Pediatr Nephrol (2008) 23:645–650
DOI 10.1007/s00467-007-0664-2

BRIEF REPORT

Use of mizoribine as a rescue drug for steroid-resistant pediatric IgA nephropathy

Yohei Ikezumi · Toshiaki Suzuki · Tamaki Karasawa · Hiroshi Kawachi · David J. Nikolic-Paterson · Makoto Uchiyama

Received: 20 August 2007 / Revised: 25 September 2007 / Accepted: 5 October 2007 / Published online: 24 November 2007
© IPNA 2007

Abstract Recent clinical trials have shown a beneficial effect of mizoribine (Miz), an immunosuppressive drug, in the treatment of new-onset pediatric IgA nephropathy (IgAN). In this study, we evaluated the efficacy of Miz treatment in three children with established steroid-resistant IgAN. The patients had IgAN featuring persistent proteinuria and diffuse mesangial proliferation and had failed to respond to 2 years of treatment with prednisolone. Based upon the second biopsy results, patients were given methylprednisolone (mPSL) pulse therapy that induced a transient reduction in proteinuria, which was reversed when the mPSL dose was tapered. Miz therapy was then instigated in place of pulse mPSL. All three patients showed a substantial reduction in proteinuria and resolution of hematuria within 5 months. A follow-up biopsy in two of the patients showed a substantial reduction in the severity of glomerular lesions and a decrease in the number of activated macrophages. In conclusion, Miz therapy was found to be a safe and effective therapy in three cases of steroid-resistant pediatric IgAN. The ability of Miz to reduce the number of activated macrophages may be an important mechanism by which this drug ameliorated renal disease in these patients.

Keywords IgA nephropathy · Childhood · Mizoribine · Activated macrophage · Heat shock protein (HSP) 60 · 14-3-3 protein

Y. Ikezumi (✉) · T. Suzuki · T. Karasawa · M. Uchiyama
Department of Pediatrics,
Niigata University Medical and Dental Hospital,
Asahimachi-dori,
Niigata 951-8510, Japan
e-mail: ikezumi@med.niigata-u.ac.jp

H. Kawachi
Department of Cell Biology, Institute of Nephrology,
Niigata University Graduate School of Medical and Dental Sciences,
Niigata, Japan

D. J. Nikolic-Paterson
Department of Nephrology, Monash Medical Centre,
Clayton, VIC, Australia

D. J. Nikolic-Paterson
Monash University Department of Medicine,
Monash Medical Centre,
Clayton, VIC, Australia

Introduction

IgA nephropathy (IgAN) is the most common form of glomerulonephritis in Japan and worldwide, and approximately 10–30% of pediatric or adult patients with this disorder develop end-stage renal disease within 20 years [1–3]. Although the pathogenic mechanisms in IgAN have yet to be fully characterized, it has been postulated that glomerular damage is secondary to the deposition of IgA-containing immune complexes, leading to the use of immunosuppressive drugs such as steroids, cyclophosphamide, azathioprine and, more recently, mizoribine (Miz) in the treatment of IgAN [4–6].

Miz is an immunosuppressant that was developed in Japan and has been reported to be effective in the treatment of kidney diseases, including idiopathic nephrotic syndrome, lupus nephritis and new-onset IgAN. Miz acts through inhibition of both T and B lymphocyte proliferation, which can suppress activation of the immune system and local inflammation. However, the mechanisms through which Miz suppresses kidney disease have not been clarified. Of relevance to this question, our recent studies have shown the importance of leukocyte infiltration, in particular macrophages, in the pathogenesis and progression of IgAN [7, 8].

Thus, suppression of macrophage activation is a potential unexplored mechanism by which Miz may suppress the progression of IgAN.

We report here on three children with IgAN that was successfully treated with Miz after long-term steroid therapy. The mechanism by which Miz induced reduction of urinary protein and red blood cell excretion is discussed.

Case reports

Three girls (patients 1, 2 and 3) aged 11 years, 8 years and 14 years, respectively, presented with microscopic hematuria or recurrent gross hematuria, which was preceded by an episode of recurrent macroscopic hematuria, and the development of sustained proteinuria that increased up to approximately 1.0 g/day. Because of sustained symptoms, renal biopsy was performed when the girls were aged 12 years, 9 years and 15 years respectively, and IgAN with diffuse segmental mesangial cell proliferation and moderate matrix expansion was diagnosed. There were 22, 29 and 30 glomeruli in the biopsy specimens, respectively, with cellular crescents in 4.5%, 3.4% and 6.7% of glomeruli (the clinical parameters and histological features are summarized in Table 1). All three patients were put onto a standard treatment protocol of prednisolone (PSL) and anti-coagulants (warfarin and dipyridamole) for 2 years. PSL was started with 1 mg/kg orally every day for 4 weeks, then followed by 1 mg/kg per 2 days for 1 month, gradually tapering to 0.5 mg/kg per 2 days during the next 10 months, and then maintained at this level for 1 year. Warfarin and dipyridamole were used at 1 mg/day to 2 mg/day and 3 mg/kg per day to 5 mg/kg per day, respectively, for 2 years. A reduction in the severity of proteinuria was observed after the beginning of the steroid-combination therapy; however, this was reversed upon recurrent upper respiratory tract infection in all three patients. Each patient underwent tonsillectomy approximately 8 months after the first biopsy, and combined angiotensin-converting enzyme (ACE) inhibitor and angiotensin II receptor blocker (ARB) was added to the treatment course. However, proteinuria remained at around 0.5 g/day. A second biopsy was performed in all three patients at the end of second year of steroid treatment and revealed 19, 42 and 21 glomeruli and the presence of active lesions (cellular or fibro-cellular crescents) in 10.5%, 4.8% and 23.8% of glomeruli,

