

IMMUNOHISTOLOGICAL OBSERVATIONS IN RAT KIDNEY ALLOGRAFTS AFTER LOCAL STEROID ADMINISTRATION

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Several immunosuppressive regimens have been used to inhibit the immune response in autoimmune disease and allograft rejection. In general the inhibition interferes mainly with the two pathways involved in cellular immune response: specific cell-mediated cytotoxicity and delayed-type hypersensitivity (DTH)¹ (1–8). In both mechanisms, lymphokine production by T cells plays a crucial role; lymphokines are essential for the proliferation and differentiation of cytotoxic T cell precursors, as well as for the effector phase of DTH reactions. Lymphokines, such as interferons, are also potent inducers of MHC class I and class II antigens (9–11), which both play a critical role in the development and augmentation of the immune response (12–16). The inhibition of lymphokine production e.g., IFN- γ and IL-2 and the modulation of MHC antigen expression was shown to be an important mode of action of immunosuppressive drugs such as steroids (17, 18) and cyclosporin A (19, 20).

So far, treatment of autoimmune disease and allograft rejection is achieved by systemic immunosuppression without specificity for the organs affected. Recently however, controversy has increased concerning the role of systemic versus local regulatory mechanisms in cellular immune response. Immune stimulation by MHC antigen expression has been seen both as a local and systemic process in autoimmune disease, as well as in allograft rejection (21–26). The same controversy holds true for the effector mechanisms during cellular immune response. Originally it was proposed that sensitized cells mature within the central lymphoid tissue and subsequently migrate as mature effector cells to the target tissue and mediate tissue destruction (8, 27–29). More recently, however, it was demonstrated that CTL could mature within sponge matrix allografts, which implies an essential role for local development of CTL within the target organ, and hence local production of lymphokines (30–32). In accordance with these observations, we recently demonstrated that intrarenal prednisolone delivery to rat renal allografts was superior to systemic administration of the drug (33). These data provide suggestive evidence for local regulation of the immune response during allograft rejection and autoimmune disease. Moreover, they indicate that

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¹ *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; MST, median graft survival time.

the local immune response within the affected organ is a favorable subject for local immunosuppressive therapy. In this study we further analyze local regulatory mechanisms in graft rejection and their response to local immunosuppressive therapy.

Materials and Methods

Animals. Inbred Brown Norway (RTⁿ) and Lewis (RT¹) male rats between 2 to 4 mo of age were obtained from the Department of Experimental Animal Services of the University of Limburg.

Experimental Model. Right kidneys were transplanted from Brown Norway (BN) to Lewis rats using microsurgical techniques according to the method of Lee (34) with modifications of Lameyer for ureter bladder anastomosis. Ischemia times were ~30 min. Recipient kidneys were both removed immediately after transplantation and graft survival was assessed by animal survival. Recipient treatment consisted of prednisolone (Centra-chemie, Etten-Leur, The Netherlands) administered by means of continuous infusion either intrarenally or intraperitoneally. Continuous intrarenal drug delivery was performed as described (33). Briefly, immediately after transplantation a catheter was introduced into the suprarenal artery of the transplanted kidney. Because the suprarenal artery in rat directly empties into the main renal artery, this method allows selective drug administration to the graft without affecting renal blood flow. After potency of the infusion system was checked the catheter was connected to an osmotic minipump (2ML2; Alza, Palo Alto, CA) filled with the drug. Pumps were implanted into the abdominal cavity and delivered prednisolone continuously from day 0 till day 13 after transplantation. The technique used for intrarenal drug delivery showed by itself no significant effect on renal allograft survival. Median graft survival time (MST) in animals treated with intrarenal infusion of saline was 7 d (one for 6 d, five for 7 d, one for 8 d, and one for 12 d), which was not significantly different from untreated controls (MST 8 d, two for 7 d, three for 8 d, and two for 9 d). For continuous intraperitoneal infusion, pumps administered prednisolone directly into the abdominal cavity. Prednisolone was dissolved in pyrogen-free distilled water without heparin. The dose of the drug (4 mg/kg body wt/d) was adjusted to body weight of the recipient the day before allografting was performed (day -1). In animals that received intrarenal treatment, correct position of the catheter was checked at autopsy.

Determination of Prednisolone Levels. Recipients were treated with prednisolone 4 mg/kg body wt/d given either by continuous intrarenal infusion or continuous intraperitoneal infusion. On day 3 after transplantation, blood was drawn and allografts were excised for analysis of prednisolone levels. Prednisolone was analyzed with HPLC. The method, as published by Rose and Jusko (35), was used with the following modifications. Separation was performed on a 250 × 4.6 mm Lichrosorb 10-DIOL[®] column (Merck, Darmstadt, Federal Republic of Germany). Eluent composition was: methylene dichloride, 73%; hexane, 25.5%; methanol, 1.5% (vol/vol). The coefficient of variation for prednisolone was ~5%, the lower limit of detection was ~10 ng/ml in serum.

