Abstract.

The relationship between chronic professional stress in nurses and immunity as well as the possible impact of psychopathology upon this relationship have been examined. Sixty subjects were selected on the basis of high/low scores on professional stress and psychopathology. Chronic professional stress appeared to be associated with immune dysfunction including signs of immune activation (increased numbers of cells expressing the interleukin-2 receptor especially CD4+CD25+ cells) and possibly immune suppression (decrease in percentage of natural killer cells). The increase in activation markers, CD3+CD16CD56+ cells and serum neopterin was most pronounced in the group with high stress/low psychopathology whereas the decrease in CD8+CDIIb+ cells was most pronounced in the group with high stress/high psychopathology. It is hypothesized that in the presence of chronic stress distinct psychological mechanisms are associated with specific immune dysfunctions.

Key words : natural killer cells, immune activation, interleukin 2, neopterin.

1. Introduction

A growing body of literature has examined the relationship between (di)stress and immunity. Research in the field of psychoneuroimmunology has revealed that acute stress as well as chronic stress in humans are associated with a reduction in cellular immunity (Kiecolt-Glaser and Glaser, 1989). A number of studies have examined the impact of professional stress upon immunity (Endresen et al., 1987; Theorell et al., 1990; Endresen et al., 1991). These studies were mainly concerned with measures of humoral immunity whereas only a small number of measures of cellular immunity were studied.
The present study intends to determine whether immune parameters discriminate between subjects that experience high professional stress and subjects experiencing low professional stress. In addition this study aims to evaluate if and to what extent psychopathology influences the impact of stress on immunity.

2. Methods

2.1. Subjects

The population that was studied consisted of 511 nurses working at a university hospital. They had to fill out a number of questionnaires regarding professional stress, psychopathology and health. A total number of 405 questionnaires were received which corresponds to a response rate of 79 %. Of these, 22 could not be taken into consideration : 6 were filled out incompletely and 16 had to be discarded on the basis of the exclusion criteria, namely the presence of an organic disorder involving the immune system or a psychiatric disorder (e.g. major depression, bipolar disorder, ...). This information was gathered by self-rated questionnaire. The total number of subjects that were finally included in the immunological study was 383.

2.2. Questionnaires

The two questionnaires that were used in the immunologic study were : the Nurse Stress Index (N.S.I.) (Harris, 1989) measuring professional stress in nurses and the Dutch adaptation of the Symptom Checklist 90-R (S.C.L. 90-R) (Arrindell and Ettema, 1986) measuring psychopathology.

2.3. Formation of extreme groups

In order to look at immunological differences between nurses experiencing high stress and nurses experiencing little stress and to evaluate to what extent psychopathology influences the relation between stress and immunity, extreme groups were formed on the basis of the total score on the N.S.I. and the S.C.L.90-R. Because neither the scores on the N.S.I. nor the scores on the S.C.L. 90-R were normally distributed, a Blom transformation (Norusis, M.J., 1993) was used in order to normalize data. The normalized scores that result from Blom transformation range from -3 to +3. In order to create a high and low stress group and a high and low psychopathology group, cut-off scores of +.75 and -.75 were used. Finally, four extreme groups were formed, combining high and low stress and psychopathology groups.
The extreme groups were defined in the following way : group 1 = high stress / high psychopathology (n = 35); group 2 = low stress / low psychopathology (n = 44); group 3 = high stress / low psychopathology (n = 8) and finally group 4 = low stress / high psychopathology, a group that contained no subjects. Of the 87 subjects in the extreme groups, 27 had to be discarded for blood sampling : 4 refused to participate whereas 23 were excluded for various practical reasons such as pregnancy, working night shifts only and being on sick leave at the time of blood sampling. Blood was finally sampled in a total number of 60 subjects of which 25 belonged to group 1, 28 to group 2 and 7 to group 3. Mean age of these 60 subjects was 34.34 (SD = 6.21) ; the majority were women (43 women/17 men) as could be expected in a nursing population. There were no significant differences with respect to age or sex between the group of 87 and the group of 60 subjects. There were also no significant differences with respect to age or sex between the 511 nurses initially addressed and the 383 subjects taken into account for the formation of extreme groups (data not shown). Because the first objective of the study was to examine if there existed immunological differences between highly stressed nurses and nurses experiencing little professional stress, regardless of their level of psychopathology, we created a high stress group by adding up group 1 and group 3 (= group 1.3 consisting of 32 subjects).

