

Antibody response to vaccination and psychosocial stress in humans: Relationships and mechanisms

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Abstract

The purpose of this review is to determine the effects of psychosocial stress on antibody response to vaccination in humans, consider possible mechanisms, and identify agenda for future research. Studies of the association between stress and vaccination response in humans were reviewed. There is evidence of a negative association between stress and antibody response to vaccination, which is most apparent with thymus-dependent vaccines and when measured at extended times after vaccination. Preliminary findings implicate the hypothalamic-pituitary-adrenal axis and sympathetic nervous system as potential mechanisms, although a role for unhealthy behaviours cannot be discounted at this stage. Results to date are sufficiently indicative to direct future research to untangling their theoretical ramifications, as well as realising their clinical implications.

Keywords: Antibody response to immunisation; Psychosocial stress; Psychoneuroimmunology.

Running head: Antibody response and stress

1. Introduction

A link between psychological stress and increased susceptibility to infectious disease has been reported anecdotally for many years. In 1884, the editor of the British Medical Journal noted that at funerals “the depression of spirits under which the chief mourners labour at these melancholy occasions peculiarly predisposes them to some of the worst effects of the chill” [1]. Since then, morbidity has been shown to be significantly increased during chronic stress in both observational [2-5] and live virus challenge [6-9] studies. These provide compelling evidence that psychological stress is associated with an impairment of the immune system’s ability to respond to infection.

This interaction between psychological stress and immunity has been a prolific area of research over the past two decades (for comprehensive review of this literature, see [10]). People experiencing periods of chronic stress have been shown reductions in the number of helper T-lymphocytes [11-13], B-lymphocytes [14], and salivary concentrations of secretory immunoglobulin A (sIgA) [15-18], compared to controls. The clinical implications of reductions in these cell counts in healthy people, and within normal ranges, are unclear [19]. Changes in cell number may just 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. In addition, absolute changes in cell number will not necessarily result in a significant change in the capacity of the lymphoid system to make an effective response to antigenic challenge [19]. It is difficult, therefore, to account for stress-related increases in disease susceptibility in terms of changes in enumerative immune measures.

In vitro measures of immune function, such as lymphocyte proliferation to mitogen, have also been shown to be susceptible to stress-induced alterations [12, 20-26]. These functional assays give a better indication of immune status than the enumerative methods, as proliferation in response to antigen is a key component of the immune response. However, studies of polyclonal stimulations do not allow conclusions to be drawn regarding the relative susceptibility to stress of particular lymphocyte subsets. It is also

difficult to generalise these *in vitro* findings to *in vivo* processes [19]. The isolated testing of any particular aspect of the network of immune cells provides scant information about the status of the highly integrated, complex immune system and, as such, has limited application to overall understanding of the relationship between stress and susceptibility to disease.

Measurement of antibody response to specific antigen challenge *in vivo* provides a model for studying integrated immune responses. Stress may alter both quantity and quality of antigen-specific antibody present at different times after immunisation by modulating a variety of processes within the immune response. Stress might affect the primary immune response in a number of broad areas, including 1) T-lymphocyte clonal expansion and maturation to T-helper-2 (Th2) primed effector lymphocytes and memory lymphocytes, 2) initial B-lymphocyte clonal expansion and production of IgM from short lived plasma cells in the secondary lymphoid tissues, and 3) germinal centre production of memory lymphocytes and plasma cells secreting high affinity IgG and IgA antibody. Stress may also impact on the long term maintenance of serum IgG levels against the priming antigen; this relies on the maintenance of a memory B-lymphocyte pool and further production of antigen specific plasma cells from germinal centre follicles where there is a long term deposit of antigen complexed with antibody bound to the surface of follicular dendritic cells. Finally, stress may affect the antibody response to a second encounter with antigen, not only by affecting B-lymphocytes, but also via alterations in the size of the antigen specific Th2 lymphocyte pool. The size and effectiveness of this pool is of particular importance to the speed and size of secondary antibody responses. As well as providing a model of the *in vivo* immune response, the existence of different types of vaccine (i.e. thymus-dependent, thymus-independent, and conjugate) allows examination of which aspects of the immune system may be influenced by stress. If, for example, the effects of stress are restricted to antibody responses to thymus-dependent and conjugate vaccines, this could suggest that T-lymphocytes are more susceptible than B-lymphocytes. Response to vaccination, therefore, provides an ideal way to assess the integrated immune response to antigen with clinically relevant outcome measures.

2. Psychological stress: concepts and measurement

Psychological stress can be conceptualised as the extent of exposure to presumed stressful environmental and social life events or as the degree to which an individual perceives such events as stressful. Most contemporary definitions of psychological stress emphasise the importance of individual perception and appraisal in determining impact. For example, Lazarus and Folkman [27] defined stress as “a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being”. The duration of the stress experience is also important. Broadly speaking, stressors can be characterised as chronic or acute. Chronic stressors are enduring, major life stressors such as bereavement or divorce. In contrast, acute stressors are the daily hassles that are experienced by everyone, ranging in severity from finding a parking space to arguments with partner. Other psychological concepts commonly reported in this literature include well-being, negative affect (the experience of aversive mood states such as anger and guilt), and anxiety. These can be categorised as state or trait; state conditions are transient, existing at a given moment in time or in response to a particular situation, whereas trait conditions are relatively stable individual characteristics [28].

Psychological stress levels are commonly measured using standard, validated questionnaires. For example, life event exposure can be assessed by asking participants to indicate which of a list of stressful life events they have experienced during a given period. The more subjective concepts, such as perceived stress and anxiety, questionnaires include items such as “how often have you felt nervous and stressed?” and provide a range of answer options (“never” to “always”). Alternatively, a common model in psychoneuroimmunological research is to compare people experiencing a chronic stress, such as spousal caregiving, with matched controls who are not experiencing that particular stressor. Although such a methodology has the disadvantage of between-subject variability, it allows the effect on immunity of a specific severe stressor to be assessed.

