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Immunology

Molecular identification of porcine interleukin 10: Regulation of expression in a kidney allograft model

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ABSTRACT **Clones encoding porcine interleukin 10 (IL-10) were isolated from a cDNA library produced from phytohemagglutinin-activated pig peripheral blood mononuclear cells. The porcine IL-10 nucleotide sequence was found to be highly homologous to the rat, mouse, and human IL-10 counterparts and to one of the open reading frames from the Epstein–Barr virus. In addition, pig IL-10 caused inhibition of γ-interferon gene transcription as determined by a bioassay. To investigate the possible immunomodulatory role of IL-10, its expression during the induction of tolerance to kidney allografts by cyclosporin A in miniature swine was also investigated. Delayed expression and higher levels of IL-10 were observed in tolerant animals compared with animals rejecting their allografts. Since tolerance is achieved by a short course of cyclosporin A, we have also studied the *in vitro* effect of this drug on IL-10 gene transcription in blood mononuclear cells and have found that cyclosporin A inhibits IL-10 gene activation in T cells but does not interfere with IL-10 transcription in lipopolysaccharide-activated cells. These results suggest that the overexpression of IL-10, observed in cell populations infiltrating grafts from tolerant animals, may be a function of monocytes and/or B cells.**

Interleukin 10 (IL-10) was originally described in the mouse (1, 2) and then in man (3) and rat (4). It is secreted mostly by monocytes (5, 6), B cells (7), and by the T_{h2} subset of CD4 cells (1). Like many others growth factors, IL-10 was found to exert pleiotropic biological effects (8). From an immunologic standpoint, the molecule has been examined most extensively for its suppressive effects on the synthesis of T_{h1}-specific cytokines (5, 9) as well as on the production of other mediators and growth factors such as IL-1a, IL-1b, IL-6, IL-8, tumor necrosis factor α, granulocyte/macrophage-colony-stimulating factor, and granulocyte-colony-stimulating factor (6). It has been suggested that IL-10 could be associated with the induction of transplantation tolerance to allografts in rodents (10, 11). Synthesis of high levels of IL-10 by donor- and host-type cells has also been reported in human severe combined immunodeficiency (SCID) patients transplanted with HLA-mismatched fetal liver stem cells (12). However, correlation between IL-10 expression and tolerance remains to be established in a preclinical model of transplantation of primarily vascularized organs into normal recipients. Our laboratory has developed a herd of partially inbred miniature swine with intra-major histocompatibility complex (MHC) recombinations in which long-term tolerance to class I-disparate renal allografts can be induced reproducibly by a short course of cyclosporin A (CsA) (13). We report here the characterization of porcine IL-10 and possible correlations between IL-10 gene regulation and the induction and persistence of long-term tolerance.‡

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MATERIALS AND METHODS

Probes. A porcine IL-10 probe, called S10.180, was derived from RNA extracted from porcine blood mononuclear cells (PBMCs) by reverse transcription (RT)–PCR and primers corresponding to regions of maximal homology between mouse and human IL-10 sequences. A 180-bp fragment was generated by using the sense primer 5′-TTAAGGGTTACT-TGGGTT-3′ and the antisense primer 5′-TTNCWYTTRT-TYTCRCANG-3′, which corresponded to positions 251–268 and 421–440 of the human IL-10 sequence, respectively (3). This fragment showed 86% nucleotide sequence homology with that of human IL-10. The pig probe for γ-interferon (γ-IFN) was generously provided by F. Lefevre (Institut National de la Recherche Agronomique, Jouy-en-Josas, France) and corresponded to an *Eco*RI–*Hin*dIII fragment of 0.75 kb encompassing the entire coding region. The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe, which was used to control the amount of RNA loaded, corresponded to a *Pst* I–*Pst* I fragment of 0.8 kb isolated from the GAPDH cDNA.

Library Screening and Sequencing. A cDNA library was prepared in the λ ZAP II vector (Stratagene) by using poly(A)$^+$ RNA extracted from peripheral blood lymphocytes activated for 3 days with phytohemagglutinin (PHA). Pig IL-10 cDNA clones were detected by colony hybridization using the S10.180 probe. Sequence analyses were performed on double-stranded inserts from clone pSW10, which contained the longest insert, by the dideoxynucleotide chain-termination method (14) using the Sequenase 2.0 kit (United States Biochemical).

