Hostility is related to clusters of T-cell cytokines and chemokines in healthy men

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1. Introduction

Hostility has been identified as a risk factor for adverse health outcomes as diverse as cardiovascular disease and post-traumatic stress disorder (PTSD). Cytokines have been suggested to mediate this relationship. We investigated whether in healthy men a relation existed between hostility and T-cell mitogen-induced cytokines and chemokines. Male Dutch military personnel (n = 304) were included before deployment. Eleven cytokines and chemokines were measured in supernatants of T-cell mitogen-stimulated whole blood cultures by multiplex immunoassay. Factor analysis was used to identify clusters of cytokines and chemokines. In a regression analysis hostility was related to the cytokine/chemokine clusters, and the potential risk factors age, BMI, smoking, drinking, previous deployment, early life trauma and depression.

Explorative factor analysis showed four functional clusters; a pro-inflammatory factor (IL-2, TNFα, IFNγ), an anti-inflammatory factor (IL-4, IL-5, IL-10), IL-6/chemokine factor (IL-6, MCP-1, RANTES, IP-10), and MIF. Hostility was significantly related to decreased IL-6/chemokine secretion and increased pro- and anti-inflammatory cytokines. There was an inverse relation between age and hostility scores. Early life trauma and depression were positively and independently related to hostility as well.

This study represents a novel way of investigating the relation between cytokines and psychological characteristics. Cytokines/chemokines clustered into functional factors, which were related to hostility in healthy males. Moreover this relation appeared to be independent of reported depression and early trauma.

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Summary Hostility is a risk factor for adverse health outcomes as diverse as cardiovascular disease and post-traumatic stress disorder (PTSD). Cytokines have been suggested to mediate this relationship. We investigated whether in healthy men a relation existed between hostility and T-cell mitogen-induced cytokines and chemokines. Male Dutch military personnel (n = 304) were included before deployment. Eleven cytokines and chemokines were measured in supernatants of T-cell mitogen-stimulated whole blood cultures by multiplex immunoassay. Factor analysis was used to identify clusters of cytokines and chemokines. In a regression analysis hostility was related to the cytokine/chemokine clusters, and the potential risk factors age, BMI, smoking, drinking, previous deployment, early life trauma and depression.

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1. Introduction

Hostility has been identified as a risk factor for the development of adverse health outcomes as diverse as post-traumatic stress disorder (PTSD; Heinrichs et al., 2005; Ouimette

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Conflict, and the association with hostility has been examined by determining levels in serum or plasma, or the release of cytokines and chemokines by stimulated monocytes. Thus, the major focus has been on the relation between hostility and the innate immune system. To date, no studies have investigated whether hostility is related to the activity of the adaptive immune system, i.e., the release of cytokines and chemokines after stimulation of T-cells. Therefore, we investigated whether cytokine and chemokine release induced by stimulation of T-cells was related to hostility as measured by the Cook-Medley Hostility Scale (Barefoot et al., 1989). Since we investigated a cross-sectional sample we explored associations rather than a possible causal relationship.

We analyzed eleven different cytokines and chemokines in supernatants of whole blood cultures stimulated with a T-cell mitogen. The cytokines measured were the pro-inflammatory cytokines IL-2, IL-6, TNFα, and interferon gamma (IFNγ), and the anti-inflammatory cytokines IL-4, IL-5 and IL-10. In addition we measured the cytokines Monocyte Chemotactic Protein-1 (MCP-1, or CCL2), Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES, CCL5), as well as Interferon-γ-Induced Protein (IP-10, CXC10). Apart from these cytokines we also determined Macrophage Migration Inhibitory Factor (MIF), a cytokine with a key regulatory role in innate immunity, which promotes the pro-inflammatory function of immune cells (Calandra and Roger, 2003).

It is known that there is a high level of interrelation between cytokines and chemokines and that there is functional redundancy in the cytokine and chemokine system. If single cytokines/chemokines show a similar relation with the psychological variable, clustering them into a limited number of factors could strengthen the relation between these clusters and the psychological variable. Therefore, we first performed an explorative factor analysis to cluster the eleven different cytokines and chemokines into groups. The factor scores of these clusters were then used in a regression analysis to analyse their association with hostility.

