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The Effects of Stress and Relaxation on the *in Vitro* Immune Response in Man: A Meta-Analytic Study

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The purpose of the present meta-analytic study was to combine and integrate the results of stress and relaxation studies for their reported changes in the in vitro *immune response. Twenty-four stress studies and 10 relaxation studies with a (quasi)-experimental design with pre- and postintervention measurements were selected. Twenty immunological variables tested in stress studies and five immunological variables tested in relaxation studies could be further analyzed. The meta-analysis of the results of the stress studies indicated that the observed changes in interleukin-2 receptor expression on lymphocytes and antibody titers against Epstein Barr virus (EBV) were consistent for the direction of change and globally significant, whereas the observed changes in percentage of natural killer (NK) cells, salivary immunoglobulin A (sIgA) concentration, and antibody titers against* Herpes simplex *virus (HSV) were not consistent and not significant. Analysis of the results of the relaxation studies indicated that the observed changes in sIgA concentration were consistent for direction of change and significant, the results for white blood cell count were consistent but not significant, and the results for percentage of monocytes were neither consistent nor significant.*

KEY WORDS: *in vitro* immune response; relaxation; stress; meta-analysis.

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INTRODUCTION

Data from a number of laboratories suggest that stress and relaxation may influence immunity in man (Ader, 1981; Ader *et al.,* 1991). Studies have assessed the impact of stress on immune function either by correlating stress measures with immunological test results (cross-sectional studies) or by assessing the changes in immunological test results in relation to an identified and specified stressor. The latter studies, use a (quasi)-experimental design and can be described as intervention studies, with the stressful event as the intervention. Studies investigating the effect of relaxation on immune function have generally used a (quasi)-experimental design, in which relaxation is the intervention.

Stress studies and, to a lesser extent, also relaxation studies have been extensively reviewed by a number of authors (Solomon, 1969; Locke, 1982; Jemmott, 1984; Evans *et al.,* 1989, Khansari *et al.,* 1990; O'Leary, 1990; Ader *et al.,* 1991). To our knowledge however, only one meta-analytic study has been published so far (Jemmott *et al.,* 1989).

The purpose of this meta-analytic study is to combine and integrate findings of (quasi)-experimental studies wherein changes in immunological and/or hematological[5] variables are assessed in relation to a stressful event or relaxation intervention.

MATERIALS AND METHODS

For this study, only those studies were selected which used a (quasi)-experimental design in which the change in the *in vitro* immune response of healthy subjects was assessed in relation to an intervention of short duration (stress or relaxation). The results of the large number of cross-sectional studies were therefore excluded from our analysis. Furthermore, studies in which the changes in immunological values were assessed in relation to, for example, the death of a spouse (Schleiffer *et al.,* 1983) or other stressors which have a great and often long-lasting impact on a person's life were also excluded. In these cases, the beginning and/or end of the stressor is not clearly fixed in time. Hence, the changes in values between baseline and stress moment cannot be determined.

To locate studies for possible inclusion in this review we used the MEDLINE system (1-8-66 until 1-8-91). The key words were *stress (psychological)* and *immunity (or subheading immunity)* and *relaxation, muscle*

[5]Whenever immunologic variables are mentioned, this should be read as immunologic and/or hematological variables.

relaxation, hypnosis, relaxation techniques or "*wit* and *humor*," and *immunity* (or *subheading immunity*). Furthermore, we used key words *humans* and *not animals* to exclude animal studies and restricted our selection to those studies written in English or Dutch. In this way 206 stress studies were detected. Of those studies, 113 investigated stress and immunity in relation to disease and 40 studies were identified as secondary studies consisting of reviews, letters, theories, and models. The cross-sectional studies and longitudinal studies for which the stressor was not specified were excluded. Finally, twenty-four studies were selected which reported change in immunological values within a group of healthy subjects in relation to a specified stressful event.

Forty-two relaxation studies were located. Thirty-three of these studies investigated relaxation in relation to disease or were exclusively concerned with the biochemical and immunological reactions associated with changes in tension of (specific) muscles. Of the remaining nine studies, three were secondary studies. The six primary studies were all included in this review. One study was identified as a stress study by MEDLINE which was a relaxation study (Jasnoski and Kugler, 1987). Furthermore, our own literature search resulted in the identification of three additional studies not identified by MEDLINE (Smith *et al.,* 1985; Olness *et al.,* 1989; Bongartz *et al.,* 1987). A total number of 10 relaxation studies was therefore, used in the present study.

