Changes in Cytokine mRNA Levels in Experimental Corneal Allografts after Local Clodronate–Liposome Treatment

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PURPOSE. Corneal allograft rejection in rats can be prevented by subconjunctival injections of liposomes containing dichloromethylene diphosphonate (clodronate–LIP), which selectively eliminate macrophages. In this study, the effect of clodronate–LIP treatment on cytokine mRNA levels in corneal allografts was examined.

METHODS. AO rats received corneal grafts of PVG rats. Rats were either not treated or injected subconjunctivally with clodronate–LIP on the day of transplantation and on postoperative days (PODs) 2, 4, 6, and 8. RNA was isolated from the graft and rim of corneas at different times after transplantation and from normal controls. Interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-6, IL-10, IL-12p40, tumor necrosis factor (TNF)-α, TNF-β/lymphotoxin (LT), interferon (IFN)-γ, monocyte chemotactic protein 1 (MCP-1), and macrophage inflammatory protein 2 (MIP-2) mRNA levels were analyzed by semiquantitative reverse transcription–polymerase chain reaction (RT–PCR).

RESULTS. Corneal rejection, observed in all untreated rats by POD 12, was associated with increased mRNA levels of all cytokines investigated in grafts and rims. Clodronate–LIP treatment prevented allograft rejection and strongly decreased the levels of IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-β/LT, MCP-1, and MIP-2 mRNA in grafts and IL-1β, IL-2, IL-4, IL-6, and IFN-γ mRNA in rims. Interleukin-12p40 mRNA levels were unaltered in clodronate-treated rats, except for a transient increase in grafts at POD 3. TNF-α mRNA levels were increased by clodronate–LIP in grafts and rims early after transplantation (PODs 3 and 7). Despite a normal appearance, long-term accepted corneal grafts (POD 100) contained mRNA for IL-10, IL-12p40, TNF-α, MCP-1, and MIP-2.

CONCLUSIONS. Clodronate-liposome treatment markedly altered the mRNA levels of all cytokines investigated in corneal allografts. These results may explain in part the mechanism by which clodronate–LIP treatment prevents corneal allograft rejection. (Invest Ophthalmol Vis Sci. 1999;40: 3194–3201)

Despite advances in surgical techniques and immuno-suppressive drug treatment, allograft rejection still occurs in 10% to 30% of the cases of penetrating keratoplasty and is the major cause of corneal graft failure. Corneal allograft rejection is characterized by infiltration of mainly T cells and macrophages into the donor tissue. Depletion of CD4+ T cells by systemic administration of specific monoclonal antibodies markedly reduced corneal graft rejection. Topical application of such anti-CD4 antibodies also reduced allograft rejection. Using a technique to eliminate macrophages, we previously showed that these cells are also crucial for the rejection process. Macrophages can be eliminated in a selective manner, due to their phagocytic activity, by liposomes containing the drug dichloromethylene diphosphonate (clodronate–LIP). After uptake by macrophages, the phospholipid bilayers of the liposomes are degraded by lysosomal phospholipases and the released drug causes cell death. In our study, five consecutive injections of clodronate–LIP into the subconjunctiva of rats early after transplantation produced 100% survival of corneal allografts for up to 100 days. This treatment was characterized by reduced cellular infiltration and reduced neovascularization of the grafted cornea. Recently, we found that clodronate–LIP prevents the induction of cytotoxic T cells and cytolytic antibodies directed against the donor tissue, which indicates that clodronate affects antigen presentation.

Although the molecular mechanisms underlying corneal graft rejection are still not completely understood, cytokines released at the graft site are thought to be involved in the
expression of IL-1 results imply that cytokines contribute to corneal graft rejection. Expression of known T cell–derived cytokines, including IL-2, IFN-γ, IL-4, IL-5, IL-6, IL-10, TNF-α, and macrophage inflammatory protein 2 (MIP-2), which were detected immediately after transplantation and during rejection.12 The expression of known T cell–derived cytokines, including IL-2, IL-4, and interferon-γ (IFN-γ), was detected after rejection occurred, underlining the central role of T cells in this process. Recently, others have also demonstrated expression of IL-2 and IFN-γ during corneal allograft rejection in mice.13,14 These results imply that cytokines contribute to corneal graft rejection. Expression of IL-1β, IL-1RA, IL-6, IL-10, MCP-1, and MIP-2 was also detected after transplantation of corneal autografts that were not rejected,12 indicating that some of these factors are already triggered by traumatic events alone.

