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Psychosocial factors are associated with the antibody response to both thymus-dependent and thymus-independent vaccines

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Abstract

The present study examined the association between psychological stress, social support and antibody response to both thymus-dependent and thymus-independent vaccinations. Stressful life events in the previous year and customary social support were measured by standard questionnaires at baseline in 75 (41 females) healthy students. Antibody status was assessed at baseline, 4 and 18 weeks following vaccination with formaldehyde inactivated hepatitis A virus and pneumococcal polysaccharides, which induce thymus-dependent and -independent antibody responses respectively. Controlling for baseline antibody status, life event stress was negatively associated with antibody response to the hepatitis A vaccine at the 18-week follow-up; participants reporting a greater number of stressful life events had a poorer antibody response. There was no relationship between psychological stress and antibody response to pneumococcal vaccination. Social support was not associated with the antibody response to hepatitis A vaccination. However, there was a significant association between support and the antibody response to the thymus-independent pneumococcal vaccine at 4-week follow-up; participants with larger social networks mounted a better response. These relationships could not be accounted for by age and sex, or by variations in health behaviours. Psychosocial factors would appear to influence the response to both thymus-dependent and thymus-independent vaccines, but not in the same manner.

Keywords: Antibody response; Hepatitis A vaccination; Life events; Pneumococcal vaccination; Psychological stress; Social support.

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1. Introduction

There is now consistent evidence showing that the antibody response to medical vaccinations requiring T-cell help in the generation of antibody by B-cells, i.e., thymus-dependent vaccines, is associated with psychological stress in humans (Burns et al., 2003; Burns et al., 2002; Kiecolt-Glaser et al., 1996; Phillips et al., 2005; Pressman et al., 2005; Vedhara et al., 1999). Less studied are the effects of stress on antibody responses to thymus-independent vaccines, i.e., polysaccharide vaccines, in which B-cells have to generate a response without T-cell help. Further, the few studies that have been conducted present an inconsistent picture. For example, spousal caregivers of dementia patients have been reported to show a poorer response to the pneumococcal polysaccharide vaccine, but only six months after vaccination (Glaser et al., 2000). In contrast, ratings of young children's problem behaviour, taken to be a measure of stress, were not associated with response to the pneumococcal polysaccharide vaccine, administered one week previously (Boyce et al., 1995). To date, only one study has examined simultaneously the influence of stress on both thymus-dependent and thymus-independent vaccine responses (Phillips et al., 2005). Whereas psychological stress was associated with the antibody response to influenza vaccination, a thymus-dependent vaccine, it was not related to the response to meningococcal A, a thymus-independent vaccine. In addition, the effects of other psychosocial factors, such as social support, have received far less attention. However, recent studies have found that social support in young adults (Phillips et al., 2005; Pressman et al., 2005) and supportive marriages in older adults (Phillips et al., 2006) were associated with a better antibody response to influenza vaccination.

The present study, then, examined the association between psychological stress and social support and antibody response to hepatitis A and pneumococcal vaccines. The

former is a thymus-dependent vaccine yet to be investigated in this context. The latter was chosen because it has produced apparently discrepant results in previous studies. It was hypothesized, first, that psychological stress would be associated with a poorer antibody response to vaccination, whereas social support would be associated with a better response. Second, it was hypothesized that the effects of psychosocial factors would be more evident with the thymus-dependent vaccine.

2. Method

2.1. Participants

Participants were 75 (41 women) University of Birmingham students. Mean age was 22.9 (*SD* = 3.89) years. In terms of ethnicity, 89% described themselves as "white," 2.7% as "Asian," 1.3% as "black," and 7% as "other". Ninety-two percent of the sample reported being non-smokers and 59% regular caffeine drinkers. Participants were excluded if they had reported receiving hepatitis A and pneumococcal vaccinations previously, were suffering from medical conditions that could affect antibody response (e.g., acute infection, glandular fever); were pregnant or taking prescribed medication, excluding the oral contraceptives. The study was approved by the appropriate Research Ethics Committees.