2.4. Procedures

2.4.1. Blood sampling and immune phenotyping

In order to reduce diurnal variability, blood was sampled between 7:00 and 8:00 AM. Blood samples were always drawn on the second day of at least 3 subsequent morning shifts. Anticoagulated blood (EDTA) was drawn and used for white blood cell enumeration, differential counts (H1 @, Technicon, Belgium) and flow cytometric studies. Lymphocyte populations were analysed with dual colour direct

immunofluorescence on a Facscan @ (Becton Dickinson, San Jose, CA) flow cytometer using the "CONSORT 30" computer software after whole-blood lysis.
Briefly, 100 p1 of whole blood was incubated with the appropriate combination of monoclonal antibodies (see further) for 25 minutes at 4°C. Subsequently the red cells were lysed using lysis buffer (Becton Dickinson) for 7 minutes, spun down and washed once with 2 ml phosphate buffered saline (PBS). After resuspension in PBS the cells were immediately analysed. The following commercially available fluorescein isothiocyanate (FITC) or phycoerythrin (PE) monoclonal antibodies were selected : Leu4-FITC (CD3), Leu 3a-FITC(CD4) and T8-FITC (CD8) were combined with HLA DR-PE, to recognise activated T cells as well as activated T cell subtypes ; Leu 4-FITC (CD3) was combined with Leu 11-PE (CD16) and Leu 19-PE (CD56) to determine the total number of CD3- natural killer (NK) cells and CD3+ large granular lymphocytes ; Leu 3a - FITC (CD4) or helper/inducer T cells were combined with Leu 18-PE (CD45RA) to measure virgin CD4 cells ; Leu 5b-FITC (CD2) or sheep erythrocyte receptor-bearing cells, Leu 4-FITC (CD3), Leu3a-FITC (CD4) and T8- FITC (CD8) were combined with IL2R1-PE (CD25) to recognise activated cells (T + NK), activated T cells, activated T helper/inducer cells and activated cytotoxic/suppressor T cells ; T8-FITC (CD8) cells were combined with Leu 15-PE (CD IIb). Finally, Leu 12-FITC (CD19) was combined with Leu 16-PE (CD20) to measure the mature B cells.
Lymphocyte gates were confirmed by the anti-CD45 FITC anti-CD 14 PE antibody combination and non-specific binding of monoclonal antibodies was in each case assessed by labeling cells with FITC or PE isotype matched control mouse Ig's. All reagents were obtained from Becton-Dickinson. Estimates of the absolute numbers of the lymphocyte cell populations that were positive or negative for the respective surface markers were determined by multiplying peripheral lymphocyte counts by the percentage of each positive or negative surface marker.

2.4.2. Soluble immune mediators and acute phase proteins
The enzyme-linked immunosorbent assay (ELISA) reagents for interleukin (IL)-2, IL-6, IL-2 receptor and IL-6 receptor measurements were obtained from Biosource (Fleurus, Belgium). Serum samples and standard dilutions of a specific cytokine or cytokine receptor were analysed according to the manufacturer's instructions. Each immunoassay has demonstrated no measurable cross-reactivity to other assayed cytokines as determined by the manufacturer. C-reactive protein (CRP) and haptoglobin were measured by nephelometry (Behring, Marburg, Germany). Neopterin levels were determined by Elisa (ICN, Asse, Belgium).

2.4.3. Statistical analyses
Statistical analyses were performed with SPSS for windows release 6.1. T -tests were used for the comparisons between groups. For those variables with a departure from normality as proven with the Kolmogoroff-Smirnov test we used a logarithmic transformation. The statistical tests were all two-tailed and were performed at the 5 % level of significance.

