Role of B Cells and Antibodies in Controlling Bacterial Pathogens

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As the workhorses of our internal defense system, we owe an enormous debt to antibodies. Responding to external threats such as disease-causing microorganisms, protein antibody molecules are released by specialized immune cells into the blood stream to disarm the threat. Their mode of attack is target recognition: millions of different molecules can be created, each with a different binding site, which can identify distinctive proteins on their specifically assigned foe with pinpoint accuracy. From Anatomy of a Killer. NobelPrize.org.

Perspective

Antibodies are the primary means by which the immune system eliminates bacterial pathogens from the human body. Antibodies clear bacteria that have gained access to circulation or normally sterile tissues, such as the liver and heart. Antibodies also protect the mucosal or "wet" surfaces of the human body, such as the lungs, oral cavity, urogenital tract, and the gastrointestinal tract, which are constantly exposed to a spectrum of opportunistic and pathogenic microorganisms.

When a host encounters a pathogen for the first time, there is an initial lag before the first wave of antibodies arrive on the scene. It is the job of the innate immune system to keep the invading bacteria at bay, while antibody-producing cells or "B cells" are enlisted and dispatched to the site of infection. The initial sortie consists of IgM antibodies. Although IgM antibodies are relatively low specificity and low affinity, they can resolve infections by working in concert with the innate immune system to lyse and engulf invading pathogens.

The second offensive, which appears 5–7 days after the start of an infection, is led by IgG or IgA antibodies, depending on the anatomical compartment. IgG antibodies predominate in the systemic compartment (e.g., blood, liver, spleen, heart), while IgA antibodies are the principal antibody type in mucosal secretions (e.g., saliva, intestinal mucus). The invading bacteria are soon confronted by swarms of IgG and IgA antibodies that fasten themselves to surfaces and appendages, such as flagella.

Antibody-coated bacteria have little chance of escape. In the systemic compartment, IgG-coated microorganisms are engulfed by professional phagocytic cells like neutrophils and macrophages. Or they are attacked by complement, a cascade of serum proteins that form pores in bacterial cell walls. In either case, IgG-coated pathogens are destroyed. In mucosal compartments, IgA-coated bacteria become entrapped in mucus and are unable to gain a foothold on host tissues. Consequently, IgA-bacteria immune complexes are eliminated from the body through normal physiologic processes such as peristalsis, mucociliary action, and coughing.

The job of B cells is not over when an acute infection is cleared. A subset of B cells known as plasma cells (effectively antibody "factories") takes up residence in peripheral tissues and bone marrow and secretes pathogen-specific IgG or IgA for months or even years. These so-called long-lived plasma cells provide a continuous supply of pathogen-specific antibodies in blood and mucosal secretions, positioned to intercept a pathogen upon its return. The term "antibody titer" refers to the levels of a given pathogen-specific antibody in a particular body fluid.

Another subset of B cells, called memory cells, patrols central and peripheral tissues, on the look-out for return visitors. Memory B cells remain quiescent until they reencounter a pathogen-derived product known as an antigen. At this point, some of the memory B cells will differentiate into plasma cells to further increase levels of circulating antibody, while others will reenter lymph nodes and undergo additional rounds of antibody gene editing through a process known as somatic hypermutation (SHM), yielding new swarms of antibodies with greater affinities for their targets. Through repeated rounds of SHM, B cells give rise to a broad and essentially infinite diversity of antibodies capable of adapting to the ever-evolving onslaught of microbial invaders.

The goal of this article is to provide an introduction to how antibodies and B cells function in immunity to bacterial pathogens. Considering the sheer number of bacteria that cause disease in humans, it is not possible to address them all in a single article. Therefore, for the sake of illustration, the discussion will be limited to the nine bacterial pathogens (and their respective toxins, when appropriate) for which there are currently approved vaccines in the United States (Table 1). For a detailed description of these vaccines and their targets, the reader is referred to The Pink Book, published by the Centers for Disease Control and Prevention (CDC). All nine of the vaccines work by stimulating pathogen-specific antibodies that target exotoxins, promote complement-dependent killing, mediate opsonophagocytosis, and/or prevent colonization of mucosal epithelial surfaces.