Selection of Data

In the present study the difference between baseline and experimental values is of main interest. Thus, if studies did not give any data or results of statistical tests on the difference between baseline and stress (or relaxation) samples, the studies were reviewed but not used for further analysis. (Dorian *et al.,* 1982; Workman and La Via, 1987; Jasnoski, 1987). In those cases where baseline values were lacking but follow-up results were available, the latter were used instead of the missing baseline values [IL2-R (Halvorsen and Vassend, 1987), HSV (Glaser *et al.,* 1985b)]. If the same hypothesis was tested on more than one sample of individuals, all the results of the independent groups were used (Green and Green, 1987; Glaser *et al.,* 1990). However, if an immunological parameter was investigated in the same experimental group in more than one fashion, such as the assessment of the percentage of NK cells with different monoclonal antibodies, then all results were presented in the tables but only the result leading to the most conservative estimate of global significance was used for further analysis (Glaser *et al.,* 1986a; Naliboff *et al.,* 1991). Hence, if the direction

of change was consistent with the majority of the results, then the least significant result was used (Naliboff *et al.,* 1991), whereas when the direction of change was not consistent with that of the majority of the results, then the most significant result was used for computation of global significance (Glaser *et al.,* 1986a). Data on white blood cell count, monocytes, granulocytes, lymphocytes, B cells, T cells, T cell subpopulations, and NK cells were generally presented as the percentage of the total sample of cells. In those instances where both percentages and absolute numbers were reported, the results of the percentages were used for further analysis. One author (Landman, *et al.,* 1984) reported only the number of cells. In this case the results expressed as the number of cells were used for further analysis.

For the purpose of combining and integrating the findings of the selected studies, data were analyzed in two different ways, namely, by comparing the direction of change of an immunological variable as reported by different authors and by assessing global significance of the reported changes of an immunological variable. Other quantitative analysis could not be performed due to the limited information reported in the studies.

Direction of Change

Information about the direction of change of an immunological variable was found in texts or tables of the primary studies. A double positive sign (++) indicates a significant rise, a single positive sign (+) indicates a nonsignificant rise, a single negative sign (−) a nonsignificant decline, and a double negative sign (−−) a significant decline during stress or relaxation as compared to baseline. A positive/negative (±) sign indicates that the baseline and experimental values were identical, i.e., the change score was zero. Furthermore, the ± sign was used to describe those cases in which no indication was given of the direction of change and the results described as nonsignificant [T helper/inducer cells (Landman *et al.,* 1984), saliva IgA concentration (sIgA) (Kiecolt-Glaser *et al.,* 1984a), IgA concentration (Vassend and Halvorsen, 1987), Ab-HSV (Fittschen *et al.,* 1990), natural killer cell activity (NKCA) (Dorian *et al.,* 1982), and white blood cell count (WBC) and monocytes (Zachariae *et al.,* 1990)].

To assess whether results are consistent for a specific immunological parameter, at least two independent observations are needed. A total of 20 immunological variables from stress studies and 5 immunological variables from relaxation studies could be compared for direction of change. Results of different authors were described as consistent when all signs

were in the same direction or if all signs were in the same direction and one was indifferent (±).

Global Significance

To compute global significance, *p* values are needed which precisely describe the hypothesis of interest of this study, i.e., differences exist between baseline and experimental values. If available, these statistical data were used. In those cases where no statistical results were available on the difference between baseline and experimental values, but the main effect of time period (MANOVA) was presented, the latter was used for further analysis. If no statistical test results were reported but the mean and standard deviations were presented, then the data were statistically analyzed (Student *t* test) by us [stress studies (Naliboff *et al.*, 1991), relaxation studies (Peavey *et al.*, 1985)]. One report (Taylor *et al.*, 1986) presented data on the percentage of change between baseline and stress samples but not the standard deviations; *p* values could therefore not be computed by us.

Almost none of the studies reported exact *p* values. For statistical analysis the most conservative (less significant) value (i.e., < is converted to =) was used.

Global significance can be reliably determined only for those immunological variables for which all *p* values are available; otherwise the results would be biased in the direction of significance. In those instances where all studies ($N > 2$) but one reported *p* values and the direction of change was ± for the *p* value not reported, the not-reported two-tailed *p* value was set at 1.00 [Ab-HSV (Fittschen *et al.*, 1990), WBC and monocytes (Zachariae *et al.*, 1990)]. If the reported or computed *p* value was larger than .50, the *p* value was also set at 1.00 [sIgA concentration (Kiecolt-Glaser *et al.*, 1984a), WBC (Peavey *et al.*, 1985)]. Nonetheless, the number of immunological variables for which global significance could be computed was very small. Global significance could be computed for only six immunological variables in the stress studies and for three of the immunological variables tested in the relaxation studies.