In the present study we have expanded our analysis of cytokine expression in corneal allografts. We examined cytokine mRNA expression patterns in rejected corneal allografts and in accepted allografts of rats treated subconjectivally with clodronate–LIP using semiquantitative reverse transcription–polymerase chain reaction (RT–PCR). Our results show that clodronate–LIP treatment markedly decreased the mRNA expression of most of the cytokines investigated in the cornea after allograft transplantation.

METHODS

Animals

Inbred male AO and PVG rats (Harlan Sprague–Dawley, Bicester, England), 10 to 12 weeks of age at the time of transplantation, were used in this study. These strains differ in their major histocompatibility complex (MHC) antigens.15 Rats were treated according to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research and the guidelines of the Animal Care and Ethics Committee of the Royal Netherlands Academy of Sciences. Rats were housed under standard conditions and were given food and water ad libitum.

Corneal Surgery and Clinical Evaluation

Orthotopic corneal transplantations were performed on one eye of AO rats as described earlier.1,4,12,16 Briefly, before corneal surgery, maximal mydriasis was induced by subcutaneous (SC) injection of atropine sulfate and topical atropine and phenylephrine drops, to prevent trauma to the iris and anterior synechiae formation. A 3.0-mm diameter trephine was used to mark both recipient and donor corneas and curved fine scissors were used to remove the buttons. Donor corneas were kept in corneal preservation medium (Eagle’s modified essential medium with 2% fetal calf serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin) until use. The donor button was sewn into the recipient cornea using eight stitches of a continuous 10–0 monofilament nylon suture. Sutures were left in place for the duration of the experiments (up to POD 100).

Transplanted rats were examined using an operating microscope every other day until POD 17 and weekly until POD 100. Opacity and neovascularization of transplanted corneas was graded using a scoring system described previously.1 Maximal opacity was graded 4, and maximal neovascularization was graded 16 (score 4 for every quadrant of the cornea). Corneal rejection was defined as a graft opacity score of 3 or higher in a previously clear graft.

Liposome Preparation and Subconjunctival Injections

Multilamellar liposomes, composed of phospholipid bilayers, containing dichloromethylene diphosphonate (clodronate; kindly provided by Boehringer Mannheim, Mannheim, Germany) were prepared as described elsewhere.17 Clodronate–LIP was suspended in phosphate-buffered saline and stored at 4°C until use. A total volume of 100 μl of clodronate–LIP was injected into the subconjunctiva near the limbal area using a 50-μl Hamilton syringe with a 30-gauge needle. Four injections of 25 μl each were given, in four different quadrants, to produce a circular bleb.

Experimental Design

Rats with technical transplantation or liposome injection failures (5 in total) were excluded from the study. Rats with successful corneal grafts (n = 42) were divided into two groups: control rats (n = 20) that received no treatment. Of this group, five rats each were killed on PODs 3, 7, 12, or 17 by intravenous injection of a lethal dose of pentobarbital. Clodronate–LIP–treated rats (n = 22) that received subconjunctival injections of liposomes at the time of corneal transplantation and on PODs 2, 4, 6, and 8. Five rats each were killed on PODs 3, 7, 12, or 17 and two rats on POD 100. In addition, 4 AO rats that did not undergo surgery were used as normal controls.

After termination, rats were perfused through the left ventricle of the heart with 500 ml sterile pyrogen-free saline to remove cells from blood vessels. Subsequently, the graft (i.e., the central corneal button) and the rim (i.e., the adjacent peripheral ring) of the recipient cornea were removed, immediately frozen in liquid nitrogen, and stored at −70°C until RNA isolation.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from all samples by a single-step extraction method.18 Corneal tissues were homogenized by vigorously vortexing in RNAzol (Cinna Biotex Laboratories, Houston, TX), and the extracted RNA was dissolved in 10 μl sterile water. For cDNA synthesis, 5 μl of total RNA was incubated with 2.5 μg oligo(dT)12–18 primer, 10 mM dNTPs (Pharmacia, Woerden, the Netherlands), and 200 U Superscript RNaseH reverse transcriptase (GIBCO-BRL, Eggenstein, Germany) according to the manufacturer’s instructions. After incubation for 1 hour at 57°C, the reaction was terminated at 65°C for 5 minutes. cDNA was stored at −70°C until use.