Antibodies, B Cells, and the Nobel Prize

The discovery of the inner workings of antibodies and B cells has gone hand-in-hand with the pursuit of vaccines to combat some of the most significant killers in public health. In the early 20th century, diphtheria caused more than 200,000 cases and greater than 15,000 deaths per year in the United States alone. Today, almost a century after implementation of a universally administered vaccine, diphtheria is essentially eradicated in the United States. The etiological agent of diphtheria is the aerobic gram-positive

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bacterium, *Corynebacterium diphtheriae*, which colonizes the human nasopharynx and secretes an extraordinarily potent and fast-acting toxin called diphtheria toxin. For physicians and scientists in the late 1800s, it was a mystery how an infection seemingly localized to the throat could be so deadly. Equally mysterious was how the immune system works to combat infectious agents like diphtheria.

It was Emil Adolph von Behring (1854–1917) and Kitasato Shibasaburo (1853–1931) who first identified a soluble substance in the blood of convalescent animals that when injected into naïve animals imparted protection against diphtheria. The protective substance, termed "antitoxin," established the concept of passive immunity and revolutionized therapies for diphtheria at the turn of the 20th century. In 1901, the first Nobel Prize in Physiology or Medicine was awarded to Emil von Behring "for his work on serum therapy, especially its application against diphtheria, by which he has opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths." Within a matter of years, public health laboratories, including the New York State Department of Health, were generating diphtheria antitoxin in horses and using it statewide in children to treat disease. An antitoxin therapy against tetanus toxin soon followed.

Of course, we now know that antibodies are responsible for the soluble "antitoxin" activity described by Emil von Behring and Kitasato Shibasaburo and characterized by other early immunologists, including Paul Ehrlich. These early discoveries are documented in the articles of "Microbe Hunters," Paul de Kruif's 1926 book that profiles the pioneers of immunology and microbiology.

A more thorough understanding of the biochemical nature of antibodies and the cells involved antibody production emerged in the early 20th century, as documented in a brief historical review of B cells by the preeminent immunologist, Dr. Max Cooper. Milestones in the field of antibodies were acknowledged by the Nobel Prize committee. For example, the 1919 Nobel Prize in Medicine or Physiology was awarded to the Belgian microbiologist Jules Bordet for the discovery of complement proteins that promote bacterial killing in conjunction with antibodies. The Australian born immunologist, Sir Frank Macfarlane Burnet, received the 1960 Nobel Prize in Physiology or Medicine for the clonal selection theory, which essentially argued that an antibody of a given specificity arises from a single B cell clone that expands in the presence of its cognate antigen (i.e., an antibody's target). The discovery that antibodies arise as a result of self-imposed genetic engineering through processes known as VDJ recombination and SHM occurred in the 1970s. In 1972, Gerald M Edelman and Rodney R Porter shared the Nobel prize for the elucidation of the structure and function of antibody molecules at the protein level. A little more than a decade later, the 1984 Nobel Prize was awarded to Niels K Jerne, Georges J.F. Kohler, and César Milstein for the ". . ..discovery of the principle for the production of monoclonal antibodies," a technology that revolutionized the field of immunology and related disciplines and has given rise to modern drugs to treat breast cancer, leukemia, and other diseases. In 1987, Dr. Susumu Tonegawa was awarded a Nobel prize for elucidating how B cells are able to generate antibody diversity. Finally, the 2018 Nobel prize in Chemistry was awarded to the American microbiologist, George P. Smith, and the British immunologist, Sir Gregory P. Winter, for the ability to genetically manipulate antibodies in bacteria and bacteriophages, which has impacted biotechnology industry and fields of cancer therapy.

Antibody-Structure and Function

Today, antibodies are arguably some of the most well studied molecules in biology. In mice and humans, there are five isotypes: IgM, IgG, IgA, IgE, and IgD. Because IgE primarily functions in allergic responses and the role of IgD in immunity is still very much an active area of research, these two isotypes will not be discussed further. Rather, the focus of this article will be on IgM, IgG, and IgA. It should be underscored that the terms "immunoglobulins" and "antibodies" are frequently used interchangeably. However, there is an operational distinction: the term immunoglobulin refers to the bulk fraction of all antibodies precipitated from serum; the term antibody refers to a subset of immunoglobulins directed against a specific target or antigenic determinant (antigen).