We included healthy male military personnel within two months before deployment to Afghanistan. We controlled for the known health risk factors as age, BMI, smoking, drinking, and depression. Furthermore, pre-deployment PTSD-related risk factors like previous deployment, and score on an early life trauma inventory were included (Stein et al., 2005; Gahm et al., 2006; Nemeroff et al., 2006).

The goal of this study was first to explore whether multiple cytokines and chemokines would form functional clusters, and second to determine whether an association exists between these clusters and hostility scores in healthy males.

2. Methods

2.1. Participants

In The Netherlands a large prospective study has started in 2005. Biological and psychological variables are assessed in military personnel before and after deployment to Afghanistan for peace keeping missions. Data collection in this cross-sectional part of the study occurred between March 2005 and October 2006. 304 participants, mean age 30.7 years (S.D. = 10.0, range 18—55 years) were recruited to participate in this project in the training period before deployment. Information about the project was provided and participants signed an informed consent. In a morning session, the participants filled out the questionnaires, and blood was collected in heparinized vacu-tubes between 8.00 and 11.00 a.m. This project was approved by the medical ethical committee of the University Medical Center Utrecht.

2.2. Questionnaires

Demographic information on age, BMI, previous deployment (yes/no), marital status, education level, military rank, smoking, alcohol use and medication use were filled out. There was no separate information for income, but as income scales per rank are fixed, we used military rank as an indicator for income.

The total score on the Cook-Medley Hostility Scale 50 item true/false version was used to measure hostility (Cronbach alpha = 0.78; Barefoot et al., 1989).

General complaints were measured with the symptom checklist 90 (SCL90; Arrindel and Ettema, 1981), 90 items on a 5-point scale (Cronbach alpha = 0.94). The SCL90 subscale depression consists of 16 items, with an internal consistency of 0.77.
The short version of the 'Early Trauma Inventory' assesses traumatic experiences before the age of 18 in four domains on a 0–1 scale; general trauma (11 items), physical abuse (5 items), emotional abuse (5 items) and sexual abuse (6 items; Bremer et al., 2000). The overall consistency of the total score of this questionnaire was good, Cronbachs alpha = 0.76.

In a subgroup of the participants (n = 180), PTSD symptoms of the past four weeks were assessed with the Self-rating Inventory for Posttraumatic Stress Disorder list (‘SRIP’, 22 items, 1–4 scale, Cronbachs alpha = 0.88; Hovens et al., 1994). The total score (mean = 26.9, S.D. = 5.3) was not different from a group of predominantly male (92%) Dutch firefighters and policemen (n = 1168, mean = 27.2, S.D. = 6.2; t = 1.03, d.f. = 1375, p = n.s.; Witteveen et al., 2006). Due to its limited sample size the SRIP was not included in the regression analysis.

2.3. Leukocyte subpopulations

The distribution of subsets of T-cells, B-cells and NK-cells within the lymphocyte population was analyzed in heparinized whole blood using dual color fluorescence analysis with a Becton Dickinson Calibur flow cytometer. Whole blood was stained using monoclonal antibodies (Becton Dickinson, Belgium) labeled with either fluorescein isothiocyanate or phyco-erythrin to quantify CD3+ (total T), CD4+ (T-helper), CD8+ (T suppressor/cytotoxic), CD19+ (B), CD16+56+ (NK), and CD3+/CD16+CD56+ (NK-T) cells. Absolute numbers of cells were calculated from a total leukocyte count.

2.4. Cytokine production

T-cell mitogen-induced cytokine secretion was measured in supernatants of whole blood cultures diluted 1:10 with RPMI-1641 (Gibco, Grand Island, NY), 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM l-glutamine after stimulation with the mitogenic anti-CD2/CD28 monoclonal antibodies (CLB, Amsterdam, The Netherlands, final concentration anti-CD2.1/anti-CD2.2 0.33 µg/ml and anti-CD28 1.33 µg/ml) for 72 h at 37 °C/5% CO2 in 96 wells round-bottom plates. Supernatants were stored at −80 °C. Cytokine production was measured using multiplex analysis to assess several cytokines and chemokines in a single sample as described elsewhere (De Jager et al., 2003). We determined the following cytokines and chemokines IL-2, IL-4, IL-5, IL-6, TNFα, IFNγ, MIF, MCP-1 (CCL2), IP-10 (CXCL10), and RANTES (CCL5).

