Brief Definitive Report

SPECIFIC CYTOTOXIC T CELLS ARE FOUND IN THE NONREJECTED KIDNEYS OF BLOOD-TRANSFUSED RATS

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In recent years, there has been much interest in the cellular events that accompany tissue graft rejection. It has become clear from the work of Loveland et al. (1), Dallman et al. (2), and from many subsequent studies (see reference 3) that the Th cell normally plays a pivotal role in the induction of the rejection response. However, the relative roles of cytotoxic cells, delayed-type hypersensitivity reactions, and other effector mechanisms in graft destruction still remains a controversial issue.

The elegant studies of Tyler et al. (4) have shown that a cloned population of MHC class I–restricted cytotoxic T cells can reject a skin graft through an epidermal antigen disparity. Similarly, cloned cytolytic T lymphocytes can destroy tumor cell grafts in mice (5). Other workers have shown that an absence of cytotoxic T cells within a renal graft correlates with graft acceptance (6, 7) and that depletion of Ly-2^+ cells in vivo, by the use of mAbs, may lead to prolonged mouse skin allograft survival (8, 9). On the other hand, using H-Y disparate skin there are certain mouse strains in which graft rejection may occur in the apparent absence of H-Y antigen–specific cytotoxic cells and where delayed-type hypersensitivity correlates well with graft rejection (10). These and many other studies (see reference 3) seem to demonstrate that in different situations a variety of effector mechanisms may be called into play in the rejection process. However, in previous studies (6, 7), the presence of specific cytotoxic cells *within* a graft has always correlated with tissue rejection and such cells have not been found in a graft that survives indefinitely in the absence of any subsequent immunosuppression.

Preoperative donor–specific blood transfusion may lead to indefinite survival of rat renal allografts (11). In this study, we have extracted cells from the kidney transplants of animals that have received a donor-specific blood transfusion preoperatively and that will retain their grafts indefinitely. The cytotoxic capacity of these cells has been compared with the activity of cells isolated from rejecting grafts. Using this system, we provide direct evidence that a high level of graft-specific T cell–mediated cytotoxic activity may be present within a tissue that is not rejected.

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Materials and Methods

Animals. Lewis-RT1^l (LEW), blood group D–Agouti-RT1^a (DA), and PVG-RT1^c (PVG) rats were obtained from Harlan-Olac UK Ltd. (Bicester, United Kingdom) or bred in the animal facility at the John Radcliffe Hospital, Oxford, United Kingdom.

Blood Transfusion. A donor-specific blood transfusion given intravenously 7 d before transplantation leads to indefinite survival of a subsequent renal allograft in all strain combinations used. The following transfusions were used: In the LEW to DA combination, 0.5 ml LEW blood; in the LEW to PVG combination, 1.0 ml LEW blood; in the DA to PVG combination, 1.0 ml DA blood. The mean survival time of untreated rats was 10 d and of blood-transfused rats >100 days in all combinations.

Renal Transplantation. Kidneys were transplanted into the left orthotopic site as described previously (12). For survival data, contralateral nephrectomy was carried out 7 d after transplantation.

Preparation of Cell Suspensions. Kidneys were removed 5 d after transplantation and infiltrating cells were released by collagenase digestion of the tissue. Kidneys were minced finely and then incubated for 30 min at 37°C in 5% CO_2 in RPMI 1640 (041-1875, Gibco Ltd., Paisley, United Kingdom) containing 1 mg/ml collagenase (C-2139; Sigma Chemical Co., Poole, United Kingdom). The digest was then pressed through a wire mesh and viable cells were obtained by centrifugation for 20 min, 800 *g*, 20°C on a single-step Ficoll gradient (Histopaque 1083-1, Sigma Chemical Co.). Cells were washed three times in a prewarmed solution of Dulbecco's A + B solution (BR14a + SR39, Oxoid Ltd., Baisingstoke, United Kingdom) containing 2% FCS (DAB/FCS), resuspended at 5×10^6 cells/ml in RPMI 1640 containing 10% FCS, 2.5×10^{-5} M 2-ME, 2 mM L-glutamine, penicillin, streptomycin, and gentamycin (RPMI complete). Serial twofold dilutions of the cell suspension were made and the cells were used immediately as effectors in the ^{51}Cr-release assay.

^{51}Cr-release Assay. A conventional ^{51}Cr-release assay (13) was set up using Con A (C-2631, Sigma Chemical Co.)–activated lymph node cells as targets (Con A blasts). These were prepared by incubation of fresh lymph node cell suspensions in RPMI complete containing 10 μg/ml Con A for 24–48 h at 37°C in 5% CO_2. Targets were harvested, washed in RPMI 1640 once, and labeled with 300 μCi ^{51}Cr (CJS.4; Amersham International, Amersham, United Kingdom) for 90 min at 37°C in 5% CO_2. Targets were then washed four times in DAB/FCS and added to 96-V-well microtiter plates (76-021-05; Flow Laboratories, Rickmansworth, United Kingdom) using 5×10^3 cells/well. Effector cells were added at an initial E/T ratio of 100:1 to make a total volume of 200 μl/well. Plates were then centrifuged for 1 min at 400 *g* and incubated for 6 h at 37°C in 5% CO_2. 133 μl supernatant was then removed for counting. All assays were carried out in duplicate. Percent specific release was calculated by the formula: % specific release = [(experimental release-spontaneous release) × 100]/[(maximum release-spontaneous release)]. Maximum release was calculated as the total counts added in the target cells. Spontaneous release was never >15% of maximum release.