3. Results
There were no significant differences in age or sex between group 1.3 (high stress) and group 2 (low stress) (data not shown). The differences in immunological measures between group 1.3 and 2 are presented in table 1. A significantly higher number of activated T-helper cells (CD4+CD25+), was found in the high stress group (1.3). The percentage of NK cells on the other hand appeared to be significantly lower in the high stress group when compared to the group reporting low stress levels.

(Table 1 about here)
In order to analyse the contribution of psychopathology to the effect of stress upon immunity we compared the immune data between the three extreme groups described earlier (table 3). Mean age and sex distribution between these three groups, are shown in table 2. There were no significant differences with respect to age or sex between these three groups.

(Table 2 about here)
When we compared group 1 (high stress/high psychopathology) to group 2 (low stress/low psychopathology) the results were largely in the same direction as the ones reported in table 1 but significances disappeared. There was however one exception to this : the percentage of CD8+CDI lb+ cells was significantly lower in group 1 than in group 2. When we compared group 1 with group 3 (high stress/low psychopathology), only the serum level of interleukin-2 was significantly higher in group 3. The comparison between group 2 and group 3 revealed CD4+CD25+ cells, CD3+CD16CD56+ cells and serum neopterin to be significantly higher in the group reporting high professional stress (both groups do not differ with respect to the dimension of psychopathology which is low in both cases).

(Table 3 about here)
We did not find any significant differences between groups with respect to CD3+, CD4+ and CD8+ cells, CD2+CD25+ cells, CD8+ activated cells (CD25+ / HLA DR+), CD4+ HLA DR+ cells, CD4+CD45RA+ and RA- cells, CD19+CD20+ cells, CD8+CD1lb- cells, **CRP, haptoglobin, IL-6,** IL-6 receptor and IL-2 receptor.

4. Discussion.
The first aim of this study was to assess the effect of chronic professional stress on immunity. According to the present study, the subjective report of high professional stress is associated with signs of systemic immune activation and possibly of immune suppression. The activity of the NK cells was not assessed in the present study so that no firm conclusion about immune suppression as a result of this type of stressor can be made.
The percentage of NK cells was significantly decreased in the high stress condition when compared to the low stress condition. This is in line with the existing literature on stress (meta-analysis in Gerits, 1996). The immune activation that was found however is in marked contrast with most of the studies on stress and immunity in which immune suppression was demonstrated. However, most early and some recent studies did not assess lymphocyte membrane activation markers. There is one recent study (Dekaris et al, 1993), assessing immune reactivity in men just released from a war prisoner camp in Bosnia, that reported a higher number and percentage of activated T cells in former war prisoners when compared to healthy controls. However, results are not fully similar since an increase in the percentage of CD8+ cells and a decrease in the percentage of CD4+ cells was reported in that study which is opposite to the findings of this study. To our knowledge, this is the first study in chronic stress in humans to report an increase in the number of activated CD4+T-lymphocytes. The immune effects of stress were not secundary to the possible immune effects of alcohol, tobacco or coffee since the use of these products did not differ among groups (data not shown).
Immune suppression has been repeatedly demonstrated in major depression (metaanalytical review Herbert and Cohen, 1993). Since we have excluded subjects with a current major depression, the reduction in NK cell percentage found here can not be attributed to depression. This is in contrast with findings in bereavement and caregiving to dementia patients, since a reduction in respectively NK cell activity, NK cell number was associated with depression in these stress conditions (Zisook et al., 1994 ; Castle et al., 1995).
In the literature on depression there is some evidence for an immune activation alongside the immune suppression. In a series of studies the group of Maes et al. reported an increase in the number and percentage of activated T lymphocytes in depression (review Maes, 1995). These studies have found an increased expression of both the CD25+ and the HLA DR+ activation marker whereas we only found an increased expression of the CD25+ activation marker. However, the subtype of activated T lymphocytes has not been assessed in major depression.