2.2. Study design

The study comprised three testing sessions: baseline, and a 4-week and 18-week follow-up. At baseline, participants completed questionnaires and provided a single venous blood sample to determine baseline antibody levels. They were then vaccinated against hepatitis A (HAVRIX™; Glaxo SmithKline; containing inactivated hepatitis A virus (HM 175 hepatitis A virus strain) of 1440 ELISA units/ml adsorbed onto aluminum hydroxide suspended in formaldehyde in a prefilled 1-mL syringe and with the 23-valent polysaccharide pneumococcal vaccine (Pneumovax II; Sanofi Pasteur MSD. The latter

included 25ug of each capsular polysaccharide serotype suspended in phenol and water in a 0.5mL vial). Both vaccines were given intramuscularly. At the follow-up sessions blood samples were again taken to assess antibody levels.

2.3. Questionnaires

2.3.1. Psychological stress

The Life Events Scale for Students (Linden, 1984) was used to assess stressful life events exposure. This is a student-specific inventory, and participants were required to select, from a list of 36, those life events that they had experienced in the previous year. It includes both major (e.g., death of your best friend) and minor (e.g., getting an unjustified low mark on a test) events. Each event also comes with a pre-determined weighting for severity (Linden, 1984), and so stress exposure can be represented in two possible ways: a simple frequency count of events and a score which is the aggregate of weighted events.

2.3.2. Social support

Participants completed the Medical Outcomes Study Social Support Survey (Sherbourne and Stewart, 1991). This provides an overall measure of structural support (number of close friends), and functional support. The questionnaire has a 5-point Likert-type format with high scores indicating high social support. Good internal consistency, Cronbach's $\alpha = .91$, and test-retest reliability, $r = .72$ to .78, has been reported (Sherbourne and Stewart, 1991).

2.3.2 Health behaviours

As in our previous studies (Burns et al., 2002; Phillips et al., 2005), typical health behaviours were assessed using a questionnaire adapted from the Whitehall II study (Marmot et al., 1991). Participants were asked, on average how much they smoked (0, 1–5, 6–10, 11–20, and 21+ cigarettes per day); how much alcohol they drank (0, 1–5, 6-10, 11–20, 21–40, and 40 + units per week); how long they slept (0–3, 4–5, 6–7, 8–9, 10–11,

and 12+ h per night). A simple categorical scoring system was used in all cases. Participants also reported how much time they spent in activities of light, moderate and vigorous exercise intensity, which were summed to yield a composite exercise score. Finally, they indicated how frequently they ate certain foods from a standard list; this yielded measures of fruit and vegetable consumption and fat intake.

2.4. Blood sampling and antibody analysis

Venous blood specimens were collected from an ante-cubital vein into two 7-ml plain tubes (BD Vacutainer, Meylan Cedex) to assess antibody titers. Samples were allowed to clot at room temperature for 1 h and centrifuged at 3500 rpm for 5 min. The separated serum was frozen at −20°C until assayed. With regard to hepatitis A, levels of IgG anti-HAV antibodies were analyzed using a commercial quantitative enzyme based assay, Enzygnost® Anti-HAV (Dade Behring, Germany). Test samples were diluted 1:10 with diluent and read at 450 nm on a ELx800 plate reader (Bio-Tek® Instruments, Inc, Vermont USA). Data are presented as the difference in optical density absorbance endpoints at this wavelength between controls and samples. Since this is a competitive assay, reciprocals of optical density values were computed, so that higher values represented higher antibody levels. Luminex technology was used to assess five pneumococcal (Pn) IgG antibody serotypes (types 1, 3, 9, 19 and 23) contained in the pneumococcal vaccine. Further details of this assay are described elsewhere (Ferraro et al., in press; Lal et al., 2005). In short, carboxyl microspheres (BioRad Labs, UK) were conjugated to the individual purified pneumococcal polysaccharides (LGC Prochem / ATCC, UK) via Poly-L-lysine. Seven four-fold dilutions of reference serum 89SF (Food and Drug Administration, Maryland, USA) (beginning at 1:20) were made with diluent buffer (PBS with 0.05% Tween-20, 1% BSA, and 5μg/ml with pneumococcus cell wall polysaccharide (Statens Serum Institute, Copenhagen, Denmark) Serum samples were diluted 1:200 in diluent buffer that additionally contained 5μg/ml purified pneumococcal serotype 22F in accordance with the WHO protocol for ELISA detection of Pn antibody (http://www.vaccine.uab.edu/#) . Conjugated microspheres (2,500 per serotype)