Immunohistologic Studies. Renal allografts were excised on days 3, 7, and 12 after transplantation. Immediately after harvesting, tissue samples were embedded in Tissue TEK II (Miles Scientific, Naperville, IL) mounting medium and snap-frozen in isopentane (-70°C). Cryostat sections of 5 μm were cut, air dried, fixed in acetone, and stored at -20°C until used. Immunohistologic studies were performed using indirect immunoperoxidase staining. Briefly, slides were thawed at room temperature for 15 min, washed for 5 min in PBS, and then incubated with the appropriate mouse mAb for 30 min at room temperature. Slides were washed again in PBS and subsequently incubated for 30 min with horseradish peroxidase-coupled rabbit anti-mouse immunoglobulin (Dako, Copenhagen, Denmark) diluted 1:100 in 10% rat serum. After washing in PBS, sections were developed with a solution of 0.5 mg/ml diaminobenzidine tetrahydrochloride (Serva, Heidelberg, Federal Republic of Germany) and 0.01% H₂O₂ in 0.05 M Tris buffer, pH 7.6. After 10 min slides were washed in tap water and counterstained with hematoxylin.

For parallel histologic observations, grafted tissue was fixed in neutral-buffered formalin, processed in a routine manner, and hematoxylin and eosin-stained sections were examined.

Monoclonal Antibodies. The following mouse anti-rat mAb were used; MRC OX6 (36) (Seralab, Crawley Down, United Kingdom) directed against rat MHC class II antigens. This antibody reacts with inbred rat strains of the haplotypes L and N; His19 (37) reacts with rat MHC class II antigens of the rat strains of haplotype L but not with antigens of rat strains of the N haplotype. The antibody was kindly provided by Dr. Stet (Dept. of Histology, University of Groningen, The Netherlands). MRC OX19 (38) (Seralab, Crawley Down, United Kingdom) reacts with all rat T lymphocytes; W3/25 (39, 40) mainly recognizes T helper cells but also stains a subpopulation of macrophages; OX8 (39, 41) reacts with T cytotoxic/suppressor cells and a subpopulation of NK cells. ED2 (42) recognizes rat macrophages; the antibody is directed against membrane antigens of tissue macrophages, and has been described in detail by Dijkstra (Dept. of Histology, Free University, Amsterdam, The Netherlands), who kindly provided the antibody. ART18 (43) is directed against an antigenic determinant of the IL-2 receptor molecule in rat. The antibody was kindly provided by Dr. Diamantstein (Dept. of Immunology, Klinikum Steglitz, Berlin, Federal Republic of Germany). DB1 (44), reacting against rat IFN- γ was kindly provided by Dr. Van der Meide (Primate Centre, TNO Rijswijk, The Netherlands). The antibody was prepared by fusing spleen cells from BALB/C mice immunized with recombinant rat IFN- γ with the myeloma SP₂O. The antibody was found to bind to rat IFN- γ in an ELISA and to block IFN- γ activity in a virus-protection assay. All mAbs were used at saturating dilutions of partially purified immune ascites. Normal kidney tissue from BN and Lewis strain stained negative with mAbs ART18 (anti-IL-2-R) and DB1 (anti-IFN- γ). Normal kidney tissue was almost devoid of ED2⁺ cells (macrophages).

Morphometric Analysis of Tissue Sections. When indicated the cellular infiltration within the grafts was quantified by morphometric analysis according to the method of McWhinnie (45). Cellular infiltration within a section was assessed by point counting using a square grid in the eyepiece of the microscope. The percentage area of each section occupied by cells of a particular antigenic specificity was calculated as follows: Percentage area infiltrated = $100 \times (\text{number of positive cells under grid intersections}) / (\text{total number of grid intersections})$. The accuracy of the technique is proportional to the number of points counted. To maintain a standard error of less than 10%, 4,250 points were counted when density of the infiltrate was less than 10%, 600 points were counted when density of the infiltrate was more than 10%. For point counting a 120 point or 850 point graticule was used, within each section five fields were chosen at random and counted by a magnification of $\times 400$.

Results

Experimental Model for Local Immunosuppression. Allografts treated locally with prednisolone 4 mg/kg body wt/d showed significant prolongation of graft survival (MST, 28 d). As demonstrated in Table I, local drug delivery resulted in a mean kidney prednisolone level of 213 ng/kidney and a mean plasma level of the drug of 53 ng/ml. To ascertain that, during intrarenal infusion, local immunosuppression within the graft was responsible for the immunosuppressive effect observed, recipients were treated intraperitoneally with the same dose of prednisolone. Systemic administration of the drug resulted in an MST of 9 d, which was not significantly different from control animals. During intraperitoneal drug delivery the mean plasma prednisolone level was 46 ng/ml. The mean kidney prednisolone level was 126 ng/kidney. These data demonstrate that the systemic plasma level reached during local drug delivery is by itself not sufficiently immunosuppressive to induce graft survival. From these observations it can be concluded that during intrarenal treatment, high kidney prednisolone levels and

