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Antibody response to vaccination as a marker of *in vivo* immune function in psychophysiological research

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Abstract

The hunt for novel tools to investigate empirical questions is ever present in psychophysiological research. Antibody response to vaccination has received increasing attention over recent years as a useful measure of *in vivo* immune function. There is now considerable evidence that the magnitude of the antibody response to vaccination is associated with a wide range of psychosocial factors. Further, there are preliminary indications that manipulating psychosocial variables, using both chronic and acute interventions, can also alter the efficacy of the vaccination. This review will discuss the theoretical and clinical relevance of the vaccine model in this context, and will address key methodological considerations for researchers considering adopting this approach. The review will also address how the strategic use of this model could help researchers further elucidate some of the remaining theoretical issues.

Key words

Antibody; social support; stress; vaccination

Introduction

Infection is one of the leading causes of death in humans (World Health Organisation 2009). Since Jenner and Pasteur first stumbled upon immunisation against smallpox and cholera, vaccines have become a major strategy in combating infectious disease. Vaccination with live attenuated or inactivated antigens manipulates the adaptive immune system into making antibodies that will protect the host against any subsequent attack from the infectious agent. Recent psychophysiological research has examined the extent of this response, as a useful *in vivo* marker of immune function. Psychological stress has been associated with a reduced antibody response to a variety of vaccinations, and in a diverse range of populations, such as young healthy adults, community-dwelling older adults, and caregivers (Burns et al. 2003; Glaser & Kiecolt-Glaser 2005). More recently, poorer antibody responses were found in participants with higher rates of neuroticism (Phillips et al. 2005b) and loneliness (Pressman et al. 2005), whereas social support (Gallagher et al. 2008a; Gallagher et al. 2008b; Phillips et al. 2005a) and positive affect (Marsland et al. 2006) both predict better antibody responses. The exact nature of these relationships seems to vary across different populations. For example, in older adults, marital satisfaction has been associated with antibody response (Phillips et al. 2006), rather than the more general measures of social support which predicted immune function in young people (Gallagher et al 2008a; Gallagher et al 2008b; Phillips et al 2005a). As such, there is considerable scope for using antibody response to vaccination as a technique for exploring the associations between psychosocial factors and immune function across the whole life span. This review will focus on the theoretical and clinical relevance of antibody response to vaccination as a marker of *in vivo* immunity, and will address some key methodological considerations for researchers considering adopting this approach. Throughout, these issues will be illustrated by selected examples of the most recent research in this field; for a more extensive review of the literature, the reader is referred to existing publications (Burns et al 2003; Cohen et al. 2001; Pedersen et al. 2009; Wetherell & Vedhara K. 2007). Further, although the current review focuses on studies conducted in humans, much can be gained from understanding the translation of research findings from the animal literature; this issue has also been reviewed elsewhere (Burns 2004)

Benefits of using antibody response to vaccination as an *in vivo* method of measuring immune function

The immune system comprises a vast array of cells and molecules, which continually intermingle in an elaborate network in order to recognize and eradicate foreign and/or dangerous entities. Further, there is now clear evidence that these immune cells and molecules interact with the structures and products of the neuroendocrine system (Ader et al. 2007), adding a new layer of complexity to an already multifarious system (for an excellent overview of the immune system and psychoneuroimmunology research, see (Segerstrom & Miller 2004) This presents a challenge to any researcher hoping to examine the effects of a particular psychosocial construct on immune function. For example, simple measures of immune cell counts may reflect changes in the dynamics of lymphocyte migration and recirculation, and other factors, such as shifts in plasma volume, rather than absolute changes in total cell numbers (Anane et al. 2009; Campbell et al. 2009). Similarly, *in vitro* immune assays may not necessarily reflect how the cells function *in vivo* (Vedhara et al. 1999). Measurement of antibody response to specific antigen challenge *in vivo* provides a model for studying integrated immune responses.

