EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Expression of Activation Markers by Lymphocytes of Patients with Infective Allergic Myocarditis

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Negative correlation between the severity of infective allergic myocarditis and the content of $CD8^+$ lymphocytes has been established. The counts of $CD25^+$ and $CD71^+$ lymphocytes increased during the early period of the disease. Attenuation of inflammation in the myocardium was associated with a tendency to normalization of these parameters.

Key Words: *infective allergic myocarditis; myocarditic cardiosclerosis; lymphocytes; surface phenotype*

The role of immune mechanisms in myocardial diseases has been widely disputed. Some immunological indices may be useful for evaluating the pathogenetic role of the immune response disorders in patients with noncoronarogenic diseases of the myocardium [5,7,8,15].

The data on the composition of the peripheral blood (PB) lymphocyte population in myocarditis are scanty and contradictory. There is evidence the total counts of T lymphocytes ($CD3^+$) and their helper-inductor subpopulation ($CD4^+$) are decreased in patients with myocarditis [2,3], while other authors report an increase in the $CD4^+$ lymphocytes in PB of patients with myocardial inflammations [5]. A transitory decrease in the counts of circulating $CD3^+$ and $CD4^+$ lymphocytes is associated with myocardial infiltration by these cells and by $CD8^+$ lymphocytes [10]. Analysis of changes in the count of $CD4^+$ lymphocytes during activation or abatement of inflammation showed an increased number of $CD4^+$ cells in PB during exacerbation of myocarditis and a decrease in their number with convalescence [9,13]. The data on the content of $CD8^+$ lymphocytes — suppressor-cytotoxic cells — are contradictory: their count was increased [2], unchanged [11,14], or decreased and normalized with convalescence [12].

High diagnostic value of the surface expression of the lymphocyte activation markers has been demonstrated for some allergic and autoimmune diseases [4,6]. However, there are no data on changes in the counts of activated lymphocytes in PB of myocarditis patients.

We studied changes in the total count of T cells (CD3), B cells (CD72), helper-inductor (CD4) and suppressor-cytotoxic (CD8) T lymphocytes, and lymphocytes carrying activation markers in patients with infective allergic myocarditis (IAM) of different duration in comparison with patients with myocarditic cardiosclerosis (MCS) and donors. The expression of the early activation markers CD25 (interleukin-2 receptor) and CD71 antigens (transferrin receptor), late activation marker HLA-DR (class II histocompatibility antigens), and B-cellular activation marker CD23 (low-affinity IgE receptor) was studied.

MATERIALS AND METHODS

Venous blood was collected from 11 donors, 25 patients with IAM, and 14 patients with MCS and sta-

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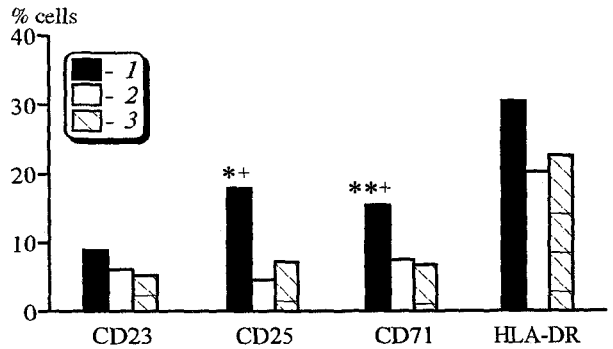

Fig. 1. Expression of activation markers by lymphocytes from patients with infective allergic myocarditis. <4 weeks (*1*) and >4 weeks (*2*) from the beginning of disease, (*3*) control. *$p<0.01$, **$p<0.05$ *vs.* *2*; +$p<0.01$ *vs.* control.

bilized with sodium ethylene diamine tetracetate. Mononuclear cells were isolated according to Boyum in a single-step Ficoll-Verograf in density gradient. Cells collected from the interphase ring were washed three times in phosphate-buffered saline, and cell suspension was then prepared (5×10^6 cells/ml). The number of cells expressing the respective membrane antigens was estimated by indirect immunofluorescence with murine monoclonal antibodies and Fab fragments of goat immunoglobulins to mouse antibodies conjugated with fluorescein isothiocyanate. The test was performed in the microvariant [1]. The preparation was examined under a water immersion microscope; the presence of fluorescence and its specificity were evaluated for at least 200 cells. Cells with the monocyte morphology were neglected. Cell viability control with trypan blue (>98%) was carried out for each test and nonspecific reaction with labeled serum (<2%) was evaluated. The results were statistically processed using Student's *t* test and Spearman's correlation *R* test.

RESULTS

In the patients of both groups (IAM and MCS), the relative counts of B lymphocytes (CD72) showed a tendency to a decrease in comparison with donors (Table 1).