The second aim of the present study was to assess the possible mediating effect of psychopathology upon the relationship between stress and immunity. Interestingly, immune activation (activated CD4+cells, neopterin) and the increase of the CD3+CD 16CD56+ subset was stronger in/restricted to the group with a high level of stress and a low level of psychopathology while the decrease in the suppressor subset (CD8+CD1lb+) was restricted to the group with a high level of both stress and psychopathology. This last finding possibly points in the direction of an auto-immune process. Both in the literature on depression and the chronic fatigue syndrome we can find anomalies pointing towards a possible auto-immune process (Maes et al., 1993; Strober, 1994). The serum level of interleukin-2 was higher in the high stress/low psychopathology group than in the high stress/high psychopathology group. The fact that immune activation apparently is stronger in the group with high levels of stress but a low degree of psychopathology is somewhat in contrast with the literature on depression and immune activation markers. Some authors (Dunbar et al, 1992; Maes et al., 1994; Maes, 1995; Sluzewska et al., 1996) have found evidence suggestive of an inflammatory response in major depression characterized by a systemic immune activation also involving an increased production of a number of interleukins, increased plasma levels of positive acute phase proteins and of neopterin.
One possible explanation for this apparent contradiction is that in the presence of chronic stress the defense against the expression and/or conscious experience of painful affects such as anxiety, depression and possibly anger is associated with immune activation. This could be the result of specific coping and/or personality styles. It is also possible that distinct immune parameters are differentially related to several psychological dimensions. However, the aforementioned results should be interpreted with caution because of the low number of subjects in the group with high stress levels and a low degree of psychopathology.

Several of the immune abnormalities reported in the high stress conditions, such as a high number and percentage of CD3+CD16CD56+ cytotoxic cells, a low percentage of CD8+CDl l b+ suppressor cells and a high number of activated T cells were also reported in some but not all studies in the chronic fatigue syndrome (CFS) (review Strober, 1994).

However, in CFS, CD8+ instead of CD4+ cells were reported to be activated. It can be concluded that the type of immune dysfunction reported in the present study appears to be similar but not identical to anomalies reported in pathological conditions such as chronic fatigue syndrome or major depression.

The finding of a significantly higher level of serum neopterin in the group reporting high levels of professional stress but a low level of psychopathology when compared to the group reporting low levels of both, is in contrast with a study by Dunbar et al. (1993) showing, under conditions of acute examination stress, a decrease in urinary neopterin `with the lowest level of neopterin coinciding with the highest level of subjectively experienced stress'. The level of serum interleukin-2 was significantly lower in the high stress/high psychopathology condition when compared to the high stress/low psychopathology condition in the present study. This is somewhat in line with Zorilla et al. (1994) who reported that in a nonpsychiatric population subclinical levels of psychological distress are associated with lower levels of circulating interleukin-2.
The present study has to be considered as an exploratorary study in need of replication. Therefore, we did not use Bonferroni corrections and did not correct for possible type 1 errors.
In conclusion, chronic professional stress in nurses appears to be associated with immune dysfunction including signs of immune activation. Mediating psychological mechanisms need further investigation. Our preliminary data suggest that distinct psychological mechanisms in the presence of stress are associated with specific immune dysfunctions.

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Table 1. t-tests of differences in immune measures between group 1.3 (high stress) and group 2 (low stress)