suspended in 25µl were added to a 1.2µm filter membrane microtiter 96-well plate (Millipore Corp., Bedford, MA) before washing and aspirating. Sera and standards (25µl per well) were run in duplicate and incubated with microspheres in the dark for 60 minutes at room temperature, with shaking. After incubation, aspiration and washing, 100µl of IgG-PE mouse anti-human secondary antibody (Southern biotech, AL, USA), with diluted 1:250 was added to the wells. This was allowed to incubate for another 30 minutes in the dark, with shaking. Contents were then aspirated, washed and resuspended in 125µl of wash buffer and read on a Luminex 100 machine (Luminex Corp, TX, USA) programmed to collect a minimum of 50 microspheres per serotype. Acquisition software (BioPlex Software Manager (version 4, BioRads, Labs, CA, USA) was used to generate serotype antibody concentrations from a 5 parameter logistic curve fit. Serum Pn IgG levels are reported in µg/ml.

2.5. Data reduction and analysis

Given the skew of the data, optical density reciprocals and antibody titres were subjected to $\log_{10}$ transformation. Repeated measures analysis of variance was used to confirm that the vaccines elicited an antibody response. Partial eta- squared (η^2) is reported as a measure of effect size. Hierarchical linear regression analyses were then applied to determine whether psychosocial factors predicted $\log_{10}$ antibody level at each follow-up. In all regression models tested, antibody levels at baseline were entered at step one. The psychosocial variables were then entered separately at step two. Further, regression analyses were undertaken to adjust for possible confounders. Age, sex and health behaviour variables were entered into these models at step two, and the psychosocial variables at step three. In line with existing research (Glaser et al., 2000), total IgG antibody titre, the sum of the individual pneumococcal strain titres, was used for pneumococcal analyses; however, in sensitivity analysis individual Pn serotypes IgG titres were examined.

3. Results

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3.1. Questionnaire data

The mean (*SD*) number of life events was 7.24 (3.36), and the life events weighted score was 306.29 (158.72). The mean (*SD*) number of close friends was 10.67 (7.07), and mean (*SD*) total functional social support score was 78.57 (14.45).

3.2. Vaccination response

The geometric mean (*95% CI*) antibody titre for HAV and Pneumococcus at each time point is displayed in Table 1. Participants responded with an initial increase in antibody titre from baseline to 4-week follow-up that in the case of hepatitis A was sustained at 18 weeks. For pneumococcus, antibody levels had declined by 18-week follow-up, but still remained well above baseline level.

[Insert Table 1 about here]

3.3. Associations between psychological stress, social support, and antibody response

Taking into account baseline antibody status, psychological stress was negatively associated with antibody response to the hepatitis A vaccine at the 18-week, although not the 4-week, follow-up (see Figure1). Participants reporting a higher number of events, $\beta = -.23$, $t = 2.15$, $p = .03$, $\Delta R^2 = .05$, and registering a higher weighted life events score, $\beta = -.22$, $t = 2.04$, $p = .04$, $\Delta R^2 = .05$, mounted a poorer antibody response. No associations emerged between life event stress and antibody response to the pneumococcal vaccine. In contrast, social support was not associated with the antibody response to hepatitis A vaccination. However, there was a significant positive association between structural support and antibody response to the thymus-independent pneumococcal vaccine at the 4-week, but not the 18-week, follow-up; participants who reported having more friends mounted a better antibody response, $\beta = .19$, $t = 2.21$, $p = .03$, $\Delta R^2 = .04$ (see Figure 1). Age and sex were not significantly associated with the antibody response to either vaccine at either follow-up. In addition, health behaviours (e.g., smoking, exercise, caffeine, and alcohol consumption) were unrelated to antibody response. It is thus

unsurprising that the associations reported above withstood adjustment for these variables in regression analyses. Finally, we undertook sensitivity analysis using the individual pneumococcal IgG antibody serotypes at 4-week follow-up. Social support was positively related to the antibody response to the Pn 3 and Pn 23 serotypes, $\beta = .19$, $t = 2.06$, $p = .04$, $\Delta R^2 = .04$ and $\beta = .27$, $t = 3.03$, $p = .003$, $\Delta R^2 = .07$, respectively. The association between support and response to the Pn 1 serotype did not quite meet the conventional criteria for statistical significance, $\beta = .18$, $t = 1.79$, $p = .07$, $\Delta R^2 = .03$.