2.5. Explorative factor analysis

The 11 cytokines and chemokines were clustered into factors using principal axis factoring with oblique (oblimin) rotation in SPSS 12.0.1. In line with the known functional relations between cytokines, bivariate correlational analyses showed that values for many cytokines and chemokines are correlated. Therefore, we chose the oblique rotation, which permits correlation between the factors, rather than the often used varimax rotation, which does not allow correlation between the factors. The cytokines and chemokines were either not (MCP-1), log 10 (IL-2, IL-4, IL-5, IL-6, IL-10, MIF) or square-root (TNFα, IFNγ, RANTES, IP-10) transformed to obtain a normal distribution. The sample size for factor analysis was sufficiently large (n = 243; Tabachnick and Fidell, 2007). The factor loadings, the correlation between the factor and a variable, varied between 0.48 (IL-10) which is considered fair (20% overlapping variance), and 0.98 (IL-4), which is considered excellent (>50% overlapping variance). The lowest rotated factor loading was 0.30 for IP-10. Communalities were high, and the four factors accounted for 65% of the variance of the variables. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.725, which is considered good (Tabachnick and Fidell, 2007). Factors were based on examination of the screen plot and had initial eigenvalues >1.0. All (transformed) variables were used to calculate the regression factor scores which were then used in the regression analysis.

2.6. Linear regression analysis

SPSS 12.0.1 was used for linear regression analysis, with hostility as the dependent variable. To prevent overfitting of the model, the regression method ‘enter’ was used, which keeps all variables in the final model (Babyak, 2004). Before analysis the data were screened for the assumptions of multivariate analysis. The hostility scale showed a normal distribution. Exclusion of a single outlier with a total hostility score of 44 did not change the outcome of the analysis. Multivariate outliers and multicollinearity diagnostics indicated no cause for concern. A p-value of 0.05 was considered significant.

In the regression analysis the presence of a single missing value results in listwise exclusion of all data for that person. In total 3% (n = 10) of the immune data were consistently missing from individuals for whom questionnaire scores and demographic variables were available. The dataset also lacked data due to handling and non-response of some questionnaire items. These missing variables were missing completely at random, and accounted for the major part of the missing variables. After listwise deletion 227 cases remained in the regression analysis, or 75% of the original total dataset.

We explored whether missing data imputation could provide a tool for a more complete dataset. Multiple Imputation (Schafer and Graham, 2002; Donders et al., 2006) was used for imputing missing data with WinMICE (www.multiple-imputation.com). Compared to the incomplete dataset, the dataset with missing data imputation showed similar correlations between related variables (e.g. cytokines); however the correlations became less strong. Comparing the regression model of the dataset with missing variables (n = 227) to the dataset with imputed variables (n = 304), the latter model explained less variance and did not fit the data as well as the smaller dataset. The imputed model showed diluted variance, therefore we chose not to report the results of the complete, imputed dataset.

3. Results

In Table 1 a description of the group is shown. The BMI showed a normal distribution, with a range from 18.5 to 35.5; 8% had a BMI >30. Military rank had a significant and high correlation with age (r = 0.732), and we chose to include only age in the regression model. Alcohol use was dichotomized into either
less than 5 units/week (0) or greater than 5 units (1). Nine percent (n = 26) reported no alcohol use at all.

Table 2 summarizes the scores on the questionnaires. The hostility scale showed a normal distribution, with a mean score of 16.8 (S.D. = 6.7). The Early Trauma Inventory (ETI) and the depression scale of the SCL90 were extremely positively skewed; most men reported no complaints or very low scores. It was not possible to obtain a normal distribution by transforming the scores. Therefore, we chose to dichotomize the scales (MacCallum et al., 2002). After examination of the frequency curve of both scales, we chose an arbitrary cut-off point which separated the peak of the distribution (the low scores) from the rest of the scores (the higher scores). For the ETI total score the cut-off point was set at ≥7 (n = 58, 19.2%), and for the depression score it was set at ≥19 (n = 74, 24.4%). The dichotomized score was used in the regression analysis.

