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Clinical response augments NK cell activity independent of treatment modality: a randomized double-blind placebo controlled antidepressant trial

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ABSTRACT

Background. Major depressive disorder (MDD) has been associated with alterations in immune function. Suppression of natural killer (NK) cell activity (NKCA) reliably characterizes immunological alterations observed in MDD. Antidepressant pharmacotherapy has been associated with modulation of NKCA. Previous investigations into antidepressant modulation of NKCA have not employed randomized double-blind placebo controlled designs. Thus, it is unknown whether treatment-associated changes in immune function are due to drug, placebo, or spontaneous remission effects. The present investigation examined the effect of antidepressant treatment on NKCA utilizing a randomized double-blind placebo controlled experimental design.

Method. Patients ($N=16$) met DSM-IV criteria for MDD and were randomly assigned to drug ($N=8$; citalopram, 20 mg/day) or placebo ($N=8$) under double-blind conditions. Severity and pattern of depressive symptoms were assessed by the Hamilton Depression Rating Scale (HDRS). NK cell function was measured using a standard chromium-release assay and NK cell number assessed by flow cytometry. HDRS scores, NK cell function, and NK cell numbers were collected at 0, 1, 2 and 4 weeks of treatment.

Results. Clinical response was associated with augmented NKCA independent of treatment condition. Failure to respond to treatment resulted in significantly reduced NKCA over treatment interval.

Conclusions. The present results suggest that alterations in the depressive syndrome, regardless of therapeutic modality, may be sufficient to modulate NKCA during antidepressant trials and thus may significantly impact on co-morbid health outcomes in MDD.

INTRODUCTION

Major depressive disorder (MDD) has been associated with immunological alterations. Considerable evidence suggests that activation of inflammatory processes (Maes, 1999) and reduction in natural killer (NK) cell activity (NKCA) (Irwin, 1999; Zorrilla *et al.* 2001) consistently accompany MDD. NK cells are a salient feature of the innate immune defence against viral infection and cancer (Herberman & Ortaldo, 1981). Immunological processes have been implicated as integral factors in the pathogenesis of MDD (Maes, 1995). Therefore, pharmacological modulation of immunological processes has been explored as a potential mechanism of antidepressant efficacy. Few studies have utilized randomized double-blind placebo controlled designs to assess the effect of antidepressant treatment on immune function. Prior investigations into the effects of antidepressant treatment on NKCA have been suggestive, but

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definitive conclusions were precluded due to non-randomization of patients, lack of placebo control, and/or absence of double-blind conditions. Thus, the effects of antidepressant treatment on NKCA remain confounded with effects due to placebo or spontaneous remission.

Investigations into the effect of antidepressant treatment on NKCA have yielded inconsistent results. Antidepressant pharmacotherapy has been associated with increases (Irwin *et al*. 1992; Kook *et al*. 1995; Frank *et al*. 1999; Mizruchin *et al*. 1999), decreases (Pariante & Miller, 1995; Ravindran *et al*. 1995), or no change (Schliefer *et al*. 1999) in NKCA or NK cell number. Several classes of antidepressant were utilized in these studies including monoamine oxidase inhibitors (Irwin *et al*. 1992), selective serotonin reuptake inhibitors (Irwin *et al*. 1992; Kook *et al*. 1995; Ravindran *et al*. 1995; Frank *et al*. 1999; Mizruchin *et al*. 1999), or tricyclics (Irwin *et al*. 1992; Pariante & Miller, 1995; Schliefer *et al*. 1999). In several studies, alterations in NKCA or NK cell number were associated with clinical response to antidepressant treatment (Kook *et al*. 1995; Pariante & Miller, 1995; Ravindran *et al*. 1995; Mizruchin *et al*. 1999) suggesting that antidepressant modulation of mood is necessary for treatment-associated changes in NK cells.

In the present investigation, a placebo control was utilized in a randomized double-blind antidepressant trial to evaluate whether clinical response, independent of treatment condition, is sufficient to alter NK cell function.

