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Attachment style and immunity: A 1-year longitudinal study

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The first author wishes to dedicate this article to the memory of his beloved father, Michele Picardi, who was a caring and reliable attachment figure and a key source of security throughout his life, and sadly passed away during the completion of this work.

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ABSTRACT

Previous cross-sectional studies suggested an association between attachment-related avoidance and altered immune function. We aimed at testing this hypothesis with longitudinal data. A random sample of 65 female nurses provided a blood sample and completed measures of perceived stress, social support, alexithymia, and attachment style. Immune assays included lymphocyte proliferative response (LPR) to Phytohemagglutinin and NK cell cytotoxicity (NKCC). State measures (perceived stress and support) and immune measures were collected again after 4, 8, and 12 months. Linear mixed effects models were used to examine the relationship between attachment and immunity. While low to moderate levels of attachment-related avoidance were not associated with NKCC, there was a significant negative association (beta −.35; $p = .005$) between high levels of avoidance and NKCC. No association was observed between NKCC and attachment-related anxiety, and between LPR and both attachment dimensions. While our findings should be interpreted with caution due to study limitations such as the relatively small sample size and the inclusion of only female participants, they corroborate the notion that attachment is linked to physiology and health.

1. Introduction

Attachment theory (Bowlby, 1969, 1973, 1980) is a lifespan developmental theory that has emerged as a major theoretical framework for studying close relationships and individual differences in emotion regulation. It postulates that humans are born with a disposition to form and conserve some important intimate relationships that are essential for survival and good health from early childhood to old age. Children rapidly build attachment bonds with their parents and maintain them across childhood and into adulthood, while in adolescence committed romantic relationships begin to develop and gradually become the primary attachment bond. The term 'attachment style' refers to individual differences in affect regulation and in perceptions of and beliefs about self and close others (Mikulincer and Shaver, 2003; Weinfield et al., 1999). Two main dimensions, named attachment-related anxiety (about abandonment or insufficient love) and attachment-related avoidance (of intimacy and emotional expression), underlie adult attachment style and conceptually correspond to similar dimensions that can be observed in infants in a laboratory procedure called 'Strange Situation' (Ainsworth et al., 1978). Relatively low levels of both attachment-related anxiety and avoidance characterize secure attachment.

In the psychoimmunology literature, it is recognized that personality and individual difference factors may exert an organizing influence on behavior and physiology and may modulate the

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immune system (Kemeny and Laudenslager, 1999; Segerstrom, 2003). Attachment style is a candidate to be one of these factors, as attachment insecurity is associated with impaired emotion regulation (Allen and Manning, 2007; Cassidy, 1994), altered physiological reactivity to stress (Feeney and Kirkpatrick, 1996; Powers et al., 2006; Rifkin-Graboi, 2008), and increased vulnerability to a variety of physical diseases, including diseases with immune system involvement (Ciechanowski et al., 2010; Janković et al., 2009; McWilliams and Bailey, 2010; Mrazek et al., 1987; Picardi et al., 2003, 2005). Two recent investigations corroborated this notion. In a cross-sectional study, we observed an association between high attachment-related avoidance and decreased natural killer cell cytotoxicity (NKCC) independent of perceived stress, social support, alexithymia, and various health-related behaviors (Picardi et al., 2007), while another study reported an association between attachment-related avoidance and higher IL-6 response to marital conflict among adults in long-term, committed relationships (Gouin et al., 2009). Our previous study (Picardi et al., 2007) had a significant limitation in the reliance on a single estimate of immune parameters and the resulting suboptimal measurement reliability. The present study aimed at establishing the reliability of the reported association between attachment-related avoidance and immunity. To this purpose, our previous study was extended to include three follow-up assessments over a 1-year period.

