INVESTIGATION OF SERUM ACID HYDROLASE ACTIVITY IN IMMUNODEPRESSION

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Despite considerable advances in transplantology in recent years, the question of adequacy of immunodepressive treatment during allografting has not yet been settled. Most immunologic tests used for this purpose require large volumes of blood, they are laborious, and they involve a long delay between taking the blood and obtaining the results of analysis. These circumstances rule out frequent tests and interfere with regular monitoring of immunodepression in the post-transplantation period. Enzymic tests using rapid micromethods of determining acid hydrolase activity in the serum of patients with a transplanted kidney may be interesting in this respect. Activation of mononuclear phagocytes, observed in the course of development of the immune response, is known to be accompanied by release of lysosomal enzymes from them [5, 8, 9]. It is not impossible that immunodepressive drugs prescribed for patients in the course of the post-transplantation period may affect the activity of these enzymes in the blood.

On the basis of this hypothesis the authors studied activity of acid hydrolases — acid RNase and acid phosphatase (AP) — both in experiments on animals and in tests on patients after kidney allografting. One aim of the investigation was to determine whether a correlation exists between activity of these enzymes and the level of intensity of transplantation immunity.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA and BALB/c mice weighing 18-20 g. Immunodepressive drugs (azathioprine and prednisolone) were given as single intraperitoneal injections in doses of 100 and 50 mg/kg body weight, respectively, in the proportion of 2:1 (the ratio used for administration to human patients). Acid RNase and AP activity in serum from the animals and patients with a transplanted kidney was determined by rapid micromethods [1]. To determine acid RNase and AP activity, 0.02 and 0.05 ml of serum, respectively, diluted tenfold, was used. The immunologic control for the patients of this group consisted of values of "active" and "total" T rosette-forming cells (A-TRFC and T-TRFC, respectively) and the A/T ratio. A-TRFC was determined by the method in [10], T-TRFC by the method in [4]. The immunologic control was repeated daily throughout the early post-transplantation period.

EXPERIMENTAL RESULTS

In the first stage of the work the time course of the serum acid RNase level was studied in mice after a single injection of the immunodepressants. Enzyme activity was determined individually in the serum of each animal before injection of the immunodepressants (background), and 4 h, and 1, 3, and 5 days after injection. The results of experiments on 35 CBA and BALB/c mice showed that acid RNase activity was 20-25% lower than the background level 4 h after injection of the drugs. Enzyme activity recovered after 24 h and was close to its initial level on the 3rd-5th days. The inhibitory effect of the immunodepressants was not connected with their direct action on the enzyme molecules, for addition of these agents to the incubation mixture did not inhibit acid RNase activity. Azothioprine, a purine base analog, is known to depress DNA synthesis in the S phase of the cell cycle [2]. In all probability, the decrease in activity of this enzyme in the serum took place as the result of depression of the cellular component of the immune response, and it could thus serve as an indirect indicator of inhibition of the immunologic reactivity of the organism.

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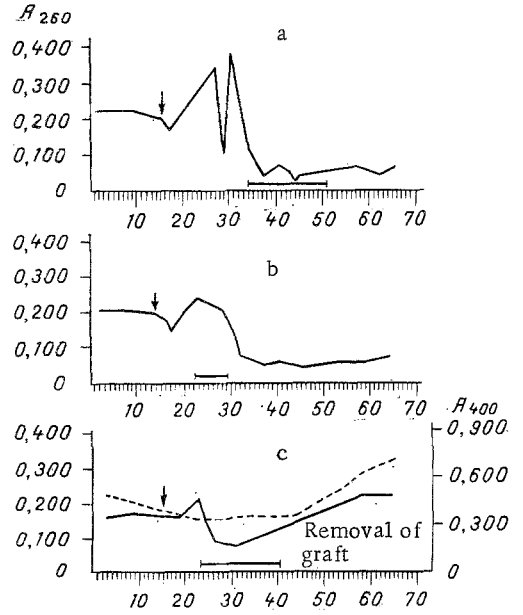

