Oral Interferon-α Treatment of Mice With Cryoglobulinemic Glomerulonephritis

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• **Cryoglobulins are associated with the development of a membranoproliferative glomerulonephritis, often referred to as *cryoglobulinemic glomerulonephritis,* particularly in the setting of hepatitis C virus infection. Parenteral interferon-α (IFN-α) commonly is used therapeutically in humans with cryoglobulinemic glomerulonephritis. We tested the therapeutic impact of oral IFN-α treatment in thymic stromal lymphopoietin (TSLP) transgenic mice, a strain that develops mixed cryoglobulinemia with glomerulonephritis closely resembling the disease that occurs in humans. A total of 41 female mice were treated for 21 days with daily ingestion of either 500 IU of Universal type I IFN or placebo. The studied groups included TSLP transgenic mice treated with IFN-α (n = 13), TSLP transgenic mice treated with placebo (n = 13), wild-type mice treated with IFN-α (n = 5), and wild-type mice treated with placebo (n = 10). A total of 39 mice completed the study; two TSLP transgenic mice treated with IFN-α died during the study period. Placebo-treated TSLP transgenic mice showed significantly increased mean glomerular tuft areas, mean glomerular areas occupied by macrophages, and mean cell numbers per glomerulus compared with wild-type controls. All three parameters were decreased in IFN-α–treated TSLP transgenic mice, although the differences compared with placebo-treated mice did not reach significance. The changes in glomerular matrix deposition were the same in IFN-α–treated and placebo-treated mice. The oral ingestion of IFN-α seemed to reduce glomerular macrophage influx, but this did not result in decreased glomerular matrix deposition. The limited positive effect provides experimental support for clinical studies that indicate the beneficial effects of IFN-α therapy observed in humans with glomerulonephritis might be attributable to its antiviral effect rather than modulation of intrarenal pathophysiologic pathways.**

INDEX WORDS: Cryoglobulins; membranoproliferative glomerulonephritis (MPGN); interferon-α (IFN-α); thymic stromal lymphopoietin (TSLP).

CRYOGLOBULINS ARE immunoglobulins or complexes of immunoglobulins that precipitate in the cold and redissolve on rewarming.[1-4] According to the components of the cryoprecipitate, three types of cryoglobulins currently are distinguished.[3] Cryoprecipitable monoclonal immunoglobulins or light chains (type I) usually are associated with lymphoproliferative disorders.[3] More common are mixed cryoglobulins, which are complexes of two or more immunoglobulins, in which IgG is bound by a second immunoglobulin with anti-IgG rheumatoid activity.[5] The antiglobulin component can be monoclonal (type II) or polyclonal (type III). Mixed cryoglobulins are associated with a wide variety of human diseases, including infections (eg, hepatitis C) and autoimmune diseases.[5-7] Hepatitis C virus infection is associated most commonly with type II cryoglobulins but also has been associated with type III cryoglobulins.[6] Extrarenal manifestations of cryoglobulinemia include inflammation after precipitation in the microvasculature of such organs as the skin, central nervous system, gut, and lung.[1]

Renal involvement (cryoglobulinemic glomerulonephritis) typically is manifest as a membranoproliferative glomerulonephritis (MPGN).[4,8] Features that help to differentiate cryoglobulin-associated MPGN from idiopathic forms of MPGN are periodic acid–Schiff (PAS)–positive deposits filling the capillary lumen (intraluminal thrombi composed of cryoglobulin complexes), endocapillary proliferation with massive accumulation of monocyte/macrophages, characteristic fibrillar-to-microtubular substructure of the deposits, vasculitis of small and medium-sized renal arteries, and demonstration of circulating cryoglobulins or rheumatoid factor activity.[8]

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Thymic stromal lymphopoietin (TSLP) is a cytokine that has been isolated from a thymic stromal cell line and cloned.[9,10] TSLP supports the growth of pre–B cell colonies and has comitogenic activity for fetal thymocytes.[10] We reported that mice transgenic for TSLP develop large amounts of mixed cryoglobulins (type III) and cryoglobulinemic glomerulonephritis.[11] Renal involvement was characterized by glomerular macrophage influx; subendothelial and mesangial immune deposits, some of which showed microtubular patterns of organization; increased glomerular matrix; and occlusion of capillary loops by PAS-positive material, which appeared to be complexes of immune aggregates when studied ultrastructurally. Overexpression of TSLP resulted in a systemic inflammatory disease involving liver, spleen, lungs, and skin.

Interferon-α (IFN-α) has antiproliferative, antiviral, and immune regulatory effects and is used widely in the treatment of neoplasia and in chronic hepatitis B and C infections.[12-14] The treatment of cryoglobulinemia with parenteral IFN-α, in *essential* and hepatitis C virus–associated cases, has been studied, but the results of these studies are still controversial.[1,4,15] Two clinical trials reported to date indicate that the efficacy of IFN-α for treatment of glomerulonephritis in hepatitis C–infected patients is limited to its antiviral effect.[16,17] Whether IFN-α can be beneficial for treatment of cryoglobulinemic glomerulonephritis independent of its antiviral effect is currently unknown. Oral use of IFN-α has been shown to have a therapeutic impact in a wide range of animal models.[18] Although the oral route of IFN-α application is still controversial in its mechanism of action and efficacy, more recent studies have shown that oral ingestion of IFN-α lowers the incidence of diabetes in nonobese diabetic mice and has antiviral and antitumor activity in mice.[19-21] We tested the potential therapeutic effect of daily oral IFN-α in TSLP transgenic mice, a system that allows evaluation of the effect of IFN-α on cryoglobulinemic glomerulonephritis independent of its activity as an antiviral agent.

MATERIALS AND METHODS

Breeding and Characterization

The establishment of the TSLP transgenic mouse strain (FF8) is described elsewhere (A. Farr, manuscript in preparation). Mice were maintained with food and water *ad libitum* and under a 12-hour light/dark cycle. All animal studies were reviewed and approved by the Animal Care Committee of the University of Washington. After backcrossing for more than eight generations to a C57Bl6 background, male TSLP transgenic animals were mated with wild-type C57Bl6 females. At 3 weeks of age, pups were weaned and tail tips were acquired for genotyping.

Study Design and Treatment

Universal type I IFN (PBL Biomedical Laboratories, New Brunswick, NJ) was purchased in a concentration of 10^7 U/mL. The stock solution was diluted to 25,000 U/mL in phosphate-buffered saline containing 0.1 mg/mL of bovine serum albumin (Fraction V, low endotoxin, Sigma Chemicals, St. Louis, MO). The solution was sterile filtered, aliquoted in 20-μL portions containing 500 U each, and kept at −70°C until used.