Four statistical methods were used to compute global significance: (I) adding *p* values and (II) testing the mean *p* (the Eddington methods), (III) adding *z* scores (the Stouffer method), and (IV), multiplying the smallest *p* value by the number of studies (the Bonferoni method) (Rosenthal, 1978). All two-tailed *p* values were converted to one-tailed *p* values before computation of global significance . The results of method I and method IV are expressed as one-tailed *p* values. The results of methods II and III

are expressed as z scores which were transformed to one-tailed p values. After computation all the results were converted to two-tailed p values.

The method of adding p values requires that the sum of the p values does not exceed unity very much. When the sum of the p values does exceed unity, the overall p tends to be too conservative. When testing the mean p the number of studies should not be less than three. No restrictions are described for the use of the method of adding z scores (Rosenthal, 1978). The Bonferoni method is not applicable when the results are not consistent for the direction of change. In case the results are not consistent for the direction of change, the results of the Bonferoni method could give, at the same time, support to the hypothesis that the immunological value is lower and that it is higher during the intervention.

Methods I and II both compute the distribution of Σp_i under H_0, whereas methods III and IV compute whether highly significant findings can still be corrected by the other findings. The results of the latter methods are strongly influenced by highly significant p values, whereas the results of methods I and II are more equally influenced by extreme and normal p values.

RESULTS

In two cases the effect of both stress and relaxation was investigated in the same study (Peavey *et al.,* 1985; Kiecolt-Glaser *et al.,* 1986). In the study by Peavey *et al.* the first part of the study was cross-sectional and led to the identification of those subjects who were high in stress and low in phagocytic activity. In the second part of the study these subjects got biofeedback-assisted relaxation training. The results of the second part of this study could be used for further analysis in our study. In the study by Kiecolt-Glaser *et al.* all students were preparing exams. Half of the group was assigned to a relaxation intervention and practiced the technique until the exam. There were no data available on the change in immunological values before and after relaxation. Furthermore, the main effect for group membership was not significant for all immunological variables tested. In this case, it was decided to use the results of the effects of stress on the immunological variables for further analysis.

Stress Studies

Of the 23 selected stress studies, 18 used an exam as the stressor and 3 used an experimental stressor (Table I). The experimental stressors were

Table I. Stress Studies

Author	Year	*N*	Stressor	Immunological variables
Palmblad	1976	8	Exper.	WBC, granulocytes (polymorphonuclear cells) and monocytes, lymphocytes, IFN prod., phagocytic activity
Dorian	1982	8	Exam	WBC, lymphocytes, B cells (surface Ig), AET rosettes (T cells), late rosettes, prolif. response to PHA, Con A, PWM, antigen-specific plaque-forming cell response, suppressor cell activity, natural killer cell activity (NKCA)
Jemmott	1983	64	Exam	sIgA
Kiecolt-Glaser	1984a	75	Exam	IgA, IgG, IgM, sIgA, NKCA
Landman	1984	15	Exper.	WBC, granulocytes, monocytes (scatter, OKM1), lymphocytes, B cells (surface Ig), T cells (Leu 1), T/B, T-helper/inducer cells (Leu3a), T-suppressor/cytotoxic cells (Leu2a), Th/Ts, NK cells (OKM1)
Ursin	1984	38	Fall	IgG, IgA, IgM, C3, C4, C1-INH
Kiecolt-Glaser	1984b	42	Exam	Lymphocyte transformation by EBV
Glaser	1985a	40	Exam	T cells (OKT3), T-helper/inducer cells (OKT4), T-suppresosr/cytotoxic cells (OKT8), prolif. response to PHA and Con A
Glaser	1985b	49	Exam	Ab-EBV (N = 49), Ab early antigens to EBV (N = 32), Ab-HSV (N = 28), Ab-CMV (N = 20), poliovirus type 2 Ab titers (N = 15)
McClelland	1985	43	Exam	sIgA
Taylor	1986	41	Space flight	WBC, granulocytes, cosinophils, neutrophils, monocytes, lymphocytes, T cells (T11), T-suppressor/cytotoxic cells (T8), T-helper/inducer cells (T4), B-cells (B1), monocytes (M3), leukocytes (HLe-1), prolif. resp to PHA
Kiecolt-Glaser	1986	34	Exam	T-helper/inducer cells (OKT4), T-suppressor/cytotoxic cells (OKT8), Th/Ts, NKCA
Glaser	1986a	40	Exam	NK cells (Leu7, LGL), NKCA, IFN-γ prod.
Glaser	1986b	40	Exam	IgG, IgM, IgA
Halvorsen	1987	8	Exam	T-suppressor/cytotoxic cells (OKT8), T-helper/inducer cells (OKT4), IL2-R expression (anti-TAC), monocytes (ID5), prolif response to PHA, *D. farinae*, IL2, and pooled allogeneic cells
Vassend	1987	10	Exam	IgG, IgM, IgA, IgE
Workman	1987	15	Exam	Proliferative response to PHA
Glaser	1987	35	Exam	IFN-γ-prod., Ab-EBV, T cell killing of EBV-transf. cells, LIF activity, cAMP level in PBL
Jemmott	1988	15	Exam	sIgA
Mouton	1989	44	Exam	sIgA
Glaser	1990	22 25 44	Exam	IL2-R express. (anti-TAC), IL2 mRNA levels, IL2 synthesis of PBL
Fittschen	1990	61	Exam	B cells (large immunocytes), Ab-HSV
Tomei	1990	14	Exam	Phorbol ester inhibition of radiation induced apoptosis in PBL
Naliboff	1991	23	Exper.	T cells (Leu4), T-helper/inducer cells (Leu3), T-suppressor/cytotoxic cells (Leu2), NK cells (Leu7, Leu11, Leu19), B cells (Leu 16), NKCA