Table 1 Role of antibodies in currently approved vaccines for bacterial pathogens.

Vaccine	*Pathogen*	*Antibody mode(s) of action*
Anthrax	*Bacillus anthracis*	Neutralize anthrax toxin
DT	*Corynebacterium diphtheriae*	Neutralize diphtheria toxin
Tetanus	*Clostridium tetani*	Neutralize tetanus toxin
aP	*Bordetella pertussis*	Neutralize pertussis toxin Inhibit bacterial adhesion to respiratory tract
Cholera	*Vibrio cholerae*	Inhibit bacterial colonization of intestine
Hib	*Haemophilus influenzae*	Complement-dependent SBA Opsonophagocytosis (OPA)
Meningococcus	*Neisseria meningitidis*	Complement-dependent SBA
Pneumococcus	*Streptococcus pneumoniae*	Opsonophagocytosis (OPA)
Typhoid	*Salmonella enterica* spp. Typhi	Opsonophagocytosis (OPA)

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IgG Antibodies

IgG is often considered the prototypic antibody isotype. IgG consists of two identical polypeptides of ~50,000 MW, known as the heavy chains (H), and two identical polypeptides of ~25,000 MW known as the light chains (L). Each heavy chain is paired covalently to a light chain in a H-L configuration. The two H chains are covalently linked to each other via a disulfide bond so that a single IgG molecule consists of HL + HL pair. Structurally, the IgG molecule is typically compared to the letter "Y" with each of the antibody's arms extending upward to engage with its cognate antigen. In reality, however, the arms have a degree of freedom due to a hinge region, such that at any given time an antibody may resemble something between a "Y" and a "T." The upward facing arms are referred to as the Fab regions and constitute the antigen binding elements. The tail or stalk is known as the Fc region. If you imagine an IgG molecule bound to the surface of a bacterium, the Fab elements would be associated with the pathogen, while the Fc region would be projecting outward.

Although the Fc elements are not interacting with the pathogen per se, they are essential for pathogen killing. Specifically, Fc elements are recognized by two components of the innate immune system: the complement system and phagocytic cells like macrophages and polymorphonuclear cells (PMN). The complement system is a collection of more than two dozen serum proteins that work in concert to promote bacterial cell lysis through the formation of pores in bacterial cell walls. Phagocytic cells recognize and engulf antibody-coated bacteria through surface expressed Fc receptors (FcR). Fc receptors specific for IgG are referred to as FcγR. Thus, bacterial pathogens coated with IgG are either killed through complement-mediated lysis or cleared by phagocytic cells that express Fc-receptors.

IgG's two identical Fab elements involved in binding to antigens (e.g., flagella, lipopolysaccharide, toxins) and a single Fc domain that serves as a molecular adaptor recognized by complement and phagocytes, can be separated from each other by proteolytic digestion. Treatment of IgG with papain, a cysteine protease from the papaya plant, cleaves IgG at its hinge region and liberates the two monovalent Fab elements, which are referred to as Fab fragments, from the Fc element. Treatment of IgG with the acid protease, pepsin, also clips IgG at its hinge and releases the stalk, but in this case the two Fab elements remain joined to each in a molecular form known as $F(ab)_2$. Thus, Fab fragments are monovalent (i.e., one antigen binding site) molecules, while whole IgG molecules and $F(ab)_2$ fragments are bivalent (i.e., two antigen binding sites).

IgG-Antigen Interactions

The interaction of a given IgG antibody (Ab) with its antigenic determinant (Ag) is non-covalent and reversible. As such, the interaction is essentially a bimolecular reaction where Ab + Ag <–> Ab-Ag. The strength of an antibody's interaction with its antigen is driven by a net negative change in the Gibbs free energy, as a result of changes in enthalpy (heat) and entropy (structure). The rate of association (k_a) is expressed as $M^{-1}\,s^{-1}$ and the rate of dissociation (k_d) is expressed as s^{-1}. The equilibrium constant or K_D (M) is defined as free [Ab] [Ag]/[Ab-Ag]. K_D is the most commonly used reference point when citing an antibody's strength.