The disease process is different between males and females, with a more severe disease course in females. Because available numbers of transgenic females at a defined time point were limited as a result of the slow breeding process in these animals, we conducted two chronologically distinct treatment series with oral administration of IFN-α. Mice were weaned at 3 weeks of age, genotyped, and treated orally with 500 U of Universal type I IFN per day or placebo over 21 days. The first series consisted of transgenic females treated with Universal type I IFN (n = 6), transgenic females treated with placebo (n = 5), and wild-type females treated with placebo (n = 8). In a second series, transgenic females were treated with Universal type I IFN (n = 7) or with placebo (n = 8), and wild-type females were treated with Universal type I IFN (n = 5) or with placebo (n = 2). Data from both study groups were combined for the final evaluation.

Preparation of DNA and Genotyping

DNA was extracted from tail tips using the Dneasy Tissue Kit (Qiagen Inc, Valencia, CA) according to the protocol of the manufacturer. Mice were genotyped using polymerase chain reaction primer 5′TGCAAGTACTAGTACGGATGGGGC3′ from the 5′ end of the coding region of the TSLP gene and 5′GGACTTCTTGTGCCATTTCCTGAG 3′ from the 3′ end of the coding region of the TSLP gene, which results in an expected 323 bp product in transgenic animals. The polymerase chain reaction contained DNA, 1X enzyme storage buffer B, 2.5 mM of magnesium chloride, 400 nM of each primer, 0.2 mM of deoxynucleotide triphosphates (dNTPs; deoxy-adenine triphosphate, deoxy-guanisine triphosphate, deoxycytosine triphosphate, and deoxythymidine triphosphate) each, and 1.25 U of Taq DNA polymerase (all from Promega Corporation, Madison, WI) in a 50-μL polymerase chain reaction. Cycling conditions were 94°C for 2 minutes, followed by 34 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and 72°C for 5 minutes.

Tissue Collection

A spontaneous urine sample was collected for the evaluation of albuminuria. After induction of deep anesthesia,

blood was drawn by cardiac puncture. Spleen, kidneys, liver, lungs, heart, thymus, and ears were removed. Tissues were fixed in part in 10% neutral buffered formalin, in methyl Carnoy's solution (60% methanol, 30% chloroform, 10% acetic acid), and in half-strength Karnovsky's solution (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0) for electron microscopy.

Light Microscopy

From all organs, 4-μm sections from formalin-fixed and paraffin-embedded tissue were stained with hematoxylin and eosin (H&E) following routine protocols. From the kidney, 2-μm sections were stained by H&E, PAS, and periodic acid methenamine silver stain (silver stain).[22]

Electron Microscopy

Tissue for electron microscopy was fixed in half-strength Karnovsky's solution, incubated in 2% osmium tetroxide for 30 minutes, dehydrated in graded ethanol, and moved into propylene oxide resin. After infiltration with Polybed (Polysciences, Warrington, PA), the tissue was polymerized at 55°C for 48 hours. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and examined with a Philips 410 electron microscope (Philips Export BV, Eindhoven, The Netherlands).

Immunohistochemistry

The protocols for immunohistochemistry have been described in detail.[23,24] Antibodies used in this study were a monoclonal antibody against CD3 (clone number CD3-2, Serotec, Raleigh, NC), which has been tested by Western blotting and immunohistochemistry by the company. It recognizes a highly conserved epitope of the CD3 molecule expressed by T lymphocytes. Glomerular macrophages were detected using a rat antimouse MAC-2 antibody (Cederlane, Ontario, Canada), which previously has been used for the detection of glomerular macrophages by immunohistochemistry.[25] A monoclonal rat antimouse CD45 RA antibody (Pharmingen, San Diego, CA) was used for the detection of B cells. This antibody has been tested for immunohistochemistry, flow cytometry, and immunoprecipitation.[26] In brief, serial 2-μm sections of formalin-fixed, paraffin-embedded tissue were used. Deparaffinized and rehydrated slides were steam heated in Antigen Unmasking Solution (Vector, Burlingame, CA) when necessary. Endogenous peroxidases were blocked by hydrogen peroxide, and endogenous biotin was blocked using the Avidin/Biotin Blocking Kit (Vector, Burlingame, CA). Primary antibodies were applied for 1 hour or overnight, diluted in phosphate-buffered saline containing 1% bovine serum albumin (Sigma Chemicals, St. Louis, MO). After subsequent washing in phosphate-buffered saline, the tissue was incubated with the biotinylated secondary antibody (rabbit antirat, Vector, Burlingame, CA). For signal amplification, the ABC-Elite reagent (Vector, Burlingame, CA) was used. 3,3′-Diaminobenzidine with nickel enhancement, resulting in a black color product, was used as chromogen. Slides were counterstained with methyl green and dehydrated, and coverslips were placed over them.

Laboratory Data

Blood urea nitrogen was measured using a standard clinical chemistry analyzer (LX-20, Beckman Laboratories, Brea, CA). Blood was stored at 37°C until separation by centrifugation. Serum was stored at 4°C and checked visually for the formation of cryoglobulins. Urine albumin-to-creatinine ratio was calculated by evaluation of albuminuria using the Albuwell (Exocell Inc, Philadelphia, PA) mouse albumin enzyme-linked immunosorbent assay and evaluation of creatinine using The Creatinine Companion (Exocell Inc, Philadelphia, PA) according to the protocol of the manufacurer.

Analysis and Statistics

Morphometry was performed on H&E-stained and silver-stained slides and on slides stained for MAC-2-positive macrophages. From each section, 15 consecutive glomerular cross-sections were photographed using an Olympus DP11 digital camera (Olympus America Inc, Melville, NY). The following parameters were measured using Image-Pro Plus (Media Cybernetics, Silver Spring, MD) software: (1) the number of glomerular nuclei and the glomerular tuft area on H&E-stained slides, (2) the area of glomerular matrix and the glomerular tuft area on silver-stained slides, and (3) the area of glomerular MAC-2-positive staining and the glomerular tuft area. Results were expressed as the cell number per glomerulus, the cell number per glomerular tuft area, the area of matrix per glomerulus, the percentage of matrix (area of matrix per glomerular area), the area of macrophages per glomerulus, and the area of macrophages per glomerular area. The statistical analysis was performed using the InStat program (Version 3.0 for Windows, Intuitive Software for Science, San Diego, CA). The means were compared using the nonparametric Kruskal-Wallis test and Dunn's multiple comparisons test. A P value < 0.05 was considered to be statistically significant. Error bars give SEM.