Antibody status following vaccination is the culmination of a series of interactions of different immune cell types in various immune compartments occurring within a dynamic neuroendocrine milieu (Roitt & Delves 2001). Antigens are recognized, internalized, and then displayed on the cell surface by antigen presenting cells (APCs). Chemokines direct these APCs to the lymphoid tissues, where the antigen is presented to T cells. If a congruent T cell is found, and the necessary co-stimulatory signals are present, it becomes activated and proliferates. Consequently, these T cells are now able to activate a B cell that has recognized, internalized and presented the same antigen. The B cell can then proliferate and differentiate into plasma cells and produce antigen-specific antibodies. These antibodies can be measured in serum, therefore yielding a quantifiable measure of the final product of this cascade of reactions. This provides an accurate reflection of the true functional status of the humoral immune system *in vivo*.

These outcomes have clear clinical implications for both vaccination efficacy and possible susceptibility to infection. Clinical evidence suggests that the greater this antibody response, the better the protection against infection; for example, the level of serum antibody against both influenza and hepatitis B vaccinations has been associated with the extent of resistance to illness (Couch & Kasel 1983; Hadler et al. 1986; Hannoun et al. 2004). Measuring antibody response to vaccination can, therefore, be used as a marker of vaccine efficacy. Studies examining the associations between psychosocial factors and antibody response to vaccination have typically reported small to medium effect sizes; elucidating the extent of the clinical implications of these effect sizes should be a priority for future research.

Antibody response to vaccination can also be used as an indicator of the immune system's general ability to respond to an antigen. Except in certain specialist circumstances, it is not possible to control contact with infectious agents; early psychoneuroimmunological research examining, for example, the effects of stress on incidence of upper respiratory tract infections was often confounded with antigen exposure. Vaccination provides an opportunity to administer a set dose of antigen at a selected time point in a safe manner, and to assess immune status both pre- and post-exposure. This has clear advantages for study design and is a further strength of the vaccine model. One study that fully utilized the advantages of this degree of control over antigen exposure was conducted by Miller and colleagues (Miller et al. 2004). Participants completed daily stress questionnaires for 13 days before, during, and after receipt of the influenza vaccination. This study demonstrated that the strongest associations between daily stress and antibody response occurred eight to 10 days after vaccine administration. This suggested for the first time that there may be a critical period during which stress influences the antibody response.

The degree of control over antigen exposure permitted by vaccination studies also permits the systematic investigation of interventions designed to enhance immune function. For example, recent studies have demonstrated that antibody response to vaccination can be enhanced by mindfulness meditation (Davidson et al. 2003) and cognitive-behavioural stress management (Vedhara et al. 2003). Some interventions, however, have had more mixed results. Although an early study suggested that

emotional disclosure, in which participants write for short periods of time about traumatic experiences, improved the antibody response to hepatitis B vaccination (Petrie et al. 1995), more recent studies have demonstrated that the efficacy of this intervention may be dependent on the cognitive processing achieved by the participants. For example, black participants who wrote about their experiences of racial discrimination actually showed poorer antibody responses to an influenza vaccination than control participants; post hoc analyses suggested that this may be due to attributional ambiguity, in which an individual is unclear about whether or not their negative experience was due to racism, and subsequent detrimental rumination (Stetler et al. 2006). Similarly, another study found benefits only in participants who showed changes in the negative affect content of their scripts and, as such, were considered to have "unburdened" (Strauman et al. 2008).

The controlled nature of vaccination studies has also been utilized to examine the impact of more acute psychological stress on antibody response to concurrent antigen exposure. This research is based on the proposition that, in the short term, immunological changes associated with the fight or flight response may be beneficial, rather than detrimental, for survival (Dhabhar & McEwen 1996; Edwards et al. 2007). For example, participants completing a 40-min socially evaluated mental arithmetic task immediately prior to vaccination showed augmented antibody responses to the influenza (Edwards et al. 2005) and meningitis A+ C (Edwards et al. 2008) vaccines; importantly, these effects were only apparent for sub-groups and strains where the corresponding control group had relatively poor responses. Similar complexities have been demonstrated in a recent report by Brydon and colleagues (Brydon et al. 2009). Acute exposure to a psychological stress task 30 minutes after vaccination was associated with a better antibody response to typhoid vaccination than control participants, but only in those who were high in dispositional optimism. This suggests that personality may influence the relationship between stress and response to vaccination. As well as providing important insights into current opinion on the interplay between psychosocial and immune processes, these examples also illustrate the range of investigations that are possible through the application of the vaccine model.

