Clin. exp. Immunol. (1991) **83**, 326–328 ADONIS 00991049100059E

Low CD4/CD8 T lymphocyte ratio in acute myocardial infarction

H. SYRJÄLÄ*†, H.-M. SURCEL* & J. ILONEN‡ *National Public Health Institute, Oulu, and Departments of †Internal Medicine and ‡Medical Microbiology, University of Oulu, Oulu, Finland

(Accepted for publication 10 September 1990)

SUMMARY

T lymphocyte subsets were analysed using monoclonal antibodies and flow cytometry to determine whether myocardial infarction and cardiopulmonary resuscitation induce changes in these. Groups of 11 infarct patients and 10 patients with past cardiopulmonary resuscitation were compared with 11 age- and sex-matched controls and 12 sepsis patients. The differences in the CD4/CD8 ratios between the four groups were significant ($F=7{\cdot}71$, $P=0{\cdot}001$). The infarct patients had lower CD4/CD8 ratios (mean $\pm$ s.d. $0{\cdot}83\pm0{\cdot}43$) than the control ($2{\cdot}12\pm1{\cdot}13$; $P=0{\cdot}001$) or sepsis cases ($1{\cdot}76\pm1{\cdot}05$; $P=0{\cdot}004$), but their ratios did not differ from those of the resuscitation group ($0{\cdot}93\pm0{\cdot}79$, $P=0{\cdot}84$). The latter group also had lower ratios than the control ($P=0{\cdot}003$) and sepsis groups ($P=0{\cdot}013$). Most infarct patients had an on admission inverted CD4/CD8 ratio which usually returned to normal in the next 2 days. A permanently low CD4/CD8 ratio may be a poor sign prognostically after both myocardial infarction and resuscitation.

Keywords myocardial infarction lymphocyte subsets

INTRODUCTION

The ratio of CD4 (helper/inducer) to CD8 (suppressor/cytotoxic) T lymphocytes in healthy persons is about 2:1 (Sprent, 1989), and a low number of CD4 lymphocytes with inversion of the normal CD4/CD8 ratio has been a well-known phenomenon since the identification of AIDS (Gottlieb *et al.*, 1981; Masur *et al.*, 1981). An inverted ratio is also found in some viral infections such as infectious mononucleosis, where a predominance of activated CD8 cells is typical of the acute phase, but the T cell subsets return to normal during the convalescent phase (Reinherz *et al.*, 1980). Significant depression of the total number of T cells and their subsets has been reported in cases of pneumonia, acute pyelonephritis and sepsis (Williams, Koster & Kilpatrick, 1983).

Increased numbers of CD8 cells and an inverted ratio of CD4/CD8 cells have been reported during the early phase of cardiac operations (Navarro *et al.*, 1988), and an inverted ratio of CD4/CD8 cells has also been reported in association with thermal injury, where the number of CD4 cells decreases (Antonacci *et al.*, 1984). After physical stress, the number of CD8 cells increases to more than that of CD4 cells causing an inverted ratio of CD4/CD8 cells (Landmann *et al.*, 1984; Edwards *et al.*, 1984).

The present work was designed to investigate whether acute myocardial infarction and cardiopulmonary resuscitation, both of which cause clear stress reaction, induce changes in the CD4/CD8 ratio.

Correspondence: Dr. H. Syrjälä, National Public Health Institute, Box 310, SF-90101, Oulu 10, Finland.

SUBJECTS AND METHODS

Subjects

Four groups of subjects (patients and three reference groups) were examined. The first group comprised 11 patients (10 men, one woman; mean age 65·1 years $\pm$8·4 s.e.m.) with confirmed acute myocardial infarction, the diagnosis being based on chest pain, changes in electrocardiography and elevated values of creatine kinase with their MB fractions at least 52 U/l. The first reference group comprised 11 age- and sex-matched hospitalized patients (mean age 65 ± 8.2) without acute cardiac events, who had been admitted to hospital for elective surgery (four), chronic heart failure (three), hypertension (one), atrial fibrillation (one), polyneuropathy (one) and pleuritis of unknown origin (one). The second reference group comprised 12 sepsis patients (seven men, five women; aged $58{\cdot}6+21{\cdot}6$). These included seven Gram-negative septicaemias (*Escherichia coli* four *Klebsiella pneumoniae* one; *K. oxytoca* one; and *Salmonella newport* one) and five Gram-positive septicaemias (*Streptococcus pneumoniae* two; *S. β*-haemolyticus group B two; and *S. aureus* one). The third reference group consisted of 10 patients who had undergone cardiopulmonary resuscitation (seven men, three women, aged $68{\cdot}1\pm10{\cdot}0$). Three of these cardiopulmonary resuscitation patients died, and only two of the other seven patients had been discharged from hospital.

