Published in final edited form as:

Curr Opin Immunol. 2018 August ; 53: 74–80. doi:10.1016/j.coi.2018.04.011.

Antibody-based vaccine strategy against intracellular pathogens

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Abstract

Historically, antibody-mediated immunity was considered effective against toxins, extracellular pathogens and viruses, while control of intracellular pathogens was the domain of cellular immunity. However, numerous observations in recent decades have conclusively shown that antibody can protect against intracellular pathogens. This paradigmatic shift has tremendous implications for immunology and vaccine design. For immunology the observation that antibody can protect against intracellular pathogens has led to the discovery of new mechanisms of antibody action. For vaccine design the knowledge that humoral immunity can be effective in protection means that the knowledge acquired in more than a century of antibody studies can be applied to make new vaccines against this class of pathogens.

Introduction

For most of the last half of the 20th century, immunology functioned within the paradigm of a division of labor for controlling pathogenic microbes whereby extracellular and intracellular pathogens were the responsibility of humoral and cellular immunity, respectively. There were many problems with this paradigm, which included the ambiguities in the definition of intracellular pathogens and the fact that inability to demonstrate antibody efficacy did not mean that there was no efficacy. The phrase intracellular pathogens included bacteria that replicated inside phagocytic cells such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, but did not include viruses, which were dependent on host cells for replication [1] (Box 1). However, by the late 1990s a series of observations challenged this view [2] and today there is widespread agreement that antibody-mediated immunity can be harnessed for vaccine design against intracellular pathogens. Evidence for this shift is apparent from the publication of several major papers describing protective antibodies to *M. tuberculosis* in the past few years [3–7]. For example, the historical uncertainty in the role of humoral immunity against *M. tuberculosis* [8] can now be explained by the finding that antibodies against mycobacteria manifest functional differences such that only some types of immunoglobulins are protective [3,6,9]. The construction and deconstruction of that

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debilitating paradigm in the fields of immunology and infectious disease is a story of experimental limitations, misinterpretations and logical errors (Box 2). In this essay, I review recent development in this field and discuss mechanisms of antibody action against so-called intracellular pathogens.

Box 1

What is an intracellular pathogen?

Intracellular pathogens are pathogenic microbes that spend all or part of their infective lifecycle inside host cells. These can be divided into obligate or facultative, depending on whether their host intracellular residence was required for replication. Obligate intracellular pathogens include *Toxoplasma gondii*, *Rickettsia* and *Chlamydia* spp. while facultative intracellular pathogens include such bacteria and fungi as *Mycobacterium tuberculosis* and *Cryptococcus neoformans*, respectively. The intracellular pathogen concept became popular in the late 20th century to describe a set of phylogenetically unrelated organisms for which certain generalities applied: replication inside cells, a requirement for cell-mediated immunity in host defense and ability to manipulate host cellular mechanisms for their survival. These generalities led to a zeitgeist that by the turn of the 21st century tended to view these organisms as different from other types of pathogenic microbes regarding their host-microbe interactions. However, as knowledge has accumulated, the concept of a sharp dividing line between extracellular and intracellular pathogens is increasingly problematic as organisms previously considered extracellular pathogens such as *Streptococcus pneumoniae*, for which antibody-mediated immunity is essential, are shown to be able to survive and replicate in macrophages [24]. Hence, the term intracellular pathogen continues to have utility with regards to conveying information on the replicative power of certain microbes in host cells but the notion that host defense against these phylogenetically diverse organisms is solely dependent on cell-mediated immunity is not tenable (See Box 2).