Results

Cytotoxic Activity in Renal Allografts. The magnitude of cytotoxic activity found in the renal transplants of transfused animals was compared with that found in similar grafts of untreated controls. Transfused animals were given an intravenous injection of allogeneic blood 7 d before a renal allograft, the blood and kidney always being from the same donor rat strain. Untreated animals only received a kidney allograft. 5 d after transplantation, the grafts were removed, and the infiltrating cells were harvested and used as effector cells in a ^{51}Cr-release assay to measure their cytotoxic activity. As may be seen, in all strain combinations tested, a high level of cytotoxicity was exhibited by cells infiltrating the renal allografts of both transfused and untreated animals (Fig. 1). In the DA

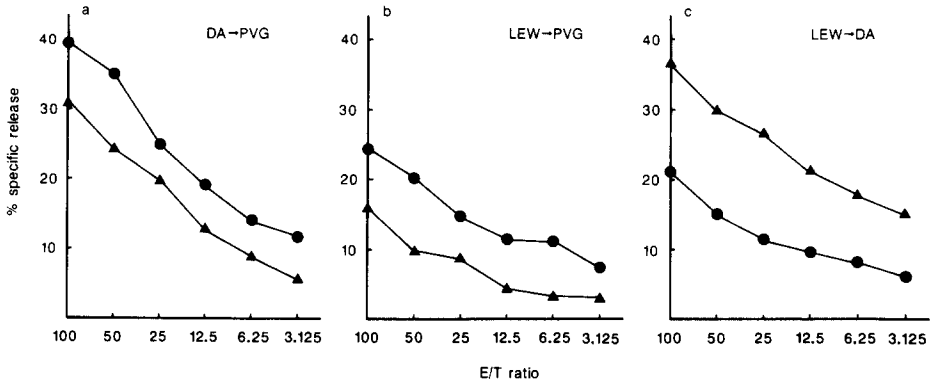

FIGURE 1. Cytotoxicity in renal allografts 5 days after transplantation. Rats were given a blood transfusion 7 d before receiving a renal allograft. The blood and kidney were from the same donor rat strain. Untreated controls were given a renal transplant only. 5 d after transplantation, the grafted kidneys were removed and the infiltrating cells were harvested after collagenase digestion of the tissue. These cells were used as effectors in a 6-h ^{51}Cr-release assay against Con A blast targets. Assays are shown for (*a*) the DA to PVG, (*b*) the LEW to PVG, and (*c*) the LEW to DA strain combinations. In each case, the activity of infiltrating cells was assayed against targets expressing the alloantigens of the kidney donor strain. All E/T ratios were performed in duplicate within an assay. The activity of cells from transfused (●) and untreated (▲) rats was compared in the same assay. The results shown are from single experiments in the three strain combinations tested. Similar results were obtained in 15, 3, and 2 other experiments in the LEW to DA, LEW to PVG, and DA to PVG strain combinations, respectively.

to PVG and LEW to PVG strain combinations, the level of cytotoxicity in transfused animals equalled or exceeded that seen in the untreated controls. A full kinetic analysis of cytotoxicity has demonstrated that, in transfused animals, an accelerated infiltration of the graft by cytotoxic T cells occurs. Further, a significant level of cytotoxic activity may be demonstrated in such grafts until at least 21 d after transplantation. This will be reported in detail elsewhere (Dallman, M. J., K. J. Wood, and P. J. Morris, manuscript in preparation). It is important to note that these assays were performed using cells immediately after extraction from the transplanted kidney and therefore are representative of effector activity present within the grafts.

Specificity of Cytotoxicity. To determine the specificity of the cytotoxic activity within the transplants, ^{51}Cr-release assays were always performed using three different target cells expressing the alloantigens of: (*a*) blood and kidney donor type, (*b*) recipient type, or (*c*) third-party type. As may be seen from Fig. 2, the cytotoxic cells obtained from either transfused or untreated animals demonstrated specificity for kidney donor alloantigens. Results are shown for the LEW to DA strain combination, but an equal degree of specificity was observed using both the LEW to PVG and DA to PVG strain combinations. Such cytotoxicity is mediated by T lymphocytes since all of the activity resides within the MRC OX19$^+$ (rat CD5) cell fraction. This has been demonstrated by separation of cells on the cytofluorograf and will be reported in detail elsewhere (manuscript in preparation). Briefly, however, in the DA to PVG strain combination, MRC OX19$^+$ cells (99.3% MRC OX19$^+$) showed 23% donor-specific cytotoxicity and

