Increase of NK–T Cells in Aged Depressed Patients Not Treated with Antidepressive Drugs

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Background: A change in number and/or activity of natural killer cells has repeatedly been reported in depressive illness. Much less attention has yet been given to the subgroup of natural killer cells that are positive for the T-cell marker CD3 (NK–T cells). These cells possibly have important immunoregulatory properties.

Methods: We compared number and percentage of NK–T cells (defined as CD3^+ and CD16^+ and/or CD56^+ by two-color flow cytometry) in the peripheral blood of control subjects and two groups of elderly depressive subjects using or not using antidepressive drugs.

Results: The number and percentage of NK–T cells were strongly elevated in elderly depressive subjects not using antidepressive drugs, as compared with control subjects and elderly depressive subjects using antidepressive drugs.

Conclusions: Depressive illness in a geriatric population is associated with a substantial increase of NK–T cells. This increase was absent in a depressive group using antidepressive drugs.

Key Words: Major depression, minor depression, immunology, NK–T cells, antidepressive drugs, aging

Introduction

In depressive illness a wide variety of disturbances in immunologic parameters have been reported, suggesting a shift in cytokine-regulated immune response, possibly of viral or autoimmunologic origin (Herbert and Cohen 1993; Maes 1995). Amongst these, changes in number and activity of natural killer (NK) cells have repeatedly been reported, though the direction of these changes was not always consistent (Frank et al 1999; Ravindran et al 1998). Part of the discrepancies may possibly be explained by differences in the definition of the NK cell. In addition to the non-T (CD3^-) NK cell, this definition in some articles also includes cells with properties of both the T cell (CD3^+) and the NK cell. This latter group of so-called NK–T cells (Musha et al 1998) has been investigated in groups of subjects with minor, major, or melancholic depression (unfortunately not completely free of psychotropic drugs) by Maes et al (1994). These authors found no differences either in absolute number or percentage of these cells, which they labeled as non–major histocompatibility complex–restricted cytotoxic T-lymphocytes, between these depressive groups and control subjects. NK–T cells might possess important immunoregulatory properties, as interleukin 4 produced by these cells can stimulate the commitment of naive T-cells to the Th2 lineage (Prussin and Foster 1997). The concomittant change in cytokine excretion pattern may play a role in the interleukin hypothesis of depression of Maes et al (1995). We therefore compared NK–T cell (expressing both CD3^+ and CD16^+ and/or CD56^+) proportion and absolute numbers between control subjects and depressive patients.

In the latter group we distinguished between subjects using or not using antidepressive medication, since antidepressive drugs have been reported to decrease the number of NK cells (Ravindran et al 1998) and to increase NK cell lytic activity (Frank et al 1999) of certain subgroups of depressive patients.

Methods and Materials

As part of a larger ongoing study in an aged population to detect vulnerability factors for depressive illness, we studied 20 depressed and 23 control subjects. The investigation of an aged population was chosen because it was concluded from a meta-analytic review that effect sizes of reported changes in numbers or percentages of B, T, helper T, and supressor/cytotoxic T-cells in a depressive population were two- to threefold greater in an older sample, as compared with the non–age-restricted population (Herbert and Cohen 1993). The subjects were recruited partly from a population-based study of noninstitutionalized persons aged 57 years or more, held in 1993, and partly from mental health outpatient clinics. The population-based control group consisted of subjects randomly selected from the highest and lowest quartile on the dimension Neuroticism of the Eysenck Psychotism Scale (Eysenck et al 1985), a notorious risk factor for...
Depression. A detailed description of the recruitment and diagnostic procedures can be found elsewhere (van den Berg et al., in press).

Depressive patients were diagnosed according to the DSM-IV (American Psychiatric Association 1994). Besides subjects with a major depressive disorder or dysthymia, we included cases of minor depression, defined as having at least two clinically significant symptoms or at least three subthreshold symptoms. Patients and control subjects were excluded in case their immunologic systems could be disturbed by a somatic disease or the use of drugs known to influence this system (i.e., infectious diseases, autoimmune diseases, and neoplasms; the use of antibiotics or corticosteroids; or subjects vaccinated for the flu; antihypertensive, anticoagulation, and antidiabetic drugs and heart, pain, vertigo, and gastric medications were permitted). In both depressed and control subjects depressive symptomatology was assessed with the Geriatric Depression Scale (GDS-15; Lesher and Berryhill 1994; Yesavage et al. 1982). Control subjects with GDS scores of >5 were excluded. All subjects gave their written informed consent.