The immune parameters are shown in Table 3. Both mean and median scores are shown since the values for cytokine and chemokine production and number of blood cells are positively skewed. The distribution of leukocytes (WBC), HB, HCT, monocytes, granulocytes and lymphocytes are well within the 95% normal range. There is no known norm-score for the range of cytokines and chemokines.

3.1. Cytokine and chemokine clustering

To bundle the effect of several related cytokines and chemokines, they were clustered by factor analysis. Interestingly, this explorative analysis resulted in extraction of four factors which represent biologically functional clusters of mediators. The first factor that emerged was an anti-inflammatory factor, which included the three anti-inflammatory cytokines determined in our assay: IL-4, IL-5 and IL-10. Second the three pro-inflammatory cytokines IL-2, TNFa and IFNg clustered into the pro-inflammatory factor. The third factor was designated ‘IL-6/chemokine’, and consisted of IL-6, MCP-1, RANTES and to a lesser extent IP-10. Macrophage Migration Inhibitory Factor, or MIF, did not load well on the other factors, and we chose to include it in the further analysis as a separate factor. In Table 4 the β-values of the factor analysis are shown, which represent the relative proportion of explained variance controlled for the other variables. In line with its role as a pro-inflammatory cytokine, IL-6 loads on the pro-inflammatory factor as well. As we used an oblique rotation, correlations between factors were maintained. We observed a negative correlation between the pro and anti-inflammatory factor (r = −0.46), which is consistent in view of the known mutual inhibition of pro- and anti-inflammatory pathways.
inflammatory cytokine production. The pro-inflammatory cytokine factor had a positive correlation with the IL-6/chemokine factor ($r = 0.34$), and negative with MIF ($r = -0.16$). Furthermore, the IL-6/chemokines factor correlated negatively with MIF ($r = -0.24$). The anti-inflammatory factor correlated weakly with IL-6/chemokines and MIF ($r < 0.08$). The factor scores for each factor were used in the regression analysis.

### 3.2. Regression analysis relation cytokine factors with hostility

Before performing a regression analysis with hostility, we explored the correlation between the hostility score and the cytokine/chemokine factors. The anti-inflammatory factor and the IL-6/chemokine factor showed significant and opposite correlations with the hostility score. The anti-inflammatory factor correlated with the hostility score ($r = 0.20$), while the IL-6/chemokine factor correlated with the hostility score ($r = -0.24$). The factor scores for each factor were used in the regression analysis.

### Table 4 Factor analysis pattern matrix

<table>
<thead>
<tr>
<th>Factor</th>
<th>Anti-inflammatory</th>
<th>Pro-inflammatory</th>
<th>IL-6/chemokines</th>
<th>MIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>-0.986</td>
<td>-0.032</td>
<td>-0.138</td>
<td>-0.072</td>
</tr>
<tr>
<td>IL-5</td>
<td>-0.913</td>
<td>-0.093</td>
<td>0.123</td>
<td>-0.062</td>
</tr>
<tr>
<td>IL-10</td>
<td>-0.487</td>
<td>0.170</td>
<td>-0.031</td>
<td>0.120</td>
</tr>
<tr>
<td>IL-2</td>
<td>-0.222</td>
<td>0.586</td>
<td>-0.018</td>
<td>-0.011</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.020</td>
<td>0.774</td>
<td>0.053</td>
<td>-0.270</td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.004</td>
<td>0.925</td>
<td>0.019</td>
<td>0.075</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.059</td>
<td>0.390</td>
<td>0.708</td>
<td>0.123</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.072</td>
<td>-0.222</td>
<td>0.831</td>
<td>-0.052</td>
</tr>
<tr>
<td>RANTES</td>
<td>-0.038</td>
<td>0.300</td>
<td>0.489</td>
<td>-0.270</td>
</tr>
<tr>
<td>IP-10</td>
<td>-0.160</td>
<td>0.120</td>
<td>0.309</td>
<td>0.001</td>
</tr>
<tr>
<td>MIF</td>
<td>-0.021</td>
<td>0.043</td>
<td>-0.009</td>
<td>-0.794</td>
</tr>
</tbody>
</table>

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Table 5  Regression analysis of hostility