RESULTS

TSLP Transgenic Mice Develop Membranoproliferative Glomerulonephritis

Mice were sacrificed at a mean age of 51 $\pm$ 0.5 days. At this time point, all TSLP transgenic females showed characteristic and generally uniform glomerular lesions (Fig 1). The glomerular mesangial area was expanded as a result of increased matrix deposition (Fig 1B, C) and accumulated immune deposits (see Figs 2 and 3). The mesangial area commonly showed a strong positivity in PAS stains (Fig 1E, F). A prominent increase of cellularity could not be detected by light microscopy. Capillary lumina were narrowed and sometimes completely occluded by PAS-positive material (Fig 1F). This situation resulted in a decreased number of patent capillaries in TSLP transgenic females with only a few peripheral lumina patent in the most severely affected glomeruli. Some glomerular capillaries

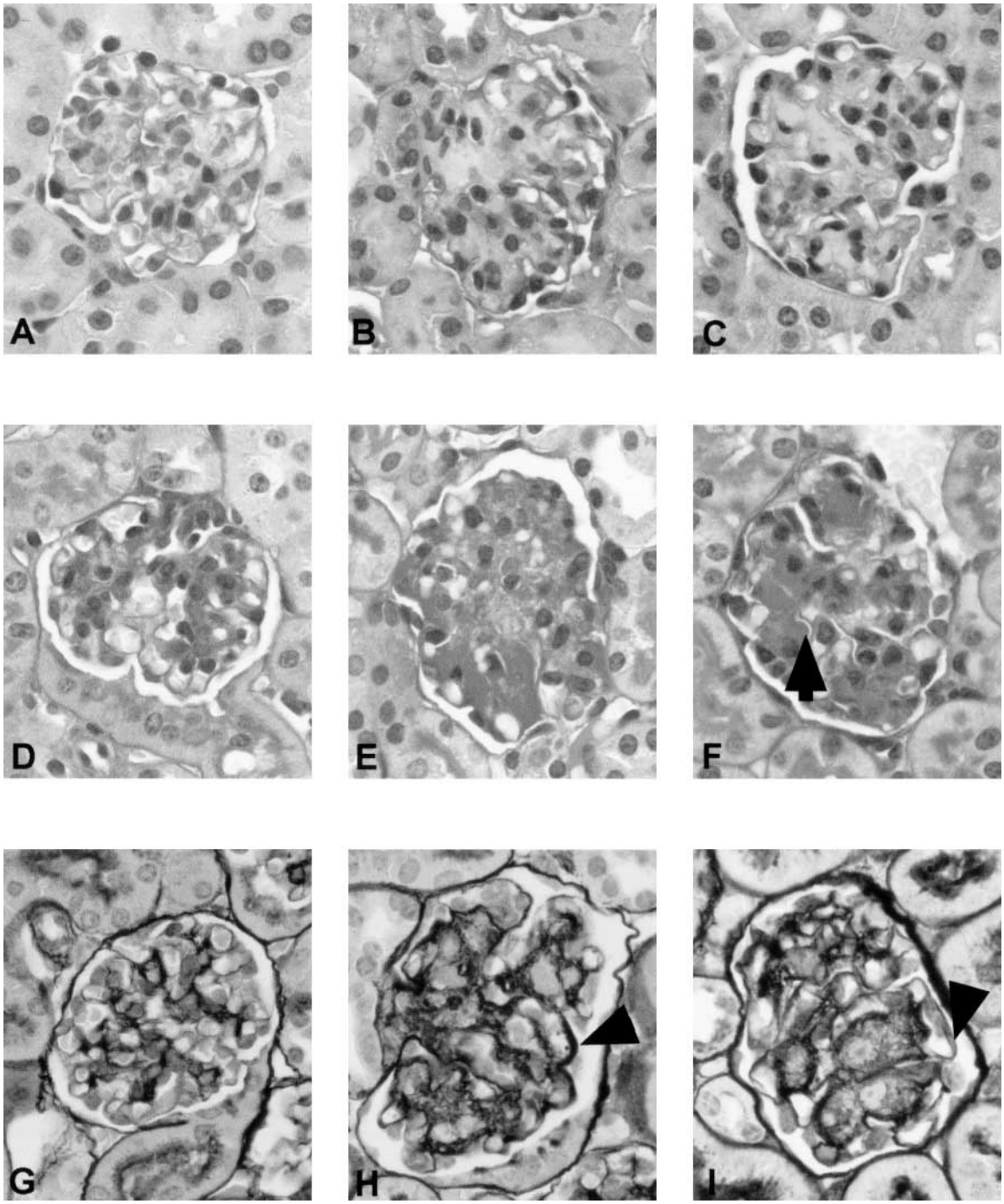

Fig 1. (A-I) Light microscopic features of the glomerular lesions. Representative glomeruli of a wild-type control mouse treated with placebo, stained with H&E (A), PAS (D), and silver (G). Representative glomeruli of a TSLP transgenic female treated with placebo, stained with H&E (B), PAS (E), and silver (H). Thickening of capillary walls is visible in H (arrowhead). Representative glomeruli of a TSLP transgenic female treated with IFN-α, stained with H&E (C), PAS (F), and silver (I). The widened mesangial area is visible in C. PAS-positive material is deposited in the mesangium and sometimes occludes the lumen of capillaries (arrow in F). The arrowhead in I marks a double contour of a capillary wall. (All original magnifications $\times$1,000.)

were filled completely by PAS-positive material, resembling *hyaline thrombi* as described in human cryoglobulinemic glomerulonephritis.[4] Thickening and splitting of peripheral capillary walls was detected on silver stains (Fig 1H, I).

The tubulointerstitium was well preserved in all cases without prominent interstitial infiltrates and without interstitial fibrosis. Intrarenal arteries showed no evidence of vasculitis.

Ultrastructural features of selected cases were studied further by transmission electron microscopy (Figs 2 and 3). TSLP transgenic mice showed a prominent increase in mesangial matrix (Fig 2A, B) compared with wild-type controls (Fig 2C). Electron-dense immune deposits were present in the subendothelial area and the mesangium (Figs 2B and 3A, C). The subendothelial deposits contributed to prominent thickening of capillary walls and narrowing of lumina (Fig 3A). At higher magnification, a *tubular* organization of the immune deposits became apparent (Fig 3C). Some capillaries were occluded completely by electron-dense material, similar to the capillary wall immune deposits, whereas in other cases only a slit of the lumen remained open (Fig 3B). Extravasation of leukocytes into the mesangium was detected in some cases (Fig 2A, B).

