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The Effect of Serum from Patients with Acute Myocardial Infarction on *In Vitro* Lymphocyte Reactivity.

I. Inhibition of Mitogen Stimulation.

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SUMMARY **Sera from 20 patients obtained within 24 hours and one week after acute myocardial infarction (AMI) were tested for their immunomodulating effect on concanavalin-A (con-A) stimulated lymphocyte cultures from 11 healthy unrelated donors. Individual control sera from 21 healthy donors and 5 pools of control sera were used for comparison. Cortisol levels were tested in patients' and controls' sera. A significantly higher suppressive effect was seen in the presence of patients' sera taken at 24 hours than corresponding sera taken one week later. However, the suppressive effect after one week was increased as compared to control sera. A significant correlation between the degree of suppression and the cortisol level in corresponding sera was observed. An increased immunosuppression was observed with increased cortisol levels.**

Patients undergoing major surgery or following accidental trauma often suffer from a decrease in immunocompetence that can predispose them to serious and even fatal infections. [1,2] Immunosuppressive serum factors were found in patients following traumatic injuries and more recently Ozkan *et al.* [3-5] detected increased levels of a specific glycopeptide in sera from patients after blunt trauma and after thermal injuries. Alterations in cell mediated immunity during stress, bereavement and depression were reported, [6] and stress associated implications in decreased cell mediated immunity were also observed in the acquired immunodeficiency syndrome. [7]

The mechanism by which psychological stress may influence immunological functions is not clear. However, evidence exists that immunological homeostasis may require not only the regulatory influence of immunocompetent cells but may also be influenced by the neuroendocrine axis. [6] The immunomodulating effect of glucocorticoids in immunologic regulation was emphasized by a number of investigators, and their important role in immuno-suppression in stressful situations was suggested. [6,8,9]

Adrenocortical response to stress in acute myocardial infarction (AMI) was reported [10] and increased levels of cortisol and other hormones were found in certain cases of acute MI. [11,12]

To our knowledge the possible immunomodulating effect of sera from patients after AMI on cell-mediated immune reactions was not investigated. The objectives of the present study was to evaluate the possible effect of these sera on the proliferative response of mitogen stimulated lymphocyte cultures from healthy controls and to observe whether this effect is reversible, time correlated, and influenced by the patients' serum cortisol levels. Sera from patients with AMI taken 24 hours and one week later were tested for their effect on mitogen stimulated lymphocyte proliferation from unrelated healthy donors and the results compared with individual patients and pooled control sera. An attempt was made to correlate the immunomodulating effect to corresponding cortisol levels in patients' and controls' sera.

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MATERIALS AND METHODS

Subjects

Twenty patients, 15 men (aged 39 to 77 years, mean age 61 years) and 5 women (aged 61 to 68 years, mean age 64.8 years) admitted to the Intensive Coronary Care Unit with acute chest pain, acute changes in the ECG: ST elevation, and/or near Q waves and increased levels of cardiac enzymes were included in this study. The clinical data of these patients are summarized in Table 1.

Sera obtained from the above mentioned patients at 24 hours and one week later were tested for their modulating effect on mitogen-stimulated lymphocyte proliferation and for their cortisol concentration. Sera from 21 healthy donors served as controls for mitogenic stimulation. Thirteen of these sera were also examined for cortisol concentration.

For mitogenic stimulation, lymphocytes from 11 healthy unrelated volunteers were used. Individual test and control sera were compared for their effect on mitogenic lymphocyte stimulation with 5 pools of control sera. For the preparation of a single pool, sera from 5 healthy donors were mixed, divided in aliquots and stored frozen at-20°C until use.

The pooling of serum from normal donors summates growth factors not always found in individual sera. By using on lymphocytes from the same donors pooled and individual control serum simultaneously with the patients' sera (taken at 2 different times), we tried to establish a uniform set of conditions to evaluate cellular reactivity in the presence of different sera.[13]

Table 1. Cardiac diagnosis of the 20 patients.