<table>
<thead>
<tr>
<th></th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adj. $R^2$</td>
<td>0.030</td>
<td>0.047</td>
<td>0.086</td>
<td>0.164</td>
<td>0.278</td>
</tr>
<tr>
<td>$\Delta R^2$% variance explained</td>
<td>3%</td>
<td>1.7%</td>
<td>3.9%</td>
<td>7.8%</td>
<td>11.4%</td>
</tr>
<tr>
<td>$F$ value</td>
<td>8.05**</td>
<td>6.61***</td>
<td>6.35***</td>
<td>6.53***</td>
<td>8.89***</td>
</tr>
<tr>
<td>$\Delta F$</td>
<td>5.02</td>
<td>5.80</td>
<td>6.13</td>
<td>12.44</td>
<td></td>
</tr>
</tbody>
</table>

Steps 1–3: factors

- IL-6 and chemokines
  - $\beta$-value: -0.0186
  - $t$-value: -2.838
- Anti-inflammatory
  - $\beta$-value: 0.146
  - $t$-value: 2.240
- Pro-inflammatory
  - $\beta$-value: 0.279
  - $t$-value: 3.402
- MIF
  - $\beta$-value: -0.006
  - $t$-value: -0.087

Step 4: demographic

- Age
  - $\beta$-value: -0.288
  - $t$-value: -4.004
- BMI
  - $\beta$-value: 0.111
  - $t$-value: 0.161
- Smoking Y/N
  - $\beta$-value: 0.076
  - $t$-value: 1.163
- Drinking Y/N
  - $\beta$-value: 0.064
  - $t$-value: 1.027

Step 5: covariates

- Previous deployment
  - $\beta$-value: -0.026
  - $t$-value: -0.410
- ETI high ($\geq 7$)
  - $\beta$-value: 0.254
  - $t$-value: 4.351
- Depression high ($\geq 19$)
  - $\beta$-value: 0.226
  - $t$-value: 3.941

<table>
<thead>
<tr>
<th></th>
<th>$\beta$-value</th>
<th>$t$-value</th>
<th>$\beta$-value</th>
<th>$t$-value</th>
<th>$\beta$-value</th>
<th>$t$-value</th>
<th>$\beta$-value</th>
<th>$t$-value</th>
<th>$\beta$-value</th>
<th>$t$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 and chemokines</td>
<td>-0.0186</td>
<td>-2.838**</td>
<td>-0.181</td>
<td>-2.782**</td>
<td>-0.297</td>
<td>-3.973***</td>
<td>-0.258</td>
<td>-3.506***</td>
<td>-0.292</td>
<td>-4.223***</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>0.146</td>
<td>2.240</td>
<td>0.275</td>
<td>3.706***</td>
<td>0.173</td>
<td>2.194**</td>
<td>0.196</td>
<td>2.652**</td>
<td>0.261</td>
<td>3.387**</td>
</tr>
<tr>
<td>Pro-inflammatory</td>
<td>0.279</td>
<td>3.402***</td>
<td>0.221</td>
<td>2.761**</td>
<td>0.235</td>
<td>3.149**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIF</td>
<td>-0.006</td>
<td>-0.087</td>
<td>-0.027</td>
<td>-0.418</td>
<td>-0.029</td>
<td>-0.463</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- $p < .05$
- $p < .01$
- $p < .001$
- $p < .0001$

Linear regression analysis was used to determine whether the variance in hostility score could be explained by cytokine and chemokine production, while controlling for age, BMI, smoking, drinking, previous deployment, early trauma and depression. To determine the relative contribution of each factor to hostility, the factors were introduced step-wise. The results are shown in Table 5. In the first step, the IL-6/chemokine factor was introduced. A lower IL-6/chemokine level is related to increased hostility, which explained 3.0% of the variance in hostility score. Subsequent introduction of the anti-inflammatory factor revealed that an increased anti-inflammatory cytokine level was related to a high hostility score. The relative contribution of each variable in the model was determined by comparing the standardized coefficient $\beta$ ($\beta$-values). When comparing the $\beta$-values of the IL-6/chemokine between step 1 and step 2, the value did not change significantly, showing that both factors have an independent relation with hostility.

In step 3 the pro-inflammatory factor and MIF were introduced. Interestingly, the pro-inflammatory factor showed a significant positive relation with hostility as well. A higher hostility score was related to a higher pro-inflammatory cytokine level.

