MICROBIOLOGY AND IMMUNOLOGY

Induced Activation of Peripheral Blood Mononuclears and Neutrophils in Recipients of an Allogeneic Kidney

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Peripheral blood mononuclears and neutrophils in recipients of an allogeneic kidney are better stimulated with zymosan and phorbol ester during the first days after transplantation and during allograft rejection. Antithymocytic globulin depresses chemiluminescence of both neutrophils and mononuclears. Antisera to human immunoglobulin suppress chemiluminescence of mononuclears but not of neutrophils.

Key Words: *allotransplantation; leukocyte activation; chemiluminescence; zymosan; phorbol ester*

Immunosuppressive therapy is aimed at preventing and suppressing the activation of recipient immunocompetent cells by allograft antigens. The success rate of transplantations of allogeneic organs and tissues rose markedly after the new immunosuppressant cyclosporin A was introduced and started to be used in combination with glucocorticoids, azathioprine, and antithymocytic globulin [9].

Regardless of the type of activating agent, transmission of a transmembrane signal involves the activation of numerous enzymes, including NADPH-oxidase, myeloperoxidase, phospholipase C, protein kinase C, flavine enzymes, etc. It is associated with the generation of superoxide anion radicals, peroxide and hydroxyl radicals, singlet oxygen, and interleukin-2, this leading to cell proliferation [2,4,8]. The level of oxidative metabolism of mononuclear and neutrophil membranes is closely related to the bactericidal activity of the recipient organism administered intensive immunosuppressive therapy after transplantation. This does not rule out the damaging effect of reactive oxygen species not only on the recipient organism, but on the transplanted organs as well [1,5]. In this connection we thought it interesting to assess the effect of immunosuppressive therapy on the oxidative metabolism of immunocompetent cells during stimulation of the "respiratory burst" by opsonized zymosan and phorbol ester, which stimulate the cellular C3b receptors and protein kinase C, respectively [4].

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MATERIALS AND METHODS

Transplantation of allogeneic cadaveric kidneys was carried out at the Transplantation Center, Moscow Region Research and Clinical Institute. Cadaveric kidney donors were compatible with recipients by AB0 and 1 or 2 HLA-A and B antigens. Immunosuppressive therapy included cyclosporin A (Sandoz) in a dose of 2 to 5 mg/kg b.w. to maintain a level in the blood plasma of 100-200 ng/ml, intravenous methylprednisolone in a dose of 250-500 mg a day for 3 to 5 days after transplantation, oral prednisolone (tablets) in a dose of 30 mg a day for 3 weeks followed by a gradual reduction of the hormone dose, and azathioprine in

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Table 1. Time Course of Luminol-Dependent CL of Peripheral Blood Mononuclears and Neutrophils in Recipients of Allogeneic Kidneys ($M \pm m$)

Group	Day post-transplantation	cpm/cell for			
		adhesion		zymosan stimulation	
		neutrophils	mononuclears	neutrophils	mononuclears
Recipients	1–10 (10)	4.8±0.6	0.16±0.04	22.2±1.2	8.4±0.8
	10–20 (14)	3.5±0.4	0.29±0.06	16.6±0.15	4.2±0.18
	21–30 (14)	2.1±0.3	0.16±0.04	13.2±1.2	6.7±0.3
	30–70 (9)	4.5±0.1	0.45±0.03	18.3±2.1	5.0±0.24
CRI patients	– (8)	3.0±0.3	0.3±0.09	16.4±1.5	6.6±0.9
Healthy subjects	– (6)	7.2±0.8	0.2±0.08	18.0±3.2	4.0±0.83

Note. The number of examinees is shown in parentheses.

a dose of 2-3 mg/kg b.w. daily. Antilympholin-Kz, goat antithymocytic globulin, was intravenously infused in a dose of 2-5 mg/kg every other day for 2 weeks, with monitoring for a drop of the peripheral blood leukocyte count. Antilympholin-Kz (manufactured by the Research Institute of Clinical and Experimental Immunology, Ministry of Health and Medical Industry of Russia, Moscow) contains antibodies to human thymocytes; its titer in the lymphocytotoxic test is 1:1024.

A total of 61 recipients of allogeneic kidneys, 15 patients with chronic renal insufficiency (CRI), and 12 normal donors were examined, 18 of these being women and 70 men. The mean age of the examinees was 31. Patients with CRI were maintained on programmed hemodialysis before kidney transplantation.

Mononuclears and neutrophils were isolated by centrifugation of blood diluted 1:2 with medium 199 in a double Ficoll-Verografin density gradient (lower solution density 1.119, upper one 1.077) for 40 min at 1500 rpm and 4°C. The suspension of isolated neutrophils was 96-98% pure, and that of mononuclears 90% pure. Zymosan was opsonized with a freshly prepared pool of sera from 12 donors and used in the final concentration of 20 mg/ml. Phorbol-12-myristate-13-acetate (Sigma) was used in a concentration of 2.5 μg/ml. Chemiluminescence (CL) was enhanced by the use of luminol in a concentration of 5×10^{-4} M and lucigenin in a concentration of 1×10^{-3} M (both manufactured by Sigma).