The main inflammatory cell type infiltrating glomeruli in TSLP transgenic mice were macrophages (Fig 4). The overall number of T cells was low (Fig 4D-F). TSLP transgenic mice showed a slightly higher number of T cells, mainly in peritubular capillaries and infiltrating the interstitium compared with wild-type animals, whereas glomerular T-cell influx was not a prominent feature. The smallest portion of the infiltrating cell population was composed of B cells, consistent with the uncommon appearance of B cells in glomerulonephritis (Fig 4G-I).

TSLP Transgenic Mice Develop Glomerular Hypertrophy and Infiltration by Macrophages That Is Reduced by IFN-α Treatment

For the quantification of glomerular cellularity, the number of glomerular nuclei and the glomerular tuft area were evaluated on H&E-stained slides. The cell number per glomerulus was increased significantly in placebo-treated TSLP transgenic mice (46 $\pm$ 4.3 cells per glomerulus versus 30.1 $\pm$ 3 cells per glomerulus; $P < 0.05$), but in TSLP transgenic animals treated with IFN-α, the mean number was not significantly different compared with wild-type controls (40.1 $\pm$ 2.9 cells per glomerulus versus 30.1 $\pm$ 3 cells per glomerulus; P = not significant) (Fig 5A). There was no difference in the cell number per glomerular tuft area between the three groups (Fig 5B). An increased glomerular size, with a normal ratio of cells per area, resulted in an overall increase of cells per glomerulus in TSLP transgenic mice. In silver stains, the area of matrix per glomerulus was significantly higher in placebo-treated (685 $\pm$ 98 μm^2 per glomerulus versus 210 $\pm$ 22 μm^2 per glomerulus) and IFN-α–treated transgenic mice (591 $\pm$ 72 μm^2 per glomerulus versus 210 $\pm$ 22 μm^2 per glomerulus) (Fig 6A). The same was true for the percentage of matrix per tuft area (27% versus 27% versus 11%) (Fig 6B).

Because the evaluation of macrophage numbers by immunohistochemistry is problematic as a result of positive color reaction product on portions of cells incompletely present in a given plane of section, we evaluated the area of macrophage staining per glomerulus and per glomerular tuft area (Fig 7). The area containing macrophages was increased significantly in placebo-treated (102.7 $\pm$ 12.6 μm^2 macrophages per glomerulus versus 8 $\pm$ 2 μm^2 macrophages per glomerulus; $P < 0.001$) and IFN-α–treated TSLP transgenic animals (67.3 $\pm$ 15.4 μm^2 macrophages per glomerulus; $P < 0.01$), but the difference between control and transgenic mice was smaller in IFN-α–treated mice (Fig 7). There was no morphologic difference in renal pathology apparent between IFN-α–treated and placebo-treated wild-type mice.

IFN-α Does Not Improve Extrarenal Manifestations in TSLP Transgenic Mice

The spleens were enlarged in all TSLP transgenic females. The weights of spleens, livers, and lungs were significantly greater in TSLP transgenic mice. There was no apparent difference between the placebo and IFN-α–treated group (Table 1). At the time of necropsy, none of the animals showed skin manifestations. The increased mean weights of livers and lungs were due to inflammatory leukocyte infiltrates without differences between placebo and IFN α–treated mice. No differences were found in the incidence