The model with all four cytokine and chemokine factors explained 8.6% of the variance in hostility score. Introduction of the pro-inflammatory cytokine factor increased the $\beta$-values of both the IL-6/chemokine and the anti-inflammatory factor. While the pro-inflammatory factor is not significantly correlated with hostility by itself, it does show a significant relation with hostility in the model. This effect is known as ‘suppression’ (Tabachnick and Fidell, 2007). The relation between the pro-inflammatory factor with the anti-inflammatory factor and the IL-6/chemokine factor ‘suppresses’ unexplained variance, which increases the relative relation of the variables with hostility. Vice versa both the IL-6/chemokine and the anti-inflammatory factor each suppress unexplained variance of the relation between the pro-inflammatory factor and hostility. It is likely that the non-significant correlation between the pro-inflammatory factors with hostility is obscured by the opposite correlation of the IL-6/chemokine factor and the anti-inflammatory factor with the pro-inflammatory factor.

After introducing the demographic (age, BMI) and health risk (smoking, drinking) variables, the model explained 15.4% of the variation in the hostility score. Age was significant and negatively related to hostility.

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In the final step of the regression analysis the binary variables previous deployment, early life trauma and depression were introduced (Table 5). Early life trauma and depression had a significant and positive relation with hostility. Being previously deployed was not related to hostility in the model. Persons reporting more early life trauma and depression were more hostile.

In the final model the three cytokine/chemokine factors showed significant relation with the hostility score. A lower IL-6/chemokine level was related to a higher hostility score. On the other hand the anti-inflammatory cytokine factor and the pro-inflammatory cytokine factor had a positive relation with the hostility score. An increased level of pro- and anti-inflammatory cytokines is related with a higher hostility score. This effect was stronger for the pro-inflammatory cytokines compared to the anti-inflammatory cytokines. The fourth factor, MIF had no relation with hostility.

3.3. Regression controlling for number of T-cells

Since we determined cytokine and chemokine production after stimulation with a T-cell mitogen, differences in number of T-cells could have affected the results. The regression model with hostility as dependent variable was controlled for the number of T-cells (log 10 #CD3+). The number of T-cells had no significant relation with hostility and controlling for this variable did not affect outcome of the regression model (data not shown).

4. Discussion

This is the first report describing a significant relation between stimulated T-cell cytokines/chemokines and hostility scores in a large group of healthy males. Explorative factor analysis revealed functional clusters of anti-inflammatory, pro-inflammatory, IL-6/chemokine and the cytokine factor MIF. Regression analysis showed that hostility was significantly and negatively related to IL-6/chemokine, and positively to both pro- and anti-inflammatory cytokine release. In other words; an increased hostility score is related to an overall decreased in IL-6/chemokines release and an increased release of pro- as well as anti-inflammatory cytokines. The relation remained intact after controlling for the significant confounders age, early life trauma, and depression.

Studies on the relation between hostility and cytokines have predominantly focused on the innate immune system. These studies observed increased pro-inflammatory cytokine levels (IL-6, TNFα) in relation to higher hostility scores (Suarez et al., 2002; Miller et al., 2003; Suarez, 2003; Kiecolt-Glaser et al., 2005; Sjogren et al., 2006). Our findings add to this field, and show that the pro- and anti-inflammatory cytokine profile after stimulation of the adaptive immune system was positively related to hostility. On the other hand, the IL-6/chemokine factor shows a negative relation with hostility. At a first glance, the observation that both pro- and anti-inflammatory cytokines are positively related to hostility is remarkable. It should be noted, however, that in a simple correlational analysis hostility was positively related to the anti-inflammatory factor and negatively to the IL-6/chemokine factor, but not to the pro-inflammatory factor. Moreover, the pro- and anti-inflammatory cytokine factors have a negative correlation of $r = -0.46$, and IL-6 loads on the pro-inflammatory factor as well. Such negative correlation between pro- and anti-inflammatory cytokines can be well understood in the physiological context, as it is known that production of pro-inflammatory cytokines can inhibit anti-inflammatory cytokines and vice versa. In addition, IL-6 has pro-inflammatory properties. In our complete model, we observed a positive correlation of both the pro- and anti-inflammatory cytokine factor with hostility, but this relation only became apparent after all factors were included in the model. We have interpreted these data as being due to ‘suppression’, the correlations among the factors ‘suppresses’ unexplained variance with hostility.