2. Methods

2.1. Participants and procedure

The study was carried out in Ancona on a random sample of female nurses from the local National Health Service Unit. Given that the staff included only few male nurses, and that small to moderate gender differences in attachment avoidance were observed in most cultures (Schmitt et al., 2003), we decided to restrict enrollment to women, as otherwise we would not have been able to control for the possible confounding effect of gender. Given that it was performed as part of the periodic occupational health examinations, the study needed no formal approval by the local ethical committee, which was nevertheless consulted and gave informal authorization. Participants were fully informed of the study objectives and procedures, were provided the opportunity to ask questions, and gave their written informed consent to take part in the study. There were no refusals, likely due to the fact that the study was incorporated into the periodic occupational health examinations. Inclusion criteria were age <60 years, at least two years in current job, absence of infectious diseases and chronic medical diseases, no history of major psychiatric disorders, and no current or recent treatment with drugs influencing the immune system (e.g., corticosteroids, cytostatics, immunosuppressors, immunomodulators). The study sample comprises the 61 nurses included in the cross-sectional study and four other nurses whose data became available later.

The participants were evaluated at baseline and 4, 8, and 12 months after baseline. All assessment sessions were conducted between 8.30 and 9.30 a.m. in a quiet and comfortable room. Participants were requested to refrain from exercising, smoking, eating, drinking alcohol, and taking medication for at least 12 h before each session. After participants had rested for at least 5 min in the sitting position, a blood sample was taken from a forearm vein and processed within 3 h at the INRCA institute laboratory, as described below. Then, participants ate breakfast and were administered a standardized form to collect information about health-related habits, and a number of self-completed questionnaires with established validity and reliability in counterbalanced order. Trait measures were completed only at baseline, whereas state measures were completed on each occasion. Therefore, except for attachment and alexithymia, all the other study variables were measured longitudinally. All immune assays were performed blinded to all other measures collected in the study.

2.2. Assessment

2.2.1. Psychometric instruments

The Experiences in Close Relationships (ECR) questionnaire is a self-report instrument with established reliability and validity in non-clinical (Brennan et al., 1998) as well as psychiatric (Picardi et al., 2011) samples, which consists of 36 items, each scored on a 7-point scale. The instrument provides scores on two dimensions, named 'Anxiety' and 'Avoidance'. Individuals scoring high on anxiety tend to be preoccupied with their romantic relationships, to worry about being abandoned, to desire a high level of closeness to their partner, and to ask the partner for more feeling and commitment. Individuals scoring high on avoidance tend to avoid emotional closeness and intimacy, to feel uncomfortable opening up to or depending on their partner, and to be reluctant to ask their partner for comfort, advice, or help.

The 20-item Toronto Alexithymia Scale (TAS-20) is a self-report questionnaire with demonstrated reliability and validity (Parker et al., 2003) which comprises 20 items, each scored on a 5-point scale. It measures the difficulty identifying and describing feelings, and the tendency to concentrate on the concrete details of external events rather than on feelings, fantasies, and other aspects of one's own inner experience. Higher scores denote higher alexithymic characteristics.

The Perceived Stress Scale (PSS) (Cohen et al., 1983) is a self-report questionnaire that has been thoroughly validated and widely used. The items are scored on a 5-point scale and summed to provide a total score. Higher scores reflect greater perceived stress. We used the 10-item version.

The Multidimensional Scale of Perceived Social Support (MSPSS) is a validated self-report questionnaire (Zimet et al., 1998) which consists of 12 items, each scored on a 7-point scale. Higher scores indicate greater perceived adequacy of support from family, friends, and significant others.