Box 2

A flawed view posits a division of labor for host defense against intracellular and extracellular pathogens

In the second half of the 20th century, a view took hold in the field of immunology that there was a division of labor between the humoral and cellular arms in providing defense against the so-called extracellular and intracellular pathogens. Three lines of evidence contributed to this intellectual synthesis: 1) immunoglobulins mediated their actions extracellularly and no role was imagined for antibody-mediated immunity against microbes inside cells; 2) it was very difficult to show a protective role for humoral immunity against such intracellular pathogens as *M. tuberculosis*; and 3) diseases caused by intracellular pathogens were increasingly associated with impaired cell-mediated immunity. Hence, many fields focused on individual intracellular pathogens neglected the study of antibody-mediated immunity for their particular microbes. However, beginning in the 1990s this synthesis unraveled primarily because of observations that it was possible to generate protective monoclonal antibodies against many pathogens for which

it was difficult to demonstrate a role for humoral immunity. Over time, it became clear that the inability of demonstrating a role for humoral immunity in host defense did not mean that there was no role for this immune arm, and that even if the role of antibody in natural host defense was minor, that it was often possible to achieve protection with certain types of antibodies. Today, the prevailing view is that both arms contribute and superior protection can follow the induction of protective humoral and cellular immune responses.

For vaccines based on protein antigens immunization elicits both humoral and cellular responses. Hence, for protein antigens it can be difficult to differentiate whether one or both arms of the immune response are mediating protection. One approach to ascertain the contribution of humoral immunity is to carry out passive protection experiments with immune sera, and conclude that antibody is important when protection is transferred by immune serum. Similarly, adoptive lymphocyte transfer producing immunity in a naïve host can provide strong evidence for protective cellular immunity. However, negative results are not conclusive since antibodies and cells may require the presence of the other arm to mediate protection. An early example of this phenomenon was the observation that for the facultative intracellular pathogen *Cryptococcus neoformans* passive administration of protective antibodies was not protective in the absence of T cells [10]. In general, attributing efficacy to any one arm can be a futile exercise when both responses are present since both passive antibody and lymphocyte adoptive transfer experiments represent conditions that are very different from those when immunization elicits both responses simultaneous. However, polysaccharide-protein conjugate vaccines consisting of a specific T cell-independent carbohydrate antigen covalently linked to an unrelated protein antigen elicit only a relevant antibody response to the sugar moiety and these mediate protection through humoral immunity [11]. Hence, conjugate vaccines that are protective against intracellular pathogens provide strong evidence that antibody-mediated immunity can be effective against this group of microorganisms.

Antibody-based vaccine strategies against Intracellular pathogens

Numerous vaccines elicit protective antibodies against a variety of diverse intracellular pathogens, including bacteria, fungi and protozoa (Table 1). Among these are several polysaccharide-protein conjugate vaccines, which are notable because they elicit only humoral immunity against the relevant microbial carbohydrate antigen that linked to a protein (Table 1). Although the majority of the vaccines listed in Table 1 are experimental vaccines, there is now definitive evidence for the clinical efficacy of antibody-based vaccine strategies against intracellular pathogens from the success of the conjugate vaccine composed of *Salmonella typhi* Vi polysaccharide conjugated to *Pseudomonas aeruginosa* exotoxin [12]. This vaccine elicits antibodies to the Vi polysaccharide and was more than 90% effective in protecting children from typhoid, as evident from the fact that *S. typhi* was isolated from 4 of 5525 immunized with the conjugate vaccine and 47 of 5566 children in the placebo group [12]. The success of this conjugate vaccine has paved the way for the development of such other vaccines against intracellular pathogens. In this regard, a polysaccharide conjugate vaccine against *Shigella flexneri* has shown efficacy in children of

certain age [13] and a new vaccine consisting of an O-antigen conjugated to *Pseudomonas aeruginosa* exotoxin is immunogenic in adults [14].

