ◆ Human doubling time ~ 20 years
(microbes 10 to 50,000 times faster)

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The microbial challenge:

We are surrounded by an unseen world of diverse, parasitic microorganisms. They outnumber us profoundly. Their rapid generation times should prevent our passing on adaptations against them to our progeny.

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How then, do we survive?

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III: The Mammalian Answer

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Microbial virulence mechanisms (examples):

- ◆ adherence
- ◆ toxigenicity
- ◆ host immune system avoidance
 - encapsulation
 - antigenic variance
 - intracellular survival
 - (resistance to phagolysosomal destruction)

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“Genome Power”

- ◆ Note that the virulence mechanisms of each pathogen are encoded within its genome.
- ◆ Mammals possess an enormous, highly complex genome which allows the existence of differentiated cells.

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Although virulent microbes:

- ◆ Threaten our individual existence and cause us substantial discomfort across the course of our lives
- ◆ Threaten the well-being of our kith and kin
- ◆ And, at times threaten whole communities, cities, and nations

Many scientists contend that they are neither a serious threat to the survival of humans (as a species), nor to the survival of other mammalian species (say, field mice for instance).

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Consider then, if you will:

- ◆ Is there a shortage of humans on planet earth on account of infectious diseases?
- ◆ When other mammalian species are threatened with extinction, are microbes thought to be the cause?

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Each cell of a mammal contains:

- ◆ 1,000 times more DNA than *E. coli*.
- ◆ Over 10,000 times more DNA than the nucleic acid found in some viruses.
- ◆ In the same way that microbial DNA underlies microbial virulence, mammalian DNA underlies mammalian resistance to microbes.



Mammalian genes are exquisitely controlled.

- ◆ Mammals turn on particular genes only in certain selected cells (and within those cells only at necessary time intervals).
- ◆ Thus, while each and every cell carries the entire genome, specific cell types express only the proteins necessary for their function.



Clinical laboratory professionals are particularly aware of cell differentiation, and can list the proteins that serve as markers for many specific cell types.

- ◆ RBCs
- ◆ PMNs
- ◆ B lymphocytes
- ◆ Myocardial cells
- ◆ Epithelial cells
- ◆ Pancreatic acinar cells
- ◆ Osteoblasts
- ◆ Osteoclasts
- ◆ Hepatocytes



Differentiated cells:

- ◆ Express **some** of their genes
- ◆ Change over time (develop)
- ◆ Play roles, benefiting other cells
- ◆ Interact as a team
 - Release chemical messages
 - Respond to chemical messages
 - (At times, by expressing different genes)

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The power of cell differentiation provides the underpinnings of non-specific immunity. (We will discuss only the last two of these.)

- ◆ Skin, tears
- ◆ Stomach acid
- ◆ Ciliated epithelium of the respiratory tract
- ◆ Blood vessel endothelium
- ◆ Genitourinary epithelium
- ◆ *Phagocytes
- ◆ *Hepatocytes, neurons, hemotopoietic cells

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PMNs are specialized for:

- ◆ amoeboid movement
- ◆ recognition of prokaryotic molecules
- ◆ communication with the endothelium as well as other WBCs
- ◆ phagocytosis
- ◆ intracellular bacterial killing

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The “acute phase reaction” (an intercellular teamwork response) towards bacterial infection.

- ◆ Monocyte-macrophage encounters bacterium, recognizes it as such, and releases cytokines (IL-1, IL-6, TNF) in response.
- ◆ The cytokines then induce the following:
 - bone marrow release of PMN reserves
 - hypothalamus directed increase in body temperature
 - liver alteration of plasma protein synthesis (less albumin, more CRP, haptoglobin, etc.)

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Differentiated cells produce environments favoring the growth of protective normal flora. (Thus, our gigantic reservoir of DNA even gives us the capability to selectively culture bacteria upon and within ourselves.)

- ◆ Outer epithelial conditions favor the growth of *Propionibacterium spp.* that release acid onto the skin.
- ◆ Cells of the female G-U tract during estrus release carbohydrates that favor the growth of *Lactobacillus spp.* which releases lactic acid, driving the pH down.
- ◆ The G-I tract favors the growth of protective normal flora as well. (Consider the effects of perturbing this flora with antibiotics.)

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The Ultimate Defensive Weapon is Specific Adaptive Immunity.

- ◆ While non-specific immunity provides a formidable barrier to infection, specific immunity represents the most powerful and “intelligent” weapon known to exist in the war between microbes and mammals.
- ◆ Specific immunity is also a product of our gigantic, exquisitely controlled mammalian genome, and cell differentiation.

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Consider the mammalian dilemma:

- ◆ The diversity of species of pathogenic microorganisms seems overwhelming
- ◆ The generation time of these organisms is miniscule compared to that of mammals
- ◆ How can mammals adapt rapidly enough to ward off such microbes?



The immune system handles this dilemma in a remarkable manner.