Strictly speaking, an antibody's affinity for its target is derived by measuring the binding kinetics of monovalent Fab fragments with its antigen using techniques like surface plasmon resonance (SPR). Fab binding affinities (K_D) range from weak (micromolar; 10^{-4}–10^{-6}) to very strong (picomolar; 10^{-10}–10^{-12}), with most falling within the nanomolar range (10^{-7}–10^{-9}). In practice, however, avidity may be a more appropriate measure of an antibody's strength than affinity, since avidity takes into account the fact that IgG is bivalent and may be able to engage two antigens simultaneously, thereby affecting the overall stability of that antibody-antigen interaction. In simple terms, avidity is equivalent to a person holding onto a vertical pull-up bar with both hands. If one hand comes off the bar temporarily, the other hand is still holding tight. Together, however, two hands make for a more stable grasp of the bar than just one hand. Likewise, antibody avidity is a measure of the strength of an interaction when both Fab arms can engage their targets simultaneously, which occurs frequently with highly repetitive bacterial surface antigens like LPS and capsular polysaccharides (CPS).

IgM and IgA Antibodies

IgM and IgA antibodies share the basic architecture of IgG but differ in several important respects. The most significant difference is valency. IgM is secreted by B cells as a pentamer in which five IgM molecules are linked via their Fc tailpieces to form a rosette-like structure with 10 Fab elements facing outwards. This unique arrangement is consequential. IgM antibodies, which are the first to arrive on the scene of a new infection, tend to have low binding affinities for their targets. However, what IgM antibodies lack in affinity, they make up for in avidity. The 10 Fab elements enable IgM to hold tight to polymeric antigens like LPS, CPS and flagella. In addition, IgM's Fc region is an extremely potent activator of the complement system. These two properties (i.e., high-avidity binding and potent complement activation) enables IgM to keep bacterial invaders at bay until IgG reinforcements arrive on the scene.

IgA is the predominant antibody type in most mucosal compartments. In the gut, for example, IgA-producing B cells (plasma cells) outnumber IgG and IgM plasma cells by approximately 10 to 1. IgA is generally produced as a dimer in which two IgA monomers are joined via their Fc tailpieces by a small protein called J chain. IgA is therefore tetravalent. Unlike IgG and IgM, IgA cannot activate the complement system. Rather, IgA functions in mucosal secretions like saliva, intestinal fluids and breast milk where serum components like complement are absent.

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Transport of IgM and IgA Into Mucosal Secretions

It is the polymeric nature of both IgA (dimer) and IgM (pentamer) that permits their transport into the secretions that bathe the alimentary tract, the upper respiratory tract, and the upper female genital tract. IgA and IgM are also actively transported by mammary epithelial cells into colostrum and breast milk. The process of antibody transport from inside to outside is most clearly exemplified in the gastrointestinal tract, where a single layer of rectangularly shaped epithelial cells are joined side-by-side to form a barrier that prevents the diffusion of macromolecules and particles (including viruses and bacteria) from the lumen ("outside") to lamina propria ("inside"). Dimeric IgA and pentameric IgM antibodies produced by B cells in the lamina propria are recognized by the so-called polymeric immunoglobulin receptor (pIgR) located on the underside (basolateral) of epithelial cells. The pIgR captures IgA and IgM and shuttles them intracellularly across epithelial cells, a process known as transcytosis. Once on the apical face (outside), the pIgR is proteolytically clipped and a fragment of the receptor, known as secretory component (SC), still bound to IgA or IgM, is released into the intestinal lumen. IgA and IgM complexed with SC are known as secretory IgA (SIgA) and secretory IgM (SIgM), respectively. SC is a large, heavily glycosylated protein that wraps around IgA and IgM's Fc elements, thereby protecting them from the harsh environment of mucosal secretions. The pIgR does not transport IgG, although there are other mechanisms by which small amounts of IgG can gain entry into mucosal secretions.