3. Psychological stress and antibody response to vaccination

The studies included in this review are summarised in Table 1. A review of the literature was performed by interrogating PubMed (National Library of Medicine) and Web of Science (Institute for Scientific Information) databases, using the search terms “vaccin*”, “immuni*” and “inoculat*”. These search terms were combined with the terms “stress”, “mood”, “psycho*”, “anxi*”, and “depress*”. Only papers with antibody titre as an outcome measure were included. In addition, the references in the articles that were generated by this search were checked.

[Insert Table 1 about here]

3.1 *Secretory IgA responses*

In early studies, participants were immunised with non-pathogenic proteins to examine their antigen-specific antibody response. Stone and colleagues [29, 30] required participants to swallow a capsule containing rabbit albumin each morning for 8-12 weeks, and then measured the daily specific sIgA response in saliva, as well as daily mood [29] and events [30]. This methodology allowed within-subject comparisons between antibody levels on high-stress and low-stress days. Antigen-specific sIgA antibody levels were lower on days with relatively high-negative mood and were elevated on days with relatively high-positive mood [29]. Subsequent research demonstrated a positive association between daily positive events and levels of antigen-specific sIgA and, conversely, a negative association between negative events and sIgA [30]. While variations in the salivary antibody levels to this antigen could be indicative of a general reduction in the ability of the immune system to respond [31], the clinical interpretation of changes in immune response to the oral ingestion of a non-pathogenic challenge remains unclear.

3.2 *Thymus-dependent vaccinations*

Primary immune response has also been assessed using keyhole limpet hemocyanin (KLH), a protein antigen that is unlikely to have been encountered previously and which elicits a thymus-dependent antibody response [32]. At eight weeks post-vaccination, but not three weeks, the KLH-specific IgG response was significantly lower in those reporting fewer positive events prior to vaccination; an increase in the number of negative events also tended to be associated with impaired antibody response. Antibody status was not, however, related to the amount of stress experienced between vaccination and follow-up. These data provide limited evidence that the antibody response to a single antigenic exposure may be susceptible to psychological influences. More recently, a small study investigating the effect of distress on antibody response to KLH failed to find any association between mood state and antibody status at three weeks [33]. However, this study did not include any of the typical measures of psychological stress, such as life event exposure and perceived stress levels.

Response to medical vaccination provides a clinically relevant model of antigenic exposure. The most commonly investigated vaccination is hepatitis B, probably due to the availability of large numbers of medical school students routinely being immunised. The complexity of the standard hepatitis B vaccination programme, consisting of three separate injections, administered at zero, one, and six months, and the low probability of prior naturalistic exposure, mean that both primary and secondary antibody responses can be assessed. Two studies have investigated the relationship between stress and primary antibody response, measured one month after the initial vaccination. In the first, those who seroconverted, defined as the presence of measurable antibody after the first vaccination, reported less mean perceived stress and anxiety over the entire course of the vaccination than those who seroconverted later [34]. While it is initially unclear why stress and anxiety levels assessed some months later may be related to seroconversion, it is likely that these questionnaires measure, to some extent, trait or dispositional tendencies to report high stress and anxiety. It seems feasible, therefore, that people who generally tend to perceive their lives as stressful would have higher mean stress scores and it is these individuals who showed lower antibody levels. In an emotional expression intervention study, there was no difference between the antibody levels of the treatment

group and control group one month after the initial vaccination [35]. However, since no measures were taken to assess the ongoing psychological impact of the intervention, it is difficult to interpret these findings. Accordingly, the evidence that stress affects the primary response to hepatitis B vaccination is limited, a conclusion also reached in another recent review [36].

Six correlational studies have investigated the relationship between stress and secondary response to hepatitis B vaccination. Again, the results are mixed. The largest study examined the relationship between self-reported life events and final antibody titre in two cohorts of students [37]. In the recently vaccinated cohort, there was no significant association between any stress measure and antibody titre. However, in the cohort vaccinated earlier, those participants reporting high levels of life events stress over the past year were two and a half times more likely to have an inadequate antibody level than those with low levels of stress. This association withstood adjustment for variations in unhealthy behaviours (smoking, alcohol consumption, exercise, sleep) and coping style. The results suggest that the immunogenicity of the hepatitis B vaccination may initially override any influence of psychological factors. Further, the larger number of individuals with inadequate antibody titres in the early vaccination cohort provides more power to detect effects. Nevertheless, this study implies that psychological stress may have its principal effects on the rate of deterioration of protection.

Jabaaij and colleagues [38] found a poorer antibody response to a low dose hepatitis B vaccination in those with a higher Stress Index at two months after vaccination. There was also a tendency for antibody titre to be related to the Stress Index at 6 months. As antibody response was not assessed at other time points, it is not possible to ascertain whether the effect of stress was predominantly on initial seroconversion or maintenance of antibody levels. It is also difficult to attribute the relationship between the stress score and antibody response to any specific aspect of stress as the Stress Index was a composite measure calculated from a life events checklist and a questionnaire assessing psychological symptoms. A similar protocol carried out by these researchers using a standard vaccine dosage yielded no significant associations

between antibody status [39]. It is worth pointing out, however, that in this latter study there was no two month assessment of stress, which had predicted antibody response previously, and the six month measure of stress used was not associated with antibody status in their earlier study either. The inconsistencies in methodology make it difficult to attribute the failure to find stress effects in the later study to the immunogenicity of the full-dose hepatitis B vaccination programme.