The study protocol was accepted by the ethical committee of the Medical faculty of Oulu University.

Methods

The controls gave one heparinized peripheral blood specimen each, and a sample was taken from each of those in the sepsis group after a Gram-stain of the blood culture had shown bacteria. In the acute myocardial infarction group, the first sample was taken on admission, and one or more specimens were taken on subsequent days from nine of the 11 patients. The first sample from the cardiopulmonary resuscitation cases was taken on admission after resuscitation, and one or more follow-up specimens were taken in the next few days after the six primarily successful resuscitations.

Mononuclear cells were separated using Lymphoprep (Nyegaard, Oslo, Norway) gradient centrifugation, washed with HBSS and diluted in RPMI 1640 medium supplemented with 5% fetal calf serum. Cells ($2{\cdot}5 \times 10^5$) were incubated for 30 min with 10 μl monoclonal antibodies conjugated with different fluorochromes in a total volume of 100 μl at 4°C. The following antibodies were used: PE- or FITC-conjugated anti-Leu-3 (CD4) and FITC-conjugated anti-Leu2 (CD8) (Becton Dickinson, Mountain View, CA). After incubation, the cells were washed with PBS at 4°C and analysed by flow cytometry (FACScan, Becton Dickinson). The percentages of cells positive for green (FITC) and red (PE) fluorescence were counted. The percentages of the lymphocyte subpopulations were defined after gating cells as lymphocytes on the basis of their light scatter parameters.

Peripheral blood leucocyte and differential counts in the myocardial infarction and control groups were analyzed using a Technicon H-1 analyser (Technicon, Stockholm, Sweden) to calculate the absolute numbers of CD4 and CD8 cells.

Statistical analysis

After logarithmic transformation of the values of CD4/CD8 ratios, one-way analysis of variance and Student's *t*-test were used.

RESULTS

Low CD4/CD8 ratios were observed in the myocardial infarction and cardiopulmonary resuscitation group (Fig. 1). The differences between the CD4/CD8 ratios in the four study groups were significant (F 7·71; $P=0{\cdot}001$). The values of the infarct group differed from those of the control group ($P=0{\cdot}001$) and from those of the sepsis group ($P=0{\cdot}004$), but did not differ from those of the resuscitation group ($P=0{\cdot}84$). The results of resuscitation group differed from those of the control group ($P=0{\cdot}003$) and those of the sepsis group ($P=0{\cdot}013$). The ratios did not differ significantly between the control and sepsis groups ($P=0{\cdot}64$). Figure 2 shows that the infarct group had on admission fewer CD4 cells and more CD8 cells than the control group, although the differences were not statistically significant ($P=0{\cdot}699$ and $P=0{\cdot}114$, respectively). Among the infarct patients, the reversed CD4/CD8 ratio returned to normal during the follow-up period in all, except for two fatal cases (Fig. 3), where the necropsies revealed rupture in the area of infarction.

Four patients in the cardiopulmonary resuscitation group had a normal CD4/CD8 ratio, all of whom had had ventricular

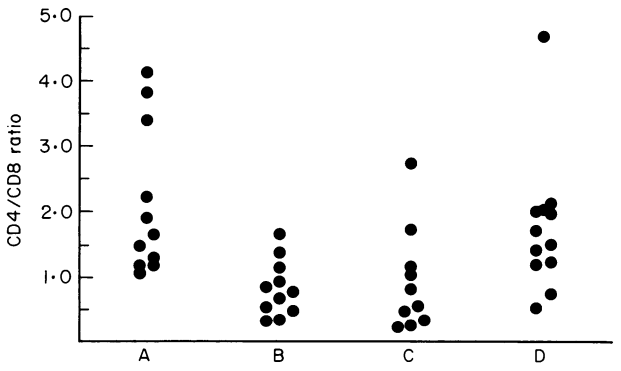

Fig. 1. CD4/CD8 lymphocyte ratios of 11 age- and sex-matched controls (A, $2{\cdot}12 \pm 1{\cdot}13$), 11 patients with acute myocardial infarction (B, $0{\cdot}83 \pm 0{\cdot}43$), 10 patients with cardiopulmonary resuscitation (C, $0{\cdot}93 \pm 0{\cdot}79$), and 12 patients with septicaemia (D, $1{\cdot}76 \pm 1{\cdot}05$). The differences of CD4/CD8 ratios of these groups were significant (F, 7·71; $P=0{\cdot}001$). The statistical differences between the four groups were: A *versus* B, $P=0{\cdot}001$; A *versus* C, $P=0{\cdot}003$; A *versus* D, $P=0{\cdot}64$; B *versus* C, $P=0{\cdot}84$; B *versus* D, $P=0{\cdot}004$; C *versus* D, $P=0{\cdot}013$.

