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Antibody-based vaccine strategies against intracellular pathogens

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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.

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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•,4,5,6,7•]. 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 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.

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

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 diving 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 regard 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 associate 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.

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 *P. 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