- ◆ Within immune cells, an ingenious manipulation of the mammalian genome allows the random generation of an almost infinite array of antibody receptor molecules.
- ◆ Thus antigen receptors are prepared *a priori* (before antigen exposure) for any possible pathogenic microbe.



Goals of the Specific Adaptive Immune Response

- ◆ Create receptors for any potential microbial protein or carbohydrate virulence factor
- ◆ Out-race all other forms of molecular evolution



These goals are accomplished by B and T lymphocytes

- ◆ B and T lymphocytes are the only cells that can specifically recognize (and distinguish between) different antigens
- ◆ This is accomplished with a stunningly diverse array of high affinity antigen receptors, surface immunoglobulin (slg) on B cells and TCRs on T cells.

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Remember, we only have time to discuss antibodies.

(The gene scrambling mechanisms used to create antibody diversity are also used to create TCR diversity.)

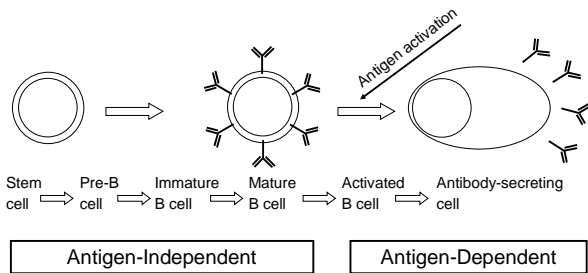
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Two awe-inspiring characteristics of Ab production account for Ab diversity, specificity, and avidity.

- ◆ Ab production is anticipatory (pre-emptive)
- ◆ Ab production has an "automatic fine-tuning" (enemy-honing) capability

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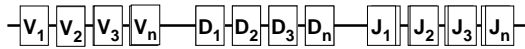
Antibody production is anticipatory



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The genes encoding the heavy and light chain Ab subunits are large and intricate.

- ◆ The heavy chain gene on chromosome 14, for example, is composed of hundreds of “V”, “D”, and “J” gene segments.



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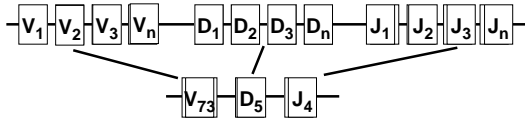
During maturation, B lymphocytes express unique DNA recombinase enzymes

- ◆ Only lymphocytes express such enzymes
- ◆ (T lymphocytes use the recombinases to synthesize TCRs)

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DNA recombinases “scramble” the immunoglobulin gene fragments

- ◆ Recombinases (in conjunction with RNA splicing enzymes) cut out one each of the V, D, and J segments and splice them together in a “purposefully error-prone” way to make the heavy chain variable region.



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Other enzyme activities (e.g., TdT) insure that no two $V_{227}-D_7-J_4$ segments are the same.

- ◆ This creates a stunning diversity of antibody variable regions when combined with an identical process occurring in the light chain gene.
- ◆ This process is referred to as *somatic recombination* (compare to *sexual recombination*)

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The generation of antibody diversity is anticipatory in that it occurs prior to any encounter with antigen.

The goal is maximization of the gene’s potential towards the synthesis of an antibody specific for any pathogen that has arisen or may arise.

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The V-D-J splicing of an Ab gene shortens the gene, thereby activating it for transcription and translation.

(This insures that Ab genes are expressed only in B lymphocytes.)

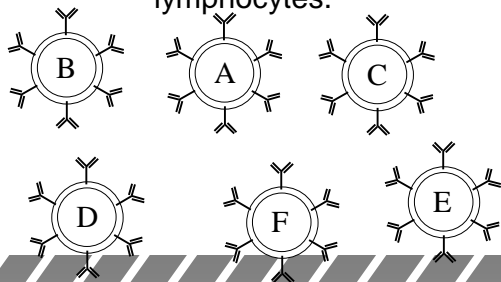
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Few B Lymphocytes Are Allowed Long Term Survival or Proliferation in This System

- ◆ Most B lymphocytes created:
 - exist for little over a week
 - never encounter antigen complementary to their sIg antigen receptors

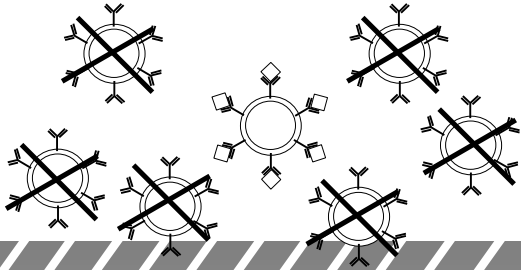
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Activated immunoglobulin gene products are expressed as cell-surface antibody on naïve, mature B lymphocytes.**



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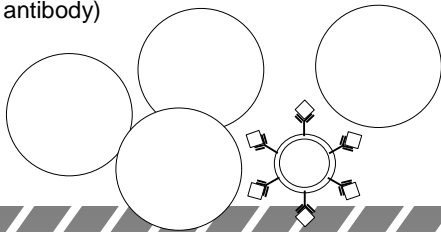
Most naive B lymphocytes survive less than two weeks.**