mental arithmetic (Naliboff *et al.,* 1991), firing an electronic rifle at small targets while hearing battle noise (Palmblad *et al.,* 1976), and cognitive conflict (Landman *et al.,* 1984). Taylor *et al.* (1986) used space flight and splash-down and Ursin *et al.* (1984) used repeated 20-m falls in a lifeboat built especially for rescue operations from oil platforms as a stressor. In the latter study there was no indication which of the two samples, day 1 or day 5, was the stress sample. However, they report a significant reduction in the Fear Index with repeated exposure to the falls. The situation at day 1 when the subjects anticipate the fall, therefore seems the most stressful.

Immunological variables which were assessed in only one study and could therefore not be further analyzed (see Materials and Methods) were the combination of polymorphonuclear leukocytes and monocytes (Palmblad *et al.,* 1976), complement components C3, C4, and C1-1NH (Ursin *et al.,* 1984), lymphocyte transformation by EBV (Kiecolt-Glaser *et al.,* 1984b), T cell proliferation to concanavalin A (Con A) (Glaser *et al.,* 1985a), antibody titers against cytomegalovirus (CMV) (Glaser *et al.,* 1985b), percentage eosinophils (Taylor *et al.,* 1986), proliferative response to *D. farinae,* IL2, and pooled allogeneic cells (Halvorsen and Vassend, 1987), serum concentrations of IgE (Vassend and Halvorsen, 1987), T cell killing of EBV-transformed cells, leukocyte migration inhibition factor (LIF) activity and cAMP level (Glaser *et al.,* 1987), IL2 mRNA levels and IL2 synthesis of peripheral blood lymphocytes (Glaser *et al.,* 1990), and phorbol ester inhibition of radiation-induced apoptosis in peripheral blood lymphocytes (Tomei *et al.,* 1990).

Immunological variables which were assessed at least twice in different studies or for different groups of subjects are listed in Table II.

Direction of Change

The results on the direction of change were consistent for WBC (Palmblad *et al.,* 1976; Landman *et al.,* 1984), percentage of granulocytes (Landman *et al.,* 1984; Taylor *et al.,* 1986), Ab-EBV titers (Glaser *et al.,* 1985b, 1987), and Ab-HSV titers (Glaser *et al.,* 1985b; Fittschen *et al.,* 1990). The values of these variables were observed to rise during the stress period. Expression of IL2 receptor (Glaser *et al.,* 1990, 4 studies) and T-cell proliferation in response to phytohemagglutinin (PHA) (Glaser *et al.,* 1985a; Taylor *et al.,* 1986; Halvorsen and Vassend, 1987) were consistently observed to be reduced during the stress period. The direction of change for the remaining immunological variables is equivocal (Table II).

Table II. Stress Studies[a]