In the Glaser study discussed earlier [34], no relationship was found between the secondary antibody response and either stress or anxiety. Similarly, Marsland and colleagues [40] found no associations between life event stress or perceived stress and antibody status five months after initial vaccination. However, those with low antibody responses did report higher levels of trait negative affect. Finally, one study has reported a positive relationship between stress and hepatitis B antibody status [41]. Final antibody status was positively related to perceived life event stress, depression, and anxiety during the first six months of the protocol. The use of a life events scale in which students were able to rate experiences as positive or negative is a strong methodology, allowing for individual differences in the interpretation of an event. In addition, the relatively large number of participants and the consistency of the associations across different psychosocial measures make it difficult to dismiss this anomalous result. The authors suggested that their unexpected finding may be a function of the relatively low levels of stress of participants. This implies a curvilinear relationship between stress and antibody status, such that moderate levels of life-change stress are associated with higher levels of antibody response to vaccination, whereas low or high levels may be detrimental.

The only study examining stress and hepatitis B antibody status that adopted an experimental, rather than correlational, design is the intervention study described earlier [35]. Participants in the emotional expression group exhibited higher antibody levels at both four and six month follow-up periods, compared to controls. This contrasts with the primary antibody response results. However, since the efficacy of the intervention was not assessed, it is difficult to definitively attribute the enhanced antibody levels in the experimental group to reduced psychological stress.

Studies of the influenza vaccination hold particular clinical relevance considering the poor efficacy of the vaccine, particularly among the elderly [42]. An early study found no relationship between life change stress or mood state and antibody response to influenza vaccination [43]. More recently, a study by Kiecolt-Glaser and colleagues [44] used the caregiver-control model in which spousal caregivers were compared to age-matched controls. Caring for an elderly spouse is acknowledged as being arduous and prolonged [45] and, therefore, it is possible to use this model to assess the effects of a severe, chronic stressor on response to influenza vaccination. Spousal care-givers and controls were administered the influenza vaccine, and their antibody responses assessed. Following vaccination, fewer care-givers achieved the four-fold increase in antibody level that can be used as a marker of vaccination success [46]. These findings were independent of variations in the measured health behaviours and medical conditions. Another study has also found that spousal care-givers displayed poorer antibody responses to influenza vaccination compared with controls [47]. However, caregivers are likely to differ from controls in a number of respects other than psychological stress, such as experiencing more physical strain. Importantly, it has been shown that former spousal care-givers display the same impaired antibody response to influenza vaccination as current care-givers [48]. While much of the physical demand of care-giving would terminate with bereavement, it is reasonable to presume that psychological stress would continue. This argues that the poorer responses to vaccination observed for the caregivers is a result of psychological stress rather than physical strain. Finally, a recent study has investigated the association between perceived stress and response to influenza vaccine in a healthy, elderly population [49]. Higher levels of perceived stress were associated with lower anti-influenza IgG titre, supporting the previous research in elderly people. However, the lack of a pre-immunisation antibody titre is a limitation of this study .

The relationship between stress and antibody response to influenza vaccination has also been assessed in young adults [50-53]. In an early study, students reporting more distress, as measured by the Profile of Mood States questionnaire, had significantly

smaller antibody responses to the vaccine three weeks later [50]. Differences between those who did and did not achieve a four-fold increase in antibody were not reported. In three recent studies [51-53], little evidence of suppression of antibody response at one month was found; indeed, two studies reported an unexpected positive association between stress and antibody response to at least one viral strain [51, 53]. However, in both studies with longer-term follow-ups, those who failed to maintain an antibody level four-times above baseline at this later time-point had significantly higher levels of psychological stress than those who maintained protective levels. Thus, there is some evidence that psychological stress exerts a deleterious influence on antibody response to influenza vaccination in younger, healthy samples, although, in the main, this does not appear until some time after vaccination.

As yet, only one study has investigated the effects of psychological factors on response to live-attenuated rubella vaccination. Among the girls who were seronegative for antibodies against rubella virus, and, therefore, for whom vaccination elicited a primary antibody response, those high in internalising (characterised by withdrawal and anxiety), high in neuroticism (emotional instability), and low in self-esteem had lower antibody titres. In contrast, in the girls who exhibited antibodies against rubella prior to vaccination, for whom this was a secondary response, no relationships between psychological status and immunity were found [54].

3.3 Thymus-independent vaccinations

The only thymus-independent vaccination to receive attention so far is that against pneumococcus, and two very different populations have been studied. The earlier study was carried out in five-year-old children, who were administered the pneumococcal vaccination one week before the start of kindergarten [55]. Ratings of problem behaviour were not associated with antibody response to the vaccine. The other study to have investigated stress and pneumococcal vaccination compared spousal care-givers, former care-givers, and age-matched controls [56]. Although there were no group differences either two weeks or one month after the vaccination, current care-givers had poorer

specific antibody titres three and six months after receiving the pneumococcal vaccination compared to the other two groups. This suggests that care-giving impacts on the rate of deterioration of protection, rather than on the initial response to vaccination.

3.4 Conjugate vaccinations

A third type of vaccination is the conjugate vaccine. A successful strategy in vaccination programmes against thymus-independent, polysaccharide antigens, has been to conjugate the polysaccharide to a protein molecule in order to invoke a thymus-dependent antibody response. The mechanisms of antibody production are, therefore, thymus-dependent, yet the antibody that is produced is against a polysaccharide thymus independent type 2-antigen. The role of psychosocial factors in the antibody response to a conjugate vaccine has only been assessed in one study [57]. Participants with high levels of perceived stress and psychological distress were more likely to have low antibody titres. Life events exposure was not predictive of antibody titre, and none of the psychological variables were significantly associated with serum bactericidal assay titre, which measures the ability of antibodies to kill meningitis bacteria. These findings suggest that the feeling that one's life is stressful and the experience of high levels of distress were more detrimental than actual exposure to stressful life events. Importantly, there was also an interaction between perceived stress and life event exposure. Those people who reported being high in perceived stress yet experiencing relatively few stressful life events were significantly more likely to have low antibody titres and serum bactericidal assay titres than any other group, including those high in both perceived stress and life event exposure. The negative influence of these context-inappropriate perceptions of stress suggest that personality factors may also be implicated in determining the adequacy of the antibody response. This resonates with the results reported for thymus-dependent vaccinations, in which higher negative affect [40], neuroticism [54], and psychological symptoms [38] are associated with poorer antibody titres, and suggests that conjugate vaccines may be similarly susceptible to psychological influence.