A chemiluminometer 3603 (Dialog Joint Venture, Moscow) loaded with computer software was used for CL, which was carried out in plastic tubes in colorless Hanks solution with Ca^{2+} and Mg^{2+}. Control tubes contained 300 μl Hanks solution and 150 μl luminol or 200 μl lucinogen; 150 μl of one of the activators was added to test tubes. 100 μl mononuclear or neutrophil suspension (2×10^5 cells) were added to experimental and control tubes and CL induced by adhesion to plastic was recorded for 60 min. Then the cells were activated with zymosan or phorbol ester and activator-induced CL was recorded for 30 min. After this, the number of pulses per cell was counted and CL was expressed in cpm. CL of mononuclear and neutrophil subpopulations was examined in parallel tests. In one of the experimental series mononuclears and neutrophils before CL recording were treated with antilympholin-Kz diluted 1:20 or with rabbit antisera (1:10) to human IgG, IgM, and IgA for 30 min at 37°C, followed by washing. Rabbit monospecific antisera manufactured by Biomed (Research Institute of Vaccines and Sera, Russian Academy of Medical Sciences, Moscow) contained antibodies to heavy and light chains of human Ig. Results were statistically processed using Student's t test.

RESULTS

Three series of experiments were carried out. In the first the time course of luminol-dependent CL of mononuclears and neutrophils in allogeneic kidney recipients was examined. In the second the activation of mononuclears and neutrophils was compared in 7 recipients with acute rejection of the allogeneic kidney (serum creatinine higher than 0.3 mmol/liter) and 8 recipients with a normally functioning allogeneic kidney. In the third series the possibility of suppressing mononuclear and neutrophil CL with specific antisera to Ig and antilympholin-Kz was assessed.

In the first series of experiments neutrophil CL induced by adhesion to plastic and by zymosan was superior to that of mononuclears (Table 1). Stimulation of mononuclears and neutrophils was maximal in the first 10 days after transplantation. An evident reduction of neutrophil adhesion

Table 2. Effects of Heterologous Antisera on CL of Peripheral Blood Leukocytes in Recipients of Allogeneic Kidneys ($M \pm m$)

Type of antisera	cpm/cell for stimulation with			
	zymosan		phorbol ester	
	mononuclears	neutrophils	mononuclears	neutrophils
ATG	1.5±0.5 (0.14±0.015)	0.14±0.03 (0.09±0.004)	0.08±0.007 (0.04±0.004)	0.05±0.003 (0.05±0.005)
to IgG	6.1±1.3 (0.76±0.23)	19.6±4.2 (4.4±1.5)	0.09±0.01 (0.04±0.004)	0.23±0.006 (0.01±0.003)
to IgM	10±2.3 (2.5±0.47)	27.0±1.7 (27±0.33)	0.17±0.008 (0.03±0.003)	0.21±0.014 (0.13±0.006)
to IgA	6.2±0.96 (1.0±0.24)	25.0±3.4 (24±1.6)	0.18±0.017 (0.04±0.003)	0.25±0.02 (0.15±0.003)
Control	8.0±2.0 (2.0±0.4)	11.5±1.8 (4.6±1.7)	0.57±0.12 (0.07±0.002)	0.22±0.07 (0.07±0.002)

Note. ATG: antithymocytic globulin. CL of leukocytes adhering to plastic is shown in parentheses.

was noted on days 21-30 after transplantation. During the same period the neutrophils were stimulated with zymosan to a lesser degree ($p<0.01$), although in the days that followed, the extent of neutrophil stimulation increased to reach the normal level. Zymosan stimulation of mononuclears was minimal on days 10-20 posttransplantation and increased by day 30.

In the second series of experiments the degree of mononuclear and neutrophil activation with zymosan and phorbol ester was higher in recipients with acute rejection of the allogeneic kidney than in recipients with a normally functioning transplant ($p<0.01$). In recipients with graft rejection zymosan-stimulated CL of mononuclears was 7.5±1 cpm/cell and phorbol ester-stimulated CL of neutrophils 0.42±0.09 cpm/cell. In patients with a good function of the transplant mononuclear and neutrophil CL stimulated with zymosan and phorbol ester was 3.2±0.56 and 0.16±0.03 cpm/cell, respectively.

The results of the third series indicate a manifest suppressive effect of antilympholin-Kz on zymosan and phorbol ester stimulation of mononuclears and neutrophils and on these cells' adhesion to plastic (Table 2, $p<0.01$). Antisera to IgG, IgM, and IgA extinguished the CL of mononuclears stimulated with phorbol ester and enhanced the CL of neutrophils stimulated with zymosan. Antiserum to IgG displayed a higher suppressive activity in comparison with antisera to IgM and IgA.

Hence, it has proved possible to assess the activation of peripheral blood mononuclears and neutrophils by means of CL in allogeneic kidney recipients with acute rejection of the graft. Phorbol ester as an activator of protein kinase C increases the CL of mononuclears and neutrophils to the same extent.

Moreover, a possible contribution of neutrophils to allotransplant rejection is confirmed by the presence of a receptor to interleukin-1 in neutrophils [8] and by the capacity of these cells for cytotoxic action *in vitro* [3]. It is possible that the *in vitro* activation of mononuclears and neutrophils with zymosan and phorbol ester is due to priming of these cells with alloantigens in the recipient organism. Priming of phagocytes is connected with polysaccharides of Gram-negative bacteria and cytokines [7]. The time course of luminol-dependent CL demonstrates a higher level of leukocyte stimulation with zymosan during the first days after transplantation. This may be due to the effect of kidney alloantigens on these cells in the initial period of immunosuppression. Antilympholin-Kz reduced the CL of mononuclears and neutrophils in our experiments, possibly due to the presence on thymocytes used for immunization of animals of antigens common for mononuclears and neutrophils. Antisera to human Ig of three classes depressed the CL of mononuclears but not of neutrophils, probably because of blocking of Ig-like antigen-recognizing receptors present in the mononuclears and absent in the neutrophils. It is worthy of note that antiserum to IgG displayed a higher suppressive activity than antisera to IgM and IgA. Antisera to Ig enhanced luminol-dependent CL of neutrophils stimulated with zymosan not only through the Fc receptor, since $F(ab')_2$ fragments of antibodies had the same effect [5].

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