Immunological variable	Author	DC	Statistics
WBC	Palmblad (1976)	±	
	Landman (1984)	+	
Monocytes	Landman (1984)		
	OKM	++	$p < .05$
	Scatter	+	
	Taylor (1986)	−	
	Halvorsen (1987)	+	
Granulocytes, neutrophils	Landman (1984)	+	
	Taylor (1986)	+	
Lymphocytes	Palmblad (1976)	−	
	Landman (1984)	+	
	Glaser (1985a)	−−	$p < .01$ [$F(1,39) = 10.86$]
	Taylor (1986)	−	
B cells	Landman (1984)	++	$p < .05$
	Taylor (1986)	+	
	Fittschen (1990)	++	$p < .001$
	Naliboff (1991)	−	
T cells	Landman (1984)	+	
	Glaser (1985a)	−−	$p < .01$ [$F(1,39) = 8.97$]
	Taylor (1986)	+	
	Naliboff (1991)	−	
T-helper/inducer cells	Landman (1984)	±	
	Glaser (1985a)	−−	$p < .05$ [$F(1,39) = 4.17$]
	Taylor (1986)	+	
	Kiecolt-Glaser (1986)	−−	$p < .003$ [$F(1,29) = 10.27$]
	Halvorsen (1987)	−	
	Naliboff (1991)	−	$p < .20$, $t = -1.3$, df = 22
T-suppressor/cytotoxic cells	Landman (1984)	+	
	Glaser (1985a)	−−	$p < .05$ [$F(1,39) = 5.90$]
	Taylor (1986)	−	
	Kiecolt-Glaser (1986)	−	$p < .20$ [$F(1,29) = 3.38$]
	Halvorsen (1987)	−	
	Naliboff (1991)	++	$p < .10$, $t = 2.01$, df = 7
Th/Ts ratio	Landman (1984)	−	
	Glaser (1985a)	+	
	Taylor (1986)	+	
	Kiecolt-Glaser (1986)	−−	$p < .03$ [$F(1,29) = 5.57$]
	Halvorsen (1987)	±	
NK cells	Landman (1984)		
	OKM1	++	$p < .01$
	Glaser (1986a)		
	Leu 7[b]	−−	$p < .0001$ [$F(1,39) = 41.73$]
	LGL	−−	$p < .01$ [$F(1,39) = 6.58$]
	Naliboff (1991)		
	Leu 7[b]	++	$p < .05$, $t = 2.34$, df = 22
	Leu 11	++	$p < .01$, $t = 2.97$, df = 22
	Leu 19	++	$p < .02$, $t = 2.69$, df = 22

Table II. Continued

Immunological variable	Author	DC	Statistics
IL2-R expression	Halvorsen (1987)	−−	$p < .01$, df = 7
	Glaser (1990)		
	Study 1	−−	$p < .001$ [$F(1,18) = 17.89$]
	Study 1[b]	−−	$p < .05$ [$F(1,21) = 5.76$]
	Study 2[b]	−−	$p < .001$ [$F(1,24) = 28.19$]
	Study 3[b]	−−	$p < .02$ [$F(1,21) = 6.53$]
sIgA	Jemmott (1983)	−−	$p < .025$
	Kiecolt-Glaser (1984a)	±	$p > .50$ [$F(1,57) = 0.05$]
	McClelland (1985)	+	$p < .06$
	Jemmott (1988)	−−	$p < .0001$ [$F(2,28) = 2.87$]
	Mouton (1989)	−−	$p < .0001$ [$F = 10.42$]
IgA	Ursin (1984)	−	
	Kiecolt-Glaser (1984a)	++	$p < .02$ [$F(1,42) = 6.05$]
	Glaser (1986b)	++	$p < .04$
	Vassend (1987)	±	
IgM	Ursin (1984)	−−	$p < .01$
	Kiecolt-Glaser (1984a)	+	$p < .20$ [$F(1,42) = 2.61$]
	Glaser (1986b)	++	$p < .05$
	Vassend (1987)	−	
IgG	Ursin (1984)	−	
	Kiecolt-Glaser (1984a)	+	$p > .50$ [$F(1,35) = 1.43$]
	Glaser (1986b)	++	$p < .002$
	Vassend (1987)	++	$p < .05$ [$F = 5.14$]
Ab-EBV	Glaser (1985b)	++	$p < .0001$ [$F(2,94) = 42.81$]
	Glaser (1987)	++	$p < .0001$ [$F(5,160) = 9.02$]
Ab-HSV	Glaser (1985b)	++	$p < .0001$ [$F(1,26) = 22.02$]
	Fittschen (1990)	±	
T cell proliferation PHA	Glaser (1985a)	−−	$p < .03$ [$F(1,39) = 5.51$]
	Taylor (1986)	−	
	Halvorsen (1987)	−	
Natural killer cell activity (NKCA)	Kiecolt-Glaser (1984a)	−−	$p < .003$ [$F(1,68) = 9.87$]
	Glaser (1986a)	−−	$p < .01$ [$F(1,39) = 8.14$]
	Kiecolt-Glaser (1986)	−−	$p < .003$ [$F(1,32) = 11.07$]
	Naliboff (1991)	+	$p < .20$, $t = -1.68$, df = 22
INF-γ production	Glaser (1986a)	−−	$p < .0001$ [$F(1,39) = 106.13$]
	Plamblad (1987)	+	
	Glaser (1987)	−−	$p < .05$ [$F(5,165) = 2.88$]

[a]DC, direction of change; p values indicate two-tailed significance.
[b]p values used for computation of global significance.

Global Significance

Global significance could be assessed for percentage of NK cells, IL2-R expression on lymphocytes, sIgA concentration, antibody titers to EBV, antibody titers to HSV, and NKCA. In Table III the results of the statistical tests are presented.