PCR Primers and Internal Control Probes

The sense and anti-sense PCR primers and internal control probes for β-actin, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, MCP-1, and MIP-2 have been described.12,19 Primers and probes for the inducible p40 subunit of IL-12 (IL-12p40) and TNF-β1/lymphotoxin (LT) were designed using sequences obtained from the GenBank database and are shown in Table 1.

PCR and Southern Blot Analysis

Semiqualitative PCR amplification and analysis of PCR products were performed as previously described.12,19 For PCR
amplification, cDNA was added to a reaction mixture consisting of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 0.2 mM dNTP, 0.2 μM sense and anti-sense oligonucleotide primers, and 1 U Taq DNA polymerase (Promega, Madison, WI) in a volume of 50 μl. The number of PCR cycles and annealing conditions for the primers have been described elsewhere, except for IL-12p40 (38 cycles, annealing at 62°C) and TNF-β/LT (35 cycles, annealing at 63°C). Before cytokine PCR analysis, the cDNA concentration of all samples was normalized to yield equivalent amounts of β-actin PCR product. Differences in the yield of β-actin PCR product were determined using an ImageMaster (Pharmacia Biotech, Uppsala, Sweden). A total of 28 samples (consisting of 1 normal button, 6 grafts of untreated and 6 grafts of clodronate–LIP–treated rats, as well as 1 normal rim, 7 rims of untreated, and 7 rims of clodronate–LIP–treated rats), with a low β-actin cDNA concentration were not used for further cytokine analysis. Subsequently, each cytokine PCR included all normalized samples of each group, as well as a titration (1/20, 1/100, 1/500) of positive control cDNA from lipopolysaccharide-, phorbol myristate acetate–, or Con A/IL-2–stimulated rat spleen cells, and a negative control, which consisted of all reagents, but without template DNA.

PCR products were analyzed by 2% agarose gel electrophoresis and ethidium bromide staining. For verification of their identity, PCR products were transferred to Genescreen-plus membranes (NEN–Du Pont, ‘s Hertogenbosch, the Netherlands), and the filters were hybridized with specific oligonucleotide probes. Internal control probes were labeled at the 5’ end with [γ-32P]ATP, using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Hybridization was carried out in 6× SSC, 0.1% sodium dodecyl sulfate (SDS), 5× Denhardt’s and 100 μg/ml sheared denatured herring sperm DNA, and 20 pmol of labeled probe overnight at 65°C. Hybridized membranes were washed with 2× SSC, 0.1% SDS at 65°C, and exposed to x-ray film.

**RESULTS**

**Clinical Features of Corneal Grafting**

Corneal transplantation caused neovascularization and changes in opacity with similar kinetics as previously described. Mild opacity (grade 1–2) was observed in some grafts of both groups on POD 2, probably due to an acute inflammatory reaction by surgical trauma. In the untreated group, opacity decreased after POD 2, and corneas remained clear until the time of rejection. In the clodronate–LIP–treated group, mild opacity (grade 1–2) was observed during the period of subconjunctival injections, but corneas regained transparency after POD 8. Corneal rejection was observed in all untreated rats within 12 days (Fig. 1). A mean graft survival time of 10.9 days was calculated using the Kaplan–Meier test. Maximal opacity (grades 3–4) was observed at the onset of rejection (Fig. 2). In contrast, graft rejection was not detected in the clodronate–LIP-treated group up to POD 100. In untreated rats, neovascularization progressed in time and reached grade 10 at POD 17. In most clodronate–LIP-treated rats, ingrowth of a few vessels in the recipient cornea up to the sutures was observed.

**Cytokine Expression in Corneas of Normal and Grafted Rats**

Cytokine mRNA levels in corneal tissues of normal and transplanted rats were analyzed using RT–PCR. This method is able to demonstrate differences in mRNA levels in a semiquantitative manner. All samples were normalized for β-actin mRNA content. Results of Southern blot analysis with cytokines expressed in corneal grafts and rims are shown in Figures 3 and 4, respectively. The cytokine expression pattern in normal and untreated rats was very similar to the results of our previous study.
constitutively expressed IL-1RA, and some animals (1/3) showed a strong signal for MIP-2, whereas all other cytokines investigated were undetectable.