No.	Patient	Sex	Age	Diagnosis
1	A.M.	M	45	A.M.I.* Inferior
2	B.Y.	M	64	A.M.I. Inferolateral
3	Z.I.	M	70	A.M.I. Inferior
4	A.M.	M	39	A.M.I. Anteroseptal
5	A.I.	M	75	A.M.I. Anteroseptal
6.	H.Y.	M	52	A.M.I. Inferior
7	H.M.	M	64	A.M.I. Anterolateral
8	N.V.	F	63	A.M.I. Inferior
9	S.A.	M	72	A.M.I. Lateral
10	Z.J.	M	55	A.M.I. Anteroseptal
11	S.J.	M	61	A.M.I. Inferior
12	P.L.	F	61	A.M.I. Inferior
13	S.D.	M	49	A.M.I. Anterior
14	B.M.	M	77	A.M.I. Anterior
15	A.M.	F	68	A.M.I. Inferior
16	G.I.	M	65	A.M.I. Inferior
17	V.H.	F	65	A.M.I. Anteroseptal
18	T.E.	F	67	A.M.I. Anteroseptal
19	M.G.	M	67	A.M.I. Anteroseptal
20	K.G.	M	74	A.M.I. Inferior

* – Acute Myocardial Infarction

Lymphocyte culture conditions

Lymphocyte cultures were prepared according to the method described previously.[14] Heparinized blood was obtained from healthy unrelated donors. The lymphocytes were separated by the Ficoll-Hypaque method.[15] Cells collected after separation on Ficoll-Hypaque were washed three times with phosphate-buffered saline and cultured in RPMI-1640 (Gibco) medium supplemented with 15% human serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Lymphocyte suspensions adjusted to 1×10^6 viable cells/ml in complete medium were distributed, 0.2 ml/well, in Falcon tissue culture plates at a concentration of 2×10^5 lymphocytes/well. According to the serum used, 4 conditions of cultures were prepared with lymphocytes from the same donor: cultures in the presence of the patients' sera (within 24 hours and 1 week after the AMI), cultures with individual control serum, and cultures with pooled control serum (fresh frozen from 5 healthy blood donors).

For each test and control serum cultures stimulated with concanavalin A (con-A) 2 mg/ml and unstimulated lymphocyte cultures were incubated for 4 days and DNA synthesis determined by addition of 1 μCi/culture of tritiated thymidine (Amersham) 5 hours before harvesting. The cultures were harvested on glass fiber filters on a semiautomatic harvester (PML Co., Yahud), and assessed for tritiated thymidine uptake by evaluation of counts per minute (cpm) culture in an LKB liquid scintillation counter. The degree of stimulation was expressed as percentage of relative response (RR%),[16] as follows :

$$RR\% = 100 \times \frac{\text{cpm con-A stimulated cultures in test serum} - \text{cpm unstimulated cultures}}{\text{cpm con-A stimulated cultures in pooled serum} - \text{cpm unstimulated cultures}}$$

Low values of RR% were obtained when the sera had a highly suppressive effect on mitogenic stimulation and increased RR% values were obtained when this effect was lower.

Cortisol determination

Due to the diurnal rhythm variation of cortisol, blood samples were always taken at the same hour in the morning. For the determination of serum cortisol levels, a solid phase radioimmunoassay was used (kits supplied by Diagnostic Products Corporation, Los Angeles, USA).

Statistical analysis

The nonparametric Wilcoxon test for paired groups and the Mann Whitney rank sum U test for unpaired groups were used. Pearson linear correlation was used to demonstrate the relationship between the degree of suppression (RR%) and the cortisol levels.

RESULTS

Effect of sera after AMI on con-A stimulated lymphocyte cultures (Fig. 1)

The serum of patients with acute MI showed a suppressive effect on con-A stimulated lymphocyte cultures. The serum suppressive effect was significantly increased ($p < 0.05$) within the first 24 hours of the AMI compared to sera from the same patients obtained one week later. As a consequence, the mean value of RR% ± SE of cultures incubated with the patients' sera during the first 24 hours of acute MI was significantly lower as compared to cultures incubated with sera obtained one week later.