4. Potential mechanisms of interaction between stress and immunity

4.1 Indirect mechanisms

An association between stress and antibody response to vaccination could arise from direct and/or indirect mechanisms. The most likely indirect mechanisms are changes in health behaviours, often associated with periods of stress, and their subsequent physiological impact on antibody formation. High levels of stress have commonly been associated with increases in unhealthy behaviours such as smoking, excessive alcohol consumption, a poorer diet, and decreased time spent exercising [58-60]. In addition, high levels of alcohol consumption have been linked to reduced vaccine efficacy [61] and alcoholics have been shown to have poorer responses to some serotypes of the pneumococcal vaccine than non-alcoholic controls [62]. Similarly, cigarette smoking has been linked to reduced response to influenza [63, 64], and hepatitis B vaccinations [65-67]. It has also been suggested that nutritional status may influence vaccine efficacy; supplementation with zinc, selenium [68], and vitamin E [69] improved the response to influenza vaccination in institutionalised elderly participants. In addition, in malnourished patients, appropriate supplementation can also improve response to the influenza vaccination [70]. In spite of these findings, some studies have failed to adequately control for health behaviours, leaving open the possibility that at least some of the observed association between stress and antibody status may be attributable to variations in unhealthy behaviours.

4.2 Direct mechanisms

More direct mechanisms of interaction between stress and immune function have also been postulated [10]. Stress is associated with alterations in sympathetic nervous system and hypothalamic-pituitary-adrenal axis activation. Functional relationships between these neuroendocrine pathways and the immune system have been acknowledged for some time (for a review see [10]). Therefore, it is feasible that changes in basal activity in these systems, or their repeated activation in response to

stress, could impact upon antibody development and maintenance. Finally, individual differences in the extent to which these systems respond to standardised stressors have been shown to be predictive of reactivity to real-life stressors [71]; those with high physiological reactivity to psychological stress may, through repeated, large magnitude responses to stressful situations, be most at risk of stress-related reductions in immune function.

4.2.1 Hypothalamic-pituitary-adrenal axis

Spousal caregivers, shown to have lower antibody responses to influenza vaccine, also had higher mean salivary cortisol values, than controls. There was also a negative correlation between mean resting cortisol concentrations and antibody response to one viral strain [47]. In contrast, basal salivary cortisol and antibody response to hepatitis B vaccination have been found to be correlated positively [72] and a null result has recently been observed for influenza vaccination [53].

The evidence regarding cortisol reactions to stress is more consistent. Cortisol reactivity is calculated as the post-stress cortisol level minus baseline. Children showing the greatest cortisol reaction to the stress of starting kindergarten had the poorest response to pneumococcal vaccination [55]. Similarly, relative to individuals with high hepatitis B antibody titres, those with low titres were characterised by more positive cortisol reactions to an acute laboratory time-pressured, socially evaluated mental arithmetic task [72]. In addition, students with the most marked cortisol reaction to the naturalistic stress of blood sampling and vaccination showed a poorer antibody response at five months to one strain of the influenza vaccine [53].

4.2.2 Sympathetic nervous system activation

The sympathetic nervous system provides another possible link between stress and immune function, either in the form of catecholamine secretion or via the direct innervation of the lymphoid organs [73]. The study discussed above [72] also addressed

the association between cardiovascular reactivity to a mental stress task, as a marker of sympathetic nervous system (SNS) reactivity, and antibody response to hepatitis B vaccination. Compared to those with high antibody titres, participants with low antibody titres had heightened SNS reactions to stress. This suggests that reactivity may be detrimental to either initial antibody response or continued antibody protection. In addition, one small study reported that individuals with relatively large sympathetically-mediated cardiovascular reactions to a standard laboratory stress task showed a more rapid decline over time in the T-lymphocyte response to influenza vaccination [53].

Marsland [40] provides further evidence of an association between physiological reactivity and antibody response to vaccination. Participants were asked to perform an evaluated speech task, during which cardiovascular and immune measures were assessed. It was found that those who exhibited greater deteriorations in lymphocyte proliferation to mitogen following an acute stressor, compared to baseline, had also responded less well to a hepatitis B vaccination programme completed two months earlier. There were significant associations between decreases in proliferative response to mitogen and stress-induced heart rate acceleration, an indirect, albeit weak, measure of sympathetic activation. It is possible, therefore, that variations in SNS activation could account for the differences in vaccine-specific antibody levels, via a decrease in the functional capacity of the lymphocytes [40].

5. Strategies/areas for future research

Overall, there is now sufficient indicative evidence to justify further, more carefully designed, studies. In designing such studies, investigators should consider whether their primary concerns lie with clinical or theoretical issues; while the vaccination model has important implications for both, future studies would almost certainly benefit from clarity of purpose.

5.1 *Clinical studies*

If stress is associated with response to vaccination, a key question for clinicians is how can this result be exploited by health care professionals for the benefit of patients? Clinical research should first focus on populations that are likely to be most susceptible to the influence of stress. Two-thirds of the studies reviewed here involved young, healthy students, who are unlikely to have poorly functioning immune systems or to be experiencing high levels of chronic stress. It is a tribute to the magnitude of stress effects on antibody response that, on the whole, effects were still found in this relatively robust population, but it would now seem sensible to turn attention to more vulnerable populations.