There are few studies which have investigated the relation between hostility and the adaptive immune system. Kemeny and colleagues found that, averaged over a six-month period, high hostility was related to a lower proportion of suppressor/cytotoxic T-cells (CD8+), but not T-helper cells (CD4+; Kemeny et al., 1989). Acute hostile marital interactions were related to increases in the number of total T-cells (CD3+) and T-helper cells (CD4+), and a decrease in T-cell function (Kiecolt-Glaser et al., 1993). However, hostility was not related to an acute stress induced increase in T lymphocytes (Mills et al., 1996). These results are difficult to extrapolate to the results observed in our study, especially since we have used a different read out, i.e. a broad spectrum of cytokines and chemokines released after T-cell stimulation rather than circulating cell numbers or T-cell proliferation.

We studied healthy male military personnel before deployment. These individuals form a homogenous group at the time of sampling to the extent that they were exposed to similar environmental factors such as training schedule, food, and vaccination. Moreover they were relatively homogenous with respect to age, BMI, education, SES, and health. A relatively large proportion (27.7%) of the total variance in hostility was explained, of which 8.6% was accounted for by the cytokine factors. This is promising for confirming these findings in a smaller sample size, if participants are chosen on the extreme ends of the hostility scale.

This is the first study to our knowledge that uses factor analysis on such a broad range of cytokines and chemokines to aggregate them into a smaller number of factors. It is important to note that the factors that emerged after factor analysis represent functional clusters of cytokines. Whole blood cultures were stimulated with a T-cell mitogen, which increases the release of several cytokines and chemokines. These, in turn, can affect the release of cytokines and chemokines by other cell types and also regulate the production of these factors by the T-cells themselves. Therefore, it is quite remarkable that functional clusters emerged. One cluster that emerged from the factor analysis consisted of the Th1-type pro-inflammatory cytokines IL-2, TNFα, and IFNγ. On the other side the Th2-type anti-inflammatory cytokines IL-4, IL-5 and IL-10 formed a cluster. The anti-inflammatory factor showed negative correlation with the pro-inflammatory, which is in accordance with the notion that pro-inflammatory cytokines or Th1 type cytokines inhibit production of Th2 or anti-inflammatory cytokines (Elenkov et al., 2005). The third factor was IL-6 with the chemokines MCP-1, RANTES...
and IP-10. IL-6 formed a separate factor with the chemokines, though IL-6 had a factor loading on the pro-inflammatory cytokine factor as well. It is known that while IL-6 has pro-inflammatory properties, it is also has a broad range of other activities, which may explain why it did load on two different factors (for review see Pedersen, 2007). Other studies have found similar results; Chan et al. (2002) and Koukkunen et al. (2001) have used factor analysis and were unable to group IL-6 and TNFα in the same factor. Macrophage Migration Inhibitory Factor formed a factor on its own. MIF is a regulator of both the innate and the adaptive immune response, and is critically involved in inflammation. Functionally it is not unexpected that MIF is loading as a separate factor, since MIF can function not only as a cytokine, but also has additional biological effects, e.g. it can act as a counter-regulator of glucocorticoid action (Calandra and Roger, 2003).

A limitation of this study is the fact that the factor analysis was explorative, and the results need to be replicated in a new sample to confirm the present findings. We had no a priori assumptions to what the outcome would be, and the clustering was based on observation of the factor analysis results. However, factor loadings were high for the anti- and pro-inflammatory variables, and IL-6/chemokine and MIF formed distinct factors as well. Moreover, the factors that emerged reflected biologically functional groups, which adds to our confidence that the clusters that were formed represent true meaningful groups, instead of chance findings. MIF did not fit well with any of the other factors and we chose to fit it in a separate factor rather than force it in with another factor or leave it out.