TABLE I
Graft Survival and Mean Prednisolone Levels in Plasma and Renal Allografts during Continuous Delivery of Prednisolone Intrarenally or Intraperitoneally

Therapy	Mean (\pm SD) prednisolone level on day 3		MST*	n
	Plasma	Renal allograft		
	ng/ml	ng/kidney	d	
4 mg/kg Continuously intraperitoneally	46 \pm 8	126 \pm 21	9	9
4 mg/kg Continuously intrarenally	53 \pm 14 [‡]	213 \pm 80 [§]	28 [¶]	9

* MST, median graft survival time as determined in graft survival studies. MST in untreated controls is 8 d.

[‡] Wilcoxon rank test for difference between plasma prednisolone levels in animals treated intraperitoneally and intrarenally is not significant.

[§] Wilcoxon rank test for difference between prednisolone levels in renal allografts of animals treated intraperitoneally and intrarenally ($p < 0.05$).

[¶] Wilcoxon rank test for difference between MST in animals treated intraperitoneally and intrarenally with prednisolone ($p < 0.01$).

hence local immunosuppression mainly are responsible for the immunosuppressive effect observed. Moreover, the model of intrarenal drug delivery offers the possibility to further analyze local regulatory mechanisms in graft rejection and their response to local immunosuppressive therapy.

The Effect of Local Immunosuppressive Therapy on Cellular Infiltration. Animals, untreated or treated with prednisolone either intrarenally or intraperitoneally, were sacrificed at days 3, 7, and 12 after transplantation, and immunohistologic studies were performed.

Untreated animals showed perivascular and interstitial mononuclear cell infiltrates at day 3 after transplantation (Table II). Most of the infiltrating cells were positive for His19, recognizing MHC class II antigens of host type. By day 7 after transplantation, cellular infiltration was clearly increased and intense infiltration of MHC class II-positive cells throughout the kidney was observed associated with marked destruction of renal tissue (Fig. 1a).

In animals treated with prednisolone intraperitoneally (MST, 9 d), cellular infiltration was somewhat postponed compared with untreated animals. Eventually, however, at day 7 after transplantation, dense infiltration of MHC class II-positive cells was observed accompanied by marked destruction of grafted tissue.

The histologic picture of animals treated intrarenally was completely different from untreated and intraperitoneally treated rats (Table II). Intrarenally treated grafts also showed distinct cellular infiltration with MHC class II-positive cells (Fig. 1b), however, extensive tissue damage and necrosis were not observed.

For further analysis, the composition of cellular infiltrates in untreated and treated animals was investigated (Table III). At day 7 after transplantation, untreated grafts showed cellular infiltrates composed of T cells and macrophages (Fig. 1, c and d). T cells were of T helper as well as T cytotoxic/suppressor phenotype; staining with the mAbs W3/25 and OX8 resulted respectively in a mean area of infiltration of 28 and 29%, which is a ratio of about 1. Similar levels and patterns of infiltration were found in intraperitoneally treated animals. Grafts treated intrarenally also showed cellular infiltrates composed of T cells

TABLE II
Immunohistological Observations in Rat Renal Allografts after Various Treatments

Therapy	Area infiltrated by MHC class II-positive host cells (His19 ⁺)*	IL-2-R on infiltrating cells (ART18 ⁺)	Presence of IFN- γ (DB1 ⁺)	MHC class II expression on tubules (OX6 ⁺)	Graft destruction, edema, hemorrhage, necrosis [‡]
BN \rightarrow BN Untreated Day 7 [§]	6 \pm 1	-	-	-	-
BN \rightarrow Lewis Untreated Day 3	15 \pm 4	+/-	+/-	+	-
Day 7	30 \pm 7	+	+	+++	+++
BN \rightarrow Lewis 4 mg/kg i.p. Day 3	6 \pm 1	-	-	-	-
Day 7	33 \pm 7	+	+	++	++/+++
BN \rightarrow Lewis 4 mg/kg intrarenally Day 3	5 \pm 1	-	-	-	-
Day 7	27 \pm 6	+/-	+/-	+	-
Day 12	20 \pm 5	-	-	-	-

Data represent consistent observations made in all rats of the experimental group ($n = 4$ or 5).