When compared to control sera (mean RR% 96.5 ± 5), the suppressive effect of the patients' sera was more evident and this could be observed within the 24 hours of the acute MI ($p < 0.03$) as well as one week later ($p < 0.04$).

Serum cortisol levels in patients after AMI (Fig. 2)

Significant increased mean cortisol levels were found in sera obtained within 24 hours of the acute MI as compared to serum levels one week later: mean serum cortisol (mg/dl ± SE) = 26.0 ± 3 vs. 18.4 ± 2, $p < 0.05$. In controls, the serum cortisol was significantly lower as compared to that of patients with AMI and this was observed within 24 hours as well as one week after

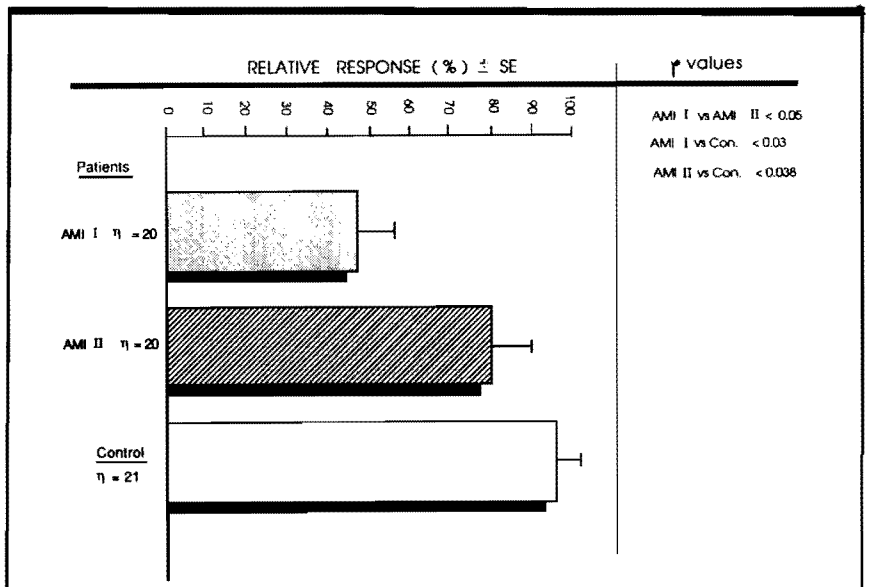

Fig. 1 Sera from patients 24 hours (AMI I) and 1 week after acute myocardial infarction (AMI II) and sera from controls (Con) were tested on the same donors' Con–A stimulated lymphocytes and their effect on cell proliferation was compared with pooled human serum for calculation of relative response % (RR%).

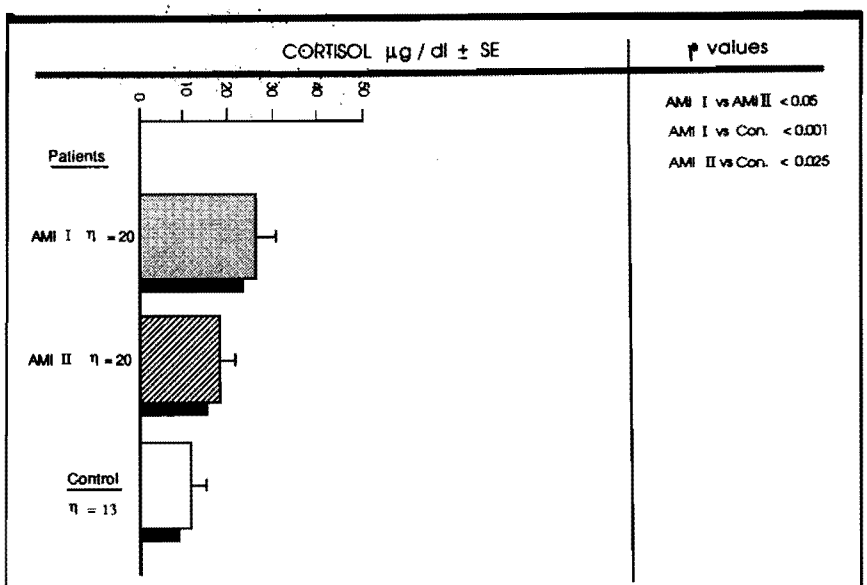

Fig. 2 Cortisol levels in sera from 20 patients and 21 controls (for details see Fig. 1).