Future studies of stress and response to vaccination should, where possible, include assessment of vaccine efficacy in terms of disease incidence and severity. As there is evidence that antibody responses to influenza vaccination in elderly populations are frequently insufficient to provide protection against disease [74, 75], further deterioration in protection due to chronic stress may be particularly detrimental to the health of the elderly. However, in the absence of direct measures of infection, it is difficult to determine the true impact of stress on the health of susceptible populations. If clinical outcomes, as well as a simple antibody count, are associated with stress exposure, more routine antibody testing of elderly recipients of the influenza vaccine is indicated, particularly in those with other risk factors, such as respiratory conditions.

Clinical research should also focus on what might be done to counteract the potentially deleterious impact of stress on antibody response. Only one intervention study has been published so far [35]. While the finding that people undergoing a trauma disclosure procedure showed better responses to hepatitis B vaccination than control participants is encouraging, it is necessary to test the efficacy of more mainstream stress management strategies, as well as assessing formally whether such strategies have ameliorated perceptions of stress. It is also worth exploring manipulations of the timing of vaccination. Randomised controlled trials of a variety of populations with predictable

periods of stress, such as students, teachers, medical, or military personnel, should be undertaken, with participants being vaccinated during either high or low stress periods. Such a simple intervention could prove informative for clinical practice.

5.2 Theoretical studies

A more theoretical goal should be to establish which aspects of the antibody response are affected by psychological stress. By differentiating between vaccines inducing thymus-dependent and thymus-independent antibody responses as outlined in this review, the relative susceptibility of different types of antibody response may be ascertained. In addition, enumerative and *in vitro* functional immune measures could be used in conjunction with the antibody response model in order to further elucidate exactly which immune parameters or reactions are sensitive to stress.

The stage at which the antibody response to vaccination is most affected by stress exposure is another important theoretical consideration. There is increasing evidence now that the detrimental effects of stress may not be apparent until several months after vaccination [37, 52, 53, 56], suggesting that the maintenance of antibody production might be more vulnerable to stress than initial antibody production. This may also explain some of the null findings [33, 39] in which only short follow-ups have taken place. Indeed, there is growing evidence that acute stress may potentiate the initial antibody response [41, 51, 53, 76]. This hypothesis is also supported by recent stress and vaccination research in animal models [77]. Future studies should involve serial blood testing during both antibody formation and long-term follow-ups in order to investigate these issues further.

Current research suggests that secondary responses may be more susceptible to stress than primary responses [36]. However, given the limited evidence concerning primary responses, this conclusion should be considered tentative. Immunisation with a novel antigen, such as KLH, avoids experimental contamination by naturalistic exposures and would allow a primary antibody response to be investigated. Booster immunisation

would then allow investigation of the impact of stress during both the primary response and the secondary response on the secondary antibody response itself. This methodology could be combined with the serial blood testing suggested above, in order to assess the dynamics of the antibody response with respect to immunoglobulin class (primarily IgM versus IgG and IgA) and affinity for antigen. This would provide further important insights into which aspects of the immune process are modulated by stress.

Studies might also pay attention to the methods used to assess stress exposure. If this is to be achieved by questionnaire, separate and validated measures of life events exposure, perceived stress, and psychological distress, are to be preferred to the composite measures of stress that have sometimes been used previously [38]. This approach would allow a better understanding of the extent to which immune function is affected by exposure to stressful life events, perceptions of stress, or an interaction between the two. In addition, it would be sensible for future studies to measure positive, as well as negative, life events; a stronger relationship with antibody response to KLH appeared for the absence of positive events than for the occurrence of negative events [32]. Finally, it is also important to differentiate more clearly between state and trait measures of psychological status. Several studies have indicated that trait-like measures of stress and anxiety may be better predictors of antibody status than more transient, state-like measures [34, 54].

Some studies have adopted a between-group comparison of high and low stress participants [44, 47, 56], which has the advantage of examining more chronic, enduring stressors than those commonly assessed in questionnaire-based studies. However, as with all between-group assessments, it is critical to control for as many extraneous variables as possible. When carefully designed though, this quasi-experimental, between-group model provides an important opportunity to study the effects of severe, chronic stress on antibody response to vaccination. Future research could exploit this method more widely, by examining a greater range of stress exposures in a wider variety of populations. However, it is also important to retain a subjective component to the

stress assessment; individual differences in the perception of the psychological demands placed on an individual by a particular situation could be key.

Finally, we would also argue for more focus on potential mechanisms. Many studies did not control for health behaviours [41, 47, 50, 54, 76]; the vast majority of the studies that do [34, 37-39, 44, 53, 56, 57] use less-than-optimal brief retrospective questionnaires. In order to fully address the potential role of health behaviours, more investigators could include detailed day-by-day assessments as were performed in the exemplary study of Pressman and colleagues [52] or objective measures of health behaviours, such as cotinine to assess cigarette smoking. Without such steps, the role of health behaviour as a mediator between stress and health may continue to be underestimated. In terms of more direct mechanisms, larger studies of basal cortisol, with more detailed diurnal profiles, or, perhaps, 24-hour urinary cortisol, are necessary to reconcile the contradictory findings reported thus far [47, 72]. Additionally, further investigations should test the stress reactivity hypothesis proposed by Cacioppo and colleagues [78]. Finally, greater range of potential mechanisms need to be examined. The potential involvement of opioids, cytokines, and neuropeptides have received relatively little attention in human psychoneuroimmunological research (for a review, see [10]), and none at all in the context of stress-induced modulation of antibody response.

6. Conclusion

There is evidence of a negative association between stress and antibody response to vaccination. This is most apparent in secondary responses to thymus-dependent vaccinations, particularly the influenza vaccine [44, 47, 52, 53], and the associations seem most convincing with stressors of a chronic, enduring nature [34, 44, 47]. There is also increasing evidence that these effects are most apparent some time after vaccination. Thymus-independent and conjugate vaccinations have received insufficient study to permit definitive conclusions. The existing preliminary evidence implicates the HPA axis and SNS as mediators of the association between stress and antibody response. Future research needs to untangle the theoretical ramifications of these data, as well as

realising their clinical implications.