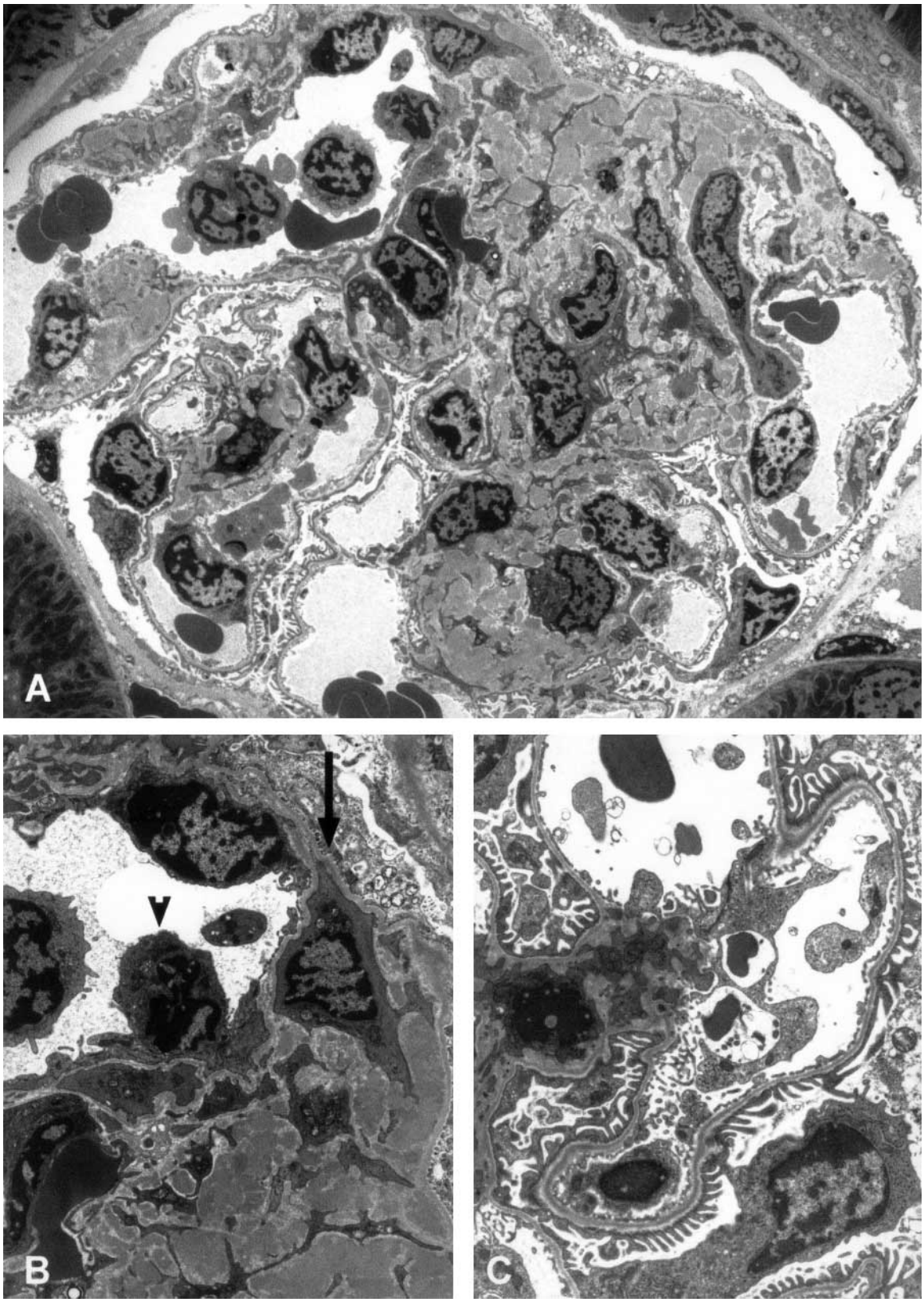

Fig 2. (A-C) Ultrastructural features of placebo-treated mice. (A and B) Transmission electron microscopy of a representative glomerulus from a TSLP transgenic female treated with placebo. (A, original magnification ×900; B, original magnification ×4,400.) Note the prominent widening of the mesangial area in A (compare with C). An early stage of cell interposition into the capillary wall (arrow) and a leukocyte, attached to the glomerular capillary endothelium (arrowhead), are visible in B. (C) Transmission electron microscopy of a representative glomerulus from a wild-type control female treated with placebo. (Original magnification ×4,400.)

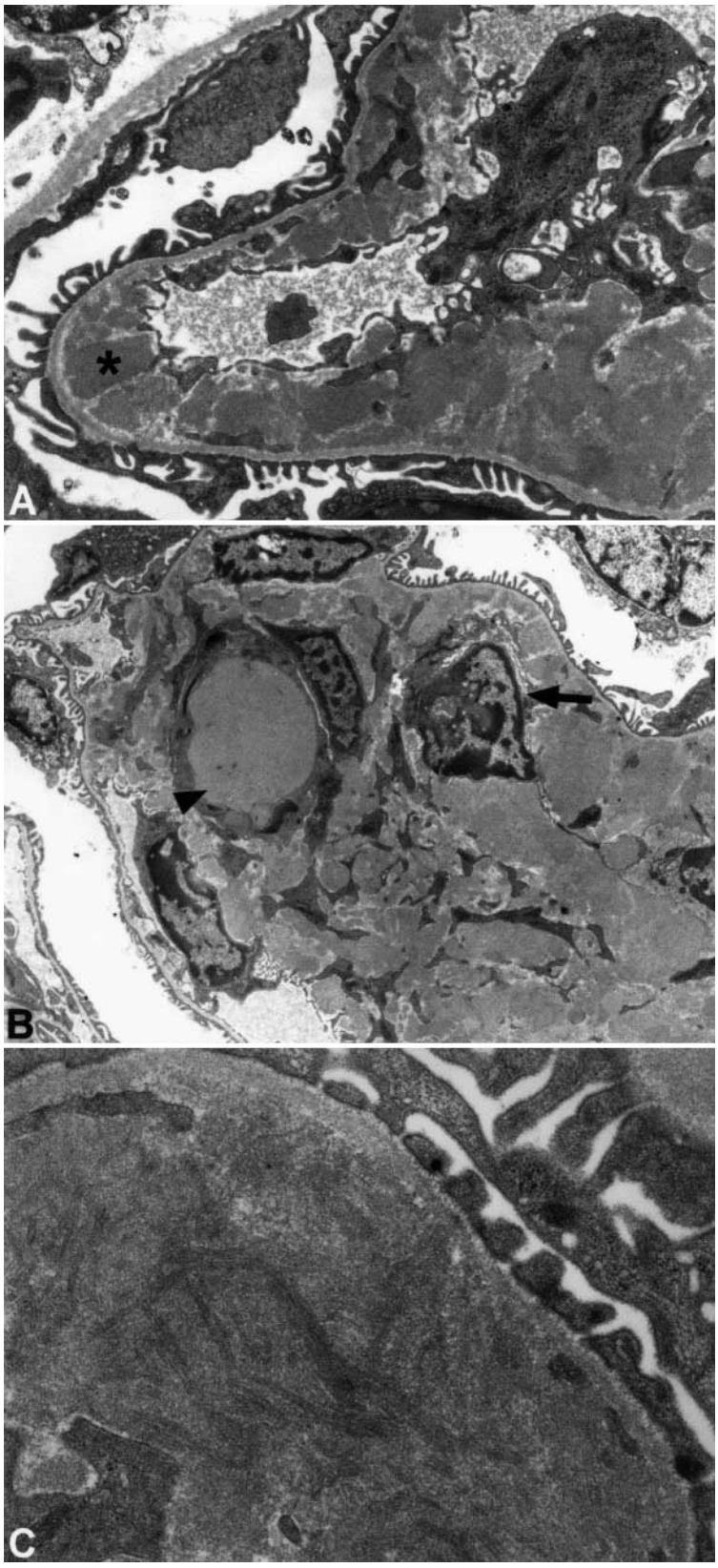

Fig 3. (A-C) Ultrastructural features of IFN-α–treated mice. (A) Transmission electron microscopy of a glomerulus from a TSLP transgenic female treated with IFN-α. (Original magnification ×4,400.) Note the strong deposition of electron-dense material in the subendothelium (asterisk). (B) Transmission electron microscopy of a glomerulus from a TSLP transgenic female treated with IFN-α. (Original magnification ×1,600.) One glomerular capillary is occluded completely by electron-dense material (arrowhead); other capillaries are compressed into thin slits (arrow). (C) Transmission electron microscopy of a glomerulus from a TSLP transgenic female treated with IFN-α. (Original magnification ×21,000.) Some of the electron-dense deposits showed a tubular ultrastructural organization at higher magification.