METHOD

Participants

Participants ($N=16$) were enrolled as outpatients at the Psychopharmacology Research Center, Department of Psychiatry of the University of Nebraska Medical Center (UNMC). Informed consent was obtained according to UNMC IRB guidelines.

Experimental design

Participants meeting DSM-IV criteria for MDD were randomly assigned to either citalopram (20 mg/day; $N=8$) or placebo ($N=8$). Under double-blind conditions, psychiatric evaluations were conducted at 0, 1, 2 and 4 weeks of treatment. Treatment began immediately after baseline assessments. Immunological assays were conducted blind to treatment condition at 0, 1, 2 and 4 weeks of treatment.

Psychiatric evaluations

All participants were assessed for MDD by structured clinical interview for DSM-IV-R (SCID-CV) (First *et al*. 1997). Demographic data, concurrent medical conditions, past/present drug use (including alcohol and tobacco use) and recent antidepressant treatment were also collected by SCID. Exclusion criteria included co-morbid alcohol and/or substance abuse, concomitant psychoactive medication use and/or medication that may modulate immune function, presence of suicidal ideation or plans, and symptoms indicative of an active viral or bacterial infection. Participants meeting DSM-IV criteria for MDD were assessed for depression severity using the composite score of the 21-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1961). Participants were further evaluated for anxiety/somatization, weight loss, cognitive disturbance, diurnal variation, retardation, and sleep disturbance (Cleary & Guy, 1977).

Materials

Citalopram hydrobromide was obtained from Forest Laboratories (New York, NY). Antibodies were obtained from Pharmingen (San Diego, CA).

Measures

Mononuclear cell (MNC) separation

Peripheral blood ($\sim$15 ml) was collected from each participant into heparinized vacutainers by venipuncture. MNC were separated from whole blood as previously described (Frank *et al*. 2002). Non-adherent MNC were utilized where indicated. Hereafter, non-adherent MNC will be referred to as MNC. Lymphoid MNC were counted and cell viability assessed by trypan blue exclusion.

NK cell cytotoxicity assay

A standard ^{51}Cr-release assay was employed as previously described (Frank *et al*. 2002). Cytotoxicity was quantified as lytic units (LU) using the non-linear equation: $Y=A(1-e^{-kx})$; where $Y=$ % cytotoxicity, $A=$asymptote of the lytic curve, $k=$slope of the lytic curve and $x=$the

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number of effector cells (Pross *et al.* 1981). One LU is defined as the number of lymphoid MNC that are required to lyse 20% of 1×10^4 target cells. Total LU were calculated as the number of LU in 10^6 lymphoid MNC with the assumption that the asymptote of the lytic curve is 100% target cell lysis. NKCA was quantitated as LU/NK cell.

Flow cytometric analyses

To quantitate percentage of NK cells, MNC (1×10^6) were incubated with an allophycocyanin (APC)-conjugated mouse anti-human CD56 (IgG_1, κ) antibody. CD56 is a cell surface antigen expressed predominately by NK cells (Lanier *et al.* 1989). Cells were exposed to antibody for 20 min at 4 °C. Cells were washed, suspended in cold PBS, and immediately analysed on a FACStar Cytometer (Becton Dickinson, San Jose, CA). Gates were set on background fluorescence of isotype controls (anti-human CD56 antibody isotype: mouse APC-IgG_1, κ) and a minimum 1×10^4 events collected. Flow cytometric data were analysed using Cell Quest software package (Becton Dickinson, San Jose, CA).