COS Cell Transfection. An *Eco*RI–*Rsa* I fragment from pSW10 that encompassed the IL-10 coding region was cloned between the *Eco*RI and *Eco*RV restriction sites of the expression vector pcDNA1/Amp (Invitrogen), resulting in the recombinant vector pSP161.5. One million COS M-6 cells (gift from B. Seed, Massachusetts General Hospital, Boston), plated in Dulbecco's modified Eagle's medium (GIBCO) containing 10% NuSerum (Collaborative Biomedical Products, Bedford, MA) were transfected with 10 μg of pSP161.5 DNA by the DEAE-dextran method (15). Forty-two hours later, transfected cells were labeled with [^{35}S]methionine (DuPont/NEN) at 0.125 mCi/ml (1 mCi = 37 MBq) for 4 hr. Supernatants were then analyzed by polyacrylamide gel electrophoresis SDS/15% PAGE. Duplicate samples were run under reducing (2-mercaptoethanol) and nonreducing conditions.

Northern Blot Analysis. Renal graft wedges (100–200 mg), obtained by open biopsies at various time points, were frozen

Abbreviations: CsA, cyclosporin A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; γ-IFN, γ-interferon; IL, interleukin; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; RT, reverse transcription.

‡The sequence reported in this paper has been deposited in the GenBank database (accession no. L20001).

in 3 ml of 4 M guanidinium isothiocyanate containing 2% (vol/vol) 2-mercaptoethanol. Tissue samples were then homogenized in a tissue homogenizer (Omni International, Waterbury, CT) and debris was eliminated by centrifugation at 3000 × *g* for 15 min. Total RNA was pelleted through a CsCl cushion (5.7 M CsCl/25 mM sodium citrate) (15). Northern blot analyses were carried out with RNA (20 μg per lane) electrophoresed in a 1.2% agarose denaturing gel (15). Gels were blotted onto Zeta-Probe membranes (Bio-Rad) in 10× standard saline citrate (SSC). Filters were hybridized overnight with ^{32}P-labeled probes (>10^9 cpm/μg) and were washed in SSC according to conditions defined in figure legends. Intensities of radioactive signals were determined by computing the volume of the spots with a computing densitometer (Molecular Dynamics). Expression indices were calculated by correcting the levels of signal observed in experimental samples according to the amount of RNA loaded as estimated by the relative intensity of the GAPDH signal. The expression index (EI) for a particular sample in a lane *l* was calculated as follows:

$$EI_l = \frac{(\text{value of sample})_l \times (\text{highest value of GAPDH})}{\text{value of GAPDH}_l}.$$

Animals and Surgery. Miniature swine were selected from our herd of partially inbred animals at 5–7 months of age. The donor/recipient combinations studied were SLA$^{g/g}$ kidney donors (MHC class I^{cc}, II^{dd}) into SLA$^{d/d}$ recipients (MHC class I^{dd}, class II^{dd}). Orthotopic kidney grafts were performed as described (16). Three experimental recipients (pigs 10349, 10511, and 10418) were treated with a short course of CsA (10 mg/kg per day for 12 days), starting on the day of transplantation, in order to induce specific tolerance across the class I barrier (13). A group of three control animals (pigs 10296, 10348, and 10625) was not submitted to this regimen and consequently all the control animals rejected the grafts within 8–11 days after transplantation. Serial open kidney biopsies, performed under general anesthesia, were taken on postoperative days 4, 8, and 11 for both groups and on days 18, 30, and 180 for the CsA-treated group. CsA at 1 μg/ml was used for *in vitro* experiments on the basis of tests demonstrating complete inhibition of γ-IFN gene transcription at this dose. In addition, this dose is equivalent to the blood levels of CsA observed during treatment (13). All procedures were performed in accordance with "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication 85-23, revised 1985) and were approved by the Massachusetts General Hospital Animal Research Committee.