Synergy of humoral and cellular immunity

Whereas reductionist approaches have attempted to identify immunological divisions of labor in host protection against infectious diseases, the fact that both the humoral and cellular arms of the innate and adaptive immune systems are highly interconnected implies that these function as a whole. Hence, whereas it is possible to protect against a specific pathogenic microbe through either humoral or cellular immunity, the presence of both can be synergistic. This emerging theme is apparent from experimental studies whereby conjugate vaccines using B cell antigens such as polysaccharides linked to T cell antigens in the form of proteins. Conjugates of type A O-polysaccharide (OPS) and manno-heptose capsular polysaccharide (CPS) antigens with carrier protein were tested against *Burkholderia pseudomallei* in a murine model of melioidosis [15]. Although vaccination with the CPS conjugate resulted in some protection, conjugating CPS to the *B. pseudomallei* recombinant protein antigen LolC elicited better protection consistent with the notion that having both humoral and cell mediated immunity was best [15]. Similarly, conjugating *M. tuberculosis* arabinomannan to *Bacillus anthracis* protective antigen elicited protective antibodies but a more protective immune response was observed when the polysaccharide was attached to mycobacterial antigen Ag85, which elicited both humoral and cellular immunity [5]. The notion that eliciting both humoral and cellular immune responses results in greater protection than either arm makes sense since these provide independent mechanisms of protection that can synergize [16]. Most clinically successful polysaccharide-protein conjugate vaccines employ a carrier protein such as cross-reacting material (CRM) of diphtheria toxin and tetanus toxoid, which elicits relevant antibody responses only to the polysaccharide moiety [17]. Hence, it should be possible to increase the efficacy of conjugate vaccines against intracellular pathogens by conjugating the B cell antigen to a T cell antigen known to elicit protective responses against the microbe in question.

Mechanisms of antibody-mediated immunity against intracellular pathogens

The classical mechanisms of antibody-mediated protection are opsonization, complement activation, neutralization of toxins and viruses, and antibody-dependent cellular cytotoxicity. These mechanisms play important roles in host defense against intracellular pathogens. For example, *M. tuberculosis* is inherently able enter macrophages and replicate intracellularly because it interferes with bactericidal effects that follow phagosomal maturation. However, ingestion through FcR-mediated opsonization leads to residence in a mature phagosome where bacterial replication is controlled (reviewed in [18]).

The study of antibody-mediated protection against the so-called intracellular pathogens has revealed new mechanisms of action. For *Listeria monocytogenes* a neutralizing antibody to the toxin listerolysin was shown to neutralize the toxin intracellularly after being taken by infected phagocytic cells and delivered to the bacterial phagosome by intracellular transport [19]. Incubation of *M. tuberculosis* ingested by macrophages with certain antibodies results

in inhibition of intracellular replication [13]. Although the mechanism for this effect is unknown, it is possible that extracellular antibody enters the cell through pinocytosis and then reaches ingested bacteria through intracellular transport to mediate direct antibacterial effects, as was described for intracellular neutralization of listerolysin [10]. Other mechanisms of antibody-mediated immunity described in recent years could be particularly important in controlling intracellular pathogens. Antibody binding to the surface of the facultative intracellular pathogens *C. neoformans* [20] and *M. tuberculosis* [5] trigger transcriptional responses that could interfere with microbial physiology. In addition to these classical and newer mechanisms of antibody action, the presence of specific antibody can contribute to protection by altering the inflammatory response through modifying the release of inflammatory mediators by triggering FcR, activating complement and neutralizing immunomodulatory microbial products.

Next horizons

In a field no longer inhibited by limiting paradigms on the role of humoral immunity it is increasingly clear that it is possible to protect against intracellular pathogens by vaccines that elicit humoral immunity. One major advantage of vaccines that protect by antibody immunity is that in contrast of other assays for immune function, the presence of antibody is easily established and serology has an excellent record of accomplishment as a reliable correlate of immunity against many infectious diseases. In fact, measuring antibody allows both quantitative assays such as determining the amount that is associated with protection and functional assays such as opsonophagocytosis, serum killing and complement activation. Furthermore, such vaccines have the added advantage that IgG transfer through the placenta can protect their infants. Maternal immunization to reduce infant mortality may be particularly effective against intracellular bacterial pathogens that cause high mortality and morbidity in young children, such as *Salmonella typhi*, *Shigella flexneri* and Chlamydia spp.