Statistical analyses

A 2×4 repeated measures analysis of variance (ANOVA) was conducted with drug condition (citalopram *v.* placebo) or clinical outcome (response *v.* non-response) as the between groups factor and treatment interval (0, 1, 2 and 4 weeks) as the within groups factor. Clinical response threshold was set at 50% reduction in HDRS scores from baseline to week 4. The criterion for clinical response utilized here is a widely accepted standard for determining clinical outcome in antidepressant trials (Fava & Davidson, 1996). Follow-up evaluations of depression severity were conducted at week 8 of treatment to assess stability of clinical response. The effect of several extraneous variables on NKCA and NK number was assessed by residualizing the dependent measure using linear regression prior to conducting ANOVA. Gender, tobacco use and alcohol use were assessed as extraneous variables based on evidence that these variables may significantly impact NK cell function and thus present as potential confounds (Irwin, 1999). A chronic allergic condition was also assessed as an extraneous variable in light of evidence that histamine, an immunological mediator of allergic responses, is a potent modulator of NK cell function (Hellstrand *et al.* 1994) and that antihistamine use has been associated with alterations in immune function in depression (Levy *et al.* 1991). If a patient reported year-round allergies, they were classified as suffering from a chronic allergic condition. Alcohol use was classifed as none (0), moderate (1) or heavy (2), based on patient's description of drinking habits obtained by SCID. Tobacco use was classified as non-smoker (0) or smoker (1). *Post hoc* comparisons were conducted using the least significant difference (LSD) method. Tests of interactions, main effects, and simple effects were conducted at $\alpha = 0{\cdot}05$ using SPSS (v.9) for Windows.

RESULTS

Patient characteristics (Table 1)

The citalopram (six males, two females; mean age = 39·12) and placebo treatment (two males, six females; mean age = 41·5) groups did not significantly differ in age ($t = -0{\cdot}686$, df = 14, $P = 0{\cdot}504$), alcohol use (Pearson $\chi^2 = 2{\cdot}786$, $P = 0{\cdot}248$) or presence of a chronic allergic condition (Pearson $\chi^2 = 0{\cdot}0$, $P = 1{\cdot}0$), however gender composition (Pearson $\chi^2 = 4{\cdot}0$, $P = 0{\cdot}046$) and tobacco use (Pearson $\chi^2 = 5{\cdot}333$, $P = 0{\cdot}021$) significantly differed between treatment groups.

Responders (five males, four females; mean age = 41·33) and non-responders (three males and four females; mean age = 39·00) did not significantly differ in age ($t = 0{\cdot}668$, df = 14, $P = 0{\cdot}515$), alcohol use (Pearson $\chi^2 = 2{\cdot}939$, $P = 0{\cdot}23$), presence of a chronic allergic condition (Pearson $\chi^2 = 2{\cdot}116$, $P = 0{\cdot}146$), tobacco use (Pearson $\chi^2 = 2{\cdot}116$, $P = 0{\cdot}146$), and gender composition (Pearson $\chi^2 = 0{\cdot}254$, $P = 0{\cdot}614$). All patients reported no recent (<2 months) antidepressant use prior to onset of treatment.

Reliability of immunological measures

Two parameters of NK cells (LU and % CD56+ cells) were correlated to assess the reliability over time of NK cell measures (Fig. 1). Previous investigations indicated that NK cell lytic activity is positively and linearly associated with number of NK cells (Maes *et al.* 1994; Frank *et al.* 2002). Likewise in the present study, lytic activity (LU) was significantly associated

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Table 1. *Patient characteristics and immunological measures*