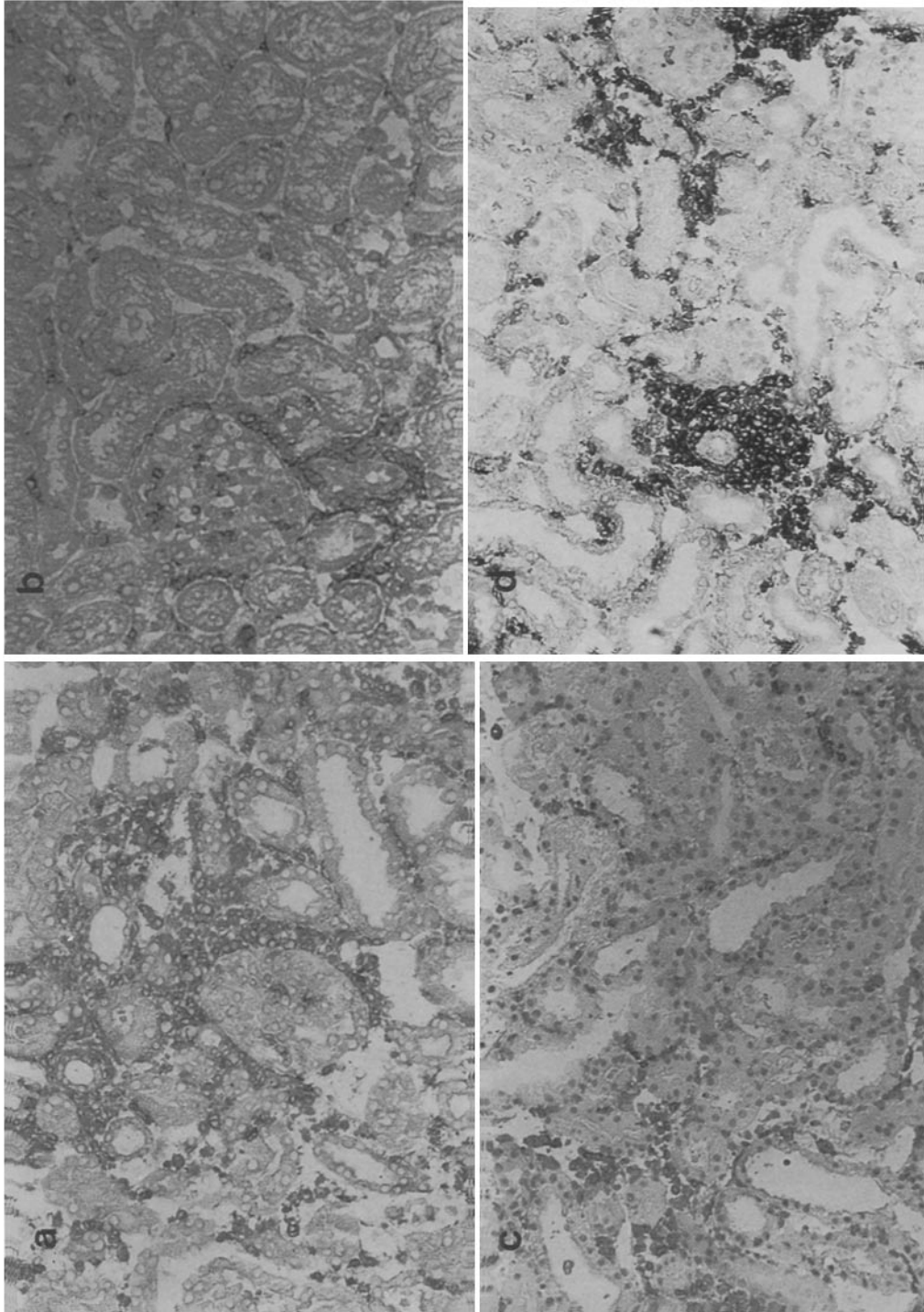
* In the BN \rightarrow Lewis strain combination, MHC class II antigens on host cells were identified with the mAb His19, which reacts with the MHC class II antigens of rat strains of the haplotype L, but not with MHC class II antigens of the rat strains of the haplotype N. In the BN \rightarrow BN strain combination, MHC class II antigens on infiltrating cells were identified with OX6, which recognizes rat MHC class II antigens of the rat strains of haplotype L and N. Results are expressed as the percentage of area of the tissue section infiltrated by MHC class II-positive cells. Data given are mean \pm SD.

[‡] Examined on routine H and E stainings.

[§] Day after grafting.

and macrophages, however, with a lower percentage of macrophages as compared with untreated animals (Table III, Fig. 1, *e* and *f*). Staining with mAbs W3/25 and OX8 resulted in a mean area of infiltration of 23 and 26%, which is comparable to untreated animals and also results in a ratio of ~ 1 . Thus except for less infiltration with macrophages, cellular infiltration in intrarenally treated animals was comparable to untreated or intraperitoneally treated rats.