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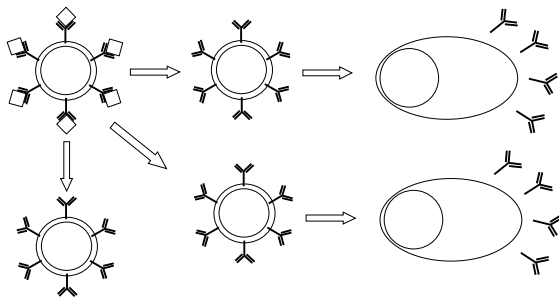
The Kiss of Life

- ◆ Only the cells which encounter an antigen match for their sIg receptors are allowed to proliferate (the cells' progeny make soluble antibody)



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The cell's progeny make soluble antibody



Memory cell

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These processes insure that the microbial environment ultimately selects the antibody specificities in a healthy individual

- ◆ 50 million B lymphocytes exit your bone marrow each day, each with different receptors.
- ◆ Only the very few which contact antigen proliferate
- ◆ B lymphocyte proliferation (called "clonal selection") is thus brought about by the microbes themselves

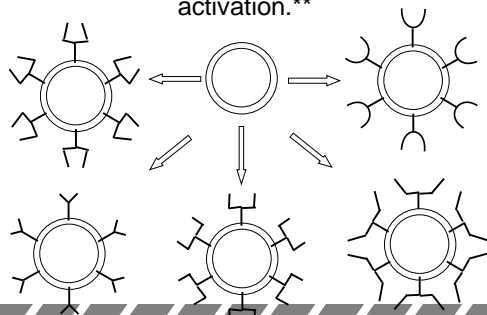
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B lymphocytes are programmed to undergo somatic hypermutation following activation

- ◆ This hypermutation is directed at the hypervariable regions within the variable segments of the heavy and light polypeptide subunits.
- ◆ Somatic hypermutation again introduces a random, error-prone mechanism into antibody production, this time amongst the progeny of an activated cell

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B lymphocyte memory cells are programmed to undergo somatic hypermutation following activation.**



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Thus when a given B lymphocyte encounters antigen and is triggered to proliferate (and its progeny to make antibody), a spectrum of similar B lymphocytes are loosed onto the battlefield.

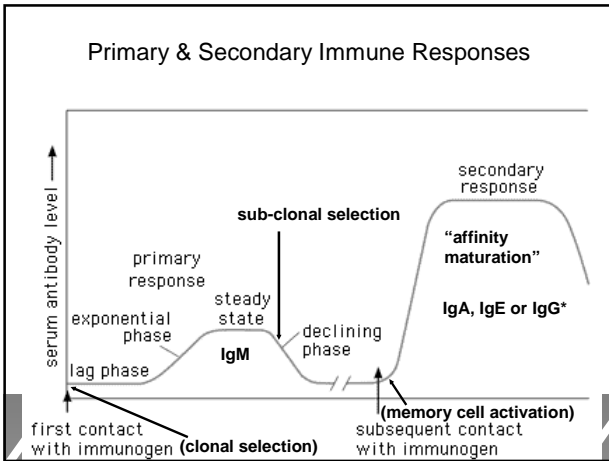
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As antigen concentration wanes, only the mutant cells that bind most tightly to antigen are triggered to proliferate in a second round of activity.

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Somatic hypermutation, followed by “sub-clonal selection” in the presence of waning antigen concentration, fine-tunes the antibody producing cells against the invader.

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These same processes are used in artificial systems to generate exquisitely specific and avid monoclonal antibodies as reagents.

Goals of the Specific Adaptive Immune Response

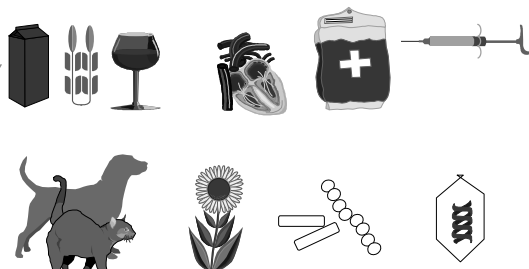
- ◆ Create receptors for any potential microbial protein or carbohydrate virulence factor
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Thus, two awe-inspiring characteristics of antibody production account for the diversity, specificity, and avidity of antibodies.

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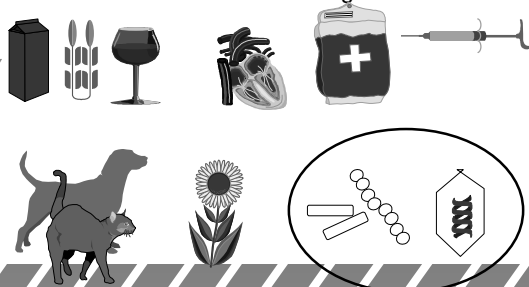
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What antigen sources are symbolized by the following line drawings?



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What is the most important difference (to the host) between the immune responses towards the encircled and non-encircled antigen sources?



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