Asian Pacific Journal of Allergy and Immunology (1991) 9 :15-19

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

II. Inhibition of IL-2 Production

C. Rudniki[1], L. Komlos[2], J. Notmann[2], S. Bass[3], M. Canetti[1], J. Hart[4] and I. Zahavi[1]

SUMMARY **Culture supernatants from concanavalin-A (con-A)-activated peripheral blood lymphocytes from healthy controls grown in the presence of sera from 20 patients 24 hours and 1 week after acute myocardial infarction (AMI) were tested for their mitogenic activity and for the presence of interleukin-2 (IL-2). Binding of exogenous IL-2 to activated lymphocytes from 10 patients was also determined. In supernatants prepared in the presence of patients' as compared to control sera, a significantly decreased mitogenic activity and IL-2 content were found. The mitogenic activity and IL-2 content in culture supernatants prepared with patients' sera collected 24 hours after the AMI (AMI I) and one week thereafter (AMI II) were significantly suppressed, and the degree of suppression in the 24-hour sera was significantly higher than in those collected after one week. No significant differences were observed in the binding capacity to exogenous IL-2 of activated patients' and control lymphocytes. The possibility is that immunosuppressive factors in the patients' sera, including cortisol, may suppress the patients' immune response acting through inhibition of IL-2 production.**

In Part I of this study,[1] we have observed that sera obtained from patients 24 hours after acute myocardial infarction (AMI) have a suppressive effect on mitogenic stimulation of lymphocytes from healthy volunteers (controls) and that this suppressive effect is significantly reduced when sera from the same patients were tested one week later.

In the present study, we have investigated the possibility that the production of lymphocyte mitogenic factors, including interleukin-2 (IL-2) in culture supernatants (conditioned medium) may also be suppressed in the presence of sera from patients 24 hours after the AMI and that an improved mitogenic activity may be achieved with culture supernatants prepared with sera of the same patients taken one week later.

We have further tested the posibility that within the first 24 hours after AMI the reactivity of the patients' lymphocytes to exogenous IL-2 may be reduced as compared to control cells.

MATERIALS AND METHODS

Sera from the same 20 patients studied in Part I obtained within 24 hours and one week after the AMI, were used for the production of mitogenic factors in supernatants. For control supernatants, 26 sera from healthy volunteers were used. In addition to 5 new control sera, 21 sera were the same as in the Part I of this study. Patients' and control sera were stored frozen at -20°C until used.

IL-2 production in supernatants

Human peripheral blood mononuclear cells from healthy volunteers were recovered by Ficoll-Hypaque fractionation of freshly drawn blood and preincubated in a 5% CO_2 atmosphere at 37°C for 48 hours to reduce suppressor cell activity.[2] For production of IL-2 in the supernatant, the method of Northoff *et al.*[2] was essentially used: the preincubated

From the [1]Department of Medicine "C" and Cardiology Service, [2]B.Gategno Research Institute, [3]Clinical Laboratory, [4]Hasharon Hospital, Golda Medical Center, Tel Aviv University Medical School, Israel

Correspondence : Prof. I.Zahavi, M.D., Department of Medicine "C" and Cardiology Service, Hasharon Hospital, Petah-Tiqva 49372 P.O.B. 121, Israel.

cells were further incubated for 24 hours with concanavalin A (Con A) 10 μg/ml in the presence of 2.5% patients' or control sera and supernatants, separated from cells, and stored at -20°C until tested for their mitogenic activity.

Assay for mitogenic activity of supernatants

IL-2 can be removed from conditioned medium only in the presence of receptors for IL-2 which appear on lectin or alloantigen activated cells. [3,4]

To obtain activated lymphocytes, the method of Larsson *et al.* [5] was used: 10^6 lymphocytes from healthy donors were cultured for 8 hours in RPMI-1640 supplemented with 10% pooled normal human serum and stimulated with 10 μg/ml Con A. After repeated washings and addition of 0.15 M α-methyl-D-manopyranoside (AMM) to block any possible residual mitogenic activity of Con A, the cells were further incubated for 4 days in the presence of 35% of the specific supernatants and their mitogenic activity measured by ^{3}H-thymidine incorporation. To prove the presence of IL-2 in supernatants, rabbit polyvalent antibody to human IL-2 (IL-2 Ab, Genzyme, Boston, MA) in concentrations able to neutralize 30 and 60 U of IL-2 respectively was added to some supernatant-containing cultures. The residual mitogenic activity was measured after 4 days by ^{3}H-thymidine incorporation and the results were compared to cultures incubated with supernatants only.