Patient number	Age	Gender	Medical condition	Alcohol use*	Tobacco use†	Drug‡	Clinical response§	Natural kill cell activity¶			
								Week 0	Week 1	Week 2	Week 4
1	36	M	None	1	0	0	1	0·55 (−0·25)	0·68 (−0·13)	1·00 (0·2)	1·21 (0·4)
2	49	M	Hypertension	0	1	0	1	0·74 (−0·44)	1·66 (0·47)	1·10 (−0·08)	2·68 (1·5)
3	44	M	Gout	0	1	0	0	1·31 (0·13)	0·89 (−0·28)	0·47 (−0·7)	0·99 (−0·18)
4	47	M	None	0	0	0	1	1·25 (0·29)	1·36 (0·39)	1·07 (0·11)	1·35 (0·39)
5	35	M	Allergies	0	0	0	1	1·59 (0·73)	0·64 (−0·22)	0·96 (0·09)	0·82 (−0·05)
6	35	F	Allergies	1	1	0	0	1·46 (0·55)	0·59 (−0·33)	0·71 (−0·21)	0·99 (0·08)
7	37	M	None	2	1	0	0	0·67 (−0·24)	0·74 (−0·17)	1·23 (0·32)	0·48 (−0·43)
8	30	F	None	0	0	0	1	0·65 (−0·21)	1·46 (0·6)	0·69 (−0·17)	0·99 (0·13)
9	44	F	Allergies	1	0	1	0	1·15 (0·42)	0·74 (0·02)	0·61 (−0·11)	0·69 (−0·04)
10	46	F	Colitis	0	0	1	1	0·87 (−0·03)	0·41 (−0·5)	0·80 (−0·11)	0·65 (−0·26)
11	29	M	None	1	0	1	0	0·74 (−0·04)	0·96 (0·18)	0·59 (−0·19)	0·36 (−0·39)
12	34	F	None	1	0	1	1	0·60 (−0·14)	1·13 (0·38)	1·36 (0·61)	1·16 (0·42)
13	46	F	None	0	0	1	0	1·16 (0·25)	0·83 (−0·08)	0·57 (−0·34)	0·39 (−0·52)
14	45	M	Diabetes	0	0	1	1	0·58 (−0·38)	0·62 (−0·34)	0·73 (−0·23)	0·47 (−0·48)
15	50	F	None	1	0	1	1	0·35 (−0·45)	0·75 (−0·05)	1·12 (0·32)	1·51 (0·71)
16	38	F	Allergies	1	0	1	0	0·34 (−0·37)	0·48 (−0·23)	0·35 (−0·35)	0·71 (0·01)

* Alcohol use: 0 = none, 1 = moderate, 2 = heavy.
† Tobacco use: 0 = non-smoker, 1 = smoker.
‡ Drug: 0 = citalopram, 1 = placebo.
§ Clinical response: 0 = non-responder, 1 = responder.
¶ Values in parentheses are the residualized NKCA controlling for the effects of extraneous variables (age, gender, chronic allergies, alcohol and tobacco use). Raw and residualized NKCA were highly correlated ($r = 0{\cdot}94$).

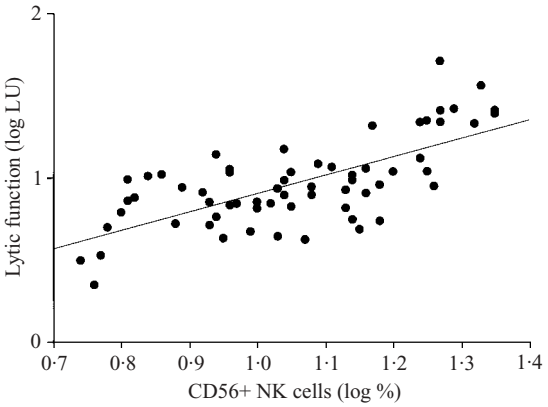

FIG. 1. Relationship between natural killer (NK) cell lytic function (lytic units; LU) and NK cell number (% CD56+ cells). NK cell parameters were assayed at each time point (week 0, 1, 2 and 4) of treatment for each participant ($N = 16$) resulting in 64 data points. NK lytic function was significantly associated with NK cell number ($r = 0{\cdot}689$, $P = 2 \times 10^{-10}$) indicating a high degree of reliability in NK cell measures across treatment interval.

($N = 64$, $r = 0{\cdot}689$, $P = 2 \times 10^{-10}$) with number of NK cells (% CD56+ NK cells). Given that NK cell number is a significant determinant of NK cell lytic activity (Maes *et al.* 1994; Frank *et al.* 2002), separate analyses were conducted for NK cell number and activity. A composite measure of NKCA (LU/CD56+ NK cell) was utilized.