References

- [1] Leader. Uncovering at funerals. *British Medical Journal* 1884; 1: 1163.
- [2] Helsing KJ, Comstock GW, Szklo M. Causes of death in a widowed population. *American Journal of Epidemiology* 1982; 116: 524-32.
- [3] Kiecolt-Glaser JK, Dura JR, Speicher CE, Trask J, Glaser R. Spousal caregivers of dementia victims: Longitudinal changes in immunity and health. *Psychosomatic Medicine* 1991; 53: 345-62.
- [4] Mackenbach JP, Looman CW. Living standards and mortality in the European Community. *Journal of Epidemiology and Community Health* 1994; 48: 140-45.
- [5] McConnochie KM, Roghmann KJ, Liptak GS. Hospitalization for lower respiratory tract illness in infants: rates among counties in New York State and areas within Monroe County. *Journal of Pediatrics* 1995; 126: 220-29.
- [6] Cohen S, Doyle WJ, Skoner DP. Psychological stress, cytokine production, and severity of upper respiratory illness. *Psychosomatic Medicine* 1999; 61: 175-80.
- [7] Cohen S, Frank E, Doyle WJ, Skoner DP, Rabin BS, Gwaltney JM. Types of stressors that increase susceptibility to the common cold in healthy adults. *Health Psychology* 1998; 17: 214-23.
- [8] Cohen S, Tyrrell DAJ, Smith AP. Psychological stress and susceptibility to the common cold. *New England Journal of Medicine* 1991; 325: 606-12.
- [9] Cohen S, Tyrrell DAJ, Smith AP. Negative life events, perceived stress, negative affect, and susceptibility to the common cold. *Journal of Personality and Social Psychology* 1993; 64: 131-40.
- [10] Ader R, Felten DL, Cohen N. *Psychoneuroimmunology*. Academic Press, 2001.
- [11] Kiecolt-Glaser JK, Fisher LD, Ogrocki P, Stout JC, Speicher CE, Glaser R. Marital quality, marital disruption, and immune function. *Psychosomatic Medicine* 1987; 49: 13-34.
- [12] Kiecolt-Glaser JK, Glaser R, Shuttleworth EC, Dyer CS, Ogrocki P, Speicher CE. Chronic stress and immunity in family caregivers of Alzheimer's disease victims. *Psychosomatic Medicine* 1987; 49: 523-35.

- [13] Futterman AD, Wellisch DK, Zigelboim J, Luna-Raines M, Weiner H. Psychological and immunological reactions of family members to patients undergoing bone marrow transplantation. *Psychosomatic Medicine* 1996; 58: 472-80.
- [14] Schaeffer MA, McKinnon, Baum A, Reynolds CP, Rikli P, Davidson LM, Fleming I. Immune status as a function of chronic stress at Three Mile Island. *Psychosomatic Medicine* 1985; 47: 85.
- [15] Jemmott JB, Magloire K. Academic stress, social support, and secretory immunoglobulin A. *Journal of Personality and Social Psychology* 1988; 55: 803-10.
- [16] Jemmott JB, Borysenko M, Chapman R, Borysenko JZ, McClelland DC, Meyer D, Benson H. Academic stress, power motivation, and decrease in secretion rate of salivary secretory immunoglobulin A. *The Lancet* 1983; 1: 1400-02.
- [17] Deinzer R, Kleineidam C, Stiller-Winkler R, Idel H, Bachg D. Prolonged reduction of salivary immunoglobulin A (sIgA) after a major academic exam. *International Journal of Psychophysiology* 2000; 37: 219-32.
- [18] McClelland DC, Alexander C, Marks E. The need for power, stress, immune function, and illness among male prisoners. *Journal of Abnormal Psychology* 1982; 91: 61-70.
- [19] Vedhara K, Fox JD, Wang ECY. The measurement of stress-related immune dysfunction in psychoneuroimmunology. *Neuroscience and Biobehavioral Reviews* 1999; 23: 699-715.
- [20] Bartrop RW, Luckhurst E, Lazarus L, Kiloh LG, Penny R. Depressed lymphocyte function after bereavement. *Lancet* 1977; 1: 834-36.
- [21] Schleifer SJ, Keller SE, Camerino M, Thorton JC, Stein M. Suppression of lymphocyte stimulation following bereavement. *Journal of the American Medical Association* 1983; 250: 374-77.
- [22] Workman EA, La Via MF. T-Lymphocyte polyclonal proliferation: Effects of stress and stress response style on medical students taking National Board examinations. *Clinical Immunology and Immunopathology* 1987; 43: 308-13.
- [23] Arnetz BB, Wasserman J, Petrini B, Brenner SO, Levi L, Eneroth P, Salovaara H, Hjelm R, Salovaara L, Theorell T, Petterson IL. Immune function in unemployed women. *Psychosomatic Medicine* 1987; 49: 3-12.