2.2.2. Cytotoxicity assay

The K562 tumor cell line was used as target. NKCC was assayed using a fluorimetric method described previously (Provinciali et al., 1992). Briefly, a stock solution (20 mg/ml acetone stored at −20 °C) of carboxyfluorescein diacetate (c'FDA, Molecular Probes, USA) was diluted in PBS to a final concentration of 75 μg/ml. K562 cells were washed twice with PBS and then labeled with c'FDA by incubation at 37 °C in a humidified 5% CO_2 incubator for 30 min Subsequently, they were washed 3 times in PBS containing 1% bovine serum albumin and resuspended at a final concentration of 1×10^5 cells/ml. C'FDA-labeled K562 cells (1×10^4) were incubated with effector cells in 200 ml total volume per well of a round microtiter plate. Effector target cell ratios from 100:1 to 12.5:1 were tested in triplicate. The plates were kept at 37 °C in a humidified 5% CO_2 incubator for 3 h and then centrifuged at 1500 rpm for 5 min The supernatant was separated by rapidly inverting the plate and flicking the supernatant out. Then, 100 ml of 1% Triton X-100 in 0.05 M borate buffer, pH 9.0, was added to each well. The plate was kept for 20 h at 4 °C to allow for solubilization and then it was read for fluorescence with a Titertek Fluoroskan II (Flow Laboratories, USA). The proportion of specific lysis was calculated as follows:

$$\%\text{Specific lyses} = \frac{F\,\text{med} - F\,\text{exp}}{F\,\text{med}}$$

where F is the fluorescence of solubilized cells after the supernatant has been removed, med = F from target cells incubated in CM medium alone, and exp = F from target cells incubated with effector cells.

Results were then normalized by conversion to lytic units, calculated as the number of effector cells required to lyse 20% of target cells and reported as the number of lytic units contained in 10^7 cells (Bryant et al., 1992).

2.2.3. Lymphocyte proliferative response

Ficoll-Hypaque enriched peripheral blood MNCs at the concentration of 1×10^6/ml in RPMI (GIBCO, USA) containing 10% FCS (GIBCO), penicillin (100 U/ml) and streptomycin (10 μg/ml), were used. Aliquots of 0.1 ml were distributed in microwells (Nunc, Denmark). Phytohemagglutinin (PHA) (Difco, USA) to reach the final concentrations of 0.0125 μg/ml and 0.05 μg/ml was added in the amount of 10 μL/well. After 48 h of incubation in a 5% CO_2-air environment at 37 °C, H3-Td (Amersham, UK: specific activity, 2 Ci/ml) was added in the amount of 1 μCi/well. After additional 18 h of incubation in CO_2 atmosphere, cultures were killed by means of a cell harvester (Skatron, Norway) and radioactivity was measured by a scintillator counter (Packard, Italy). All cultures were performed in quadruplicate. The results, referring to the highest responses regardless of the mitogen concentration used, are expressed in counts per minute (cpm) per culture (Mocchegiani et al., 1994).

The INRCA institute laboratory is ISO 9000 certified, and the inter-assay reliability of NKCC and lymphocyte proliferation assays as calculated from the replicate samples is consistently higher than 95% as previously reported (Provinciali et al., 1992). In the present study, the inter-assay reliability of NKCC was 96%.

2.3. Statistical analysis

The analysis were based on Linear Mixed Effects (LME) models, and was performed with STATA software, version 12 (Stata Corporation, College Station, Texas, USA) and with R software version 2.14.2 (R Foundation for Statistical Computing, http://www.R-project.org). All tests were two-tailed, with alpha set at .05. Prior to analysis, data were screened for outliers. Two outliers with extremely high and fluctuating NKCC values were found and excluded from the analysis. Time was coded as 0, 1, 2, 3 at baseline, 4-, 8-, and 12-month follow-up, respectively. The effect of time was not statistically significant when considered as a categorical variable in the model. Thus, in the reported results time was treated as a continuous variable, assuming that the change in the response variable is linear in time in order to gain statistical power. As a few missing data were deemed to be inevitable with this study design, no attempt was made to impute missing values; all collected data were included in the analyses. Longitudinal data were analysed by jointly considering all four follow-up measurements (baseline, 4-, 8- and 12-month data) and using a random intercept model to accommodate within-subject correlation across time. NKCC was regressed upon attachment-related anxiety and avoidance, time, age, NK cell number per mm^3, TAS-20 score at baseline, quality of sleep, exercise, NSAIDS intake,