Evolution of Antibody Responses

As alluded to above, microbial pathogens that successfully breach the body's natural defenses and gain access to normally sterile environments like the blood or any of the visceral organs will soon come under attack by swarms of antibodies. The first wave consists of low-affinity, high-avidity IgM antibodies capable of triggering the complement system and bacterial lysis. The second wave consists of high-affinity IgG antibodies, which also recruit complement and phagocytic cells to sites of infection. If the infection originates in a mucosal compartment, then the second wave of antibodies consists mainly of IgA, rather than IgG. The generation of long-lived IgG or IgA secreting plasma cells in response to a particular infection guarantees a steady reserve of circulating antibodies should the microbial invaders return. As further reinforcements, memory B cells are poised to proliferate and produce new swarms of IgG and IgA antibodies on demand. But how do antibody responses originate in the first place and what drives the formation of plasma cells and memory cells? These are central concepts in immunology and well beyond the scope of this article. However, it is important to appreciate three basic tenets.

Tenet 1: Antibody Affinities Evolve With Time

The first tenet is that antibodies evolve during the course of an active infection from low- to high-affinity and tailor their specificity to available targets on the surface of the invading pathogen. B cells originate in the bone marrow and migrate into the periphery where they develop into so-called naïve mature B cells that express IgM (and IgD) on their surfaces. When naive mature B cells "recognize" a foreign antigen the will secrete large amounts of IgM for immediate use in combatting an infection. Alternatively, they will enter the germinal centers and undergo switching to IgG- or IgA-positive B cells.

Broadly speaking, the conversion from IgM-positive to IgG-positive (or IgA-positive) B cells occurs in two stages. Class switch recombination (CSR) is a process where the DNA encoding the IgM Fc region is swapped for IgG or IgA Fc region. The choice between IgG and IgA Fc elements is dictated by the local environment. SHM is the process whereby the DNA encoding the antibody variable regions (V_H and V_L) are edited to generate a better "fit" with its antigenic determinant. This process is also referred to as affinity maturation, since the antibody's affinity for its target can increase by orders of magnitude as a result of SHM. Affinity maturation requires help from a number of other immune cell types, most notably follicular dendritic cells and T cells.

Tenet 2: Evolution is Driven SHM and Clonal Selection

The second tenet is that the process of SHM gives rise to an infinite number of antibodies against virtually any microbial protein or carbohydrate target. As defined earlier above, SHM is a process in which the DNA encoding an antibody's variable regions (V_H and V_L) are edited in an effort to improve overall binding affinity to its target. To be clear, any given B cell will produce antibodies of only one specificity (i.e., B cell "x" produces antibody "x," while B cell "y" produces antibody "y"). Therefore, the collective diversity of the antibody response to a given pathogen is dictated by the ensuing number of B cells that arise following infection. The term B cell "repertoire" refers to the total diversity of B cell specificities. However, B cell clones can give rise to progeny that carry minor variations in their variable regions that alter target specificity. B cell clone "x" can give rise to subclones x.1, x.2, x.3. which bind the same or nearly the same epitope but with slightly different affinities. The constant release of new generations of antibodies capable of saturating a given target has given rise to the concept of an antibody "swarm."

Tenet 3: B Cell Memory Guarantees Long-Term Immunity

The third tenant is that upon re-exposure to a pathogen, the recall antibody response is faster, more specific, and of higher magnitude than the first exposure, due to the existence of memory B cells. The preeminent immunologist Antonio Lanzavecchia

refers to memory B cells as the "…repository of an immune experience of an individual." In other words, memory B cells are the equivalent of an antibody hard drive that can be accessed on demand upon re-encounter with a pathogen. Concurrent with the formation of memory B cells are long-lived IgG or IgA plasma cells that can sustain circulating antibody levels over a period of years or even decades. In fact, modern day vaccines are predicated on the ability of a surrogate antigen or attenuated pathogen to elicit a memory B cell response capable of responding to an actual infection over a period of years or even decades.

In summary, a bacterial infection in a mucosal (e.g., lung, gut) or systemic (e.g., liver, kidney) compartment will elicit a two-phased antibody response (IgM followed by IgG and/or IgA) that will continue to mature or evolve until the pathogen is cleared. Long lived plasma cells will maintain circulating antibody titers for years to ward off a recurrent infection, while memory B cells will lie in waiting until called into action.