IL-1β mRNA Expression

Albeit weakly, IL-1β was detected from PODs 3 to 17 in 9 of 14 grafts and from PODs 3 to 12 in 6 of 10 rims of untreated rats. IL-1β was not detected in grafts of liposome-treated rats but was found in rims of these animals at POD 3 (in 2 of 3 samples) and in POD 3 (in 2 of 3 samples). Interleukin-1β mRNA was not detected in long-term grafts (POD 100).

IL-6 mRNA Expression

Interleukin-6 mRNA was detected from PODs 3 to 17 after transplantation in all 14 grafts and 13 rims of untreated rats. Interleukin-6 mRNA levels in grafts and corneal rims were steady over time. The induction of IL-6 expression was strongly reduced in clodronate-treated rats from PODs 7 to 17; IL-6 was found only in grafts at POD 3 (in 2 of 4 samples) and POD 7 (in 1 of 3 samples) and in rims at POD 3 (in 2 of 3 samples). Interleukin-6 mRNA was not detected in long-term grafts (POD 100).

IL-10 mRNA Expression

Similar to IL-6, IL-10 mRNA was detected from PODs 3 to 17 after transplantation in all 14 grafts and in 12 of 13 rims of untreated rats. The highest level of IL-10 mRNA was detected on POD 12. Clodronate–LIP treatment decreased IL-10 mRNA expression in grafts, but IL-10 was detected from PODs 3 to 100 in 10 of 16 samples. Clodronate–LIP did not affect IL-10 mRNA expression in peripheral rims; it was detected from PODs 3 to 17 in 10 of 13 samples as well as at POD 100 (in 2 of 2 samples).

IL-12p40 mRNA Expression

Like IL-6 and IL-10, IL-12p40 mRNA was present from PODs 3 to 17 in 13 of 14 grafts and in 11 of 13 corneal rims of untreated rats. Clodronate–LIP treatment caused a transient increase in IL-12p40 expression in grafts at POD 5 but did not affect mRNA levels at later time points (PODs 7 to 17). Interleukin-12p40 mRNA was detected from PODs 3 to 17 in 12 of 14 grafts, as well as in the two long-term grafts (POD 100). Interleukin-12p40 mRNA was detected in all 15 rims of clodronate-treated at similar levels compared with untreated rats.

IFN-γ mRNA Expression

In grafts of untreated rats, a minor expression of IFN-γ mRNA was detected at POD 3 in 1 of 4 samples. A strong IFN-γ signal was detected from PODs 7 to 17 in 9 of 10 grafts and in 8 of 10 corneal rims. Clodronate–LIP treatment markedly decreased IFN-γ expression in grafts and rims; mRNA was detected in none of the grafts and only at POD 12 in 1 of 3 rims tested.

TNF-α mRNA Expression

In untreated rats, TNF-α mRNA was detected from PODs 7 to 17 in 7 of 10 grafts and from PODs 3 to 17 in 10 of 13 rims. The highest mRNA levels were found on POD 17. Clodronate–LIP induced an increase in TNF-α mRNA levels in grafts and rims early (PODs 3 and 7) after allograft transplantation. At POD 100, all animals showed a strong signal for TNF-α mRNA.

TNF-β/LT mRNA Expression

TNF-β/LT mRNA was detected at PODs 12 and 17 in 6 of 7 grafts and at slightly lower levels in all 6 rims of untreated rats.
Similar to IL-2, IL-4, and IFN-γ, liposome treatment completely blocked TNF-β/LT mRNA expression in grafts. In rims of clodronate–LIP–treated rats, however, TNF-β/LT mRNA was readily detected from PODs 7 to 17 in 3 of 10 samples. At POD 100, a faint signal for TNF-β/LT was found in 1 of 2 rims of clodronate-treated rats.

**MCP-1 mRNA Expression**

Like IL-6, MCP-1 mRNA was detected at similar levels from PODs 3 to 17 in all 14 grafts and 13 rims of untreated rats. Expression of MCP-1 was decreased in grafts from PODs 7 to 17 but not in rims of clodronate–LIP–treated rats. In these rats, MCP-1 was found from PODs 3 to 17 in 12 of 14 grafts and in all 13 rims, as well as on POD 100 in all samples.