Effect of citalopram treatment on NK cell number

Drug treatment failed to significantly interact with treatment interval ($F = 0{\cdot}954$, df = 3, 42, $P = 0{\cdot}424$) neither did the main effect of drug treatment ($F = 0{\cdot}414$, df = 1, 14, $P = 0{\cdot}53$) significantly alter NK cell number. Within-group comparisons between baseline (week 0) NK number and week 1, 2 and 4 NK number also showed that treatment did not significantly modulate NK cell number in citalopram-treated patients (NK cell number: week 0 *v.* week 1, $P = 0{\cdot}356$; week 0 *v.* week 2, $P = 0{\cdot}345$; week 0 *v.* week 4, $P = 0{\cdot}316$) and placebo-treated patients (NK cell number: week 0 *v.* week 1, $P = 0{\cdot}341$; week 0 *v.* week 2, $P = 0{\cdot}269$; week 0 *v.* week 4, $P = 0{\cdot}119$).

The effect of extraneous variables (i.e. age, gender, chronic allergies, alcohol and tobacco use) was extracted from NK cell number using linear regression. The repeated measures ANOVA was rerun to assess whether the explanatory power of these variables obscured the effect of citalopram treatment on NK cell number. Elimination of the effect of extraneous variables failed to alter the interaction between drug condition and treatment interval ($F = 0{\cdot}954$, df = 3, 42, $P = 0{\cdot}424$) and the main effect

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of drug on NK cell number ($F=0{\cdot}013$, df = 1, 14, $P=0{\cdot}912$). Results of within-group comparisons were comparable to those obtained with non-residualized NK cell number in citalopram-treated (week 0 *v.* week 1, $P=0{\cdot}339$; week 0 *v.* week 2, $P=0{\cdot}315$; week 0 *v.* week 4, $P=0{\cdot}311$) and placebo-treated patients (week 0 *v.* week 1, $P=0{\cdot}279$; week 0 *v.* week 2, $P=0{\cdot}231$; week 0 *v.* week 4, $P=0{\cdot}06$).

Effect of citalopram treatment on NKCA

The repeated measures ANOVA indicated that drug treatment failed to significantly interact with treatment interval ($F=0{\cdot}428$, df = 3, 42, $P=0{\cdot}734$) to modulate NKCA. Within-group comparisons showed that drug treatment did not significantly alter NKCA in citalopram-treated (week 0 *v.* week 1, $P=0{\cdot}449$; week 0 *v.* week 2, $P=0{\cdot}243$; week 0 *v.* week 4, $P=0{\cdot}283$) or placebo-treated patients (week 0 *v.* week 1, $P=0{\cdot}458$; week 0 *v.* week 2, $P=0{\cdot}399$; week 0 *v.* week 4, $P=0{\cdot}449$).

Control of extraneous variables did not modulate these effects of drug treatment on NKCA in citalopram-treated (week 0 *v.* week 1, $P=0{\cdot}444$; week 0 *v.* week 2, $P=0{\cdot}258$; week 0 *v.* week 4, $P=0{\cdot}269$) and placebo-treated patients (week 0 *v.* week 1, $P=0{\cdot}46$; week 0 *v.* week 2, $P=0{\cdot}399$; week 0 *v.* week 4, $P=0{\cdot}451$).

Effect of clinical outcome on NK cell number

Clinical outcome (nine responders and seven non-responders) did not significantly interact with treatment interval ($F=0{\cdot}598$, df = 3, 42, $P=0{\cdot}62$) to alter NK cell number. Main effect of clinical outcome also did not attain significance ($F=0{\cdot}296$, df = 1, 14, $P=0{\cdot}595$). Within-group comparisons indicated that neither clinical response (week 0 *v.* week 1, $P=0{\cdot}415$; week 0 *v.* week 2, $P=0{\cdot}363$; week 0 *v.* week 4, $P=0{\cdot}209$) nor non-response (week 0 *v.* week 1, $P=0{\cdot}236$; week 0 *v.* week 2, $P=0{\cdot}299$; week 0 *v.* week 4, $P=0{\cdot}232$) significantly altered NK cell number over baseline values.