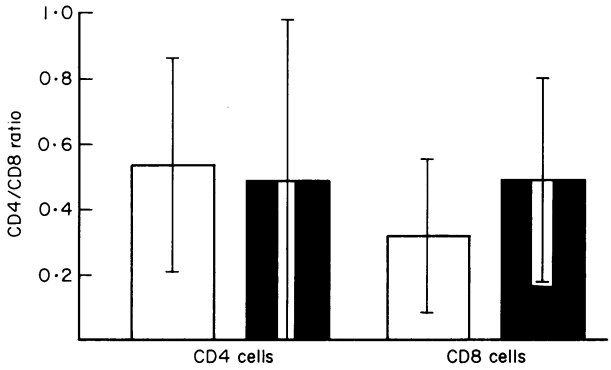

Fig. 2. The absolute CD4 and CD8 numbers (× 109/*l*) on admission for the myocardial infarct group (■) and their age- sex-matched controls (□) (mean + SD). The differences between the groups were not significant (for CD4 cells, $P=0{\cdot}699$; for CD8 cells, $P=0{\cdot}114$).

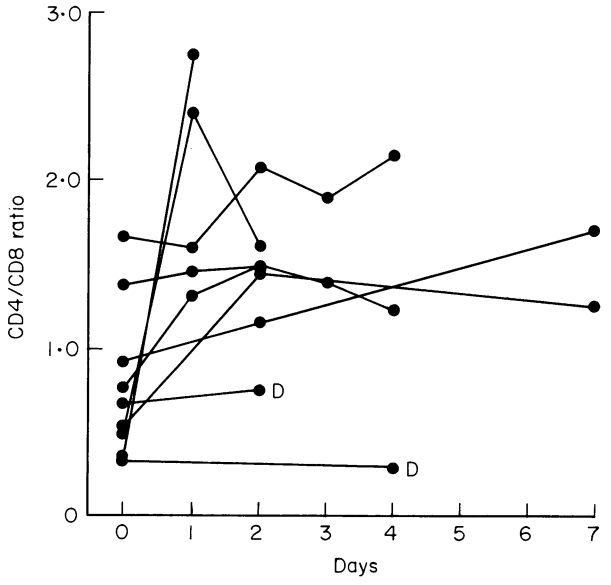

Fig. 3. CD4/CD8 ratio on admission and on subsequent days in nine patients with acute myocardial infarction. D, died.

fibrillation when transferred to the emergency room. Two of them survived, and no myocardial infarction could be demonstrated by ECG or by the MB fraction of creatine kinase. The other two died and their relatives did not allow necropsy. At least one of these did not have any chest pain before the fatal episode. Six patients had a reversed CD4/CD8 ratio after resuscitation, and autopsy revealed myocardial infarction in two of these. The necropsies of the other two did not show infarction, although one had had chest pain before resuscitation and the other had had a new lateral elevation of the S–T segment in his ECG after resuscitation. The last two patients with reversed CD4/CD8 ratio without autopsy had a high CK–MB fraction of creatine kinase as a sign of myocardial damage.

A permanently low CD4/CD8 ratio seemed to be a poor sign prognostically even after resuscitation. A 73-year-old woman who had a CD4/CD8 ratio of 0·25 after resuscitation on admission died the next day, when her CD4/CD8 ratio was 0·34. Necropsy revealed a large area of acute infarction (3 × 5 cm) in the posterior wall of the left ventricle. A 77-year-old man who had a low CD4/CD8 ratio continuously (on admission, 0·23; on day 1, 0·56; on day 4, 0·32; on day 7, 0·59; and on day 11, 0·64) was unconscious during the whole period of hospitalization. He died some 3 weeks later. A 55-year-old man for whom we could not analyse the admission specimen after resuscitation still had a low CD4/CD8 ratio of 0·47 on day 3, when he died. Necropsy revealed a lateral, posterior infarction of the left ventricle.

DISCUSSION

The results show that a reversed CD4/CD8 ratio is a common phenomenon in acute myocardial infarction and usually returns to normal rapidly; however, a permanently low CD4/CD8 ratio may be a poor sign prognostically, as it is after cardiopulmonary resuscitation. If the CD4/CD8 ratio is normal immediately after cardiopulmonary resuscitation, this may be due to primary ventricular fibrillation rather than myocardial infarction being the cause of the emergency attack.