Effect of Local Immunosuppressive Therapy on IL-2-R Expression, IFN- γ Production and MHC Class II Antigen Induction on Renal Tissue. To further analyze the positive effects of local immunosuppression on renal allograft rejection, grafts were stained for IL-2-R expression, the presence of IFN- γ , and the expression of MHC class II antigens on renal tissue (Table II). In untreated animals weak staining with ART18 and DB1 could be seen on cells in areas with cellular infiltration by the third day after transplantation. Serial sections stained with OX19 indicated that the cells positive for DB1 were T lymphocytes, as were the vast majority of the cells positive for ART18 (data not shown). On day 7 after



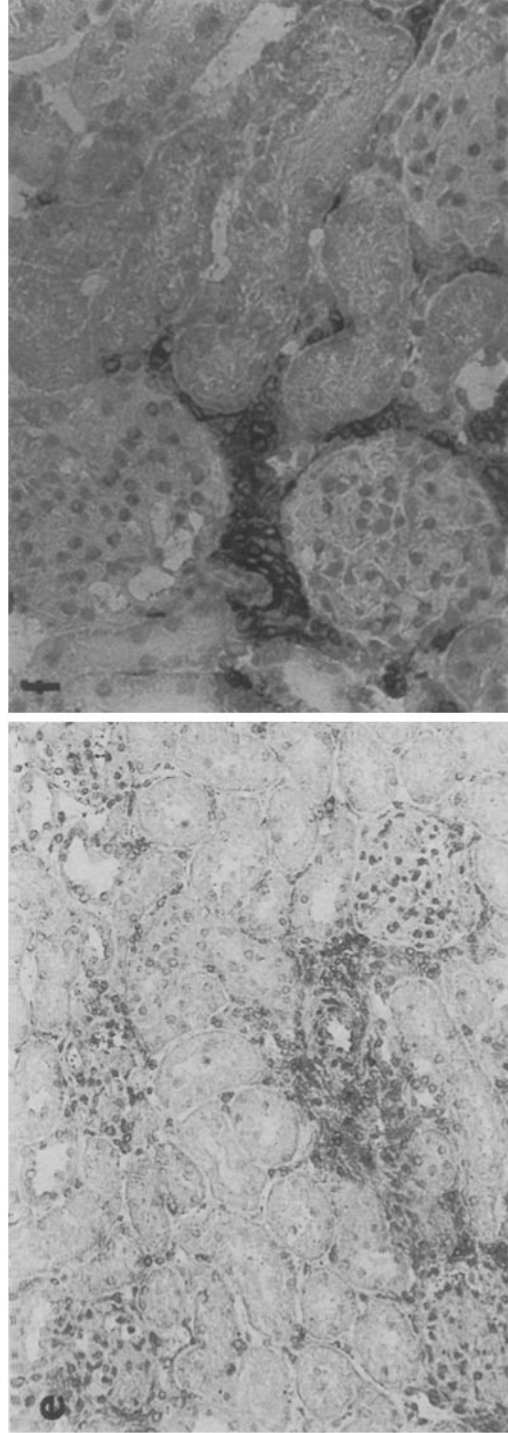


FIGURE 1. Immunohistologic studies for characterization of infiltrating cells in renal allografts from untreated recipients and from recipients treated with intrarenal prednisolone. (a) Untreated graft at day 7. Staining with His19 shows infiltrating cells positive for MHC class II antigens of host type. $\times 170$. (b) Intrarenally treated graft at day 7 stained with His19. Note distinct staining of infiltrating cells positive for MHC class II antigens of host type. $\times 190$. (c) Untreated graft at day 7 stained with OX19-recognizing T cells. $\times 150$. (d) Untreated graft at day 7, staining with ED2 shows the presence of infiltrating macrophages. $\times 140$. (e) Intrarenal treated graft at day 7, staining with OX19 identifies infiltrating T cells within the graft. $\times 150$. (f) Intrarenal treated graft at day 7 stained with ED2. Infiltrating macrophages are observed within the graft. $\times 240$.

TABLE III
*Phenotypic Composition of the Cellular Infiltrate in Rat Renal Allografts
 after Various Treatments*

Therapy	Percentage of area infiltrated by positive cells*				
	OX19 T cells	W3/25 Th	OX8 (Tc, Ts)	W3/25 + OX8 [†] (Th, Tc, Ts)	ED2 (mac- rophages)
BN → Lewis untreated	24 ± 6	28 ± 5	29 ± 7	37 ± 6	38 ± 11
BN → Lewis 4 mg/kg i.p.	21 ± 5	25 ± 6	27 ± 7	37 ± 9	31 ± 8
BN → Lewis 4 mg/kg intrarenally	20 ± 4	23 ± 4	26 ± 6	32 ± 8	18 ± 6

Immunohistological studies were performed on grafts removed on day 7 after transplantation.

* Data given are mean values ± SD; *n* = 5.

† Stained simultaneously with W3/25 and OX8.

transplantation the amount of positive cells was significantly increased (Fig. 2, *a* and *b*). At that time, positive cells were scattered throughout the kidney and were present as isolated cells in the interstitium as well as in cellular infiltrates. The time course of the induction of MHC class II antigens on renal tissue was in accordance with the stainings for IFN- γ . On day 3, several foci of MHC class II expression on renal tubules were demonstrated. By the seventh day after transplantation, MHC class II expression was markedly increased, and distinct staining of MHC class II antigens on renal tissue was observed (Fig. 2*c*).