RESULTS

Cloning and Sequencing of Porcine IL-10 cDNA. A phagemid library derived from PHA-activated pig splenocytes was screened with the porcine IL-10 probe S10.180 as described in *Materials and Methods.* Two clones, from 6 × 10^5 screened, were identified by strong hybridizing signals, and restriction mapping analysis confirmed that they contained an identical insert of 1.3 kb. The insert from clone pSW10 was sequenced in both directions by the dideoxynucleotide chain-termination method (14), initially by using M13 primers and then by using primers derived from previous sequencing. The full-length cDNA sequence had a size of 1336 bp and contained an open reading frame corresponding to a protein of 175 aa (Fig. 1). The predicted amino acid sequence was 3 aa shorter than its human and murine counterparts (1, 3), giving rise to a protein with a projected molecular weight of 19,921. There was one putative N-glycosylation site in position 130 corresponding to that described in position 134 for human and mouse IL-10. The overall homologies between pig and man and between pig and mouse IL-10 were 82% and 75% at the nucleotide level, and

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   1 ggcacgagcaggggcttgctcttgcaaaaccaaaccacaagtccgactcaacgaagaagg  60

  61 cacagctctacc ATG CCC AGC TCA GCA CTG CTC TAT TGC CTG ATC TTC 108
   1             M  P  S  S  A  L  L  Y  C  L  I  F              12

 109 CTG GCA GGG GTG GCA GCC AGC ATT AAG TCT GAG AAC AGC TGC ATC 153
  13  L  A  G  V  A  A  S  I  K  S  E  N  S  C  I               27

 154 CAC TTC CCA ACC AGC CTG CCC CAC ATG CTC CGG GAA CTC CGA GCT 198
  28  H  F  P  T  S  L  P  H  M  L  R  E  L  R  A               42

 199 GCC TTC GGC CCA GTG AAG AGT TTC TTT CAA ACG AAG GAC CAG ATG 243
  43  A  F  G  P  V  K  S  F  F  Q  T  K  D  Q  M               57

 244 GGC GAC TTG TTG CTG ACC GGG TCT CTG CTG GAG GAC TTT AAG GGT 288
  58  G  D  L  L  L  T  G  S  L  L  E  D  F  K  G               72

 289 TAC CTG GGT TGC CAA GCC TTG TCA GAG ATG ATC CAG TTT TAC CTG 333
  73  Y  L  G  C  Q  A  L  S  E  M  I  Q  F  Y  L               87

 334 GAA GAC GTA ATG CCG AAG GCA GAG AGT GAT GGG GAG GAT ATC AAG 378
  88  E  D  V  M  P  K  A  E  S  D  G  E  D  I  K              102

 379 GAG CAC GTG AAC TCC CTG GGG GAG AAG CTG AAG ACC CTC AGG CTG 423
 103  E  H  V  N  S  L  G  E  K  L  K  T  L  R  L              117

 424 AGG CTG CGG CGC TGT CAT CAA TTT CTG CCC TGT GAA AAC AAG AGC 468
 118  R  L  R  R  C  H  Q  F  L  P  C  E  N  K  S              132

 469 AAG GCC GTG GAG GAG GTG AAG AGT GCC TTT AGC AAG CTC CAA GAG 513
 133  K  A  V  E  E  V  K  S  A  F  S  K  L  Q  E              147

 514 AGG GGT GTC TAC AAA GCC ATG GGT GAG TTT GAC ATC TTC ATC AAC 558
 148  R  G  V  Y  K  A  M  G  E  F  D  I  F  I  N              162

 559 TAC ATA GAA GCC TAC ATG ACG ATG AAG ATG AGG AAG AAC TGA agca 604
 163  Y  I  E  A  Y  M  T  M  K  M  R  K  N  *                  176