**MIP-2 mRNA Expression**

In agreement with our previous studies, a variable expression of MIP-2 mRNA was found in normal corneas. After transplantation, MIP-2 mRNA was detected from PODs 3 to 17 in all 14 grafts and 13 rims of untreated rats. Clodronate–LIP completely blocked the induced MIP-2 mRNA expression in 8 of 14 grafts from PODs 3 to 17, but did not alter the expression in the grafts of the other 6 rats. MIP-2 mRNA was detected from PODs 3 to 17 in all 13 rims of liposome-treated rats, at levels slightly above those of untreated rats. A strong signal for MIP-2 was also found in the two long-term accepted grafts (at POD 100).

**DISCUSSION**

The results presented here demonstrate that prevention of corneal allograft rejection by subconjunctival clodronate–LIP treatment is associated with a marked alteration of the cytokine mRNA expression profile in the allograft and surrounding recipient cornea. mRNA levels of all cytokines investigated were increased during allograft rejection compared with levels in normal controls. Clodronate–LIP treatment caused a strong decrease of IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-β/LT, MCP-1, and MIP-2 mRNA levels in grafts and IL-1β, IL-2, IL-4, IL-6, and IFN-γ mRNA levels in corneal rims. In contrast, TNF-α mRNA levels in grafts and rims were increased by clodronate–LIP early after transplantation. Clodronate–LIP did not alter IL-12p40 mRNA levels, except for a transient increase in grafts at POD 3. Despite a normal appearance, on clinical grounds, long-term corneal grafts still showed detectable mRNA for IL-10, IL-12p40, TNF-α, and MCP-1, which were not detected in any of the normal control corneas. MIP-2 mRNA, which was occasionally present in normal corneas, also showed a strong signal in long-term accepted grafts. The prolonged cytokine expression in these corneas may have been triggered by the graft, by the sutures that were still present, or by a long-lasting effect of the clodronate injections. Experiments evaluating cytokine expression in normal rats treated subconjunctivally with clodronate and longitudinal studies in rats receiving an autograft could resolve this issue.
The pattern of cytokine expression in corneas of untreated rats, which rejected the allograft by POD 12, was largely consistent with the results of our previous study, except that expression of IL-2, IL-4, and IFN-\(\gamma\) was detected at an earlier time in the present study. This suggests that the rejection process was slightly accelerated in the present study. The reason for the discrepancy between these results is unknown but may be related to improved surgical procedures or to differences between batches of rats. The observed expression of IL-2, IL-4, IFN-\(\gamma\), and TNF-\(\beta\)/LT during allograft rejection is compatible with the cytokine expression pattern of T helper 0 (Th0) cells, or of a combination of Th1 cells (which produce IL-2, IFN-\(\gamma\), and TNF-\(\beta\)/LT) and Th2 cells, which produce IL-4. This suggests that corneal graft rejection in this model is driven either by Th1 cells or by a mixture of Th1 and Th2 cells.

Our results are in agreement with a recent study of Sano et al., who demonstrated by enzyme-linked immunosorbent assay and immunohistochemistry that IL-1\(\alpha\), IL-2, TNF-\(\alpha\), and IFN-\(\gamma\) protein levels in the cornea were increased during graft rejection. The latter results further imply that increased cytokine mRNA expression in the cornea, as we have found, results in increased cytokine protein levels.

Van der Veen et al. showed that local administration of clodronate-LIP strongly reduced the number of macrophages and T cells in the graft and recipient cornea. The almost complete abrogation of IL-1\(\beta\), IL-2, IL-4, IFN-\(\gamma\), and TNF-\(\beta\)/LT mRNA expression in allografts by clodronate-LIP suggests that during rejection these cytokines are derived from macrophages and T cells. The presence of IFN-\(\gamma\) and TNF-\(\beta\)/LT mRNA in a number of rims, but not grafts, of clodronate-treated rats can be explained by differences in T-cell infiltration of these sites. Macrophages are able to produce a variety of cytokines and are likely a source of IL-1\(\beta\) and IL-6 in the cornea in this model. A marked reduction of IL-1\(\beta\) and IL-6 expression by clodronate-LIP-mediated depletion of macrophages has been found in several studies. The fact that mRNA levels of other macrophage-derived cytokines (TNF-\(\alpha\), IL-10, IL-12, MCP-1, and MIP-2) were not completely reduced may be related to higher mRNA levels for these cytokines or to a higher sensitivity of the PCR for these factors. Alternatively, these cytokines could be produced by clodronate-LIP-resistant macrophages, or by other (resident) cells in the cornea.