The reactivity of the patients' lymphocytes to exogenous recombinant IL-2

To express IL-2 receptors, lymphocytes from patients and controls were activated as described above for 8 hours, washed and further incubated in medium containing 0.15 M AMM, 10% pooled human serum and two different concentrations of 10 and 5 U respectively, of recombinant human IL-2 (Genzyme, Boston, MA). After 4 days of incubation the degree of lymphocyte proliferation in the presence of IL-2 was measured by ^{3}H-thymidine incorporation in patients' and control cultures.

Statistical evaluation

For paired groups the nonparametric Wilcoxon test was used. The Mann-Whitney nonparametric U test was used for comparison between control and test groups.

RESULTS

Mitogenic activity of supernatants

The mitogenic activity of supernatants containing sera from the 20 patients obtained during the first 24 hours (AMI I) and one week after the AMI (AMI II) were compared on the same lymphocytes to 26 supernatants containing control sera (Fig. 1).

A significantly increased mitogenic activity, expressed in counts/minute (cpm) of ^{3}H-thymidine incorporation, was observed with supernatants prepared with AMI II sera as compared to supernatants prepared with AMI I sera ($p < 0.02$).

The mitogenic activity of supernatants prepared with control sera was significantly increased as compared to supernatants obtained from the patients' sera ($p < 0.0011$ vs. AMI I and $p < 0.0032$ vs. AMI II).

IL-2 in the supernatant

When the mitogenic activity of supernatants containing sera from patients after AMI was tested on lymphocytes in the presence of IL-2 Ab, part of the IL-2 present in the supernatant was neutralized by the specific antibody and a significant reduction in the proliferative response was observed.

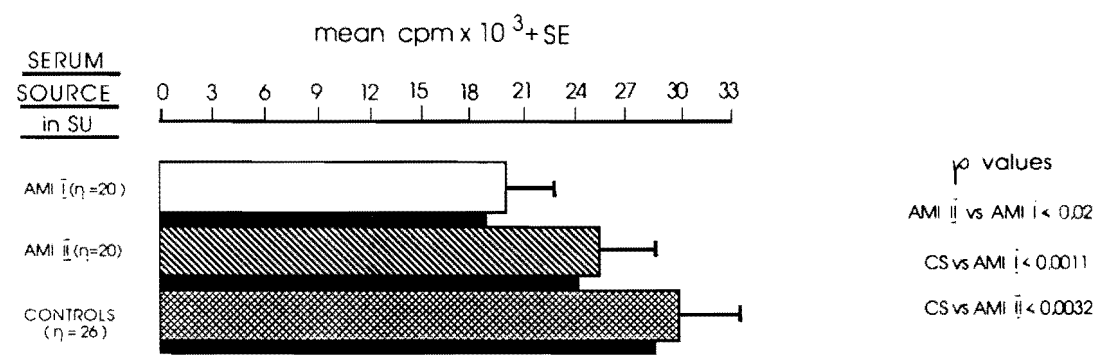

Fig. 1 Activated lymphocytes from healthy donors incubated in the presence of 35% supernatant (SU) containing wera from patients 24 hours (AMI I) and 1 week (AMI II) adter the AMI. Activated lymphocytes containing 35% suppernatants prepared with control sera (CS). Cultures were incubated for 4 days.s

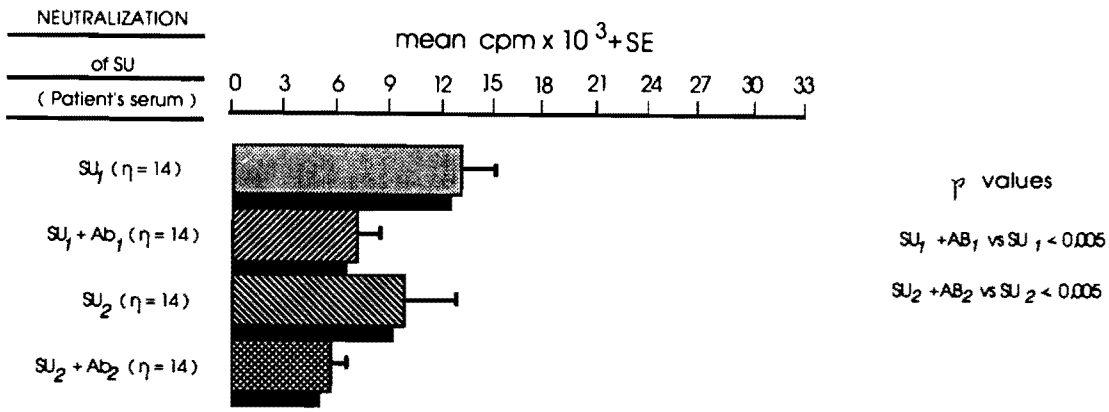

Fig. 2 Cultures inclubated with 2 different concentrations of supernatants containing patients' Sera. SU_1 = 35% supernatant and SU_2 = 18% supernatant. The same cells were also incubated in the presence of supernatants (SU_1 and SU_2) and anti–human IL–2 antibody and were able to neutralize 60 U (Ab_1) and 30 U (Ab_2), respectively, of IL–2. The duration of incubation was 4 days.