the AMI: mean serum cortisol in controls (mg/dl ± SE) = 11.8 ± 8, $p < 0.001$, and $p < 0.025$ (within 24 hours and after one week, respectively).

Correlation between the cortisol levels and the suppressive effect of sera after AMI on con-A stimulated lymphocytes (Fig. 3, 4)

When RR% in cultures incubated with sera from the patients within 24 hours and one week after acute MI were correlated with corresponding cortisol levels, a significant inverse correlation between increasing RR% and decreasing cortisol levels was observed ($r = -0.493$, $p < 0.0027$, Fig. 3).

For control sera from 13 healthy donors, the inverse correlation between increasing RR% and decreasing cortisol levels was not significant: $r = -0.361$, $p < 0.21$ (Fig. 4).

DISCUSSION

The present results suggest that during the first 24 hours after the AMI, changes in the suppressive effect of the patients' sera on con-A stimulated unrelated lymphocytes occur, and that in the majority of cases a good correlation can be observed with the patients' serum cortisol levels.

The exact turning point for the decreased serum suppressive effect was not determined in this study. However, a significantly increased mean RR% ($p < 0.05$) was observed with the sera taken one week after the AMI as compared with the sera taken within the first 24 hours after the AMI. Although other undetermined factors like heart reactive antibodies which may combine with circulating cardiac autogens in the presence of elevated levels of C_3d complement, forming soluble complexes,[17] or specific catecholamines which were found to be elevated during myocardial infarction[18] could influence our results, a significant relationship between increased cortisol levels and increasing suppression,

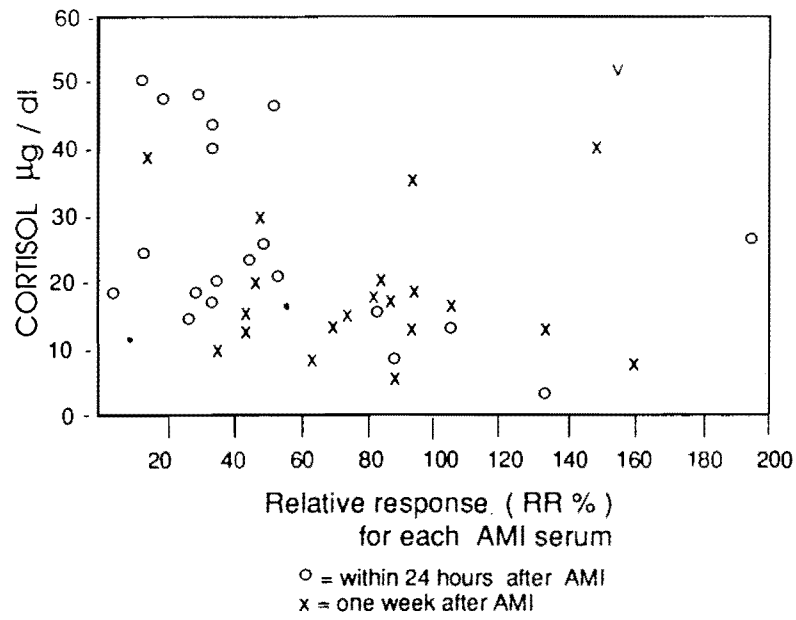

Fig. 3 The inverse correlation coefficient according to Pearson between increasing RR% and decreasing cortisol levels in sera from patients after acute MI was significant ($r = 0.493$, $p < 0.027$.