[Insert Figure 1 about here]

4. Discussion

This is the first study that we know of to examine the effects of psychosocial factors on the antibody response to hepatitis A vaccination. Life events stress was significantly associated with the response at 18-weeks follow-up; the greater the stress exposure the poorer the response. This result is consistent with the growing literature showing negative associations between psychological stress and antibody responses to a range of thymus-dependent vaccines (Burns et al., 2002; Marsland et al., 2001; Miller et al., 2004; Pressman et al., 2005). On the other hand, social support was not reliably associated with hepatitis A vaccination response. Previous investigations have observed that participants reporting less social support or more loneliness mounted a poorer antibody response to at least one strain of the influenza vaccine (Phillips et al., 2005; Pressman et al., 2005). Strain specificity in the effects of psychosocial factors on the response to influenza vaccination have been explained in terms of novelty, with relatively novel vaccine strains being vulnerable to the impact of life events stress and the response to less novel strains being related to social support (Phillips et al., 2005). Since the hepatitis A vaccine was almost certainly a novel challenge for the majority of our participants, this could explain the present pattern of results. The current result for life events also indicates that psychosocial influence on antibody response to vaccination is not restricted to vaccines that elicit a secondary response.

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The antibody response to the pneumococcal vaccination was positively associated with social support; the larger the support network the better the antibody response. This result is at odds with our previous null findings for another thymus-independent vaccine, meningococcal A (Phillips et al., 2005). It would appear that not all thymus-independent vaccination responses are as insensitive to psychosocial factors as meningococcal A. In addition, our failure to find an association between psychological stress and pneumococcal antibody response would seem to contrast with the finding of a poorer pneumococcal response in spousal caregivers than in matched controls (Glaser et al., 2000). However, it is perhaps worth noting that the caregivers in this study did not differ significantly from controls on perceived stress; they did, however, differ in social support, with caregivers reporting poorer support. Accordingly, this discrepancy in results may be more apparent than real.

Although small sample size can be regarded as a potential limitation, the present sample was the same order of magnitude as that tested in other vaccination studies (Burns et al., 2002; Marsland et al., 2001; Pressman et al., 2005). In addition, given that this is a correlational study, it remains possible that some confounding variable is driving the associations observed. However, statistical adjustment for age, sex, and health behaviours failed to attenuate these relationships.

In summary, the present study extends previous research on psychosocial factors and antibody response to medical vaccination: first, by showing that psychological stress was negatively associated with the antibody response to a thymus-dependent vaccine yet to be examined in this context; second, by demonstrating that social support was positively related to the response to a thymus-independent vaccine.

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References

Boyce, W.T., Adams, S., Tschann, J.M., Cohen, F., Wara, D., Gunnar, M.R., 1995. Adrenocortical and behavioral predictors of immune responses to starting school. Pediatr Res. 38, 1009-1017.

Burns, V.E., Carroll, D., Drayson, M., Whitham, M., Ring, C., 2003. Life events, perceived stress and antibody response to influenza vaccination in young, healthy adults. J Psychosom Res. 55, 569-572.

Burns, V.E., Drayson, M., Ring, C., Carroll, D., 2002. Perceived stress and psychological well-being are associated with antibody status after meningitis C conjugate vaccination. Psychosom Med. 64, 963-970.

Ferraro, A.J., Drayson, M.T., Savage, C.O.S., MacLennan, I.C.M., in press. Autoantibodies, unlike antibodies to all extrinsic antigen groups, fall following B cell depletion with Rituximab. European J Immunol.

Glaser, R., Sheridan, J., Malarkey, W.B., MacCallum, R.C., Kiecolt-Glaser, J.K., 2000. Chronic stress modulates the immune response to a pneumococcal pneumonia vaccine. Psychosom Med. 62, 804-807.