Methodological considerations in using the vaccination model

The vaccination model is an attractive technique for examining *in vivo* immunity, but there are a number of methodological issues that must be considered. Some of the key considerations will be discussed, along with examples of the types of research questions that could be addressed by exploiting different methodologies.

Choice of vaccination

A multitude of different vaccinations are available for use in psychophysiological research and the choices made by the researcher can have direct implications on both the theoretical and clinical conclusions that can be drawn from a particular study. Firstly, vaccines differ in terms of the nature of antibody response that they elicit, depending on the type of antigen. Protein antigens, which comprise the majority of vaccinations including influenza, induce a thymus-dependent response; this means that T helper lymphocytes are an essential stimulus for successful B lymphocyte proliferation and maturation to antibody-secreting plasma cells. Other vaccines, such as those containing polysaccharide capsules that coats bacteria such as pneumococci and meningococci, are able to evoke antibody responses without T lymphocyte help, known as thymus-independent antibody responses. There is also a third type of vaccination, in which a polysaccharide antigen, which alone would produce a thymus-independent response, is conjugated to a protein molecule in order to invoke a thymus-dependent, and therefore more robust, antibody response. By comparing the relative susceptibility of these different types of vaccination, the investigator can start to elucidate which aspects of the immune system are most influenced by stress in the *in vivo* response to antigen. For example, if the antibody responses to vaccines eliciting thymus-dependent and conjugate, but not thymus-independent, responses are associated with psychosocial processes, then this would imply that the stress-vulnerability may lie with T cells. Alternatively, if all types of vaccination are equally susceptible, then it could be surmised that stress affects more general processes such as antigen presentation or B-lymphocyte clonal expansion and production of immunoglobulins. The first study to compare the relative susceptibility to stress of these different types of vaccines found that the effects of psychosocial factors were limited to antibody response to the influenza vaccine, which

elicits a thymus-dependent response, and was not evident in the response to the thymus-independent meningococcal A vaccine (Phillips et al 2005a). However, there is more recent evidence that the antibody responses to both types of vaccines are associated with psychosocial factors (Gallagher et al 2008a). Moreover, young caregivers of children with developmental disabilities, who show high rates of psychological morbidity, showed a diminished antibody response to both influenza (Gallagher et al. 2009a) and the polysaccharide pneumococcal (Gallagher et al. 2009b) vaccinations, compared to parents of normally developing children. This suggests that not all thymus-independent vaccination responses are insensitive to psychosocial influence. Whether the somewhat less consistent findings in thymus-independent vaccines reflect a relative robustness to psychosocial influence warrants further attention.

A second difference between vaccinations that should be considered is the likelihood of previous vaccination and naturalistic exposure to the antigen. Most people have already been exposed to common viruses and bacteria, such as prevalent strains of influenza and pneumococcus, and, therefore, the vaccines are likely to induce secondary antibody responses. In contrast, less prevalent antigens, such as hepatitis B, are unlikely to have been naturalistically encountered previously; this makes them more likely to effect a primary immune response. Further, as the hepatitis B vaccine comprises a three-dose protocol, it provides an interesting opportunity to examine both primary and secondary immune responses, by measuring the antibody response to each of the three vaccinations. It should be noted, however, that the likelihood of previous exposure will depend not only on the vaccination, but also on the population being investigated. For example, young, healthy adults in the United Kingdom, where hepatitis B vaccination is only distributed to those in high risk professions, are largely seronegative at recruitment. In contrast, more participants will be seropositive at recruitment in populations in which naturalistic exposure to blood-borne antigens is more common, such as intravenous drug users, or in cultures where hepatitis B vaccination is provided during childhood, such as in the United States. Some researchers attempt to circumvent such issues, through the use of a non-clinical antigen, such as keyhole limpet hemocyanin (KLH) (Smith et al. 2004a; Smith et al. 2002; Smith et al. 2004b). KLH is a novel copper-containing protein, derived from the giant keyhole limpet mollusc. It is non-pathogenic and is not

encountered naturally, but when given as a vaccine it induces a pronounced primary immune response in humans. The lack of naturalistic exposure has clear benefits for study design and the avoidance of possible confounding factors; however, there are also questions over the clinical relevance of measuring the antibody response to a non-pathogenic protein. Overall, by selecting vaccinations according to whether they induce a primary or secondary response, it is possible to investigate which aspects of the immune response are most susceptible to psychophysiological processes.

