T Cells Mediating Early Acute Kidney Allograft Rejection in the Rat Are Different From Those Responsible for Chronic Rejection

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IT HAS been proposed that recognition of alloantigens by $CD4^+$ T cells can be achieved through two different pathways.[1] The direct pathway where T cells directly recognize the polymorphism of major histocompatibility complex (MHC) class II alloantigens displayed at the surface of allogeneic stimulators, and the indirect pathway where alloantigens are seen in the same way as nominal protein antigens, that is, as processed peptides associated with self-MHC class II molecules. The hypothesis was that, in the process of allograft rejection, the involvement of direct pathway-sensitized T cells was strictly dependent on the presence within the allograft of donor-genotype "passenger" leukocytes. If early rejection episodes were inhibited or suppressed, for example, by immunosuppressive drugs or MHC matching, and the survival of the graft extended, replacement within the graft of donor passenger leukocytes by cells of recipient origin could take place. It was postulated, that, in such an event, donor-passenger leukocyte-depleted allografts would lose their ability to stimulate direct pathway-sensitized CD4 T cells, and that if subsequent rejection episodes occurred, the only route available for sensitization of recipient's $CD4^+$ T cells to donor alloantigens would be the indirect pathway.

In order to verify this hypothesis, rat alloreactive T-cell line and clones sensitized through the direct pathway of allorecognition were tested for their ability to reject kidney allografts that either harbored donor passenger leukocytes or were depleted of these cells.

The AS ($RT1.A^l.B^l$) anti-AUG ($RT1.A^c.B^c$) rat alloreactive T-cell line, L10, and clones, L12.4 and L12.8, were isolated in mixed lymphocyte culture (MLC) by stimulating AS spleen T cells with irradiated AUG spleen stimulators.[3] The clones and the cell line were $CD4^+$, noncytotoxic, and were shown to specifically react to $RT1.B^c$ molecules displayed by AUG spleen stimulators. The ability of these cells to reject kidney allografts that either harbored donor passenger leukocytes or were depleted of these cells was tested. The following protocol was applied (Fig 1): AS recipients that received 5 Gy irradiation from a cobalt source were orthotopically transplanted 24 hours later with normal left August kidneys or with August kidneys previously depleted of donor passenger leukocytes. On the completion of the renal transplant operation the animals were injected with the AS anti-August T-cell line or clones, and the survival of the grafts was regularly monitored thereafter by measuring the blood urea. The passenger leukocyte-depleted allografts were obtained by parking for 30 to 50 days August kidneys in AS rats that received cyclosporine (CyA) treatment (10 mg/kg/d) for 10 days following transplantation. Only kidneys with normal function were used as passenger leukocyte-depleted grafts.

When no cells were injected into the irradiated AS recipients, the normal August kidney allografts survived more than 50 days with normal function. By contrast, normal August kidneys transplanted into irradiated AS recipients reconstituted with normal AS spleen T cells were rejected with a median survival time of 8 days. Reconstitution with various number of L10 cells caused rejection of normal August kidney grafts. As few as 1 million L10 cells were able to bring about rejection with a graft median survival time of 11 days. However, when donor passenger leukocyte-depleted August kidneys were used as allografts, L10 cells were unable to cause rejection and all the grafts survived beyond 50 days. Even injecting a dose of 10 million cells did not affect the long-term survival of the grafts. These results were repeated with the AS anti-August T-cell clones, L12.4 and L12.8, that is, the clones mediated the acute rejection of normal August grafts, but failed to cause the rejection of passenger leukocyte-depleted grafts.

The failure of passenger leukocyte-depleted kidneys to stimulate the cell line and the clones cannot be explained by the lack of expression of August MHC class II products within the grafts.[2] Indeed, rat renal tubular epithelial cells do constitutively express MHC class II molecules. In the absence of donor passenger leukocytes, have these cells the capacity to stimulate direct pathway-sensitized alloreactive $CD4^+$ T cells? To answer the question, August renal tubular epithelial cells were isolated and cultured, and then tested for their ability to stimulate AS anti-August alloreactive T cells. Cultured rat renal epithelial cells did not express MHC class II molecules; however, expression could be induced by culturing the cells in interferon (IFN)-γ-containing medium. The clone L12.4 was tested for proliferation in response to August $RT1.B^+$ epithelial cells or to normal August spleen cells. A proliferative response was only observed when spleen cells were used as stimulators.