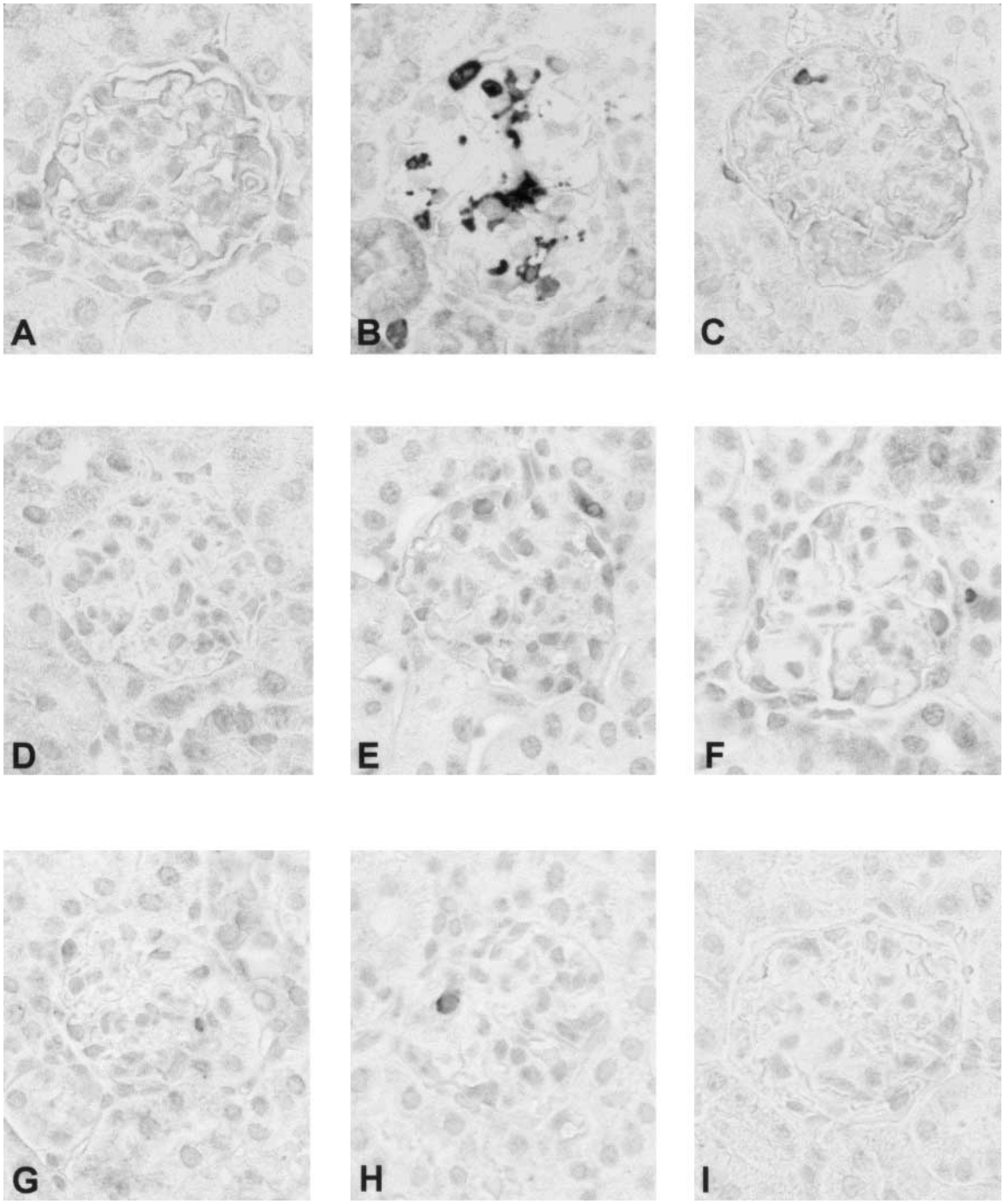

Fig 4. (A-I) Characterization of the infiltrating leukocytes by immunohistochemistry. Immunohistochemistry of representative glomeruli of a wild-type control treated with placebo, for macrophages (A), T cells (D), and B cells (G). Immunohistochemistry of representative glomeruli of a TSLP transgenic female treated with placebo, for macrophages (B), T cells (E), and B cells (H). Immunohistochemistry of representative glomeruli of a TSLP transgenic female treated with IFN-α, for macrophages (C), T cells (F), and B cells (I). The placebo-treated TSLP transgenic mouse shows a prominent macrophage infiltrate (B), which is not present in the IFN-α–treated transgenic female (C) or the wild-type control (A).

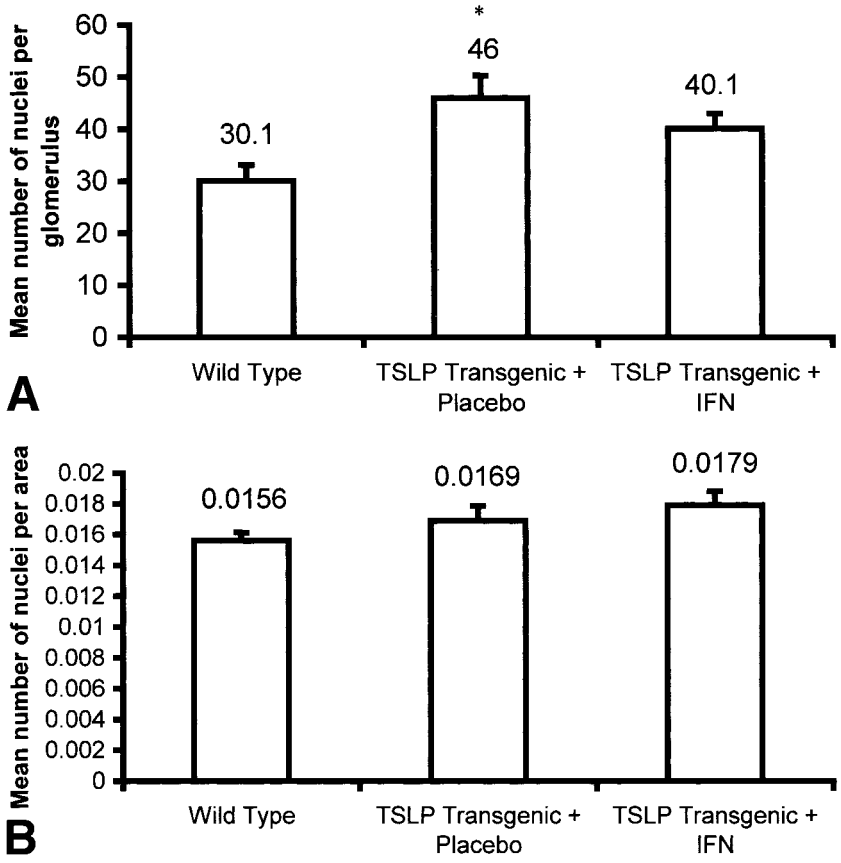

Fig 5. Mean cell number per glomerulus (A) and per μm^2 of glomerular tuft area (B) in wild-type mice and TSLP transgenic mice treated with placebo or treated with IFN-α. (Bars represent SEM, *$P < 0.05$.)

and amount of visible cryoglobulin and the amount of albuminuria.