Immunological variably	Croup 1.3 (n - = 32) M (SD)	Group 2 (n .._ 28) m (S U)	t - statistic		(If p - value
CD25+ (number)	364.41 (148.47)	292.54 (117.13)	2.09	57.43	< 0.05
CD25+ (%)	15.56 (5.06)	13.65 (4.59)	1.53	58	NS
CD3+CD25+ (number) L	2.28 (0.24)	2.19 (0.15)	1.96	52.79	NS
CD3+CD25+ (%) L	0.93 (0.20)	0.86 (0.12)	1.49	58	NS
CD3+CD16CD56+(number)	97.56 (70.81)	66.25 (48.13)	1.97	58	NS
CD3+CD16CD56+ (%) L	0.50 (0.36)	0.38 (0.29)	1.42	58	NS
CD3-CD16CD56+ (number)	231.47 (154.65)	260.89 (136.66)	-0.78	58	NS
CD3-CD16CD56+ (%)	9.43 (4.69)	12.16 (5.70)	-2.03	58	< 0.05
CD4+CD25+ (number)	280.44 (119.43)	223.46 (91.02)	2.09	56.99	< 0.05
CD4+CD25+ (%)	11.98 (4.13)	10.50 (4.07)	1.40	58	NS
CD8+CDIlb+ (number)	131.06 (82.28)	145.71 (67.43)	-0.75	58	NS
CD8+CDIlb+ (%)	5.43 (2.92)	6.76 (2.53)	-1.88	58	NS
IL-2 L	-0.40 (0.30)	-0.39 (0.39)	-0.12	58	NS
Neopterin	2.53 (0.52)	2.46 (0.43)	0.57	58	NS

CD25+ = activated cells
CD3+CD25+ = activated T-cells
CD3+CD 16CD56+ = subset Cytotoxic T-cells
CD3-CD 16CD56+ = NK-cells
CD4+CD25+ = activated Helper/Inducer T-cells
CD8+CD1lb+ = Suppressor T cells

L = logarithmic transformation

Table 2. Age and sex distribution for group 1 (high stress & high psychopathology), group 2 (low stress & low psychopathology) and group 3 (high stress & low psychopathology)

	Group 1 (n -'2-5)	Group 2 (ti 28)	Group 3 (n 7)
Age '	34.80 (7.46)	34.21 (5.22)	33.00 (5.40)
Sex b			
Female	18	21	4
Male	7	7	3

'Mean (standard deviation) b Frequency

Table 3. t - tests of differences in immune measures between group 1 (high stress & high psychopathology), group 2 (low stress & low psychopathology) and group 3 (high stress & low psychopathology)

Immunological variable	Croup I (n = 25) M (SD)	Group 2 (n = 28) lwl (SIB)	Group 3 (n = 7) M(SD)
CD25+ (number)	352.72 (145.97)	292.54 (117.13)	406.14 (161.41)
CD25+ (%)	15.13 (4.44)	13.65 (4.59)	17.10 (7.05)
CD3+CD25+ (number) L	2.27 (0.25)	2.19 (0.15)	2.34 (0.19)
CD3+CD25+ (%) L	0.92 (0.20)	0.86 (0.12)	0.97 (0.19)
CD3+CD16CD56+(number)	91.48 (72.59)	66.25 (48.13)	119.29 (64.22)
CD3+CD16CD56+ (%) L	0.46 (0.36)	0.38 (0.29)	0.64 (0.30)
CD3-CD16CD56+ (number)	226.76 (161.03)	260.89 (136.66)	248.29 (139.35)
CD3-CD 16CD56+ (%a)	9.43 (5.05)	12.16 (5.70)	9.43 (3.41)
CD4+CD25+ (number)	270.52 (115.36)	223.46 (91.02)	315.86 (136.34)
CD4+CD25+ (%)	11.61 (3.51)	10.50 (4.07)	13.30 (6.01)
CD8+CDIlb+ (number)	119.40 (68.04)	145.71 (67.43)	172.71 (117.76)
CD8+CD1lb+ (%)	4.97 (2.18) ~	6.76 (2.53) ~ *	7.07 (4.57)
IL-2 L	-0.47 (0.25)	-0.39 (0.39)	-0.17 (0.36)
Neopterin	2.44 (0.52)	2.46 (0.43)	2.84 (0.45)

CD25+ = activated cells
CD3+CD25+ = activated T-cells
CD3+CD 16CD56+ = subset Cytotoxic T-cells
CD3-CD16CD56+ = NK-cells
CD4+CD25+ = activated Helper/Inducer T-cells
CD8+CD1lb+ = Suppressor T cells

logarithmic transformation

$p < 0.05$ $p < 0.01$