It therefore appeared that August kidney epithelial cells were not able to stimulate a proliferative response in alloreactive $CD4^+$ T cells. Instead, they were shown to

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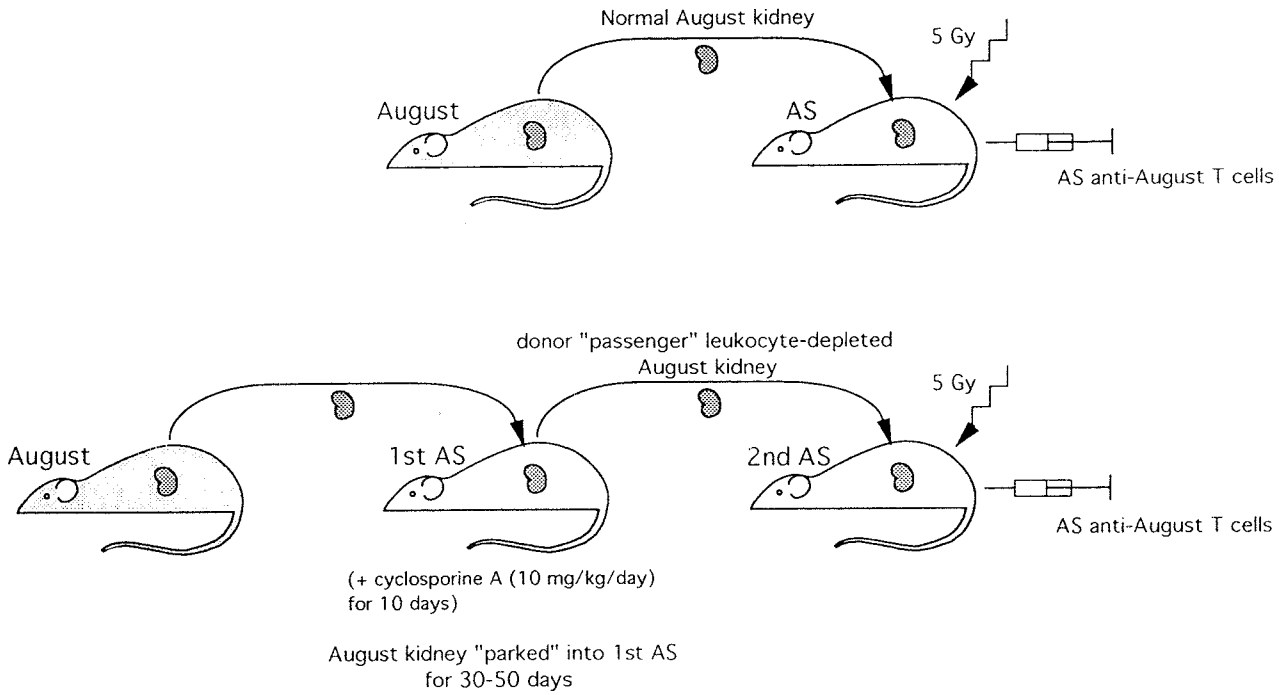

Fig 1. Experimental protocol used for testing the ability of direct pathway-sensitized alloreactive AS $CD4^+$ T cells to cause rejection of normal and passenger leukocyte-depleted August kidney allografts.

induce a state of unresponsiveness among L12.4 cells. It was observed that overnight incubation in the presence of irradiated $RT1.B^+$ August epithelial cells rendered L12.4 cells refractory to subsequent alloantigen challenges. Although preincubation with syngeneic AS epithelial cells did not affect the ability of L12.4 cells to respond to August spleen cells, coculturing L12.4 cells with August epithelial cells expressing class II products inhibited their capacity to respond to August spleen cells. A similar observation was made with the clone L12.8.

Thus, direct pathway-sensitized alloreactive AS $CD4^+$ T cells cause acute rejection of normal August kidney allografts, but fail to mediate the rejection of August kidneys depleted of donor passenger leukocytes. August renal epithelial cells, expressing class II MHC alloantigens, fail to stimulate direct pathway-sensitized AS anti-August T-cell clones to proliferate in vitro; in contrast they induce a state of specific nonresponsiveness. These results indicate that delayed or chronic rejection of kidney allografts occurring after the loss of donor passenger leukocytes must be induced by a cell population different from direct pathway-sensitized $CD4^+$ T cells, possibly self-restricted $CD4^+$ T cells sensitized by the indirect pathway.

REFERENCES

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