 605 ttctagggaaaccaaagaaaaaccttccaggatgacgactctactaaactctaggataca 664

 665 aattggagactttcaaaatctgctccaaggttcccggggagcagaaccagcaccctggga 724

 725 aaccctgctgtacctctcccctgagatatttattacctctgatacctcagctcccatttc 784

 785 tatttatttaccgagcctctctgtgaactatttagaagaagaacagtattatacttttt 844

 845 cagtatttattattttcacctgtgtttaagctttccatagggtgtgccctatggtgttca 904

 905 actgttttaagagaaattgtaagttatataaggggggaaaaaatgttccttcaggagcca 964

 965 actgcagcttccattccaagcctacccacccgggaaagctagtgggctatttgtcctgac 1024

1025 tgcctcccactttctcttgtccctgggctggggcttccggagtgtgacaaagtcgcttac 1084

1085 actcataggaagagaaactagggagccccttgacagctaatattccggtggccctgagg 1144

1145 gattcccctgacctcattccccaaacacttcattcttgaaagctgtggccagcttgttat 1204

1205 ttaaaacaacctaaaattggttctaatagaactcggttttaactagaagcaattcaattc 1264

1265 ctctgggaatgttacattgtttgtctgtcttcatagcagattttaattttgaataaataa 1324

1325 atggtcttattc                                                 1336
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FIG. 1. Porcine IL-10 cDNA and predicted protein sequences. Complete sequence was established in both directions. A potential N-glycosylation site and polyadenylylation signal are underlined. Nucleotide numbering starts with the beginning of the sequence.

74% and 68% at the amino acid level, respectively. Alignments of amino acid sequences of these three species (Fig. 2) confirmed that the N-2 terminal part of the protein (aa 20–68 of the pig sequence) was more polymorphic than the C-terminal part, as evidenced by the absence of large blocks of homology in the former region. To preserve the best possible alignment of the three sequences, a gap of 4 aa, corresponding to aa 20–23 of the human sequence, was introduced in the peptide leader sequence of the swine sequence. Independent RT–PCR and sequence analysis carried out on three different porcine RNA samples extracted from PBMCs confirmed the sequence presented in Fig. 1 and strongly suggested that the gap observed was not due to a cloning artifact.

Expression and Biological Activity of Recombinant Porcine IL-10. An *Eco*RI–*Rsa* I fragment from plasmid pSP161.5, which encompassed the IL-10 coding region as well as a portion of 134 nt from the 3′ untranslated region, was inserted into the expression vector pcDNA1. Transient transfections were carried out in COS cells and culture supernatants were analyzed after 48 hr in medium with [^{35}S]methionine. SDS/PAGE under reducing as well as nonreducing conditions revealed the expected band for IL-10 at 18 kDa as well as a second band at 15 kDa (Fig. 3), neither of which was observed in the control (mock). These bands may correspond to two

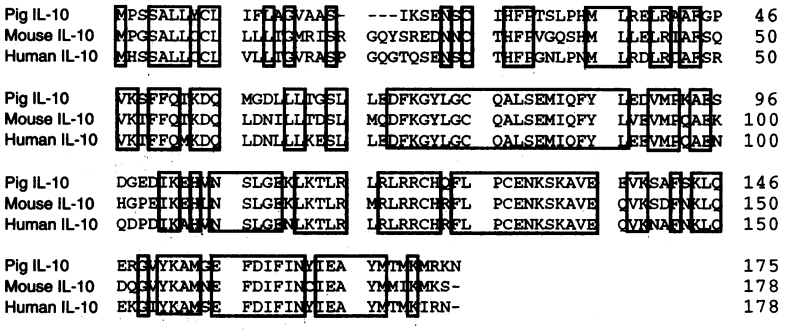

FIG. 2. Alignment of the pig, mouse (3), and human (1) IL-10 amino acid sequences. Boxed residues indicate identity between the three species. Hyphens denote gaps introduced for maximum alignment.

distinct IL-10 transcripts which were detected in these transfected cells by Northern analyses (result not shown). IL-10 has been shown to inhibit γ-IFN gene transcription in human T cells activated by PHA or anti-CD3 antibodies (3). To know whether the recombinant porcine IL-10 was biologically active, a bioassay was developed to examine its effect on γ-IFN transcription in porcine T cells activated with Con A. A representative test clearly indicated that γ-IFN transcription was reduced in a dose-dependent fashion by the presence of recombinant porcine IL-10 (Fig. 4, lanes 3 and 4 vs. lanes 5 and 6). Levels of control γ-IFN transcription observed in the presence of COS supernatants were higher than those obtained without COS supernatants (lanes 5 and 6 vs. lane 2).

***In Vivo* Expression of IL-10.** Two groups of three pigs each were used as experimental and control groups in studying putative correlations between IL-10 expression and induction of transplantation tolerance in our renal allograft model. Results presented in Fig. 5 typified the data obtained on IL-10 and γ-IFN transcription in serial biopsies from pig 10348 (rejector, not treated with CsA) and from pig 10349 (tolerant, treated with CsA). As indicated in the bar graph, IL-10 transcription rate was moderate in the rejector, which rejected at day 9, as compared with that in the CsA-treated animal, which developed increasing levels of IL-10 mRNA. In addition, no detectable transcription of the gene encoding the immunostimulatory cytokine γ-IFN was observed in tolerant animals compared with rejectors, an example of which is also shown in Fig. 5.