Fig. 1

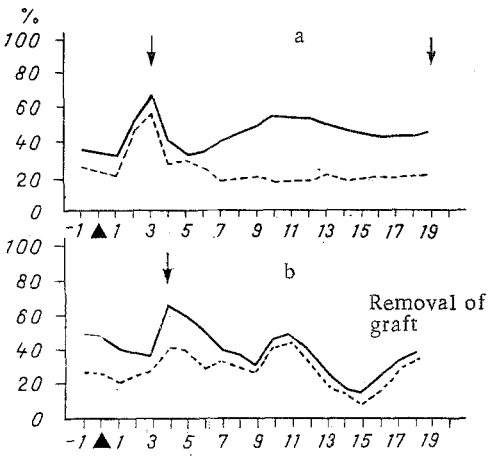

Fig. 2

Fig. 1. Time course of serum acid hydrolase activity in patients after kidney allografting (typical cases). Continuous line — acid RNase activity; broken line — AP activity. a, b) Reversible crises; c) irreversible crisis. Abscissa, time (days); ordinate, enzyme activity. Horizontal line indicates period of crisis; arrow, beginning of immunosuppressive therapy.

Fig. 2. Kinetics of parameters A-TRFC and T-TRFC during period of crises. Abscissa, days before and after transplantation; ordinate, values of A-TRFC and T-TRFC (%). a) Reversible crisis (patient Ch.); b) irreversible crisis (patient G.). Continuous line, T-TRFC; broken line, A-TRFC. Arrows indicate beginning and end of crisis; triangles, day of kidney transplantation.

The results obtained on animals provided a basis for the study of serum acid hydrolase activity in patients during kidney allografting. Examination of 40 patients in the course of the postoperative period showed that, during adequate immunosuppressive therapy, activity of both acid RNase and AP falls. Comparative determination of the activity of these enzymes showed that acid RNase activity is the most stable parameter. Immediately after the start of immunosuppressive therapy its activity fell by 50-75% compared with the background level (Fig. 1). In the period preceding the crisis (Fig. 1a) or at its height (Fig. 1b) an increase in enzyme activity was observed to its initial level, and in some cases this was exceeded by 30-50%. Potentiation of immunosuppression during reversible rejection crises again caused a fall in acid RNase activity (Fig. 1a, b). During irreversible crises activity of the enzyme continued to rise, despite potentiation of immunosuppression (Fig. 1c).

Simultaneous immunological testing revealed synchronization of changes in acid RNase activity with the time course of some parameters of cellular immunity: A-TRFC, T-TRFC, and the A/T ratio. In the early post-transplantation period, uncomplicated by rejection crises, values of TRFC were observed to fall compared with their preoperative level. For instance, T-TRFC fell by 30% during the first 2 weeks, A-TRFC by 20%. Toward the end of the first month the two parameters were reduced by 20%, toward the end of the 2nd month by 15%, but by the end of the 3rd month they varied within the limits of their original values. Conversely, the time course of the A/T ratio changed only slightly, within limits of 5% from its original level.

A completely different kinetics of A-TRFC and T-TRFC was observed during crises of rejection of the kidney allograft. During reversible rejection crises the high values of TRFC following intensive adequate immunosuppression began to fall, and under these circumstances a more marked decrease was observed in A-TRFC (Fig. 2a). Evidence of this was given by values of the ratio A/T, which in 70% of cases was 0.2 or less. On the following days, on recovery of function of the grafts, both A-TRFC and T-TRFC rose within the limits of values

characteristic for these times of the uncomplicated post-transplantation period. In the presence of irreversible rejection crises (Fig. 2b) values of A-TRFC either increased in a stable fashion both on the days preceding the crisis and during the period of frank clinical manifestation of the crisis, or they changed within the limits of values corresponding to T-TRFC. In irreversible rejection crises the A/T ratio rose to 1.0.

In the modern view A-TRFC is the fraction of most mature T lymphocytes which can come into contact rapidly with marker cells, and thus reflect most faithfully the degree of intensity of transplantation immunity [3, 6, 7]. It can be tentatively suggested that the fall in the values of A-TRFC during reversible rejection crises is connected with accumulation of these cells in the graft and a decrease in their activity in the peripheral blood under the influence of adequate doses of immunosuppressive drugs. As a result of this redistribution, contact of A-TRFC with macrophages is local in character, and this is manifested by fluctuating changes in serum acid RNase activity. In the case of irreversible crises, however, a generalized increase in the values of A-TRFC probably takes place. Immunosuppression in the patients of this group does not give the required effect despite its potentiation. Activation of phagocytic cells rises sharply, with the result that acid hydrolase activity increases. The possibility cannot be ruled out that these enzymes, together with other factors liberated by mononuclear cells, may play an important role in cellular interactions.

The results indicate the possibility of using this enzyme test as one of a group of methods of immunologic monitoring to assess the adequacy of immunosuppressive treatment of patients after kidney allografting.

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