DISCUSSION

Mixed cryoglobulinemia with renal involvement in the form of MPGN constitutes an important systemic disease, especially in areas of a high prevalence of hepatitis C virus infection.[27] The prognosis of this disease is poor.[27] The optimal treatment of mixed cryoglobulinemia in hepatitis C–associated cases and the rare *essential* cases is still under debate.[1,4,15] Treatment options in humans are glucocorticoids, cytotoxic drugs such as cyclophosphamide, and the parenteral administration of IFN-α.[4]

Parenteral IFN-α has been used widely in mixed cryoglobulinemia for its antiviral and antiproliferative activity. Although parenteral IFN-α is an expensive treatment, which commonly has significant adverse effects, controlled studies are limited.[17,28,29] Parenteral IFN-α has been tested in cryoglobulinemia with and without renal involvement. The few controlled studies showed that improvement of extrarenal manifestations, such as decreased cryocrit and decreased clinical symptoms for the duration of therapy, occurred in close association with reduced levels of viremia.[17,28,29] Withdrawal of the drug resulted in disease relapse in most treated patients who exhibited an initial response to therapy.[29] The studies of patients with renal involvement showed conflicting results. One study indicated improvement of proteinuria without improvement of renal function in patients with suppressed viremia,[16] whereas other authors described improvement of renal function.[30,31] As pointed out by D'Amico,[4] the positive effect seems to correlate best with the improvement of viremia in patients with hepatitis C virus infection.

It has been shown that the oral administration of IFN-α also results in antiviral and antiproliferative effects.[21] Omitting the necessity of parenteral drug administration and exchanging it with oral treatment with decreased side effects would

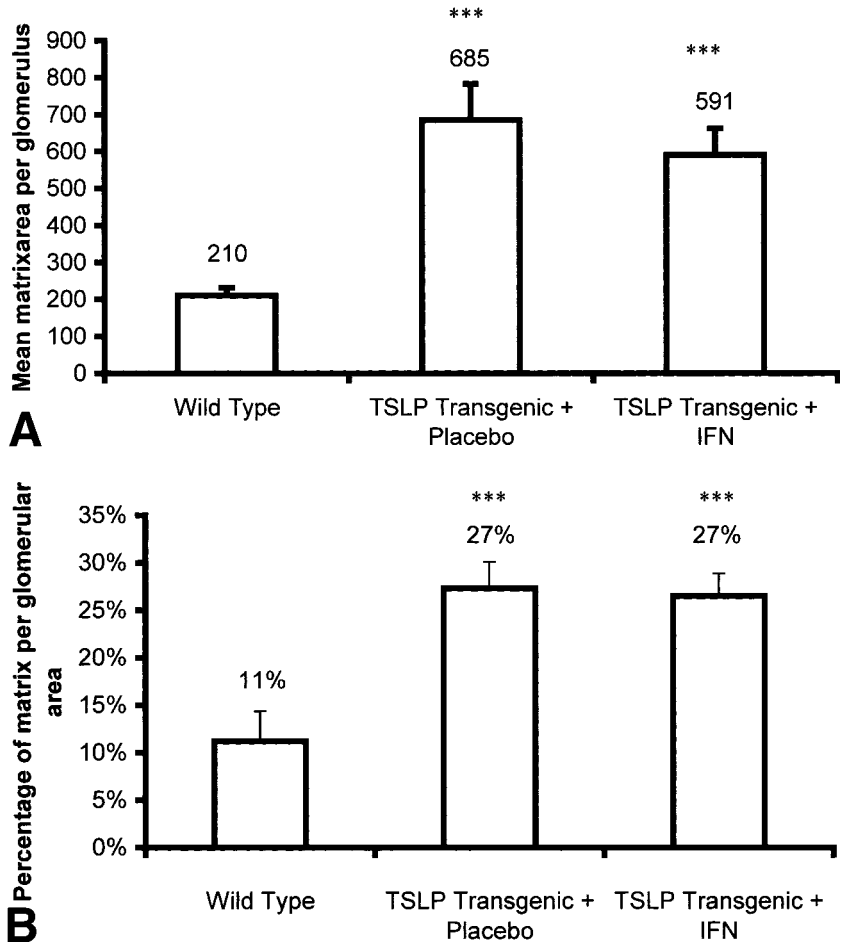

Fig 6. Mean area of matrix per glomerulus (μm^2) (A) and percentage of matrix per glomerular tuft area (B) in wild-type mice and TSLP transgenic mice treated with placebo or treated with IFN-α. (Bars represent SEM; ***$P < 0.001$.)

improve available therapeutic options for cryoglobulinemia. We tested the potential therapeutic impact of a daily oral dose of 500 IU of IFN-α in a transgenic mouse model of mixed cryoglobulinemia with renal involvement. This model enables detailed studies on type III cryoglobulinemia associated with MPGN. Because cryoglobulinemia in this model is not a consequence of hepatitis C virus infection, the direct effect of IFN-α on cryoglobulinemia and renal injury can be separated from its antiviral effect. Renal vasculitis is not a feature in TSLP transgenic mice, and treatment options for this consequence of cryoglobulins that occurs in some patients cannot be addressed in this model.

The main finding resulting from this intervention was a change in the glomerular pathology characterized by a decrease in glomerular macrophages and a slight decrease in glomerular cellularity. This effect was not associated with a decrease of glomerular matrix expansion because matrix area per glomerulus and percentage of matrix per tuft area were the same in placebo-treated and IFN-α–treated TSLP transgenic mice. We found no differences in the incidence of cryoglobulins and no benefit on the extrarenal manifestations, such as lung and liver involvement. Although the lethal outcome in two IFN-α–treated mice is not of clear importance with respect to the other groups, it cautions that oral treatment might not be as well tolerated in some instances as previously described.[21]

The limited benefit of oral IFN-α needs to be discussed from the perspective of studies in humans with parenteral administration and from the special perspective of the pathogenesis in TSLP transgenic mice. Consistent with our data, parenteral IFN-α is of limited benefit in cryoglobulinemic patients in whom viremia does not improve during the treatment.[4] The antiprolifera-