***In Vitro* Effects of CsA on IL-10 Gene Activation.** Since induction of tolerance in our porcine model is mediated by a short course of CsA, which targets T cells, and since IL-10 is produced by B cells, monocytes, and T cells, the respective contribution of T cells and non-T cells to IL-10 expression was assessed in comparison to expression of the T-cell (and natural killer cell)-restricted lymphokine γ-IFN. Northern blot analysis was carried out on total RNA purified from selectively activated PBMCs in the presence or absence of CsA. Comparison of IL-10 and γ-IFN transcription rates revealed a marked decrease of both IL-10 and γ-IFN gene transcription in Con A-activated cells when CsA was added during the stimulation phase (Fig. 6, lanes 5). In contrast, IL-10 gene activation induced by lipopolysaccharide was not modified by the addition of the drug (lane 7).

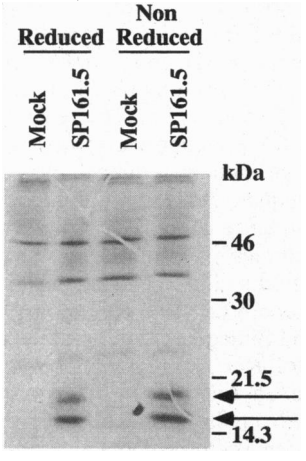

FIG. 3. SDS/PAGE of recombinant pig IL-10 protein. COS cells transfected either with pSP161.5 IL-10 recombinant plasmid or with the empty expression vector were cultured for 4 hr in [^{35}S]methionine medium. Supernatant from experimental (pSP161.5) and control (mock) transfectants were analyzed by SDS/15% PAGE in reducing and nonreducing conditions. Arrows indicate specific bands observed after experimental transfection.

DISCUSSION

By using oligonucleotide primers corresponding to conserved regions of rodent and human IL-10 sequences, a 180-bp fragment was derived by RT–PCR amplification of porcine

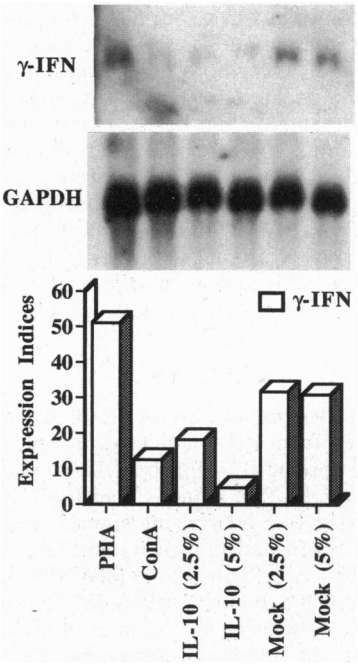

FIG. 4. Effect of recombinant IL-10 on γ-IFN transcription *in vitro*. RNA was prepared from pig PBMCs stimulated *in vitro* by Con A (5 μg/ml) in presence of 2.5% or 5% culture supernatant from IL-10-transfected (IL-10) or nontransfected (Mock) COS cells. RNA was analyzed by Northern blot and the same filter was successively hybridized with the γ-IFN and GAPDH probes (*Upper*). Autoradiographic signals were then processed on a computing densitometer and expression indices were determined by adjusting signal intensities to the amount of RNA loaded as described in *Materials and Methods* (*Lower*).