Gazing at the horizons in this field allows me to discern areas that may be particularly fertile. The experimental demonstration that a bivalent vaccine protected against both anthrax and plague suggests that many such combinations may be possible [21]. Given that polysaccharide-protein, conjugate vaccines include two components it may be feasible to protect against one pathogen by eliciting antibodies through the carbohydrate moiety and against a second pathogen with the protein component. Alternatively, employing both the carbohydrate and protein antigen from one pathogen can elicit both humoral and cellular responses, which can be synergistic and provide stronger protection than that conferred by one arm alone. Enhanced vaccine efficacy can also be achieved by providing more than one antigen that elicits protective antibodies as was evident with observation that strong protection against *Y. pestis* required expression of both the F1 capsular antigen and the LcrV protein in a live attenuated Salmonella vaccine [22]. Another exciting area is the use of defined oligosaccharides in conjugate vaccines to elicit protective antibody responses. In contrast to microbial polysaccharides that are antigenically complex and can elicit both good and bad antibodies, oligosaccharides present a chemically defined epitope that can theoretically elicit only protective antibodies. The recent development of O-linked glycosylation systems to generate conjugate vaccines could provide additional options for

the synthesis of conjugate vaccines for microbial polysaccharides where chemical conjugation is chemically difficult [23].

Apart from the utilitarian aspects involved in harnessing antibody-mediated immunity to generate new vaccines the study of mechanisms by which humoral immunity protects against intracellular pathogens has ushered a renaissance in studies of antibody action. Whereas the discovery of antibody-mediated opsonization, complement activation and toxin neutralization dates to the early days of immunology, the realization that antibodies can have direct antimicrobial activity and can modify the host-cell microbe interaction to affect intracellular events have come from studies with intracellular pathogens (Table II; also reviewed in [16]). Similarly, the presence of specific antibody is known to modulate inflammatory responses for some time but when this is coupled with the discovery of multiple FcRs with activating and inhibitory functions one can envision the possibility that humoral immunity can make a decisive contribution against intracellular pathogens by modulating cellular immunity.

In summary, there is overwhelming evidence from experimental systems (Table 1), from the clinical success of a conjugate vaccine against typhoid [12] and from the varied mechanisms of action by which antibody can protect against intracellular pathogens (Table 2), that humoral immunity can be harnessed by vaccines against this class of pathogenic microbes. As a class, intracellular pathogens present more complex challenges than those microbes for which vaccines can protect through the neutralization of toxins or by simply providing opsonic antibodies because of their capacity to inhabit and replicate in host cells. However, the versatility inherent in antibodies, with their variation in specificity, affinity and isotype combined with consequences of FcR activation provide a bewildering set of possibilities that combine to create a rich arsenal against intracellular pathogens.

Acknowledgments

AC was supported by 5R01HL059842, 5R01AI033774, 5R37AI033142, and 5R01AI052733.

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Highlights

- Antibody can protect against bacterial, fungal and protozoal intracellular pathogens
- Vaccines that protect against bacterial intracellular pathogens are available
- Antibody protection against intracellular pathogens relies on diverse mechanisms

Table 1

Examples of intracellular pathogens from different kingdoms for which vaccines can elicit protective antibody responses (not a complete list).