Since only the results of two studies were available for the assessment of global significance for Ab-EBV and Ab-HSV, the method of testing the

Table III. Global Significance for the Variables Tested in the Stress Studies[a]

Immunological variable	(i) Adding p values	(ii) Testing mean p value	(iii) Adding z scores	(iv) Smallest p value
NK cells ($N = 3$)	.36	.35	.76	Not done
IL2-R expression ($N = 4$)	$2{\cdot}10^{-7}$	$6.8{\cdot}10^{-4}$	$6{\cdot}10^{-7}$	4.10^{-3}
sIgA ($N = 5$)	.12	.11	$3.2{\cdot}10^{-4}$	Not done
Ab-EBV ($N = 2$)	10^{-8}	Not done	10^{-7}	2.10^{-4}
Ab-HSV ($N = 2$)	.25	Not done	$5.2{\cdot}10^{-3}$	2.10^{-4}
NKCA ($N = 4$)	.057	.059	$3.2{\cdot}10^{-4}$	Not done

[a]Two-tailed global significance as computed by (i) adding p values, $(\Sigma p)^N/N!$; (ii) testing mean p value, $[.50-(\Sigma p/N)]\ \sqrt{12N}$; (iii) adding z scores, $(\Sigma z)/\sqrt{N}$; and (iv) the Bonferoni method, smallest p value $\times N$.

mean p value was not used (see Materials and Methods). The Bonferoni test was not performed for percentage of NK cells and sIgA concentration, since these results were not consistent for the direction of change (see Materials and Methods).

The results of adding p values and testing the mean p were very close for percentage of NK cells, sIgA concentration, and NKCA. The results of the method of adding z scores for sIgA concentration, Ab-HSV and NKCA depart considerably from the results of the other methods. This may be due to the fact that the method of adding z scores is, as the Bonferoni method, very sensitive to extreme p values, i.e., high z scores.

Summarizing, the results of the stress studies were observed to be consistent for the direction of change and globally significant for IL2 receptor expression on lymphocytes and antibody titers against EBV. The observed changes in percentage of NK cells, sIgA concentration, and NKCA were not consistent and not significant and the antibody titers against HSV were consistent but not significant.

Relaxation Studies

The interventions used to obtain a pleasant or relaxed state in the experimental subjects were progressive muscle relaxation, focused breathing and imagery (Jasnoski and Kugler, 1987), Benson's relaxation response, guided visualization, massage, and lying quietly with eyes closed (Green and Green, 1987), lying quietly with eyes closed, focused breathing, and repeating "hum" with each exhalation (Green *et al.*, 1988), guided imagery (Zachariae *et al.*, 1990), biofeedback-assisted relaxation (Peavey *et al.*, 1985), hypnoses with (Smith *et al.*, 1985; Olness *et al.*, 1989) or without (Bongartz *et al.*, 1987; Olness *et al.*, 1989) specific suggestions to alter the

Table IV. Relaxation Studies

Author	Year	*N*	Method	Immunological variables
Peavey	1985	16	Biofeedback	WBC, neutrophils (band), neutrophils (segmented), monocytes, lymphocytes, basophils, eosinophils, phagocytic activity (nitroblue tetrazolium test)
Kiecolt-Glaser	1985	27 (12)	Relaxation	NKCA, Ab-HSV, PHA, PWM
Smith	1985	1	Hypnosis	Lymphocyte proliferation to *Varicella zoster* Virus
Dillon	1985	9	Humorous film	sIgA
Jasnoski	1987	10	Relaxation	sIgA
		10	Relaxation + imagery	sIgA
Green, R.	1987	50	Relaxation	sIgA
Bongartz	1987	12	Hypnosis	WBC, granulocytes (PMN), monocytes, lymphocytes
Green, M.	1988	40	Relaxation	sIgA, IgA, IgG, IgM
Olness	1989	19	Hypnosis	sIgA, sIgG
		19	Hypnosis + specific suggestions	sIgA, sIgG
Zachariae	1990	10	Relaxation	HT, HB, WBC, lymphocyte, T cells (T11, T3), T-helper/inducer cells (T4), T-suppressor/cytotoxic cells (T8), B cells (B1), monocytes (My4), NK cells (N901), NKCA

immune response, and viewing a humorous film (Dillon *et al.,* 1985) (see Table IV).