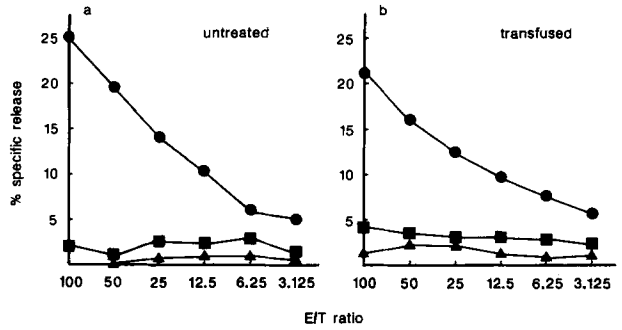

FIGURE 2. Specificity of cytotoxic activity. DA animals were given an intravenous injection of 0.5 ml LEW blood or were not treated, and 7 d later they were transplanted with a LEW kidney. 5 d after receiving the kidney graft, the transplants were removed and the infiltrating cells were recovered by collagenase digestion of the tissue. The cells from untreated (*a*) or transfused (*b*) rats were then used as effectors in a 6-h ^{51}Cr-release assay. The Con A blast target cells in the assay were of three types: LEW, i.e., blood and kidney donor type (●); DA, i.e., recipient type (■); or PVG, i.e., third-party type (▲). All E/T ratios were carried out in duplicate. The results of a single experiment are shown and similar results were obtained in 15 other experiments using the LEW to DA strain combination. A similar degree of specificity was observed in the lysis of target cells expressing donor alloantigens by effectors from transfused and untreated animals in both the DA to PVG and LEW to PVG strain combinations in a total of five experiments.

the unfractionated cell population (67% MRC OX19$^+$) showed 20%, both at an E/T ratio of 50:1.

Discussion

We have demonstrated, using a functional analysis, that 5 d after transplantation the grafted kidneys of blood transfused animals, which survive indefinitely, contain a high level of T cell–mediated cytotoxic effector activity that has specificity for kidney donor alloantigens. This activity is present within the harvested cell population immediately after extraction from the transplanted organ and therefore should accurately reflect cytotoxicity present within the graft. Despite the presence of such specific cytotoxic T cells within the transplant, the grafts of transfused animals are not rejected. There are at least four possible explanations for this finding. First, although much experimental evidence supports the notion that the specific cytotoxic cell (defined by its in vitro activity) plays a critical role in graft rejection, it is possible that cytotoxicity as assayed in the in vitro system is irrelevant to the rejection process in vivo. Indeed, it may be argued that the Con A blast is not a suitable target on which to assay a population of cells that will effect kidney graft rejection. It is difficult, however, to develop a target that is more appropriate to kidney graft rejection since the target cell within the kidney whose destruction leads to graft necrosis is not clearly defined.

Second, it has been suggested that organ graft rejection may be severely impeded unless MHC class I and II antigens are induced on the target tissue (14). It is apparent, however, that sufficient target antigens are present within the transplanted kidney since we have shown, by immunohistology and quantitative absorption, that both MHC class I and class II antigens are induced equally

in the kidney of transfused and untreated recipients (Wood, K. J., M. J. Dallman, and P. J. Morris, manuscript in preparation).

Third, it is possible that there are qualitative differences in the cytotoxic cells present in transfused and untreated recipients. Wood and Streilein (15) have recently demonstrated that qualitative differences may occur in lymphocyte populations, since cytotoxic cells derived from neonatally tolerant animals may interact with their target cells with lower avidity than cytotoxic cells from their normal counterparts. We are currently investigating this possibility.

Finally, it is possible that the action of cytotoxic cells in transfused animals is blocked within the transplant. Such blocking may occur by the presence of enhancing antibodies, although other workers (16) have found it difficult to demonstrate significant levels of such antibody in blood-transfused animals. Alternatively, it has been suggested that a suppressor cell may block effector activity. Infiltrating cells with suppressor activity as demonstrated in adoptive transfer have been isolated from nonrejecting grafts shortly after transplantation (17). However, it seems unlikely that such cells are responsible for inhibiting the action of cytotoxic cells in the present experiments, as suppressor cells are not usually effective at blocking fully activated cells, but rather act on the afferent arm of the immune response (18). Further, if suppressor cells could block the effector activity of cytotoxic cells one would not expect to see the high levels of cytotoxicity so readily demonstrable in the assays reported here. We are currently examining these alternatives.

These studies demonstrate that the survival of transplanted kidneys in transfused rats cannot be explained by an absence of specific cytotoxic cells within the recipient, and that the infiltration of specific cytotoxic effector cells into a transplant does not necessarily result in rejection of that tissue.

Summary

Preoperative, donor-specific blood transfusion leads to indefinite survival of rat renal allografts in the strain combinations used. ^{51}Cr-release assays have shown that the level of specific cytotoxic effector activity in the grafts of transfused (nonrejected kidney) animals is very high and may equal or exceed that seen in the grafts of untreated (rejected kidney) recipients. Such cytotoxicity demonstrates specificity for the alloantigens of the kidney, is T cell–mediated, and may persist within the transplant.

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