Control of extraneous variables failed to alter the interaction ($P=0{\cdot}62$) and main effect ($P=0{\cdot}726$). Within-group comparisons showed that the effect of extraneous variables did not obscure the effect of clinical response (week 0 *v.* week 1, $P=0{\cdot}398$; week 0 *v.* week 2, $P=0{\cdot}334$; week 0 *v.* week 4, $P=0{\cdot}192$) or clinical non-response (week 0 *v.* week 1, $P=0{\cdot}249$; week 0 *v.* week 2, $P=0{\cdot}269$; week 0 *v.* week 4, $P=0{\cdot}142$) on NK cell number.

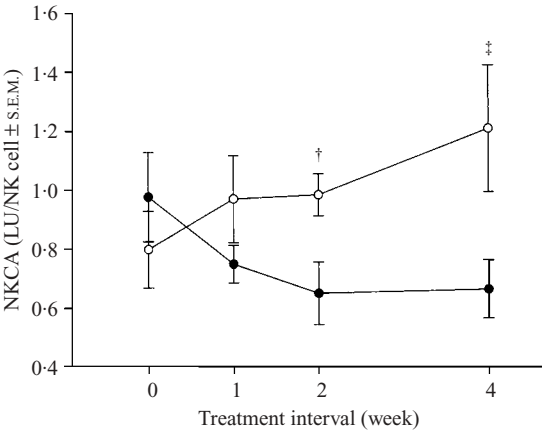

FIG. 2. Effect of clinical outcome on natural kill cell activity (NKCA). Clinical outcome significantly interacted with treatment interval to alter NKCA ($P=0{\cdot}0428$). Clinical responders (○) exhibited significantly greater NKCA at week 2 and week 4 of treatment compared to clinical non-responders (●). † $P=0{\cdot}008$; ‡ $P=0{\cdot}027$.

Effect of clinical outcome on NKCA

Clinical outcome significantly interacted with treatment interval to modulate NKCA ($F=2{\cdot}865$, df = 3, 42, $P=0{\cdot}048$) (Fig. 2). Simple effects analysis revealed that clinical response was associated with increased NKCA at week 2 ($P=0{\cdot}008$) and week 4 ($P=0{\cdot}027$) of treatment compared with clinical non-response. Within-groups comparisons indicate that clinical response linearly augmented NKCA over baseline values (mean NKCA = 0·797) at week 1 (mean = 0·97, $P=0{\cdot}2$), week 2 (mean NKCA = 0·98, $P=0{\cdot}11$) and week 4 (mean NKCA = 1·2, $P=0{\cdot}06$) of treatment. In contrast, clinical non-response linearly suppressed NKCA over baseline values (mean NKCA = 0·975) at week 1 (mean NKCA = 0·74, $P=0{\cdot}09$), week 2 (mean NKCA = 0·64, $P=0{\cdot}05$) and week 4 (mean NKCA = 0·65, $P=0{\cdot}05$).

Control of extraneous variables failed to attenuate the interaction between clinical outcome and treatment interval ($P=0{\cdot}048$). Likewise, simple effects remained significant at week 2 ($P=0{\cdot}025$) and week 4 ($P=0{\cdot}022$) of treatment, while within-groups effects were partially strengthened in clinical responders (week 0 *v.* week 1, $P=0{\cdot}19$; week 0 *v.* week 2, $P=0{\cdot}13$;

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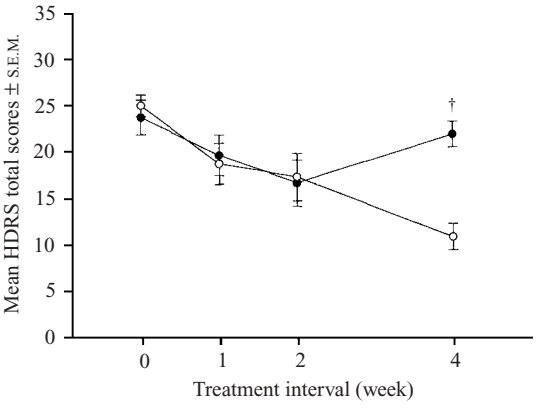