Immunological variables which were tested in only one study were percentage basophils, eosinophils, phagocytic activity, band granulocytes, segmented granulocytes (Peavey *et al.,* 1985), antibody against HSV, and lymphocyte proliferation in response to PHA and pokeweed mitogen (PWM) (Kiecolt-Glaser *et al.,* 1985). Lymphocyte proliferation to *Varicella zoster* virus was tested only in a quasi-experimental case study (Smith *et al.,* 1985). The ratio of the T-helper/inducer and T-suppressor/cytotoxic cells (Kiecolt-Glaser *et al.,* 1986), granulocytes not differentiated (Bongartz *et al.,* 1987), serum immunoglobin A, G, and M concentrations (Green *et al.,* 1988) and saliva immunoglobulin G concentrations (Olness *et al.,* 1989), hematocrit, hemoglobin, differential count not further specified, percentage T cells, and percentage T helper/inducer cells, T suppressor/cytotoxic cells, B cells, and NK cells (Zachariae *et al.,* 1990) were also all tested once and therefore not further analyzed.

A total of five immunological parameters, namely, WBC, percentage of monocytes, lymphocytes, sIgA concentration, and NKCA, was measured twice or more (see Table V).

Table V. Relaxation Studies[a]

Immunological variable	Author	DC	Statistics
WBC	Peavey (1985)	−	$p > .50$, $t = -.17$, df = 15
	Bongartz (1987)	−−	$p < .00001$ [$F(1,11) = 50.87$]
	Zachariae (1990)	±	
Monocytes	Peavey (1985)	+	$p < .50$, $t = 1.02$, df = 15
	Bongartz (1987)	−−	$p < .01$ [$F(1,11) = 17.25$]
	Zachariae (1990)	±	
Lymphocytes	Peavey (1985)	+	$p < .50$, $t = 1.18$, df = 15
	Bongartz (1987)	−−	$p < .001$ [$F(1,11) = 27.17$]
	Zachariae (1990)	+	
sIgA	Dillon (1985)	++	$p < .026$, $t = 2.26$, df = 8
	Green, R. (1987)		
	Relaxation	++	$p < .05$ ($F = 4.34$)
	Visualization	++	$p < .05$ ($F = 6.67$)
	Massage	++	$p < .01$ ($F = 20.55$)
	Lying down	+	
	Green, M. (1988)	++	$p < .001$ [$F(1,39) = 103.62$]
	Olness (1989)		
	Hypnosis	+	$p = .94$ ($F = .053$)
	Hypnosis + specific suggestions	++	$p = .007$ ($F = 5.53$)
Natural killer cell activity (NKCA)	Kiecol-Glaser (1985)	++	$p < .05$ [$F(2,35) = 3.63$]
	Zachariae (1990)	+	

[a]DC, direction of change; p values indicate two-tailed significance.

Direction of Change

The results are consistent for a reduction in WBC (Peavey *et al.*, 1985; Bongartz *et al.*, 1987, Zachariae *et al.*, 1990) and a rise in sIgA concentration and NKCA (Kiecolt-Glaser *et al.*, 1985; Zachariae *et al.*, 1990) during or after a relaxation intervention.

Global Significance

Global significance was determined for WBC, percentage of monocytes, and sIgA concentration (see Table VI). One p value of the sIgA concentrations was missing. Since a relative large number of results was available, it was nonetheless decided to compute global significance for this immunological parameter. The results were not significant for WBC and percentage of monocytes but were highly significant for sIgA concentration. The result of adding the z score for WBC depart considerably from the results of adding p values and testing the mean p. These differences are due to the fact that the reported p value by Bongartz was highly significant (see Materials and Methods).

Table VI. Global Significance for the Variables Tested in the Relaxation Studies[a]

Immunological variable	(i) Adding p values	(ii) Testing mean p value	(iii) Adding z scores	(iv) Smallest p value
WBC ($N = 3$)	.33	.32	.0093	Not done
Monocytes ($N = 3$)	.66	.62	.27	Not done
sIgA ($N = 7$)	$54 \cdot 10^{-7}$	$14 \cdot 10^{-5}$	$2 \cdot 10^{-7}$	$7 \cdot 10^{-3}$

[a]Two-tailed global significance as computed by (i) adding p values, $(\Sigma p)^N/N!$; (ii) testing mean p value, $[.50-(\Sigma p/N)]\sqrt{12N}$; (iii) adding z scores, $(\Sigma z)/\sqrt{N}$; and (iv) the Bonferoni method, smallest p value $\times N$.

Summarizing, the results for sIgA concentration were consistent for direction of change and the overall result was highly significant, the results for WBC are consistent but not significant, and the results for percentage of monocytes are not consistent and not significant.

Comparison of the Stress and Relaxation Studies

All variables which were studied under the relaxation condition were also studied under the stress condition. Comparison of the results revealed that WBC increases during stress and decreases during relaxation, although global significance could not be determined or was not significant (see Table VII). sIgA concentration and NKCA are observed to increase consistently and for sIgA concentration also significantly after relaxation. Under the stress condition the results are not consistent for the direction of change.