- [24] Kiecolt-Glaser JK, Glaser R, Cacioppo JT, MacCullum RC, Snyder-Smith M, Kim C, Malarkey W. Marital conflict in older adults: Endocrinological and immunological correlates. *Psychosomatic Medicine* 1997; 59: 339-49.
- [25] Kiecolt-Glaser JK, Malarkey W, Chee M, Newton T, Cacioppo JT, Mao H, Glaser R. Negative behaviour during marital conflict is associated with immunological down-regulation. *Psychosomatic Medicine* 1993; 55: 395-409.
- [26] Linn BS, Linn MW. The effects of psychosocial stress on surgical outcome. *Psychosomatic Medicine* 1987; 49: 210.
- [27] Lazarus RS, Folkman S. *Stress, appraisal, and coping*. New York: Springer, 1984.
- [28] Spielberger CD. *State-trait anxiety inventory for adults*. Palo Alto, California: Consulting Psychologists Press, 1983.
- [29] Stone AA, Cox DS, Valdimarsdottir H, Jandorf L, Neale JM. Evidence that secretory IgA antibody is associated with daily mood. *Journal of Personality & Social Psychology* 1987; 52: 988-93.
- [30] Stone AA, Neale JM, Cox DS, Napoli A, Valdimarsdottir HB, Kennedy-Moore E. Daily events are associated with a secretory immune response to an oral antigen in men. *Health Psychology* 1994; 13: 440-46.
- [31] Stone AA, Cox DS, Valdimarsdottir H, Neale JM. Secretory IgA as a Measure of Immunocompetence. *Journal of Human Stress* 1987; 136-40.
- [32] Snyder BK, Roghmann KJ, Sigal LH. Effect of stress and other biopsychosocial factors on primary antibody response. *Journal of Adolescent Health Care* 1990; 11: 472-9.
- [33] Smith AJ, Bennett B, Vollmer-Conna U, Hickie IB, Lloyd AR. The effect of distress on the development of a primary immune response. *Psychosomatic Medicine* 2002; 64: 166-Abstract.
- [34] Glaser R, Kiecolt-Glaser JK, Bonneau RH, Malarkey W, Kennedy S, Hughes J. Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. *Psychosomatic Medicine* 1992; 54: 22-29.
- [35] Petrie KJ, Booth RJ, Pennebaker JW, Davison KP, Thomas MG. Disclosure of trauma and immune response to a hepatitis B vaccination program. *Journal of Consulting and Clinical Psychology* 1995; 63: 787-92.

- [36] Cohen S, Miller GE, Rabin BS. Psychological stress and antibody response to immunization: A critical review of the human literature. *Psychosomatic Medicine* 2001; 63: 7-18.
- [37] Burns VE, Carroll D, Ring C, Harrison LK, Drayson M. Stress, coping, and hepatitis B antibody status. *Psychosomatic Medicine* 2002; 64: 287-93.
- [38] Jabaaij L, Grosheide PM, Heijntink RA, Duivenvoorden HJ, Ballieux RE, Vingerhoets AJJM. Influence of perceived psychological stress and distress on antibody response to low dose rDNA hepatitis B vaccine. *Journal of Psychosomatic Research* 1993; 37: 361-69.
- [39] Jabaaij L, Hattum Jv, Vingerhoets JJM, Oostveen FG, Duivenvoorden HJ, Ballieux RE. Modulation of immune response to rDNA hepatitis B vaccination by psychological stress. *Journal of Psychosomatic Research* 1996; 41: 129-37.
- [40] Marsland AL, Cohen S, Rabin BS, Manuck SB. Associations between stress, trait negative affect, acute immune reactivity, and antibody response to hepatitis B injection in healthy young adults. *Health Psychology* 2001; 20: 4-11.
- [41] Petry J, Weems LB, Livingstone JN. Relationship of stress, distress and the immunologic response to a recombinant hepatitis B vaccine. *Journal of Family Practice* 1991; 32: 481-86.
- [42] Patriarca PA. A randomized controlled trial of influenza vaccine in the elderly: Scientific scrutiny and ethical responsibility. *Journal of the American Medical Association* 1994; 272: 1700-01.
- [43] Greene MA, Betts RF, Ochitill HN, Iker HP, Douglas RG. Psychosocial factors and immunity: A preliminary report. *Psychosomatic Medicine* 1978; 40: 87.
- [44] Kiecolt-Glaser JK, Glaser R, Gravenstein S, Malarkey WB, Sheridan J. Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proceedings of the National Academy of Science of the United States of America* 1996; 93: 3043-47.
- [45] Wallsten SM. Comparing patterns of stress in daily experiences of elderly caregivers and non-caregivers. *International Journal of Aging and Human Development* 1993; 37: 55-68.

- [46] Levine M, Beattie BL, McLean DM, Corman D. Characterization of the immune response to trivalent influenza vaccine in elderly men. *Journal of the American Geriatric Society* 1987; 35: 609-15.
- [47] Vedhara K, Cox NKM, Wilcock GK, Perks P, Hunt M, Anderson S, Lightman ST, Shanks NM. Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. *Lancet* 1999; 353: 627-31.
- [48] Glaser R, Kiecolt-Glaser JK, Malarkey W, Sheridan JF. The influence of psychological stress on the immune response to vaccines. *Annals of the New York Academy of Sciences* 1998; 840: 649-55.
- [49] Kohut ML, Boehm GW, Moynihan JA. Moderate exercise is associated with enhanced antigen-specific cytokine, but not IgM antibody production in aged mice. *Mechanisms of Ageing and Development* 2001; 122: 1135-50.
- [50] Bovbjerg DH, Manne SL, Gross PA. Immune response to influenza vaccine is related to psychological state following exams. *Psychosomatic Medicine* 1990; 52: 229 (Abstract).
- [51] Larson MR, Treanor JJ, Ader R. Psychosocial influences on responses to reduced and full-dose trivalent inactivated influenza vaccine. *Psychosomatic Medicine* 2002; 64: 113 (Abstract).
- [52] Pressman S, Cohen S, Miller G, Rabin B. Stress and antibody response to immunization in college freshman. *Brain, Behavior and Immunity* 2002; 16: 208 - Abstract.
- [53] Burns VE, Carroll D, Drayson M, Whitham M, Ring C. Psychological stress, cortisol, and antibody response to influenza vaccination in a young, healthy cohort. *Brain, Behavior and Immunity* 2002; 16: 173 - Abstract.
- [54] Morag M, Morag A, Reichenberg A, Lerer B, Yirmiya R. Psychological variables as predictors of rubella antibody titers and fatigue - a prospective double blind study. *Journal of Psychiatric Research* 1999; 33: 389-95.
- [55] Boyce WT, Adams S, Tschann JM, Cohen F, Wara D, Gunnar MR. Adrenocortical and behavioral predictors of immune response to starting school. *Pediatric Research* 1995; 38: 1009-16.