The reduction in the mRNA levels of these cytokines after clodronate treatment may result indirectly from the lack of IL-1\(\beta\) and IL-6.

Surprisingly, clodronate-LIP induced a transient increase in the levels of TNF-\(\alpha\) and IL-12p40 mRNA in grafts, but not of other macrophage-derived cytokines, early after transplantation. Moreover, TNF-\(\alpha\) and IL-12p40 mRNA levels in rims from PODs 3 to 100 were steady over time and not markedly altered by clodronate, which was injected in the adjacent conjunctiva. Together, these results suggest that TNF-\(\alpha\) and IL-12p40 were
not produced by macrophages. Activated dendritic cells are another source of TNF-α and IL-12. Dendritic cells (i.e., Langerhans cells), which are present in low numbers in the peripheral cornea normally and in the central cornea after transplantation, may be triggered to produce TNF-α and IL-12p40 after activation by sutures or by the massive destruction of macrophages in clodronate–LIP–treated rats. Because of their limited phagocytic activity, dendritic cells are presumably not eliminated by clodronate–liposomes. Currently, we are investigating the fate of local dendritic cells in this model.

Interleukin-1RA is constitutively produced in rat and human corneas. In the latter, IL-1RA is expressed mainly by epithelial cells. The presence of IL-1RA in the cornea implies the existence of an inherent control mechanism for IL-1–mediated responses. Recently, Dana et al. found that IL-1RA strongly reduced centripetal Langerhans cell migration in the cornea. Interleukin-1RA mRNA expression was not markedly decreased in untreated corneal allografts and autografts. However, IL-1RA mRNA expression in grafts, but not in the recipient corneas, of clodronate-treated rats was completely abolished immediately after transplantation. Interleukin-1RA mRNA levels returned to normal by POD 17. Although the epithelial cell layer on the graft of these rats appeared normal early after transplantation (Ref. 4. P. Torres, personal observation), this finding suggests that clodronate may affect the normal activity of epithelial cells on the graft.

Our study clearly reveals that prolonged expression of IL-10, IL-12p40, TNF-α, MCP-1, and MIP-2 in corneal grafts is not correlated with rejection. This is consistent with our previous study, in which we showed IL-10, MCP-1, and MIP-2 mRNA expression in nonrejected autografts. It has been hypothesized that IL-10 may contribute to graft survival by blocking cytokine production and downregulating MHC class II expression. Several studies showed that topical treatment with IL-10 reduced corneal inflammation after herpes simplex virus infection. However, local treatment with IL-10 during corneal transplantation did not prolong allograft survival. Although TNF-α, MCP-1, and MIP-2 are considered to be inflammatory mediators, and IL-12 a pivotal factor in the development of Th1 responses, their role in graft rejection remains largely unknown. A number of studies have indicated that IL-12 and TNF-α may have a beneficial role in graft acceptance. Interleukin-12 expression was found in long-term cardiac allografts after rapamycin treatment. Moreover, IL-12 antagonism, by antibodies or an IL-12p40 homodimer receptor antagonist, exacerbated cardiac allograft rejection. Interestingly, Voest et al. have reported that IL-12 is capable of blocking corneal neovascularization. In view of this activity, expression of IL-12 in the cornea after transplantation may serve to limit angiogenesis. With regard to TNF, it has been shown that systemic administration of this cytokine decreased skin allograft rejection. This tissuerenegative effect may result from a strong inhibition of the antigen presenting capacity of dendritic cells by TNF.

In summary, clodronate–LIP treatment markedly altered the cytokine mRNA expression profile in corneal allografts of rats. Whether the absence or the long-term presence of certain cytokines in the cornea is necessary to acquire definite graft acceptance remains to be clarified.

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