The total count of T lymphocytes ($CD3^+$), counts of the helper-inductor ($CD4^+$) and suppressor-cytotoxic ($CD8^+$) cells in myocarditis patients were virtually the same as in donors, although tended to decrease. In MCS patients the total count of T lymphocytes was significantly decreased, while the content of $CD8^+$ lymphocytes increased in comparison with donors ($p<0.05$). Negative correlation ($R=-0.40$, $p=0.007$) was established between the severity of hemodynamic disorders (according to NYHA classification) and content of $CD8^+$ lymphocytes in PB of IAM patients.

In IAM patients, the number of the early activation markers CD23, CD25, and CD71 on PB lymphocytes showed a tendency toward an increase. All examined parameters of the lymphocytes from MCS patients were virtually the same as those of donor cells (Table 2).

Analysis of case histories and clinical status of IAM patients showed that expression of antigenic activation markers was much higher in the patients who had clinical symptoms of acute inflammation of the myocardium (pain in the heart, and augmentation of circulatory failure: dyspnea, edemas, enlargement of the liver, and ascites) for 2-4 weeks before blood sampling (Fig. 1). The difference in CD25 expression in patients with short duration of myocardial inflammation (group 1) and in those who had developed clinical symptoms of myocarditis more than 4 weeks before the study (group 2) was significant.

The counts of CD25 lymphocytes increased 2.4 times in group 1 patients with IAM in comparison with that in donors. The count of $CD71^+$ cells in group 1 patients was two times higher than in healthy subjects. By contrast, in group 2 IAM patients the counts of $CD25^+$ and $CD71^+$ lymphocytes were virtually the same as in donors.

Thus, study of surface antigenic markers showed high diagnostic validity of the early activation antigens CD25 and CD 71 in IAM. During the initial period of myocarditis the counts of lymphocytes with signs of early activation in PB are increased. This points to important role of immune mechanisms in the pathogenesis of inflammatory diseases of the myocardium and the relevance of immunocorrective therapy of these diseases.

TABLE 1. Population and Subpopulation Composition of PB Lymphocytes in Donors and Patients with IAM and MCS ($M\pm m$)

Antigen	Donors	IAM	MCA
CD3	69.71±1.15	66.13±2.15	63.61±2.15*
CD4	38.34±2.21	33.86±3.00	36.29±3.12
CD8	25.95±1.32	22.51±2.49	30.58±2.56**
CD72	9.39±2.23	6.09±1.17	5.54±0.86

Note. $p<0.05$: *vs.* donors, **vs.* patients with IAM.

TABLE 2. Content of Lymphocytes Expressing Activation Markers in PB of Patients with IAM and MCS ($M\pm m$)

Antigen	Donors	IAM	MCS -
CD23	5.20±1.81	7.18±1.00	6.01±1.52
CD25	7.21±2.36	9.91±1.86	7.24±1.67
CD71	6.73±2.41	10.75±2.01	7.16±1.72
HLA-DR	22.68±3.25	24.26±3.32	22.60±5.37

REFERENCES

1. A. Yu. Baryshnikov, *Gematol. Tranfuziol.*, No. 8, 4-7 (1990).
2. N. N. Kipshidze, V. B. Chumburidze, M. F. Demetrashvili, and L. M. Dzidziguri, *Ter. Arkh.*, No. 4, 136-139 (1988).
3. N. N. Kipshidze, V. B. Chumburidze, L. M. Dzidziguri, and M. N. Detashidze, *Ibid.*, **56,** No. 10, 56-58 (1984).
4. G. V. Poryadin, Zh. M. Salmasi, and A. I. Makarkov, *Immunologiya,* No. 3, 4-8 (1997).
5. M. Yu. Samsonov, E. L. Nasonov, and V. P. Masenko, *Ter. Arkh.*, No. 4, 40-43 (1994).
6. A. N. Cheredeev and L. V. Kovalchuk, *Rus. J. Immunol.*, **2,** No. 2, 85-90 (1997).
7. B. Devaux, D. Scholz, A. Hirche, *et al., Eur. Heart J.*, **18,** No. 3, 470-479 (1997).
8. R. Ferrari, T. Bachetti, R. Confortini, *et al., Circulation,* **92,** 1479-1486 (1995).
9. T. Kanda, T. Yokoyama, S. Ohshima, *et al., Clin. Cardiol.*, **13,** No. 9, 617-622 (1990).
10. C. Kishimoto, A. Matsumori, N. Tomioka, *et al., J. Cardiogr.* Suppl., **9,** 19-25 (1986).
11. C. Kishimoto, N. Tomioka, S. Tamaki, and C. Kawai, *Jpn. Circ. J.*, **52,** No. 1, 94-98 (1988).
12. Y. Koga, Y. Miyazaki, H. Toshima, *et al., Ibid.*, **53,** No. 1, 78-86 (1989).
13. T. Saito, A. Shiokawa, and S. Inoue, *Ibid.*, 1-6.
14. T. Takamoto, Y. Hori, M. M. Yokoyama, *et al., J. Clin. Lab. Immunol.*, **19,** No. 3, 113-116 (1986).
15. G. Torre-Amione, S. Kapadia, C. Benedict, *et al., J. Am. Coll. Cardiol.*, **27,** 1201-1206 (1996).