DISCUSSION

For the present meta-analytic study we selected all available quasi-experimental studies which reported data on the change in immune

Table VII. Combined Results[a]

Immunological variable	Stress condition		Relaxation condition	
	DC	Global significance	DC	Global significance
WBC	+		−	$p < .33$
Monocytes	±		±	$p < .66$
Lymphocytes	±		±	
sIgA	±	$p < .12$	+	$p < .007$
NKCA	±	$p < .59$	+	

[a]DC, direction of change; +, consistent positive; −, consistent negative; ±, inconsistent results.

function of healthy subjects under conditions of stress or relaxation. Generally, it is desirable to include all studies for which there is not good evidence of biased findings (Jackson, 1980). Furthermore, selection of only those studies which are methodological and statistical adequate would have reduced the already small number of selected studies drastically.

Meta-analytic studies use the data presented by the authors of the primary studies. The less precise the data of the primary studies, the less reliable the results of the meta-analytic study. In this study, it was observed that nine of the primary stress studies and three of the relaxation studies did not report p values when observed changes were not significant. Consequently, for many of the immunological variables, global significance could not be determined. Furthermore, in some studies only the main effect for time was given. A significant main effect for time was often taken as evidence that indeed there was a significant difference between the data sampling moments of interest. However, a significant main effect for time does not necessarily have to indicate that the differences between stress and baseline samples are significant (Glaser *et al.,* 1985b). The probability that significant changes in immunological variables over time can be explained by some alternative parameters, such as month-related variability in immunological test results (van Rood *et al.,* 1991), is increasing with increasing time intervals. The results of Mouton (1989), who measured sIgA on four occasions over 2 academic years, could very well be influenced by month- or season-related variability in sIgA concentration.

The results of the meta-analysis, especially the lack of consistent findings for the direction of change, could be due partly to the use, as described by the authors of the primary studies, of different immunological techniques (coulter counter and flow cytometry), and/or different monoclonal antibodies for the determination of the percentage of monocytes (moab: ID5, My4, M3), B cells (surface Ig, B1, large immunocytes, Leu 16), T cells (Leu 1, OKT3, T11, Leu 4, T3), T helper/inducer cells (Leu3a, OKT4, T4, Leu3), T cytotoxic/suppressor cells (Leu2a, Leu2, OKT8, T8), and NK cells (OKM1, Leu7, large granular lymphocytes, Leu 19, N901). Even the technique for the assessment of the number of leukocytes is not always the same. Taylor *et al.* (1986) used the monoclonal antibody "HLe-1," whereas all other authors used the coulter counter.

The variety of monoclonal antibodies and techniques used in the studies partly reflects the development and hence availability of new techniques and more cell subset-specific monoclonal antibodies.

Another source of variability is the type of intervention. Although most stress studies (N = 18; 78.3%) used an academic exam as the stressor and 6 relaxation studies (54.5%) used a specific relaxation method, a total of 6 different stress interventions and 11 different relaxation interventions

was reported. It could be argued that certain specific characteristics of the relaxation techniques or stressors could have influenced the effect of the intervention on the immune response. As yet, it is not known which part of the total variability in immunological test results *between* studies can be accounted for by the use of different stressors.

In our study we have analyzed 26 subgroups. These subgroups, possibly, differ for population characteristics such as country (culture), sex, age, and educational level. The observed differences between studies might well be explained by a difference in effect of a particular stressor on different subpopulations. The variability as the result of the use of different subpopulations could even be larger than the variability caused by the use of different stressors. Observe that we analyzed 26 subgroups associated with only 6 different stressors.

By a completely different line of reasoning, it can be argued that not the objective characteristics of the stressor-stimulus, such as duration (acute or chronic) and intensity, but the subjective evaluation and interpretation of the situation by the individual[6] determines the stress level. Consequently, stress is more a characteristic of the subject than a characteristic of the event, i.e., rather subject specific than situation specific. As a result it is expected that a larger proportion of the variability in immunological test results between studies will be accounted for by between-subject variability than by between-stressor variability.

In the stress studies the samples are generally collected on the day the subject is exposed to the stressor, i.e., exam. In the relaxation studies there is considerably more variability in the choice of data sampling. Some authors collect their samples directly after the relaxation intervention, whereas others point out that, for example, serum immunoglobulin concentrations cannot be expected to change so quickly. They therefore also collected samples 22 days after the relaxation intervention (Green *et al.*, 1988).

The results of this study underline the need for more extensive reports of primary data and for more strict replication studies. We therefore propose that future studies test the same immunological parameters and use the same immunological tests as have been used in the methodologically sound studies done so far.

[6]Stress can be defined as the process by which individuals realize and identify problems and appraise, evaluate, and interpret the necessity to react upon the problems as well as their evaluation of their own coping resources to solve the problems (Lazarus, 1966).

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