There was a clear difference between CD4/CD8 ratios of the infarct patients and their age- and six-matched controls, and between the cardiopulmonary resuscitation group and the controls. However, the CD4/CD8 ratios of the control group did not significantly differ from those of the sepsis patients, while the mean CD4/CD8 ratio of our sepsis patients ($1{\cdot}8 \pm 1{\cdot}1$) was similar to that reported by Williams *et al.* (1983) ($1{\cdot}7 \pm 1{\cdot}4$). The results of determination of CD4/CD8 ratios seemed to be repeatable; in one subject CD4/CD8 ratio did not change remarkably in three successive days (2·3, 2·1, and 2·5). Rapid changes in CD4/CD8 ratios have also been reported after cardiac operations (Navarro *et al.*, 1988).

It has been shown (Soppi *et al.*, 1984) earlier that patients recovering from cardiopulmonary resuscitation show anergy with respect to skin test antigens and the responses of their lymphocytes to mitogenic stimulation are lower than those of controls. Soppi *et al.* (1984) suggested that this anergy may be due to increased suppressor activity, but the subsets of T lymphocytes were not analysed. After physical stress the number of CD8 cells has been reported to increase more than that of CD4 cells causing an inverted ratio of CD4/CD8 cells (Landmann *et al.*, 1984; Edwards *et al.*, 1984). Our results are in accordance with the earlier reports, although CD4 and CD8 cells did not differ statistically between our infarct and control groups.

The mechanisms that caused inverted CD4/CD8 ratios of the infarct patients are unclear. Stress-induced changes can explain at least some of our findings (Landmann *et al.*, 1984; Edwards *et al.*, 1984). The drug therapy at the time of study did not explain our results. Three infarct patients had not received any treatment before myocardial infarction and two of them had an inverted ratio of CD4/CD8 ratio. The others had used different combinations of drugs for cardiovascular or other diseases.

If further studies confirm our preliminary findings, measurement of the CD4/CD8 ratio may have some clinical importance even in critical care medicine.

REFERENCES

Antonacci, A.C., Calvano, S.E., Reaves, L.E., Prajapati, A., Bockman, R., Welte, K., Mertelsmann, R., Gupta, S., Good, R.A. & Shires, G.T. (1984) Autologous and allogenic mixed-lymphocyte responses following thermal injury in man: the immunomodulatory effects of interleukin 1, interleukin 2 and a prostaglandin inhibitor, WY-1825. *Clin. Immunol. Immunopathol.* **30,** 304.

Edwards, A.J., Bacon, T.H., Elms, C.A., Verardi, R., Felder, M. & Knight, S.C. (1984) Changes in the populations of lymphoid cells in human peripheral blood following physical exercise. *Clin. exp. Immunol.* **58,** 420.

Gottlieb, M.S., Schroff, R., Schanker, H.M., Weisman, J.D., Fan, P.T., Wolf, R.A. & Saxon, A. (1981) *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men. Evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* **305,** 1425.

Landmann, R.M.A., Müller, F.B., Perini, C.H., Wesp, M., Erne, P. & Bühler, F.R. (1984) Changes of immunoregulatory cells induced by psychological and physical stress: relationship to plasma catecholamines. *Clin. exp. Immunol.* **58,** 127.

Masur, H., Michelis, M.A., Greene, J.B., Onorato, I., Stouwe, R.A.V., Holzman, R.S., Wormser, G., Brettman, L., Lange, M., Murray, H.W. & Cunningham-Rundles, S. (1981) An outbreak of community-acquired *Pneumocystis carinii* pneumonia. Initial manifestation of cellular immune dysfunction. *N. Engl. J. Med.* **305,** 1431.

Navarro, M., Lozano, R., Larrad, L., Roman, A., Suarez, J. & Armijo, J. (1988) Variation in T helper cell/T cytotoxic-suppressor cell index during cardiac operations. *J. thorac. cardiovasc. Surg.* **96,** 926.

Reinherz, E.L., O'Brien, C., Rosenthal, P. & Schlossman, S.F. (1980) The cellular basis for viral-induced immunodeficiency: analysis by monoclonal antibodies. *J. Immunol.* **125,** 1269.

Soppi, E., Lindroos, M., Nikoskelainen, J. & Kalliomäki, J.L. (1984) Effect of cardiopulmonary resuscitation-induced stress on cell-mediated immunity. *Intensive Care Med.* **10,** 287.

Sprent, J. (1989) T lymphocytes and the thymus. In *Fundamental Immunology* (ed. by W. E. Paul) P. 69. Raven Press, NY.

Williams, R.C., Koster, F.T. & Kilpatrick, K.A. (1983) Alterations in lymphocyte cell surface markers during various human infections. *Am. J. Med.* **75,** 807.