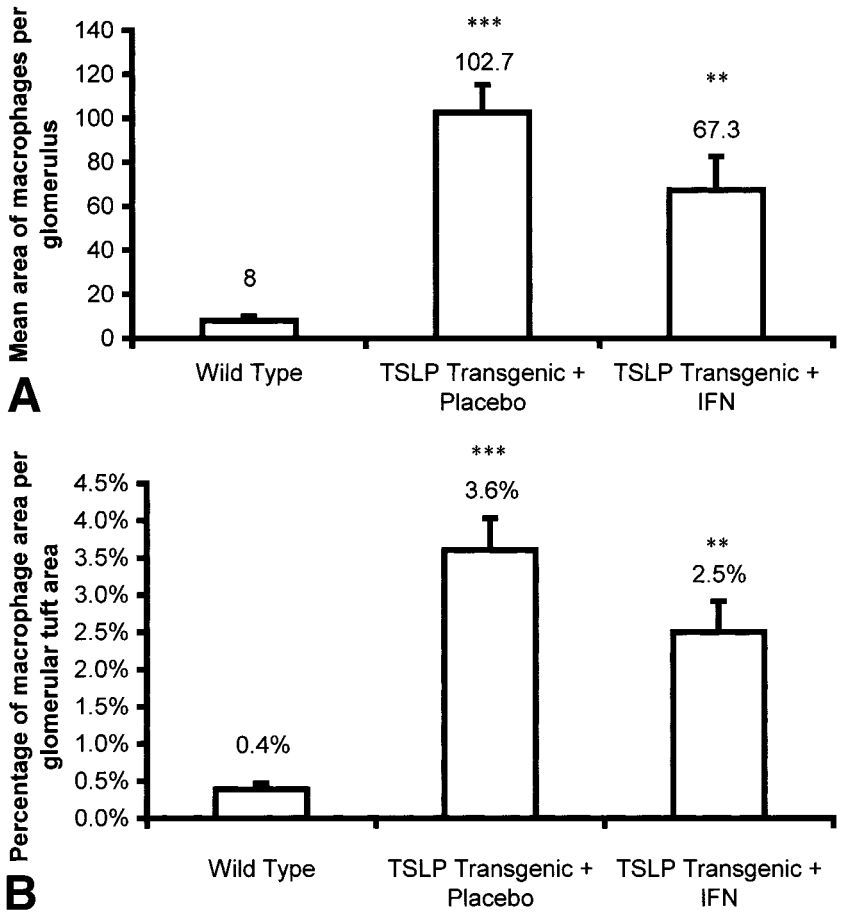

Fig 7. Mean area of macrophages per glomerulus (A) and per μm^2 of glomerular tuft area (B) in wild-type mice and TSLP transgenic mice treated with placebo or treated with IFN-α. (Bars represent SEM; **$P < 0.01$; ***$P < 0.001$.)

tive effect of IFN-α might not be sufficient for successful treatment as long as the stimulus for the production of cryoglobulins in the form of hepatitis C virus remains unchanged. We cannot exclude the possibility that the small benefits on glomerular pathology after 3 weeks of treatment might result in a clinically significant improvement of renal survival in long-term treatment studies.

The limited beneficial effect in our model might be due to the special pathophysiologic situation in TSLP transgenic mice. TSLP supports the differentiation of IgM-positive B cells in cocultures with fetal liver cells and long-term growth of B cells.[9] Although TSLP shares some functions with interleukin (IL)-7, this long-term B-cell growth could not be achieved by IL-7 and could not be blocked by anti-IL-7 antibodies.[9] Proliferation of a pre–B cell line in response to TSLP was inhibited by anti-IL-7 receptor α antibodies, implying that TSLP functions by way of this receptor.[32] IFN-α has been shown to inhibit IL-7-induced growth and survival of B-cell precursors in vitro.[33] Parenteral IFN-α treatment of newborn mice resulted in strong reduction of bone marrow cellularity, splenic cellularity, and B-cell numbers.[33] We hypothesized that oral treatment might have similar effects and might be beneficial in TSLP transgenic mice. This hypothesis was not confirmed by our study because the extrarenal disease manifestations were not improved by the therapy. Because TSLP is involved in earlier steps of B-cell development than IL-7, the blockade of IL-7 effects might occur downstream in the pathogenic cascade and may not be sufficient to result in a strong beneficial effect. It is currently unknown how oral IFN-α treatment influences B-cell development and whether its actions are similar to the parenteral treatment.

The lack of a beneficial effect might not be due to the absence of a positive effect but to a worsened disease course in some animals. A few

Table 1. Summary of Clinical Data

	Wild-Type Mice	TSLP Transgenic Treated With Placebo	TSLP Transgenic Treated With IFN-α
Body weight (g)	15.1 ($\pm$ 0.6)	15.1 ($\pm$ 0.7)	14.8 ($\pm$ 0.6)
Cryoglobulins (no./studied sera)	0/15	12/12	10/10
Blood urea nitrogen (mg/dL)	22.7 ($\pm$ 2)	29.6* ($\pm$ 1.6)	27.4 ($\pm$ 2)
Albuminuria (μg albumin/mg creatinine)	25 ($\pm$ 4.6) (n = 11)	89 ($\pm$ 34) (n = 11)	70 ($\pm$ 30) (n = 9)
Spleen weight (g)	0.06 ($\pm$ 0.002)	0.37† ($\pm$ 0.02)	0.39† ($\pm$ 0.03)
Liver weight (g)	0.7 ($\pm$ 0.05)	0.99† ($\pm$ 0.037)	0.99† ($\pm$ 0.037)
Lung weight (g)	0.11 ($\pm$ 0.004)	0.29† ($\pm$ 0.013)	0.27† ($\pm$ 0.014)
Kidney weight (g)	0.21 ($\pm$ 0.006)	0.21 ($\pm$ 0.01)	0.21 ($\pm$ 0.01)

NOTE. Values are mean ($\pm$SEM).
*$P < 0.05$ versus wild-type.
†$P < 0.001$ versus wild-type.

IFN-α–treated animals had surprisingly severe glomerular lesions, and two deaths occurred in the treatment group. Oral IFN promotes a T-helper type I immune response.[18] It is likely that an increased T-helper type I response is counterproductive in many forms of glomerulonephritis.[34]

In summary, the oral administration of IFN-α in a murine model of mixed cryoglobulinemia was of limited benefit to glomerular pathology. The treatment seemed to decrease the accumulation of macrophages and glomerular hypertrophy but not glomerular matrix deposition. Although oral IFN-α treatment would be an attractive alternative treatment option in humans, our data do not indicate that this agent effects a major improvement in non–hepatitis-associated type III cryoglobulinemia–associated MPGN.

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