Post-print, not final published version. Cite this article as: Gallagher, S., Phillips, A.C., Ferraro, A.J., Drayson, M.T., & Carroll, D. (2008). Psychosocial factors are associated with the antibody response to both thymus-dependent and thymus-independent vaccines. *Brain, Behavior, and Immunity*, 22, 456-460. http://dx.doi.org/10.1016/j.bbi.2007.10.018

Kiecolt-Glaser, J.K., Glaser, R., Gravenstein, S., Malarkey, W.B., Sheridan, J., 1996. Chronic stress alters the immune response to influenza virus vaccine in older adults. Proc Natl Acad Sci USA. 93, 3043-3047.

Lal, G., Balmer, P., Stanford, E., Martin, S., Warrington, R., Borrow, R., 2005. Development and validation of a nonaplex assay for the simultaneous quantitation of antibodies to nine Streptococcus pneumoniae serotypes. J Immunol Methods. 296, 135-147.

Linden, W., 1984. Development and initial validation of a life event scale for students. Can Counsellor. 18, 106-110.

Marmot, M.G., Dowey Smith, G., Stansfeld, S., Patel, C., North, F., Head, J., White, I., Brunner, E., Feeney, A., 1991. Health inequalities among British civil servants: the Whitehall II study. Lancet. 337, 1387–1393.

Marsland, A.L., Cohen, S., Rabin, B.S., Manuck, S.B., 2001. Associations between stress, trait negative affect, acute immune reactivity, and antibody response to hepatitis B injection in healthy young adults. Health Psych. 20, 4-11.

Miller, G.E., Cohen, S., Pressman, S., Barkin, A., Rabin, B.S., Treanor, J.J., 2004. Psychological stress and antibody response to influenza vaccination: when is the critical period for stress, and how does it get inside the body? Psychosom Med. 66, 215-223.

Phillips, A.C., Burns, V.E., Carroll, D., Ring, C., Drayson, M., 2005. The association between life events, social support, and antibody status following thymus-dependent and thymus-independent vaccinations in healthy young adults. Brain Behav Immun. 19, 325-333.

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Phillips, A.C., Carroll, D., Burns, V.E., Ring, C., Macleod, J., Drayson, M., 2006. Bereavement and marriage are associated with antibody response to influenza vaccination in the elderly. Brain Behav Immun. 20, 279-289.

Pressman, S.D., Cohen, S., Miller, G.E., Barkin, A., Rabin, B.S., Treanor, J.J., 2005. Loneliness, social network size, and immune response to influenza vaccination in college freshmen. Health Psych. 24, 297-306.

Sherbourne, C.D., Stewart, A.L., 1991. The MOS social support survey. Soc Sci Med. 32, 705-714.

Vedhara, K., Cox, N.K., Wilcock, G.K., Perks, P., Hunt, M., Anderson, S., Lightman, S.L., Shanks, N.M., 1999. Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. Lancet. 353, 627-631.

Table 1. Geometric mean and (95% confidence intervals) reciprocal optical densities for hepatitis A and antibody titres (ug/ml) for pneumococcal at baseline, 4-week and 18-week follow-up

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Vaccine	**Baseline**	**4-week follow-up**	**18-week follow-up**	**ANOVA**
Hepatitis A	1.10	2.63[a]	2.69 [b]	F (2, 66) = 65.31, $p < .001$
	(0.2- 6.9)	(0.3- 21.8)	(0.2- 35.4)	$\eta^2 = 0.664$
Pneumococcal	14.9	99.1[a]	84.6 [b,c]	F (2, 66) = 107.79, $p < .001$
	(1.6- 144.5)	(7.2- 1348.9)	(2.7- 549.5)	$\eta^2 = 0.769$

[a] Significant difference between baseline and 4-week follow-up.

[b] Significant difference between baseline and 18-week follow-up.

[c] Significant difference between 4-week and 18-week follow-up.

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Figure 1. Hepatitis A (A upper) and Pneumococcal total IgG (B lower) mean and standard error of antibody levels for those with high and low life events at 18-weeks and high and low social support networks at 4-weeks: median splits were used to generate the binary psychosocial variables.