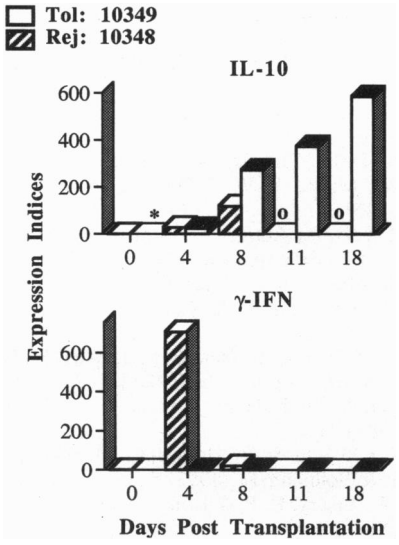

FIG. 5. IL-10 and γ-IFN gene expression in renal biopsy samples from rejected and accepted kidneys. Wedge biopsies were taken at days 0, 4, and 8 for the rejector animal 10348 (Rej) and at days 0, 4, 8, 11, and 18 for the tolerant animal 10349 (Tol). Northern blot filters were successively hybridized with the S10.180 and γ-IFN probes and were washed in 0.1× SSC/0.5% SDS at 60°C for 25 min. O, The rejected kidney was removed at day 9 because of profound renal failure; *, RNA sample from day 0 of pig 10349 was degraded. Expression indices, indicative of the density and volume of radioactive signals, were calculated as described in the legend of Fig. 4.

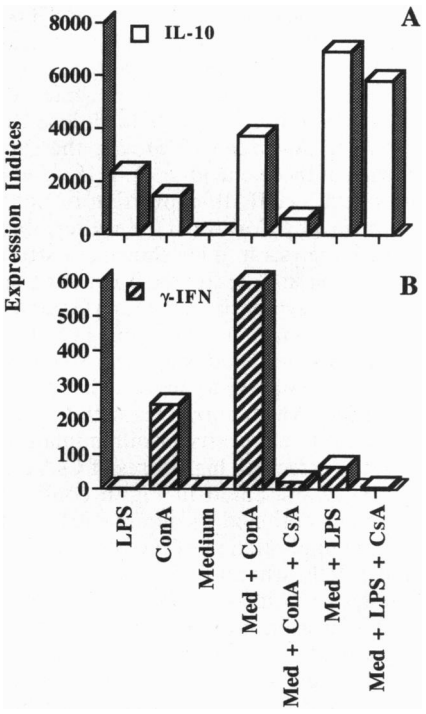

FIG. 6. Effect of CsA on IL-10 transcription in PBMCs activated by Con A or lipopolysaccharide (LPS). Cells were activated by ConA (5 μg/ml) or LPS (1 μg/ml) for 48 hr in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. CsA was solubilized into the Cremaphor/ethyl alcohol medium (Med) and was added, at 1 μg/ml, as a suspension in Med where indicated. Total RNA was prepared from harvested cells and was tested on a Northern blot filter hybridized successively with the IL-10, γ-IFN, and GAPDH probes. Expression indices were determined as described in the legend of Fig. 4.

total RNA purified from PBMCs. Sequencing of this fragment confirmed that it encompassed a part of the coding region of porcine IL-10. It also allowed the screening of a pig cDNA library from which the full-length IL-10 clone, pSW10, was selected and sequenced. When compared with its human and mouse homologs, the porcine IL-10 predicted protein sequence showed a 3-aa deletion starting in position 19 (Fig. 2). Because the overall IL-10 cDNA nucleotide sequence is highly conserved among mammals, especially between human and pig (82%), we assume that the organization of the IL-10 gene is conserved among these species as well. If this assumption is correct, it predicts that the deletion observed in the porcine IL-10 sequence would have occurred in a region corresponding to the leader peptide and would not have affected the overall structure of the 18-kDa mature porcine IL-10 protein.

It has been reported that the BCRFI open reading frame of the Epstein–Barr virus encodes a protein sequence homologous to the mature IL-10 sequence of mouse and human (3). Comparison between the pig IL-10 and the Epstein–Barr virus sequences also revealed 77% nucleotide homology with BCRFI. Nucleotide sequence alignments of BCRFI sequence with either human, mouse, or pig IL-10 indicated a higher conservation between human and BCRFI sequences (94%) than between BCRFI and any other IL-10 sequences (average homology, 77%). Therefore, our results are in agreement with the hypothesis which proposes that the BCRFI sequence originated from the human IL-10 sequence, giving obvious selective advantages to the resulting viral genome (2).