FIG. 3. Change in severity of depression over treatment interval in clinical responders and non-responders. The interaction between clinical outcome and treatment interval ($P=0{\cdot}001$) indicates that clinical responders (○) and non-responders (●) diverged in depressive symptom severity at week 4 of treatment. (HDRS = Hamilton Depression Rating Scale; † $P=0{\cdot}0008$.)

week 0 *v.* week 4, $P=0{\cdot}05$) and non-responders (week 0 *v.* week 1, $P=0{\cdot}07$; week 0 *v.* week 2, $P=0{\cdot}04$; week 0 *v.* week 4, $P=0{\cdot}03$).

Treatment-associated changes in depressive syndrome

Citalopram and placebo treatment exhibited similar effects on the depressive syndrome over treatment interval. Neither the interaction of drug and treatment interval ($F=0{\cdot}857$, df = 3, 42, $P=0{\cdot}471$) nor the main effect of drug ($F=0{\cdot}258$, df = 3, 42, $P=0{\cdot}619$) attained statistical significance. Comparison of responders and non-responders over treatment interval revealed that clinical response significantly interacted with treatment interval ($F=6{\cdot}597$, df = 3, 42, $P=0{\cdot}001$) (Fig. 3). Simple effects analysis demonstrated that responders and non-responders significantly diverged in severity of depressive syndrome at week 4 of treatment ($t=14{\cdot}317$, $P=0{\cdot}00084$). To address whether clinical response at week 4 of treatment was stable over time, thus reflecting a fundamental change in depressive syndrome, a follow-up assessment of depressive symptom severity was conducted in 15 of 16 patients at week 8 of treatment. Clinical response at week 4 was highly predictive of clinical response at week 8 of treatment ($r=0{\cdot}681$, $P=0{\cdot}005$).

Change in overall depressive syndrome or any particular symptom pattern was not correlated with changes in NK cell number or NKCA over treatment interval.

DISCUSSION

Consistent with prior reports (Kook *et al.* 1995; Mizruchin *et al.* 1999), the present results suggest that clinical response to treatment may be necessary for enhancement of NKCA. Unlike prior studies, the present investigation included a placebo control to assess whether clinical response, independent of treatment condition, is sufficient to modulate NKCA. Indeed, clinical response was associated predominately with increased NKCA over treatment interval irrespective of treatment condition. Clinical responders exhibited augmented NKCA at week 1, 2 and 4 of treatment compared to pre-treatment values with week 4 values attaining statistical significance. In contrast, failure to respond to treatment, whether it be drug or placebo, resulted in robust reductions in NKCA at weeks 1, 2 and 4 compared with baseline values. These results suggest that the mode of alleviating depressive symptoms is less important than clinical outcome in modulating NKCA. Moreover, the present findings indicate that persistence or non-abatement of depressive symptoms throughout a treatment regimen may confer considerable risk for suppression of NKCA. The effect in non-responders reported here is consistent with the findings of Pariante & Miller (1995), who demonstrated that failure to respond to tricyclic antidepressant treatment resulted in reduced NKCA from baseline to follow-up. Interestingly, tricyclic levels did not differ between responders and non-responders suggesting, much like the present results, that persistence of the depressive syndrome in non-responders is a critical factor in suppressing NKCA. However, unlike the present findings, clinical response to tricyclic treatment failed to alter NKCA over treatment interval. Several methodological and experimental differences between the present study and that of Pariante & Miller (1995) render comparisons between results tenuous. In contrast to the present study and others (Kook *et al.* 1995; Pariante & Miller, 1995; Mizruchin *et al.* 1999; Schleifer *et al.* 1999), Irwin *et al.* (1992) demonstrated that antidepressant treatment (predominately