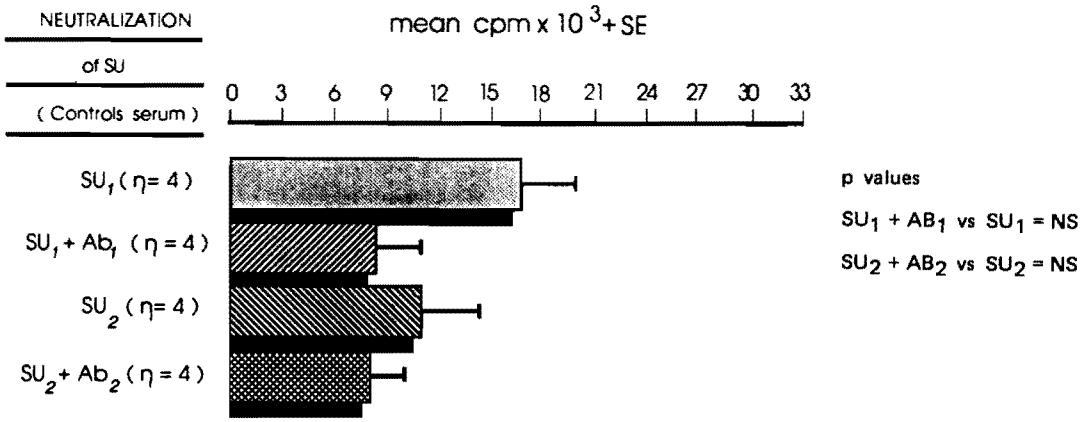

Fig. 3 For details see Fig. 2. Supernatants (SU_1 and SU_2) prepared in the presence of control sera. Differences were not statistically significant probably due to the small number of suppernatants.

Table 1. Effect of recombinant human IL–2 on the mitogenic activity of patients' and controls' lymphocytes.

Lymphocyte source	No.	Mean CPM values ± SE 5 U IL–2	10 U IL–2	p values
Patients*	10	20.497 ± 3787	34.012 ± 5953	N.S.**
Controls	7	19.144 ± 4998	32.408 ± 5812	

* First 24 hours after the AMI

** Patients vs Controls

In the presence of two different concentrations of the patients' supernatant and IL-2 Ab a significant reduction ($p < 0.005$) was observed in cultures incubated in the presence of supernatant and anti IL-2 Ab, as compared to cultures incubated with supernatant only (Fig. 2).

Although the number of tests is small, neutralization of IL-2 activity in the presence of anti IL-2 Ab could also be observed in the presence of supernatants prepared with control sera with a reduction in the mitogenic reactivity of 51% and 28%, respectively, when compared to cultures incubated with supernatants only (Fig. 3).

The reactivity of patients' lymphocytes to IL-2

The results are summarized in Table 1. In the presence of 5 or 10 U of IL-2, respectively, the mitogenic activity of previously activated lymphocytes from patients and controls was not significantly different and mean cpm values ± SE were very similar.

DISCUSSION

In the present study, it was observed that the mitogenic activity of lymphocyte culture supernatants prepared in the presence of sera from patients after AMI was significantly depressed as compared to supernatants prepared in the presence of control sera, and the suppressive effect of AMI I sera was significantly more pronounced than that of AMI II sera. Addition of anti IL-2 Ab at two different concentrations suppressed significantly the mitogenic effect of the supernatants. Such specific inhibition by anti IL-2 Ab suggest that IL-2 present in different amounts in the supernatants may contribute to mitogenic reactivities.