Grafts from animals treated intraperitoneally with prednisolone showed immunohistological stainings on day 7 comparable to untreated rats.

Completely different results however were obtained in animals treated intrarenally (Table II). Grafts from these rats showed almost no staining for IL-2-R expression and IFN- γ , despite the presence of cellular infiltrates (Fig. 3, *a-c*). Except for some weak staining on day 7, all stainings with ART18 and DB1 were negative on day 3 as well as on day 12 after transplantation. These findings correspond well to the observations made in stainings with OX6. On day 3 and day 12 after transplantation all kidneys examined failed to show an increase in MHC class II antigen expression on renal tissue as compared with stainings of normal nongrafted kidneys (Fig. 3*d*). Only on day 7 after transplantation were several small foci of MHC class II antigen expression on renal tissue observed. However, compared with intraperitoneally treated or untreated grafts this staining was very weak. From the observations, as summarized in Table II, we conclude that intrarenal prednisolone levels interfere with the expression of IL-2-R, the production of IFN- γ , and the induction of MHC class II antigen expression on renal tissue.

Discussion

In this study we demonstrate that intrarenal prednisolone delivery to rat renal allografts results in high drug levels within the graft combined with low systemic drug levels. Systemic drug levels were, by themselves, not immunosuppressive, and local prednisolone levels within the graft proved to be responsible for the immunosuppressive effect obtained. This experimental model was used to ana-

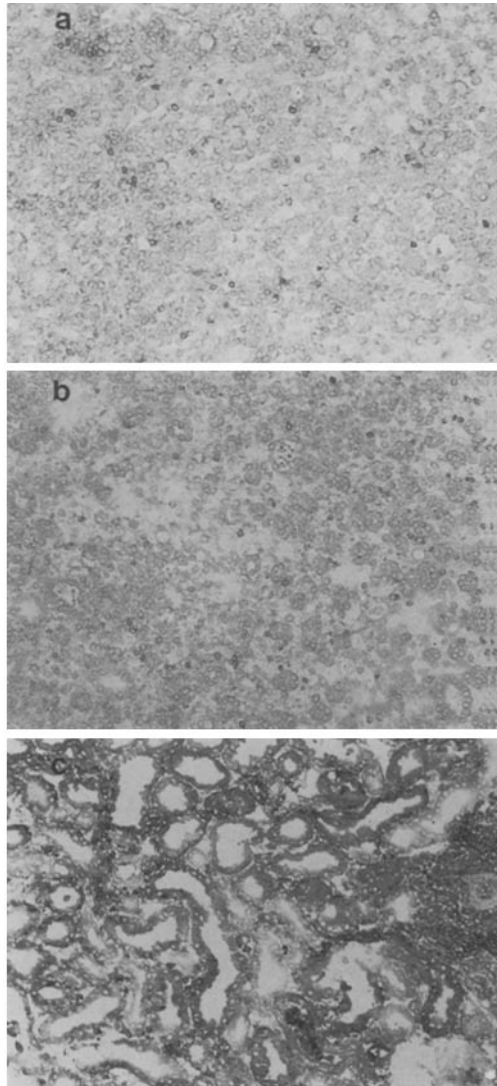


FIGURE 2. Cryostat sections of untreated grafts removed on day 7 after transplantation. Sections were stained with mAbs (a) ART18, (b) DB1, and (c) OX6. Infiltrating cells positive for IL-2-R (a) and IFN- γ (b) are scattered throughout the graft. $\times 100$. Staining with OX6 (c) shows strong induction of MHC class II antigens on renal tubule cells associated with marked destruction of renal tissue. $\times 100$.

lyze local events in allograft rejection and their response to immunosuppressive therapy.

The role of local versus systemic immune processes in cellular immune response has been studied in allograft models (30, 32, 46). These studies report extensive white cell traffic between the lymphoid tissue and the graft in the early days after transplantation. After a time, however, the rejection process becomes independent of the host, and lymphocytes mature and proliferate within the graft, which implies an essential role for local regulatory mechanisms and hence local production of lymphokines.