A further consideration should be the relative immunogenicity of the vaccination. Vaccines vary in their efficacy, and there is preliminary evidence that this may impact upon their susceptibility to psychosocial factors such as stress. Studies using the trivalent influenza vaccine often find that only one strain appears to be affected by stress (Miller et al 2004; Phillips et al 2005a). Moreover, it has also been argued that strains which evoke robust antibody responses, i.e. are more immunogenic, are less susceptible to psychosocial influence whereas less antigenic strains are more vulnerable to such effects (Cohen et al 2001). The use of different vaccine doses would enable a more systematic examination of this proposition.

The clinical relevance of the vaccination for the prospective study population should also be a consideration. While any vaccination will act as a theoretical model of *in vivo* immunity, the clinical implications are enhanced where the vaccine is carefully chosen in order to reflect pressing concerns in the selected group of participants. For example, the influenza virus causes high levels of morbidity and mortality in older adults and, therefore, studies finding an influence of psychosocial stress in this context have a particular resonance. Similarly, examples of stress-induced decrements in hepatitis B vaccine efficacy in medical students may have ramifications for clinical practice.

Finally, the use of the trivalent influenza vaccine warrants a special note. This is the most common vaccine used in this literature, due to its clinical relevance, wide availability, and often relatively low immunogenicity. However, its use is complicated by the fact that at least some of the three strains contained in the vaccine change each year to reflect their current naturalistic prevalence. This makes it difficult to conduct a study over more than one season and combine the data, particularly if the analysis uses the absolute antibody levels, the magnitude of which will vary dependent on strain. If it

is necessary to test over longer periods, researchers could consider using a more categorical outcome measure; for example, it is possible to assess the number of strains to which the participant achieved an accepted clinical cut off level, such as a four-fold increase from baseline or a hemagglutination inhibition assay titre of 40. In sum, these various theoretical and clinical implications should be considered when the choice of vaccination is made, in order to maximize the eventual impact of the research findings.

Timing of assessment and antibody subclass

Vaccination studies are typically designed to measure the antibody levels against the specific antigen contained within the vaccine at baseline, and then at a specified time post-vaccination, in order to assess the magnitude of the response. The baseline measurement is a crucial element in all situations where previous exposure to the antigen is likely, as pre-vaccination antibody levels are a strong predictor of the subsequent response. The timing of the post-vaccine sample or samples can vary according to the interests of the researcher, and again these choices will dictate the conclusions that can be drawn from the data. The most common time point chosen for follow-up antibody results is four to six weeks post-vaccination. This coincides with the peak antibody response and is, therefore, a useful outcome measure for the majority of vaccination studies.

Another interesting time point, that has received considerably less attention to date, is approximately one week post-vaccination, which coincides with the peak immunoglobulin (Ig) M response. The humoral response to most pathogenic challenges is characterised by an early rise in IgM, followed by affinity maturation, isotype switching and the rising elicitation of IgG, IgA, and IgE antibodies. Clinically, IgM plays a key role in clearance of infection by enhancing IgG production and promoting an efficient neutralizing IgG response (Baumgarth et al. 2000). Recent research has demonstrated that social support is positively associated with the IgM (Gallagher et al 2008b), as well as the IgG (Gallagher et al 2008a), response to the polyvalent pneumococcal polysaccharide vaccine. This may be clinically relevant, as IgM activates part of the innate immune system called the complement cascade, which is an important early defense against bacterial infection. In addition, chronic self-regulatory failure, in which individuals see themselves as failing to make progress towards their goals, has

been shown to be associated with the IgM, but not the IgG, response to influenza vaccination (Strauman et al 2008); the clinical implications of such a finding for this viral antigen is less clear.

As well as early and peak responses, it is important to assess the maintenance of antibody levels over time. When vaccinating patients, an assumption is made that the vaccine will provide long lasting protection against the relevant disease. In fact, there is evidence that there is considerable individual variation in the degree to which antibody levels are sustained, and that psychosocial factors are associated with this maintenance. For example, a study of two cohorts of students vaccinated against hepatitis B, either within the last year or earlier than that, only found associations between antibody levels and psychological stress in those participants vaccinated more than a year ago (Burns et al. 2002a). Similarly, a study comparing pneumococcal vaccine efficacy between caregivers and controls found group differences at three and six months post-vaccination, but not at two weeks or one month (Glaser et al. 2000). By assessing early, peak, and long term antibody levels, the impact of psychosocial processes on different aspects of immune protection can be investigated.