Kingdom	Organism	Comment	Reference
Bacteria	<i>Burkholderia pseudomallei</i>	Conjugates of type A O-polysaccharide (OPS) and manno-heptose capsular polysaccharide (CPS) antigens with protein were tested protect in murine model of melioidosis	[15]
	<i>Chlamydia</i> spp.	Recombinant chlamydial major out protein elicits neutralizing antibodies against <i>C. pecorum</i> in koalas	[25]
		Multivalent heterologous immuno-repeat 1 (Hirep1) of major outer membrane protein variable domain elicited neutralizing antibodies that protected against experimental infection in mice	[26]
	<i>Francisella tularensis</i>	Intranasal Immunization of mice with bacterial-antibody complexes elicited antibody-mediated protection	[27]
		A conjugate vaccine composes of O-antigen linked to <i>Pseudomonas aeruginosa</i> carrier protein exotoxin A elicits protective antibodies in mice	[28]
	<i>Legionella pneumophila</i>	Mice immunized with B cell antigens had reduced bacterial burden	[29]
	<i>Salmonella</i> spp.	Live oral <i>Salmonella enterica</i> induces opsonic antibodies in humans	[30]
		Conjugate vaccines against <i>Salmonella typhimurium</i> and <i>Salmonella enteritidis</i> elicit bactericidal antibodies against both	[31]
		Vi capsular polysaccharide from <i>Salmonella typhi</i> conjugated to pneumococcal surface protein A (PspA) results in vaccine that protects against both pathogens	[32]
		Capsular polysaccharide of <i>Salmonella typhi</i> conjugated to recombinant exoprotein A of <i>Pseudomonas aeruginosa</i> protein carriers elicits antibody responses that protected children	[33,34]
	<i>Shigella flexneri</i>	O-specific polysaccharide (O-SP) conjugates against <i>Shigella flexneri</i> induces protective antibodies	[13,35]
	<i>Mycobacterium tuberculosis</i>	Conjugation of <i>M. tuberculosis</i> arabinomannan to <i>Bacillus anthracis</i> protective antigen or <i>M. tuberculosis</i> Ag85 elicited protective antibody responses in mice	[5]
	<i>Yersinia</i> spp.	Antibody-mediated protection against F1 antigen of <i>Y. pestis</i> also requires T cells	[36]
		A bivalent chimeric protein rVE elicits protective antibodies against <i>Yersinia enterocolitica</i>	[37]
Fungi	<i>Cryptococcus neoformans</i>	Capsular Polysaccharide-tetanus toxoid conjugate vaccine elicits protective antibody response	[38]
	<i>Candida albicans</i>	Synthetic conjugate vaccine composed of oligosaccharides and peptides found in cell wall induced protective antibody response	[39]
	<i>Histoplasma capsulatum</i>	Monoclonal antibodies to <i>H. capsulatum</i> surface antigens are protective in murine models of infection	[40]
Protista	<i>Plasmodium falciparum</i>	A voluminous body of literature supports that specific antibody can protect against malaria	[41]
	<i>Toxoplasma capsulatum</i>	Passive antibody administration protects guinea pigs against lethal infection	[42]
		Monoclonal antibodies against <i>T. gondii</i> nucleoside triphosphate hydrolase-II reduce parasite replication	[43]

Table 2

Potential mechanisms of antibody-mediated protection against intracellular pathogens

Mechanism	Outcome	Microbe	Reference
Opsonization	Phagocytosis by FcR	<i>M. tuberculosis</i>	[5]
		<i>Coxiella burnetii</i>	[44]
		<i>Yersinia pestis</i>	[45]
		<i>Salmonella typhi</i>	[30]
	Reduced microbial ability to modulate pH	<i>H. capsulatum</i>	[46]
	Increased phagolysosomal fusion	<i>L. pneumophila</i>	[47]
	Enhanced oxidative burst	<i>M. tuberculosis</i>	[48]
	Enhanced oxidative burst	<i>Leishmania major</i>	[49]
	FcR dependent T cell enhancement	<i>F. tularensis</i>	[50]
	Enhanced intracellular killing	<i>M. tuberculosis</i>	[51]
Toxin neutralization	Interference with intracellular replication	<i>L. monocytogenes</i>	[19]
Direct effects	Antibody-binding to microbe alters microbial physiology	<i>C. neoformans</i>	[20]
		<i>M. tuberculosis</i>	[5]
	Interference with yeast budding	<i>C. neoformans</i>	[52]
Complement activation	Serum-mediated killing	<i>S. enterica</i>	[53]
Enhancement of inflammatory response	FcR engagement promotes Th1 response	<i>Chlamydia spp.</i>	[54]
Interference with adhesion function	Antibody binding to invasin protein prevents cell infection	<i>Coxiella burnetii</i>	[55]