Depressive patients were divided into two subgroups. Group D(+) consisted of 10 depressive patients (six major depressions, two minor depressions, and two dysthymias) on psychoactive medication: four patients used paroxetine, one fluvoxamine, one mirtazapine, one desipramine, one trimipramine, and two moclobemide; in addition, five of the patients used benzodiazepines, whereas six also used other medications (two male patients, eight female patients; mean age = 72 ± 7 years [SD], range 61–85 years; mean duration of the depressive episode: 1372 ± 1606 days [SD]; mean interval between assessment of diagnosis and blood sampling: 21 ± 9 days [SD]).

Group D(−) contained 10 depressive patients (four major depressions, five minor depressions, and one dysthymia) not using psychoactive medication. Eight of them used other medications (three male patients, seven female patients; mean age = 76 ± 7 years [SD], range 64–82 years; mean duration of the depressive episode: 304 ± 526 days [SD]; mean interval between assessment of diagnosis and blood sampling: 30 ± 8 days [SD]).

Group C was a control group that consisted of 23 subjects without depressive illness and not using psychoactive medication; 11 of them were using other drugs (16 male subjects, seven female subjects, mean age = 70 ± 7 years [SD], range 63–83 years). Neuroticism was scored by nine subjects in the highest quartile and by 14 subjects in the lowest quartile.

Blood was collected in EDTA-containing vacuum tubes between 8:00 AM and 9:00 AM. Two-color flow cytometry (Facstar, Becton Dickinson, San Jose, CA) was performed with a whole blood technique with paraformaldehyde fixation, in which 0.1 mL of whole blood was mixed with 0.01 mL CD3 fluorescein isothiocyanate/CD16 phycoerythrin (PE)/CD56 PE (Immuno Quality Products, Groningen, The Netherlands). Approximately 4000 lymphocytes (purity control with CD45/CD14 gating) were analyzed by forward/sideward scattering and NK–T cells were defined by the presence of the T-cell marker CD3 and one or both of the NK cell markers CD16 and CD56. The technician performing the immunologic assays was not informed about the status of the subjects who provided the blood.

Results

Analysis of variance (ANOVA) revealed that the severity of depression did not differ between the two depressive groups: D(−) = 7.4 ± 2.3 and D(+) = 7.1 ± 3.1 (means ± SDs). The GDS score for the control group was 0.6 ± 1.0. The three groups did not differ in distribution of gender or the use of nonpsychotropic medication (Fisher exact probability tests). Moreover, the three groups tended to differ in age [ANOVA: F(2,40) = 2.73, p = .077]. The lower age of control subjects, as compared with D(−) patients, could account for this effect [F(1,40) = 5.42, p = .025].

The D(+) group tended to have suffered longer from depression than the D(−) group [ANOVA: F(1,18) = 4.00, p = .061]. Moreover, the interval between the assessment of the diagnosis and blood sampling was significantly shorter for the D(+) subjects, as compared with the D(−) subjects [ANOVA: F(1,18) = 4.74, p = .043].

Figure 1 shows the numbers and percentages (with respect to the total number of lymphocytes) of NK–T cells for the C, D(−), and D(+) subjects. Means ± SDs for the different groups were, for number (10⁶ cells/L), 129 ± 65, 320 ± 158, and 78 ± 59 and, for percentage, 5.9 ± 5.9, 14.3 ± 6.5, and 4.8 ± 3.4, respectively. We found that the three groups differed in levels of NK–T cells both in numbers and in percentages [ANOVA: main effect numbers, F(2,40) = 8.02, p = .001; main effect percentage, F(2,40) = 9.61, p = .0004]. Strongly elevated numbers and percentages of NK–T cells were found in the D(−) group, as compared with C [number, F(1,40) = 11.83, p =
.001; percentage, $F(1,40) = 15.82, p = .0003$. In addition, the numbers and percentages of the D(+) group were lower than those of the D(−) group [number, $F(1,40) = 13.60, p = .0007$; percentage, $F(1,40) = 14.71, p = .0004$]. In contrast, the D(+) group did not differ significantly from the C subjects.