Antibody Function: Finding a Pathogen's Achilles' Heel

Just as the proverbial well-placed arrow to Achilles' heel led to the death of the mythic Greek warrior, antibodies eliminate bacterial pathogens by targeting sites of microbial vulnerability. To fully appreciate the shrewd nature of the immune system, it is important to recall the strategies used by pathogens to cause disease: exotoxins, evasion of innate defenses, tissue colonization, cellular invasion, and proliferation in the blood stream. B cells respond in kind to each of these insults by releasing their equivalent of poison arrows.

Bacterial Toxins, Virulence Factors, and Surface Polysaccharides as Antibody Targets

Without question, exotoxins are the most potent virulence factors associated with bacterial pathogens. Two prime examples are diphtheria toxin produced by *C. diphtheriae* and cholera toxin secreted by *V. cholerae* (Table 1). Diphtheria toxin is released by *C. diphtheriae* after the bacterium has colonized the human upper respiratory tract, while cholera toxin is produced when *V. cholerae* has set up shop in the small intestine. Diphtheria toxin kills mammalian cells by inhibition of protein synthesis as a result of ADP-ribosylation of elongation factor 2 (EF-2). Cholera toxin causes severe watery diarrhea by perturbing cyclic AMP levels in intestinal epithelial cells as a consequence of ADP-ribosylation of small G proteins. Strains lacking these toxins are essentially avirulent, attesting to the singular contributions of cholera and diphtheria toxins to disease. Three other pathogens listed in Table 1 produce exotoxins; *B. anthracis* (anthrax toxin), *C. tetani* (tetanus toxin) and *B. pertussis* (pertussis toxin).

Bacterial pathogens also express an array of other virulence factors involved in attachment to and invasion of mammalian hosts. Surface appendages like fimbriae and pili enable bacterial adherence to specific cell types. For example, both *B. pertussis* and *V. cholerae* express adhesins required for colonization of human epithelial cells. Other pathogens express macromolecular "syringes" or secretion systems on their surfaces that inject "effector" proteins into mammalian cells that facilitate bacterial entry into intracellular compartments. In fact, *Salmonella* species, including *S. enterica* serovar Typhi, employs two different so-called type-three secretion systems (T3SS) during infection of the gastrointestinal tract.

LPS, capsular polysaccharides (CPS), and exopolysaccharides (EPS) are classified as virulence factors by virtue of their ability to serve as shields against components of the innate immune system. The importance of CPS in pathogenesis is best exemplified by *H. influenzae*, a leading cause of bacterial meningitis in young children (Table 1). *H. influenzae* is a small, nonmotile Gram-negative bacterium that adheres to epithelial cells and mucins in the human upper respiratory tract. In some individuals, the bacterium becomes invasive and breaches epithelial defenses and surmounts the innate immune system to gain access to the blood stream. The invasive phenotype and ability to evade phagocytosis is associated with a specific CPS consisting of polyribosyl-ribitol-phosphate (PRP). Strains of *H. influenzae* that lack this specific CPS are associated with invasive disease much less frequently. Three other pathogens listed in Table 1 rely on capsule production to evade innate immunity and replicate within the human host: *S. pneumoniae*, *N. meningitidis*, and *S.* Typhi.

As a rule, immune responses to microbial pathogens, such as those listed in Table 1, are complex and involve antibodies targeting multiple different bacterial-associated antigens. However, when a single virulence factor like a toxin is central to disease manifestation, the most important antibody response may be against a single target. The list below demonstrates the strategies used by antibodies to exclude and/or clear bacterial pathogens from systemic and mucosal compartments.

Direct Neutralization of Toxins and Other Virulence Factors

The current anthrax, diphtheria, pertussis, and tetanus vaccines listed in Table 1 are designed specifically to elicit anti-toxin antibody responses. The primary mode by which antibodies neutralize toxins is by blocking attachment to host cell receptors. Antibodies that recognize epitopes on the binding subunits of anthrax, diphtheria, pertussis, and tetanus toxins have been described. However, toxin-neutralizing antibodies do more than just interfere with receptor binding events. In the case of anthrax, there are antibodies that prevent toxin oligomerization, pore formation, proteolytic cleavage, and subunit assembly. In fact, Raxibacumab is an FDA approved human IgG monoclonal antibody against anthrax toxin protective antigen (PA) designed as a pre-exposure prophylaxis in the event of anthrax being used in bioterrorism. Raxibacumab doesn't interfere with PA from binding to receptors on target cells, but it does prevent PA from associating with its toxic components, lethal factor and edema factor. Thus, antibodies are capable of interfering with essentially all the steps in the intoxication process.