Assay type

A final practical consideration for researchers considering using the vaccination model is the type of assay used to assess antibody levels. A common method for assessing the antibody response to some viral vaccines, notably influenza, is the hemagluttination inhibition assay (World Health Organisation 2002). Influenza virus particles are able to bind erythrocytes together into a lattice-like structure, via a surface protein known as hemagglutinin. Specific antibodies in serum can prevent this process occurring. The HAI assay compares the ability of a range of serum dilutions to inhibit the hemagglutination; the highest dilution of serum that prevents hemagglutination is the antibody titer. For example, a serum antibody titer of 40 means that hemagglutination was blocked at a dilution of 1:40, but not at further dilutions. The HAI assay is a clinically relevant, widely accepted tool for assessing antibody levels to influenza vaccination. One shortcoming, however, is that this assay can only assess total antibody, rather than distinguishing between immunoglobulin subclasses.

An enzyme-linked immunosorbent assay (ELISA) is perhaps the most common method used to assess antibody levels against a specific antigen (Janeway et al. 2005). This assay is simple and easy to perform, and can be adapted depending on the priorities of a particular study. For example, by altering the secondary antibody used in the assay, the ELISA can be used to distinguish between IgG and IgM antibodies. However, as with the hemagluttination inhibition assay, antibodies against only one antigen at a time can be assessed using an ELISA, making assessment of the response to polyvalent vaccines relatively time consuming.

An important advance has been the development of multiplex systems, such as the Luminex Platform. This technology also allows the simultaneous assessment of the antibody response to multiple antigens. Instead of adhering the antigen to the base of a microtiter plate, as in an ELISA, this system uses up to 100 color-coded bead sets, each of which can be conjugated with a different specific antigen. A "cocktail" of relevant beads can then be mixed with the serum, allowing an interaction between the bead-bound antigen and the antibodies in the sample. Captured antibodies are detected using a biotinylated detection antibody and streptavidin-phycoerythrin (S-PE); again, this can be modified to assess either IgG or IgM levels. The sample is passed through the dual laser analyzer; one laser identifies the bead, and therefore the antigen, and the other determines the magnitude of the PE-derived signal which is in direct proportion to the amount of analyte bound. The benefits of this type of assay are clearly demonstrated by the work of Gallagher et al (Gallagher et al 2008a; Gallagher et al 2008b; Gallagher et al 2009b) who assessed antibody responses against multiple pneumococcal serotypes in a single assay. As psychosocial effects have largely been found with polyvalent vaccinations where a number of similar acting antigenic strains are administered in one vaccine, this type of technology is likely to be crucial in comparing the relative susceptibility of different strains.

Finally, it is also possible to take a more functional approach to vaccine response assessment. For example, with vaccines against bacterial antigens, a serum bactericidal antibody (SBA) assay can be performed. In this assay, serial dilutions of human sera are incubated with appropriate bacterial target cells and complement. Activation of the antibody-dependent classical complement pathway ultimately results in lysis of the target

cell. The SBA titre for each serum is expressed as the reciprocal serum dilution yielding ≥50% killing as compared to the number of target cells present before incubation with serum and complement (Maslanka et al. 1997). SBA activity has been shown to highly correlate with immunity to meningococcal disease (Goldschneider et al. 1969). Although the only study to use this assay in this context found no associations with psychosocial factors (Burns et al. 2002b), the clinical relevance of the measure would argue that future studies should consider incorporating this sort of approach.

Conclusion

The assessment of the antibody response to vaccination provides a useful measure of *in vivo* immune function for psychophysiological research. There is now an abundance of literature demonstrating that these markers are associated with a wide range of psychosocial factors. The vaccine model confers a multitude of options for the researcher. Strategic methodological choices will enable further investigation of underlying mechanisms of these relationships, and exploration of the role of psychosocial interventions in augmenting antibody response to vaccination, both in terms of improving vaccine efficacy and as a marker of a more generalized improvement in immune system function.

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