Because (nearly) significant differences existed between the various groups in age (D[−] > C), in interval between diagnosis and blood sampling (D[+] < D[−]), and in episode duration (D[+] > D[−]), we reanalyzed the groups that revealed significant differences in NK–T measures. Now analyses of covariance (ANCOVAs) were performed, using these three variables as covariates. The above results with respect to the NK–T measures remained significant: D(+) subjects had still lower levels of NK–T cells than the D(−) subjects [number, $F(1,15) = 11.19, p = .004$; percentage, $F(1,15) = 8.12, p = .012$]. Moreover, age differences between D(−) and C could not explain that D(−) subjects showed higher levels of NK–T cells than C [ANOVA with age as covariate: number, $F(1,39) = 9.74, p = .003$; percentage, $F(1,39) = 11.20, p = .002$].

In addition, we found no indication that major depressives, minor depressives, and dysthymia cases within one group differed with respect to number or percentage of NK–T cells (ANOVA, three diagnostic classes).

**Discussion**

To our knowledge this is the first report of an increase in NK–T cells in depressive illness in a geriatric population. Also, the finding that this increase is absent in patients using antidepressive medication is very intriguing. It should be noticed that this apparent “normalization” of the number of NK–T cells in patients using antidepressive drugs cannot be explained by differences in severity of depression, as the average GDS scores in both depressive groups were equal. Moreover, results could not be explained by gender, age, or diagnostic subclass.

It is interesting that the normalizing effect of the antidepressive drugs was present in all structurally different classes of drugs used. This suggests that the effect is attained via a common, yet unknown, pathway.

Speculations about the role of these NK–T cells in depressive illness await further characterization of these cells and replication of these results in a nongeriatric population. The investigation of a possible relationship of these NK–T cells with the human analog of murine NK1 T-cells might be interesting, as these cells are said to possess important immunoregulatory properties (Prussin and Foster 1997).

In contrast to our findings in a geriatric population, Maes et al (1994) found no differences in number and percentage of the same cell type between three nongeriatric depressive groups and a control group. This might suggest that our results are specific for an older population. Alternatively, these authors may have found artificially low values for their depressive groups, as these groups were not completely free of the use of psychotropic drugs. The results of Ravindran et al (1998) may be interpreted in support of our findings. They reported increases in CD16+/CD56+ cells in depression, followed by a decline after treatment with antidepressant medication. As the measurements of these authors probably included CD3+/CD16+/CD56+ cells (NK–T cells), our findings suggest that their results could well be attributed to these NK–T cells.

The interpretation of the present results should be considered with caution. The increase of NK–T cells may not be specific for depressive illness. An increased percentage of NK–T cells has indeed been reported in subjects suffering from migraine in attack-free periods (Mosnaim et al 1998) and in ovarian cancer (Schroeder et al 1997). A long-lasting expansion of NK–T cells also followed autologous bone marrow transplantation (Parrado et al 1997). Moreover, McNerlan et al (1998) found a significant increase of both proportion and absolute count of NK–T cells with age, whereas Musha et al (1998) showed that the proportion of NK–T cells is very low in cord blood, but increases with age after birth. It should also be acknowledged that the number of subjects per group is still small and that a selection bias cannot be excluded in this older population: the exclusion criteria led to a large reduction in number of the subjects who were included in the ongoing study (only 17% of the included subjects).

Our finding that the number of NK–T cells is increased in depression but shows normal values in a depressive population using antidepressive medication provides further support for an association of depressive illness and immune status. Whether these changes in immune status play a role in the etiology of depression remains to be investigated in future longitudinal research. Our results also support the suggestion of Frank et al (1999) that antidepressants may modulate immune function and thus may possibly be useful for wider clinical application.

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