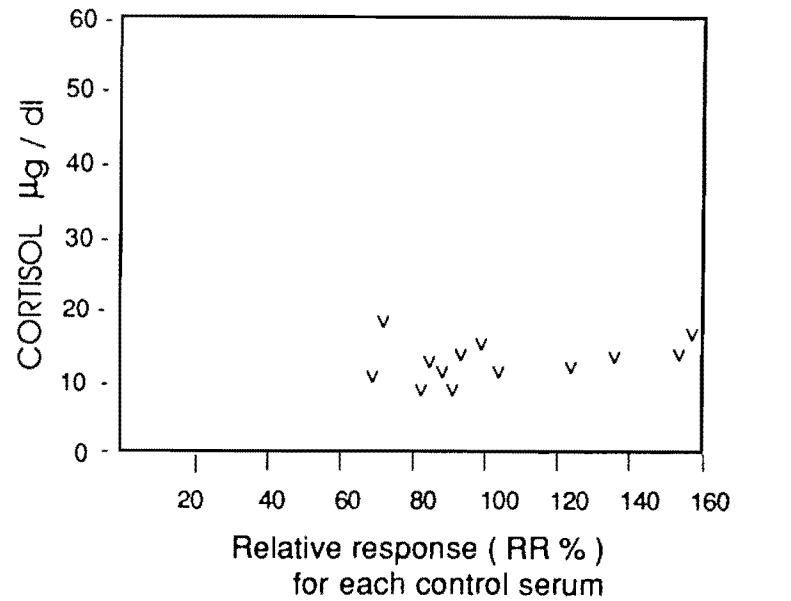

Fig. 4 For control serum the inverse correlation coefficient according to Pearson between increasing RR% and decreasing cortisol levels was not significant.

and the opposite-decreased cortisol levels and decreasing suppression was observed.

The firm correlation between serum cortisol levels and immunosuppression could also be observed when control sera were compared to patients' sera obtained one week after the AMI with a signifcantly lower suppressive effect in the former, associated with corresponding significantly lower cortisol levels.

The increased serum cortisol levels in AMI were suggested to be a response to stress, [10] and sometimes high concentrations as observed in Cushing's syndrome were reported. [12] Prakash *et al.* [19] showed that in complicated cases of AMI the cortisol levels may remain elevated until death. In our series, 7 out of 20 patients (35%) had morning serum cortisol levels higher than 25 mg/dl and only in 3 of them the levels remained consistently high also one week later. These 3 patients and 2 from the other 4 patients had decompensated hemodynamics manifested by severe congestive heart failure, complicated arrhythmias and infections-pneumonia, pseudomonas sepsis-complications in the course of their illness.

The importance of glucocorticosteroids in immunologic regulation was emphasized by a number of investigators, [6,8,9,20] who suggested that stress induced increased levels of glucocorticoids may have a counterregulation effect on immune functions and may suppress potentially dangerous overactive immune reactions during stress situations. [20,21] The immunosuppressive effects of glucocorticoids are directed mainly at cell mediated immunity, and mitogenic induced lymphocyte proliferation was reported to be reduced in the presence of corticosteroids. [21]

Corticosteroid receptors are present on both neutrophils and lymphocytes, [8] and preferentially helper T cells were reported to be more affected, while suppressor T cell functions remain intact. [22,23]

Glucocorticoids were reported to inhibit cytokine production and interleukin receptor expression including IL-1, [24,25] IL-2, [25,26] IL-3, [27] and interferon. [25]

In most of these studies exogenous corticosteroids were added at high pharmacological doses. In the present study, however, the results were not influenced by exogenous corticosteroids since none of the patients received steroid treatment.

In conclusion, the results of this study indicate that sera from patients after acute MI have an immunosuppressive effect on mitogen stimulated unrelated lymphocytes. This effect is time-correlated and the degree of suppression is significantly increased in the presence of sera obtained during the first 24 hours after the acute MI, compared with the sera taken one week later. Moreover, a significant correlation between the degree of suppression and the serum cortisol levels was also observed.

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