T cells express the gene encoding the lymphokine IL-2, previously termed T-cell growth factors, after T cell activation by antigens, mitogens or antibodies.[2] To exert its biological effects, IL-2 must interact with specific high affinity receptors which appear within hours after polyclonal activation of T cells.[2,6]

In this study, the capacity to bind exogenous IL-2 was found to be similar for activated patients' and control lymphocytes. These results suggest that the expression of IL-2 receptors on the patients' lymphocytes and their binding capacity are not affected and that the decreased mitogenic reactivity may be correlated to decreased IL-2 production. Inhibition of IL-2 production by human sera was reported. Nicholas *et al.*[7] observed that pregnancy serum can inhibit the production of IL-2 and suggested that during normal pregnancy decreased glucocorticoid levels may contribute to this suppressive effect.

In the present study we tested the supernatants from the same sera used in the accompanying report,[1] in which a significant correlation between a depressed mitogenic response and increased cortisol levels was shown. Gillis *et al.*[8,9] observed that immune suppression by glucocorticoid was mediated via T cell growth factors (TCGF), since addition of exogenous TCGF to glucocorticoid-treated mitogen-stimulated T lymphocytes overcame the glucocorticoid suppressive effects. Our results showing reduced IL-2 production in the presence of AMI I sera concurrent with increased cortisol levels would be in agreement with the findings reported by Gillis *et al.*[8,9]

To our knowledge, the results of this study suggest for the first time that immunosuppressive factors in sera from patients after AMI can act through inhibition of IL-2 production. These suppressive serum factors seem to be time-correlated, since the inhibitory effect of the patients' sera on IL-2 production was decreased significantly within one week after the AMI. Stress related immune suppression was reported by several investigators[10-16] as to suggest that the severe physical and psychological stress after AMI may contribute to a transient immune suppression.

REFERENCES

1. Rudniki C, Komlos L, Notmann J, Bass S, Canetti M, Hart J, Zahavi I. The effect of serum from patients with acute myocardial infarction on *in vitro* lymphocyte reactivity. I. Inhibition of mitogen stimulation. Asian Pac J Allergy Immunol 1991; (in press).
2. Northoff H, Carter CH, Oppenheim JJ. Inhibition of Concanavalin A - induced human lymphocyte mitogenic factor (interleukin-2) production by suppressor T lymphocytes. J Immunol 1980; 125 : 1823-8.
3. Waldmann TA. The interleukin-2 receptor on normal and malignant lymphocytes. Adv Exp Med Biol 1987; 213 : 129-37.
4. Mier JW, Gallo RC. Purification and some characteristics of human T-cell growth factor from phytohemagglutinin-stimulated lymphocyte-conditioned media. Proc Natl Acad Sci USA 1980; 77 : 6134-8.
5. Larsson EL, Continho A. The role of mitogenic lectins in T cell triggering. Nature 1979; 280 : 239-41.
6. Cantrell DA, Smith KA. Transient expression of interleukin-2 receptors : consequences for T cell growth. J Exp Med 1983; 158 : 1895-912.
7. Nicholas NS, Panagi GS, Nouri AME. Human pregnancy serum inhibits interleukin-2 production. Clin Exp Immunol 1984; 58 : 587-95.
8. Gillis S, Crabtree GR, Smith KA. Glucocorticoid-induced inhibition of T-cell growth factor production. I. The effect on mitogenic-induced lymphocyte proliferation. J Immunol 1979; 123 : 1624-31.
9. Gillis S, Crabtree GR, Smith KA. Glucocorticoid-induced inhibition of T-cell growth factor production. II. The effect on the in vitro generation of cytolytic T cells. J Immunol 1979; 123 : 1632-8.
10. Rodrick ML, Wood JJ, O'Mahony JB, *et al.* Mechanisms of immunosuppression associated with severe non thermal traumatic injuries in man: production of interleukin 1 and 2. J Clin Immunol 1986; 6 : 310-8.
11. Abraham E, Regan RF, Chang YH. Lack of effect of exogenous recombinant

interleukin 2 on trauma-induced abnormalities in lymphocyte proliferation. Crit Care Med 1986; 14 : 847-51.

12. Ninnermann JL. Trauma, sepsis and the immune response. J Burn Care Rehabil 1987; 8 : 462-8.
13. Gupta S. Immune response following surgical trauma. Crit Care Clin 1987; 3 : 405-15.
14. Glaser R, Kiecolt-Glaser J. Stress associated depression in cellular immunity: implications for acquired immune deficiency syndrome (AIDS). Brain Behav Immun 1987; 1 : 107-12.
15. Glaser R, Rice J, Sheridan J, *et al.* Stress related immune suppression: health implications. Brain Behav Immun 1987; 1 : 7-20.
16. Baker GH, Byrom NA, Irani MS, *et al.* Stress, cortisol, and lymphocyte subpopulations. Lancet 1984; 1 : 574.