According to these data, we observed that local treatment of allografts does not prevent the accumulation of activated white cells within the graft, as occurs

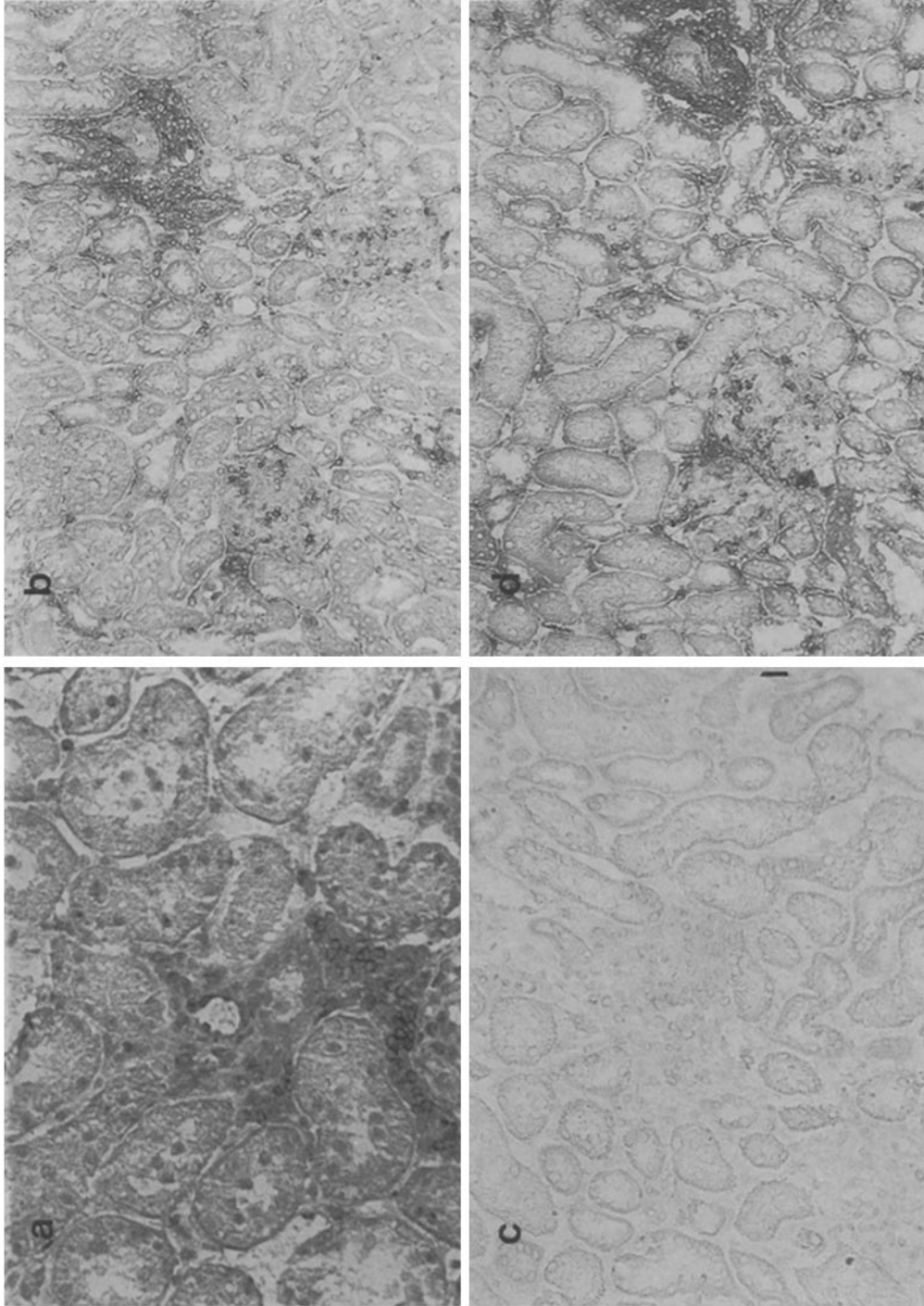


FIGURE 3. Cryostat sections of intrarenally treated grafts removed on day 12 after transplantation. Sections were stained with mAbs (a) OX19, (b) His19, (c) DB1, and (d) OX6. (a) Staining with OX19 demonstrates the presence of infiltrating T lymphocytes within the graft. $\times 215$. (b) Staining with His19 shows infiltrating cells positive for MHC class II antigens of host type. $\times 170$. (c) Staining with DB1, recognizing IFN- γ , is completely negative. $\times 160$. (d) Staining with OX6 shows that only infiltrating cells are positive for MHC class II antigens, while grafted tissue stains negative. $\times 140$.

in the early days after transplantation. White cells positive for MHC class II antigens of host type were equally present in untreated and intrarenally treated rats. Moreover, the composition of the cellular infiltrate in intrarenally treated grafts was comparable to the untreated grafts, except for a lower percentage of infiltrating macrophages. The ratio of W3/25⁺:OX8⁺ cells, which is variably reported to be changed during successful systemic immunosuppressive therapy (4, 45, 47, 48), was not affected by local treatment. These data show that during local treatment the systemic responsiveness of the host is normal. Moreover, the findings confirm that the model of local prednisolone delivery allows the analysis of intragraft events and their response to local immunosuppressive therapy.

Local treatment was associated with lack of IL-2-R expression, absence of IFN- γ , and inhibition of MHC class II induction on renal tissue. Inhibition of these intragraft events most likely prevented T cells and macrophages present within the graft from destroying the grafted tissue.