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Antibodies can also "neutralize" virulence factors associated with bacterial attachment and invasion of mammalian cells, as best exemplified by the acellular pertussis (aP) vaccine (Table 1). Whooping cough is caused by the Gram-negative bacterium, *Bordetella pertussis*, which colonizes the human upper respiratory tract where it secretes pertussis toxin. The aP vaccine consists of a collection of recombinant proteins that stimulates antibodies against pertussis toxin, plus several of the fimbriae and adhesins involved in bacterial attachment to the respiratory epithelium. Thus, antibodies against fimbriae and adhesins interfere with bacterial attachment to mucosal surfaces of the airway, while anti-toxin antibodies neutralize pertussis toxin that gains access tissues and circulation.

Activation of the Classical Complement Pathway

Simply stated, the complement system is a collection of more than two dozen serum proteins that function as a bridge between the innate and adaptive immune systems. The so-called classical complement pathway is initiated when two or more IgM or IgG antibodies are deposited in close proximity to each other on the surface of a pathogen. Clusters of IgM or IgG are recognized by the complement component, C1. C1 has an affinity for the Fc elements of IgM and IgG (namely IgG1, IgG2, and IgG3 subclasses), but not IgA. IgM, by virtue of its pentameric star-like structure, is particularly effective at recruiting C1. Once bound, C1 recruits a cascade of additional complement proteins to the bacterial cell surface, resulting in the assembly of the membrane attack complex (MAC). The MAC is a molecular channel or pore in the bacterial cell wall that perturbs cellular osmolarity and results in cell death by lysis.

Antibody-dependent, complement-mediated killing is a significant biological activity associated with the *H. influenzae* and *N. meningitidis* vaccines (Table 1). The *H. influenza* vaccine consists of the type B capsular polysaccharide (PRP) chemically linked to a carrier protein like the non-toxic variant of diphtheria toxin CRM 197 or inactivated tetanus toxin. The *N. meningitidis* vaccines in the United States consist of polysaccharide protein conjugates and various combinations of recombinant protein surface antigens. In all cases, vaccination stimulates serum IgG of antibodies capable of decorating (opsonizing) the surfaces of *H. influenzae* and *N. meningitidis* and subsequently activating complement-mediated killing. In fact, efficacy of *N. meningitidis* vaccines correlates with how well antibodies from a vaccinated individual perform in a standardized serum bactericidal assay (SBA) (McIntosh et al., 2015).

Killing by Opsonophagocytosis

Opsonophagocytosis is the engulfment, by macrophages and other phagocytic cells like neutrophils, of bacteria opsonized with antibodies and/or complement proteins. Thus, microbial cells in the blood or within a specific tissue that become coated with IgG are soon recognized by patrolling macrophages and neutrophils. Recognition occurs through specific surface-expressed FcγRs on the phagocytes. When multiple FcγRs are engaged simultaneously, it cues the phagocyte to envelope its prey and confine it within an intracellular compartment known as the phagosome. The phagosome is a dead-end for most microbial pathogens. Within minutes, the encased bacteria are exposed to reactive oxygen species, acidic pH and a barrage of degradative enzymes.

Opsonophagocytosis is a primary means by which antibodies promote the clearance of *S. pneumoniae* and accounts for the biological activity associated with the successful pneumococcal vaccines in widespread use around the globe (Table 1). In fact, one measure of vaccine efficacy is the opsonophagocytosis killing (OPK) assay, which involves the engulfment of *S. pneumoniae* by human neutrophils in the presence of serum from a vaccinated individual. Antibodies also coordinate opsonophagocytosis in the clearance of *H. influenzae* and *S*. Typhi.