Both factor analysis and multiple cytokine determination in a single sample via multiplex analysis have been used predominantly in cardiovascular research. Martins et al. (2006) performed a multiplex cytokine assay of 8 plasma cytokines. Coronary artery disease was positively related to IL-2, IL-4, IL-6, IL-12 and IL-18, however factor analysis was not used to confirm the a priori group assignment of the cytokines (Martins et al., 2006). Tziakas et al. (2007) used factor analysis in 320 patients with acute coronary syndrome. Three clusters were identified; a “systemic inflammation” cluster with CRP and fibrinogen, a “local inflammation-endothelial dysfunction” cluster with IL-18 and ICAM-1 and an “anti-inflammation” cluster comprising IL-10 and HDL cholesterol. The anti-inflammatory cluster was a significant predictor of adverse cardiac events (Tziakas et al., 2007). In the study of Chan et al. (2002) factor analysis in a group of 107 persons revealed 6 factors related to components of the metabolic syndrome, a risk factor for CVD. The cytokines IL-6 and TNFα were independently and significantly related to several metabolic syndrome related factors. These studies further underline that a combination of multiple cytokine determinations and factor analysis, as used in our study, can be a valid tool to improve our understanding of the relation between psychological factors, cytokine/chemokines and health.

As hostility is a personality factor, a stable trait, it can be suggested that the observed relations with cytokine/chemokine release represent an intrinsic relation. This could render persons with a high hostile profile more vulnerable to adverse health related outcomes as their adaptive immune system responds in a different manner than in persons with a low hostile profile. Indeed it has been found that hostility is a risk factor for adverse health outcomes like PTSD (Quinette et al., 2004; Heinrichs et al., 2005; Orth and Wieland, 2006), atherosclerosis and CVD (Siegmam et al., 2000; Kop, 2003; Everson-Rose and Lewis, 2005; Bunde and Suls, 2006), and disturbed immune functions have been described for adverse health outcomes as diverse as autoimmune diseases, infectious diseases, cardiovascular disease, and psychosomatic conditions like depression and PTSD, presumably mediated by stress (Everson-Rose and Lewis, 2005; Kemeny and Schedlowski, 2007; Kendall-Tackett, 2007).

We have analyzed a cross-sectional dataset, which does not give us any information on the causality of our findings. The studied group will be followed after deployment, which provides a chance to determine whether the relation between hostility and cytokines before deployment as we describe it in this study is related to adverse health outcomes.

The relation between cytokine factors and hostility we report here remained intact after controlling for age, BMI, smoking, drinking, and previous deployment, early life trauma and depression. It has been suggested before that hostility is related to CVD via risk factors as BMI, smoking and drinking (Bunde and Suls, 2006). Hostility has been related to PTSD risk factors as early trauma and depression (Brewin et al., 2000; Stein et al., 2005; Gahm et al., 2006), which is confirmed in our study. Depression has been related to pro-inflammatory cytokines like IL-6 and TNFα (Elenkov et al., 2005; Sjogren et al., 2006). When controlling for depression and early life trauma the relation between cytokines and hostility increased slightly (the standardized coefficient β increased), but the larger part of the relation remained unaffected. This suggests first of all an independent underlying mechanism in the relation between cytokines and hostility on the one hand and hostility and depression or early trauma on the other hand. Our depression and early trauma scores were arbitrarily divided in two, and do not represent clinical scores. Nevertheless, it can be hypothesized that an increased depression or early trauma score may enhance the relationship between hostility and cytokines/chemokines factors. This is in line with the study of Suarez and colleagues, who observed an additional positive effect of the combination of high hostility and high depression scores in relation to plasma IL-6 levels (Suarez, 2003). Miller et al. (2003) on the other hand rather observed a relation between plasma IL-6 and high hostility, but low depression. We can, however, not directly compare our data to these studies since we used ex vivo cytokine release rather than plasma cytokines and since scores on hostility and depression were lower in our sample.

The implications of our findings may become apparent in our follow-up studies when all the analyses will be repeated after deployment of the military personnel in Afghanistan. Our results imply an intrinsic relation between cytokines and hostility, which may be part of a preexisting vulnerability of hostile persons. Therefore, based on our findings and previous research we predict that higher levels of hostility and T-cell cytokine release before deployment are related to the development of adverse health outcomes.

In conclusion T-cell mitogen-induced cytokines and chemokines formed functional clusters as identified by factor analysis. These factors were significantly related to hostility scores in healthy men.
Hostility and stimulated T-cell cytokines

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Conflict of interest

The authors Eric Vermetten and Elbert Geuze are employed by the Ministry of Defense of The Netherlands. All other authors are employed by the University Medical Center Utrecht. All authors declare that they have no conflicts of interest.

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