PERIPHERAL BLOOD AND INTRARENAL PHAGOCYTIC CHEMILUMINESCENCE DURING ACUTE KIDNEY GRAFT REJECTION

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Abstract—During organ graft rejection, soluble mediators of inflammation are released into the polymorphs (PMNs) and monocytes recruited from the blood. One functional capacity of polymorphs and monocytes/macrophages is the production of cytotoxic activated oxygen species upon stimulation, which may contribute to the rejection process. Nothing is known about the influence of allograft rejection on this inflammatory cell property. Chemiluminescence (CL) allows measurement of respiratory burst capacity in small cell samples. Zymosan-induced and luminol-amplified CL of diluted whole blood, separated PMNs, and mononuclear cells from peripheral venous blood, as well as of intragraft phagocytes was measured after allogeneic and autologous kidney transplantation in untreated dogs. CL of separated PMNs, mononuclear cells, and intragraft phagocytes was significantly elevated during allograft rejection. In autologous kidneys transplanted to recipients of allografts, CL was also increased in the autologous grafts during rejection of the allogeneic ones, indicating a systemic alteration in phagocyte function.

INTRODUCTION

Phagocytic cells, among them polymorphonuclear leukocytes (PMNs) and monocytes/macrophages, react to a number of stimuli with the generation of activated oxygen species (O₂, H₂O₂, ·OH, etc.) in a respiratory burst. A method to quantify this metabolic activity is luminol-amplified chemiluminescence (CL), introduced by Allen and Loose (1). Some mediators (interleukin-1, gamma-interferon) involved in the foreign antigen recognition and cytotoxic cell recruiting process, which ultimately leads to allograft destruction, have been shown to also modulate granulocytic and monocytic functions (2-8).

We measured CL to study whether the respiratory burst activity of PMNs and monocytes is modified during untreated canine kidney graft rejection. As a
control group, we used bilaterally autotransplanted dogs. The CL of graft infiltrating leukocytes was also measured. To compare the influence of the "specific" inflammatory process in the allogeneic kidney to that of unspecific concomitant changes in autologous kidneys in the same animal, all allotransplanted dogs simultaneously received kidney autotransplants to the contralateral side. CL was also registered from aspirates of these grafts. As a clinically standardized technique of rejection diagnosis, fine-needle aspiration cytology (FNAC) was used.

MATERIALS AND METHODS

Animals and Transplantation. Ten mongrel dogs (body weight = 21.8 ± 3.4 kg) were transplanted with kidneys from five unrelated donors to one fossa iliaca and autotransplanted to the contralateral one. Six control animals were bilaterally autotransplanted. Kidneys were perfused with cold (4°C), heparinized (5000 IU/liter, heparin–natrium, B. Braun, FRG) Euro-Collins (Bio-test Pharma, Dreieich, FRG) solution immediately after dissection of the vessels, kept on ice until implantation, and continuously cooled during implantation (mean cold ischemia time 1.5 h). The renal vessels were anastomosed to the common iliacal vessels of the recipient in a standard technique (9). Ureterovesicostomy was performed using a modified Gregoir technique (9). During the surgical procedure, the animals were kept under a combined halothane/nitrous oxide anesthesia and levomethadone analgesia (Polamivet, Hoechst AG, FRG). Starting on day 1 postoperatively, all animals received a penicillin/streptomycin preparation (Tardomycel comp III, 0.1 ml/kg subcutaneously, Bayer, FRG).

Fine-Needle Aspiration Cytology and Blood Cell Counts. Five milliliters of heparinized (100 IU/ml) venous blood was drawn daily. Leukocytes were counted with a Coulter counter (Clay Adams, Runcorn, UK). Transplanted kidneys were biopsied daily with a spinal needle (22 gauge, Becton & Dickinson, Spain) and cells aspirated into heparinized (100 IU/ml) Hanks' medium. Aspirates and blood cells were transferred to slides in a cytocentrifuge, stained with May–Grunwald–Giemsa (Merck, FRG) and morphologically differentiated. The corrected increment, a numerical evaluation of graft infiltrating cells, was calculated according to the method described by Häyry et al. (10). In short, percentages of contaminating blood leukocytes are subtracted from intragraft leukocytes and every single increment multiplied with an empirically found corrective factor. The corrected increments were added up. Only aspirates with a parenchymal cell number of more than 5 per 100 leukocytes were evaluated.

Cell Separation. PMNs and mononuclear cells were separated from peripheral blood, diluted 1:10 in Hanks', by Ficoll-Isopaque density gradient centrifugation (1.075g) (11). After separation, the erythrocyte-containing PMN-enriched pellet was lysed by 30 sec of hypotonic exposure followed by double isotonic replenishment. Both mononuclear cells and PMNs were resuspended in Hanks' and washed twice. Purity of the PMN suspension was 97%. The mononuclear cell suspension contained between 30% and 50% monocytes. Percentage of monocytes did not differ in both groups. Viability was tested by trypan blue exclusion and was always above 96%.

Chemiluminescence. PMNs, mononuclear cells, and fine-needle aspirates were adjusted to 5 × 10^5/ml in Ca^2+-, Mg^2+-free phosphate-buffered saline. Heparinized blood (100 IU/ml) was diluted 1:10 in Ca^2+-, Mg^2+-free PBS. Then 650 μl of veronal-buffered saline were added to 100 μl of Luminol (200 μmol, Sigma, FRG) and 200 μl of the respective cell suspension (final concentration 1 × 10^6 cells/ml) or diluted blood (1:50 in the probe) in polystyrene vials (Berthold, FRG). The samples were preincubated for 10 min at 37°C in the dark. The luminescent reaction