Downregulation of MHC class II expression on grafted tissue is postulated to be an essential mechanism during successful systemic immunosuppressive therapy (49). In this study the induction of MHC class II expression on renal tissue was inhibited by local treatment, which strongly suggests local regulation of MHC class II expression within the graft. Local regulation of MHC class II expression in cellular immune response was also postulated by others (50, 51). For example, Nathan et al. (51) showed that intradermal injection of IFN- γ induced local effects similar to DTH-like hypersensitivity, including MHC class II induction. The physiologic role of the induction of MHC class II antigens on grafted tissue is most likely the enhancement of antigen presentation to T helper lymphocytes (15, 16). Inhibition of MHC class II induction may therefore interfere with the effector mechanisms in graft rejection such as DTH and cell-specific cytotoxicity.

The induction of MHC class II antigens on nonlymphoid tissue is assumed to result from IFN- γ (9–11), although recently also non-IFN- γ MHC class II-inducing factors have been described (52, 53). Steroids were reported to inhibit the production of IFN- γ (18), which explains the absence of this lymphokine in the presence of high intrarenal drug levels reached in intrarenally treated grafts. By the inhibition of IFN- γ production, prednisolone may indirectly affect the induction of MHC class II antigens on grafted tissue.

It has to be noticed that the absence of IFN- γ within the graft may also contribute to prolongation of graft survival in an other way apart from the postulated effect on MHC class II expression. IFN- γ has been described as an activating agent for macrophages (54), and the absence of the lymphokine in certain immune reactions has been associated with reduced macrophage infiltration and function (51). We observed a lower percentage of macrophages within the intrarenally treated grafts; concerning their function however, no direct data were obtained. It has been postulated that steroids interfere with macrophage function, e.g., inhibit the production of IL-1 and tumor necrosis factor (55, 56). To what extent these mechanisms play an essential role during local immunosuppressive treatment has to be examined further.

We observed that high local drug levels of prednisolone did not affect the accumulation of activated (MHC class II-positive) white cells within the graft, while the expression of IL-2-R on these cells was clearly inhibited. This obser-

vation is in concordance to Gillis (17), who demonstrated *in vitro* that steroids did not prevent cell activation but interfered with IL-2 production. Because IL-2-R expression is strongly induced by IL-2 (57), high local drug levels within the graft may interfere with IL-2-R expression by the inhibition of IL-2 production. The inhibition of IL-2-R expression may be an important mechanism to inhibit cellular immune response, as is suggested by immunosuppressive therapy with mAbs against the IL-2-R (58, 59).

In conclusion, intrarenal drug levels most likely did not affect the systemic immune responsiveness against the graft, since cellular infiltration in intrarenally treated grafts was hardly affected. Intrarenal drug levels, however, inhibited intragraft events such as IL-2-R expression, IFN- γ production, and MHC class II induction on grafted tissue, and in this way prevented rejection. These data strongly support local independence of the cellular immune response within the graft after a certain time, as suggested by others. Second, the data indicate that local treatment of allografts can result in effective immunosuppression. Local manipulation of the immune response by steroids has proven successful in skin disease and inflammatory lung disease. Although the use of steroids in transplantation is also based on their antiinflammatory effects, which may be of local as well as systemic origin (60), local delivery of the drugs to vascularized organ allografts has hardly been studied, since in man local steroid infusion into the renal artery of kidney transplants was seriously limited by infections and thrombosis (61, 62). Recently, however, new types of oral drugs have been developed that allow selective treatment of target organs, e.g., liver or kidney, either by specific tissue binding of the drug or tissue-specific activation (63, 64). The usefulness of immunosuppressive drugs that fulfill these pharmacologic properties has to be further examined.

Summary

In this report we investigated local regulatory mechanisms in graft rejection and their response to local immunosuppressive therapy. For this purpose local immunosuppression was induced in rat kidney allografts by intrarenal infusion of prednisolone. Intrarenal drug delivery resulted in high drug levels within the graft and low systemic drug levels. Systemic drug levels were by themselves not sufficiently immunosuppressive to induce graft survival, and local prednisolone levels within the graft proved to be responsible for prolongation of graft survival. During intrarenal drug delivery, systemic responsiveness to the renal allograft proved normal, since intrarenally treated grafts were infiltrated by MHC class II-positive host cells and, except for a somewhat lower percentage of macrophages, cellular infiltration in intrarenal treated grafts was comparable to untreated grafts. However, T cells and macrophages present in intrarenally treated grafts were not able to destroy the grafted tissue. Local immunosuppressive therapy resulted in inhibition of IL-2-R expression, absence of IFN- γ , and prevention of MHC class II induction on grafted tissue. These observations strongly indicate the presence of local regulatory mechanisms in graft rejection. The experimental model described can be used for further analysis of these intragraft events. Moreover, the results demonstrate that local immunosuppres-

sive therapy can contribute to effective inhibition of cellular immune response in graft rejection.

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