- [56] Glaser R, Sheridan JF, Malarkey W, MacCullum RC, Kiecolt-Glaser JK. Chronic stress modulates the immune response to a pneumococcal pneumonia vaccine. *Psychosomatic Medicine* 2000; 62: 804-07.
- [57] Burns VE, Drayson M, Ring C, Carroll D. Perceived stress and psychological well-being are associated with antibody status following meningitis C conjugate vaccination. *Psychosomatic Medicine* 2002; 64: 963-70.
- [58] Schacter S, Silverstein B, Kozlowski LT, Herman CP, Liebling B. Effects of stress on cigarette smoking and urinary pH. *Journal of Experimental Psychology* 1977; 106: 24-30.
- [59] Steptoe A, Lipsey Z, Wardle J. Stress, hassles, and variations in alcohol consumption, food choice and physical exercise: A diary study. *British Journal of Health Psychology* 1998; 3: 51-64.
- [60] Heslop P, Davey Smith G, Carroll D, Macleod J, Hyland F, Hart C. Perceived stress and coronary heart disease risk factors: The contribution of socio-economic position. *British Journal of Health Psychology* 2001; 6: 167-78.
- [61] Gluckman SJ, Dvorak VC, MacGregor R. Host defenses during prolonged alcohol consumption in a controlled environment. *Archives of Internal Medicine* 1977; 137: 1539-43.
- [62] McMahon BJ, Parkinson AJ, Bulkow L, Davidson M, Wainwright K, Wolfe P, Schiffman G. Immunogenicity of the 23-valent pneumococcal polysaccharide vaccine in Alaska native chronic alcoholics compared with nonalcoholic native and non-native controls. *American Journal of Medicine* 1993; 95: 589-94.
- [63] MacKenzie JS, MacKenzie IH, Holt PG. The effect of cigarette smoking on susceptibility to epidemic influenza and on serological responses to live attenuated and killed subunit influenza vaccines. *Journal of Hygiene* 1976; 77: 409-17.
- [64] Finklea JF, Hassleblad V, Riggan WB, Nelson WC, Hammer DI, Newill VA. Cigarette smoking and hemagglutination inhibition response to influenza after natural disease and immunization. *American Review of Respiratory Disease* 1971; 104: 368-76.
- [65] Winter AP, Follett EAC, McIntyre J, Stewart J, Symington IS. Influence of smoking on immunological responses to hepatitis B vaccine. *Vaccine* 1994; 12: 771-72.

- [66] Shaw FE, Guess HA, Roets JM, Mohr FE, Coleman PJ, Mandel EJ, Roehm RR, Talley WS, Hadler SC. Effect of anatomic injection site, age, and smoking on the immune response to hepatitis B vaccination. *Vaccine* 1989; 7: 425-30.
- [67] Horowitz MM, Ershler WB, McKinney P, Battiola RJ. Duration of immunity after hepatitis B vaccination: Efficacy of low-dose booster vaccine. *Annals of Internal Medicine* 1988; 108: 185-89.
- [68] Girodan F, Galan P, Monget A-L, Boutron-Ruault M-C, Brunet-Lecomte P, Preziosi P, Arnaud J, Manuguerra J-C, Hercberg S. Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients. *Archives of Internal Medicine* 1999; 159: 748-54.
- [69] Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R, Thompson C, Pedrosa MC, Diamond RD, Stollar BD. Vitamin E supplementation and *in vivo* immune response in healthy elderly subjects: A randomized controlled trial. *Journal of the American Medical Association* 1997; 277: 1380-86.
- [70] Chandra RK, Puri S. Nutritional support improves antibody response to influenza virus vaccine in the elderly. *British Medical Journal* 1985; 291: 705-06.
- [71] Parati G, Pomidossi G, Casadei R, Groppelli A, Ravogli A, Trazzi S, Cosana B, Mancia G. Limitations of lab stress testing in the assessment of subjects' cardiovascular reactions to stress. *Journal of Hypertension* 1986; 4: S51-53.
- [72] Burns VE, Ring C, Drayson M, Carroll D. Cortisol and cardiovascular reactions to mental stress and antibody status following hepatitis B vaccination: A preliminary study. *Psychophysiology* 2002; 39: 361-68.
- [73] Felten DL, Felten SY, Carlson SL, Olschowka JA, Livnat S. Noradrenergic and peptidergic innervation of lymphoid tissue. *Journal of Immunology* 1985; 135: 755-65.
- [74] Keren G, Segev S, Morag A, Zakay-Rones Z, Barzilai A, Rubinstein E. Failure of influenza vaccination in the aged. *Journal of Medical Virology* 1988; 25: 85-89.
- [75] Bernstein E, Kaye D, Abrutyn E, Gross PA, Dorfman M, Murasko DM. Immune response to influenza vaccination in a large, healthy elderly population. *Vaccine* 1999; 17: 82-94.

[76] Locke SE, Hurst MW, Heisel SJ, Kraus L, Williams M. The influence of stress and other psychosocial factors on human immunity. 36th Annual Meeting of the Psychosomatic Society 1979;

[77] Viswanathan K, Bilbo SD, Dhabhar FS. Acute stress administered either before primary immunization or before challenge enhances skin-cell-mediated immunity to Keyhole Limpet Hemocyanin (KLH). *Brain, Behavior and Immunity* 2002; 16: 222 - Abstract.

[78] Cacioppo JT, Berntson GG, Malarkey WB, Kiecolt-Glaser JK, Sheridan JF, Poehlmann KM, Burlinson MH, Ernst JM, Hawkley LC, Glaser R. Autonomic, neuroendocrine, and immune responses to psychological stress: The reactivity hypothesis. *Annals of the New York Academy of Sciences* 1998; 840: 664-73.