Using transient transfection of COS cells with an IL-10 recombinant expression vector, we have also demonstrated that a specific polypeptide chain of the expected molecular mass, 18 kDa, was secreted into the culture medium (Fig. 3). However, a second band of MW of 15 kDa was also observed in the same samples (Fig. 3, lanes 2 and 4) that may be due to a selective cleavage of the mature protein followed by degradation of the smallest fragment and/or to an artifact in the splicing process in COS cells. To evaluate the biological activity of the secreted products a bioassay for pig IL-10 was developed. The test aimed to measure the effects of IL-10 on activation of the γ-IFN gene in Con A-activated T cells. This assay clearly demonstrated that the engineered pig IL-10 possessed the expected activity in specifically blocking the transcription of the γ-IFN gene (Fig. 4). Taken together, the biochemical and functional data confirm that the recombinant porcine IL-10 molecule synthesized by the transfected COS cells is the porcine equivalent of the IL-10 cytokine.

The use of MHC-homozygous as well as recombinant pigs as an experimental model gave the unique opportunity to study organ transplantation in reproducible conditions (17). This large-animal model also allows the performance of repeated open biopsies on the same kidney in order to obtain enough material for RNA purification. Total RNA was first used as a crude screening approach for detecting lymphokine transcription, and positive signals were obtained on Northern blots for various lymphokine messages, including that for IL-10 (G.B., unpublished work). Kidney biopsy specimens taken on the day of transplantation, at a time when there is no recipient cell infiltration of the graft, did not show positive IL-10 signals, suggesting that IL-10 production is controlled by recipient PBMC-derived cells that colonize the transplanted organ. Northern blot analysis is not as sensitive a technique as the RNase protection assay or RT–PCR; nevertheless, obvious variations of IL-10 gene transcription were observed between tolerant and rejector animals, making the observation highly significant (Fig. 5). As previously described in human patients rejecting kidney allografts (18), IL-10 signals can be detected in samples from rejector animals following long exposure of Northern filters (results not shown). However, these signals were weaker and peaked at earlier time points than those from tolerant animals (G.B., unpublished work). The effect of IL-10 on transplantation tolerance is therefore likely to depend on a quantitative balance between various cross-regulating cyto-

kines, such as those synthesized by either T_{h1} or T_{h2} helper T-cell subsets, rather than on its exclusive expression in tolerant recipients.

As previously described, tolerance can be achieved in our swine model by the use of a short course of high-dose CsA. This drug has been shown to downregulate *in vitro* the expression of cytokine genes such as those encoding IL-2, IL-4, and γ-IFN (19, 20). CsA did not affect IL-10 expression of murine T_{h2} clones stimulated *in vitro* by Con A (21); however, those data were contradicted by analysis in mice showing a strong inhibition of IL-10 expression in CsA-treated T cells and concomitant activation of IL-10 synthesis in monocytes and B cells (22). Upregulation of IL-6 and transforming growth factor β expression following CsA treatment were reported in humans (23, 24). It is therefore tempting to suggest that the upregulation of IL-10 synthesis, which correlates with the induction of tolerance toward renal allografts in our miniature swine model, is related to the effects of high doses of CsA on B cells and monocytes. Results presented in Fig. 6 confirmed and extended previous data obtained in mouse (22) by showing that CsA inhibited the upregulation of the porcine IL-10 gene in Con A-activated T cells, whereas it had no effects on IL-10 expressed by lipopolysaccharide-stimulated cells (Fig. 6, compare lanes 5 and 7). However, note that the dose of CsA used in these *in vitro* assays may have exceeded the local concentration of CsA used *in vivo*; consequently, IL-10 gene activation in tolerant swine may still be controlled in part by T cells, as suggested by others (25).

It is conceivable that in the induction phase of tolerance T_{h1}-like cells would be repressed by high doses of CsA leading to a low expression of IL-2 and γ-IFN, which are, in part, responsible for the differentiation and proliferation of cytotoxic T lymphocytes. Experiments in mice treated with various suppressive regimens have indeed shown downregulation of expression of IL-2 and γ-IFN (26–28) and IL-2 receptor (29). This initial imbalance of lymphokine expression would lead to persistent suppression of T_{h1} cells through expression of IL-10 by macrophages, B cells, and possibly T_{h2}-like and $CD8^+$ T cells even after the CsA treatment has ceased. Our data present evidence in favor of the intervention of IL-10 in the induction phase of transplantation tolerance to kidney allografts in a preclinical large-animal model. A report showing high levels of IL-10 in patients with genetic severe combined immunodeficiency who are tolerant to allogeneic fetal liver hematopoietic stem cells (12) are in agreement with our observations. Further investigations will be required to elucidate the precise mode of action of IL-10 in these models.

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