Secretory IgA Paralyze and Entrap Bacteria in Mucosal Secretions

In mucosal compartments, secretory IgA (SIgA) plays by different rules. As noted earlier in the article, IgA is actively transported by the pIgR into intestinal secretions in the form of SIgA. SIgA does not activate complement, nor does it promote opsonophagocytosis. Instead, SIgA functions by a process known as immune exclusion in which pathogens are aggregated by antibodies and entrapped in mucus. Immune complexes between SIgA and microbial invaders are not only restricted in their ability to diffuse through mucus but can also be cleared from the body through normal physiologic processes such as cilia movement in the respiratory tract and peristalsis in the intestinal tract.

The concept of immune exclusion is best exemplified in the gut where pathogenic bacteria like *V. cholerae* set up shop and cause the disease cholera (Table 1). The bacteria employ flagellar-based motility to penetrate intestinal mucus and then deploy pili to secure themselves onto epithelial cells. Local production of cholera toxin results in a deadly bout of watery diarrhea that can kill an otherwise healthy adult within a matter of hours. Thus, the battle between host (antibodies) and microbe occurs in the intestinal lumen. As it turns out, antibodies against LPS are associated with immunity to *V. cholerae* infection, presumably because they are able to entrap the bacteria in mucus well before *V. cholerae* can gain a foothold on the epithelium. In contrast, anti-cholera toxin antibodies fail to protect against disease because the toxin is released only when the bacterium is in intimate contact with host epithelial cells, where there is little time or space for antibodies to intercept their target.

Anti-LPS SIgA likely functions by more than one mechanism. On the one hand, they are highly effective at promoting bacterial agglutination to the point where "rafts" of bacteria are so large that they are visible to the naked eye when the assay is performed in a

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test tube. Experimentally, agglutination has also been observed in the intestinal lumen, so there is credence for immune exclusion play a role in vivo. On the other hand, recent evidence has demonstrated that anti-LPS IgA antibodies are potent inhibitors of *V. cholerae* motility. In fact, certain IgA preparations have been shown to completely paralyze *V. cholerae* within just a matter of minutes. This has led to the hypothesis that SIgA might work at two levels: paralyze, then handcuff. The whole cell-killed and live attenuated cholera vaccines in current use are administered to children and adults by the oral route as a means to stimulate *V. cholerae*-specific SIgA in the intestinal tract.

Additional Modes of Antibody Function

There are certainly under-explored or yet to be discovered mechanisms by which antibodies function in clearing bacterial pathogens, beyond complement activation, opsonophagocytosis, and immune exclusion (Lu et al., 2018). For example, there are reports describing monoclonal IgM and IgG antibodies against outer surface proteins of *Borrelia hermsii* (relapsing fever) and *B. burgdorferi* (Lyme disease) that cause direct bacterial lysis, independent of complement. There are also examples of antibodies that playing a central role in immunity against obligate intracellular pathogens like *Ehrlichia chaffeensis*, the causative agent of tick-borne disease ehrlichiosis. The recent discovery of an intracellular Fc receptor known as TRIM-21 has already challenged the conventional notions about antibody function. Finally, in the past several years has it become apparent that subtle differences in Fc glycosylation patterns on human IgG can have profound effects on antibody functionality.

Back to the Future With Monoclonal Antibodies (MAbs)

With the global crisis in antibiotic resistance, there is a desperate need to find alternative measures to control bacterial pathogens. As we look forward, new generations of vaccines are an obvious avenue to pursue. But an equally exciting prospect is the use of recombinant monoclonal antibodies (MAbs) in prophylactic and therapeutic applications. Historically, MAbs have been generated from mice; B cells from immunized animals are immortalized by hybridoma technology and then isolated as individual clones the secrete antibodies of a single specificity. Hence, the term "monoclonal antibody." In the past decade, however, it has become technologically feasible to generate MAbs from humans, using recombinant antibody technology as described by Corti and Lanzavecchia (2014). Basically, the genes encoding the variable regions of antibodies of interest are cloned and expressed in mammalian cell lines. In the case of HIV, Ebola and other viruses, recombinant antibodies have been shown to afford short term immunity. It is not inconceivable that MAbs will be used as a supplement or even as a replacement for antibiotics for some hard to treat bacterial infections. In a back to the future-like scenario, the use of high tech, recombinant "antitoxins" may soon be routine in the clinic.

Further Reading

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Relevant Websites

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