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tricyclics) uniformly increased NKCA from baseline to follow-up in older male hospitalized patients to levels comparable to controls. Similar to the Schleifer *et al*. (1999) study, Irwin *et al*. (1992) did not classify patients as responders or non-responders, which precludes comparisons with the present results. Clearly, heterogeneity of sample composition (age and gender make-up), substance use, and treatment regimen (SSRI *v*. tricyclic) among the studies cited above obscures the identification of reliable relationships between antidepressant treatment and modulation of NKCA. Several extraneous variables have been previously identified (Irwin, 1999), which strongly influence NKCA including age, gender and substance use. Unlike prior studies, the present investigation evaluated the effect of drug treatment and clinical response on NKCA prior to and after extracting variance in NKCA due to several extraneous variables (i.e. age, gender, chronic allergic condition, alcohol and tobacco use). The effect of clinical outcome on NKCA was robust to control of extraneous variables as evidenced by the effect of clinical outcome on residual NKCA. Several studies (Kook *et al*. 1995; Pariante & Miller, 1995; Mizruchin *et al*. 1999) are consistent with the present finding that clinical response may be a salient variable modulating NKCA. The evaluation of a placebo control in the present study provides additional support that response to treatment moderates, in large part, treatment effects on NKCA.

Although suggestive, these findings should be considered tentatively in light of the small sample size. Conclusions regarding the effect of spontaneous remission on NKCA are precluded due to the absence of an untreated (neither drug nor placebo) group. The possibility is not excluded that spontaneous remission accounted for the observed changes in NKCA apart from treatment conditions. Although the modality of change in depressive symptoms is confounded, the common endpoint of clinical response, independent of therapeutic modality, appears particularly salient in modulation of NKCA. In addition, NKCA was not evaluated in these patients prior to onset of MDD. Therefore, it is unknown whether the changes in NKCA represent normalization to or substantial deviation from pre-morbid NKCA levels. The health consequences of altered NKCA or other immune parameters in depression have yet to be determined (Zorrilla *et al*. 2001). Furthermore, the functional redundancy of the immune system may render the observed changes in NKCA inconsequential to overall health. However, alterations in NKCA have been associated with increased risk of viral infection in depression (Zorrilla *et al*. 1996).

The effect of clinical response on NKCA, independent of treatment condition, suggests that exposure to drug is neither necessary nor sufficient to modulate NKCA. Rather, the effect of placebo response on NKCA suggests that alteration in the depressive syndrome, regardless of therapeutic modality, may be sufficient to modulate NKCA during antidepressant pharmacotherapy. However, the present data do not exclude the possibility that direct antidepressant effects on NKCA in drug responders may mediate, in part, treatment effects. Direct antidepressant effects on NKCA have been reported (Miller *et al*. 1986; Kook *et al*. 1995; Xiao & Eneroth, 1996; Frank *et al*. 1999), however no consistent trend has emerged.

The present results are obscure to the mechanism of action in drug and placebo responders that underlie the effect of clinical outcome on NKCA. However, a handful of studies have addressed whether response to placebo reflects an altered pattern of central nervous system (CNS) functioning compared with drug response. Mayberg *et al*. (2002) demonstrated that clinical response was associated with a common pattern of functional alterations in the CNS of antidepressant and placebo responders. However, Leuchter *et al*. (2002) demonstrated that placebo and drug responders exhibited quite distinct alterations in CNS function, whereas placebo and drug non-responders displayed no change in CNS function. Considerable evidence indicates that the CNS and immune system communicate via bidirectional circuits (Black, 1994). Neuroendocrine and autonomic pathways mediate CNS modulation of immune function (Besedovsky & Del Rey, 1992). Therefore, the present results provide a basis for characterizing whether different mechanisms and pathways mediate immunological effects in drug and placebo responders.

These findings provide further evidence that the clinical course of the depressive syndrome affects the immune system and therefore may

have significant impact on co-morbid health outcomes in the treatment of MDD.

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