Table 1 Clinical and histological features of the three patients

Patient		1	2	3
Gender		Female	Female	Female
Age at onset (years)		11.2	8.3	14.1
At first biopsy	Age (years)	12.8	9.6	14.8
	Proteinuria (g/day)	0.34	0.62	0.60
	Hematuria (score)	1	4	4
	Glomerular lesion (score)	1.6±1.1	5.3±1.7	3.0±1.3
	Interstitial lesion (score)	1.7±0.8	0.3±0.6	1.8±1.1
	Glomerular Sn+MQ (/gcs)	0.2±0.4	1.4±1.0	0.2±0.4
	Interstitial Sn+MQ (/hpf)	4.7±1.7	11.6±2.7	7.2±1.7
At second biopsy	Age	15.1	11.9	16.9
	Proteinuria	0.46	0.26	0.38
	Hematuria	1	1	1
	Glomerular lesion	5.8±1.1	5.0±1.0	7.3±0.8
	Interstitial lesion	2.0±0.8	1.8±0.8	2.1±0.7
	Glomerular Sn+MQ	2.0±1.0	1.9±0.9	3.3±1.6
	Interstitial Sn+MQ	9.7±2.7	10.5±3.6	10.5±3.1
Age at which Miz therapy was started		16.1	13.5	17.9
At third biopsy	Age	18.0	14.8	ND
	Proteinuria	0.14	0.04	
	Hematuria	0.5	0.5	
	Glomerular lesion	1.6±0.8[a]	2.7±0.5[a]	
	Interstitial lesion	1.7±1.0	2.0±0.7	
	Glomerular Sn+MQ	1.0±0.8[c]	0	
	Interstitial Sn+MQ	5.0±1.8[b]	4.3±0.82[b]	

At least 12 glomeruli or nine high-power fields in the interstitial area were scored or counted, and the mean values were compared by Mann–Whitney test (*gcs* glomerular cross-section, *hpf* high-power field, *Sn* sialoadhesin, *MQ* macrophage).
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ compared with parameters at the second biopsy

respectively. Based upon the biopsy results, methyl prednisolone (mPSL) pulse therapy (1 g/day, once in 2 weeks) was given to patients 1 and 3 for 6 months. A transient improvement in proteinuria was seen during the mPSL pulse therapy; however, this was reversed when the dose of mPSL was tapered. Because of the sustained proteinuria and hematuria, Miz therapy was stared when the girls were 16 years, 13 years and 17 years of age, respectively—more than 3 years after the start of steroid therapy. Although the original Miz administration was 2.5–4.0 mg/kg (maximum 150 mg) per day, orally, divided into two or three doses [9], our patients were given 150 mg once a day to keep their maximum blood concentration at more than 1.0 μg/ml. A significant decrease in proteinuria and hematuria was seen in all three patients within 5 months of Miz treatment (Fig. 1), which allowed further reduction in the dose of PSL. A third renal biopsy was performed 2 years or 18 month after the commencement of Miz therapy in patients 1 and 2, respectively. A third biopsy was not performed in patient 3 because of the benign course after the Miz treatment.

Quantification of histologic damage in renal biopsies

Periodic acid–Schiff (PAS) and periodic acid–methenamine silver stained paraffin-embedded sections were analyzed by an independent anatomy pathologist who was blinded to both clinical data and the quantification of macrophage accumulation. Glomeruli were scored for proliferative lesions, segmental sclerotic lesions, and crescents, as previously described [7]. The degree of interstitial fibrosis/tubular atrophy was scored according to area involved: 0 (none), 1 (0 to 5%), 2 (6 to 25%), 3 (25 to 50%) and 4 (>50%). At least nine high-power fields were evaluated for each patient and expressed as mean ± SD.

Antibodies

Antibodies used in this report were: HSn7D2, anti-sialoadhesin (Sn) (mouse IgG1; Serotec, Oxford, UK); Y1/82A, anti-CD68 (mouse IgG2b; BD Biosciences Pharmingen, San Diego, CA, USA); anti-14-3-3ε (mouse IgM; BD Biosciences Pharmingen); N–20, goat anti-heat shock protein (HSP) 60 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); fluorescein isothiocyanate (FITC)-conjugated antibodies to mouse IgG1, mouse IgG2a, mouse IgM, goat IgG, and tetramethyl-rhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG2b (all from Southern Biotechnology Associates, Birmingham, AL, USA).

Immunofluorescence

Macrophages were detected in 3 μm frozen sections by indirect immunofluorescence. The number of cells stained for Sn or CD68 antigens were counted at least in 12 glomeruli per patient. Interstitial cells stained for Sn or CD68 antigens were counted in at least in nine high-power fields (×400). Scoring was performed on blinded slides.

Localization of the Miz binding proteins, 14-3-3 and HSP60, was also examined by immunofluorescence staining. Renal tissue from the uninvolved portions of adult renal carcinoma nephrectomies was used as the normal tissue control.

Results

Proteinuria and hematuria

The clinical course of cases 1 to 3 are summarized in Table 1 and illustrated in Fig. 1. Both proteinuria and hematuria were significantly reduced after the commencement of Miz treatment compared with the mean range for the 6 months prior to Miz treatment. All three patients showed a significant response within 3 months of their beginning Miz treatment.

Renal histology

Significant renal damage was evident on the second biopsy in all three patients, with severe glomerular and interstitial lesions (Fig. 2a,b). Two of the patients, patients 1 and 2, underwent a third biopsy 18 months and 24 months,

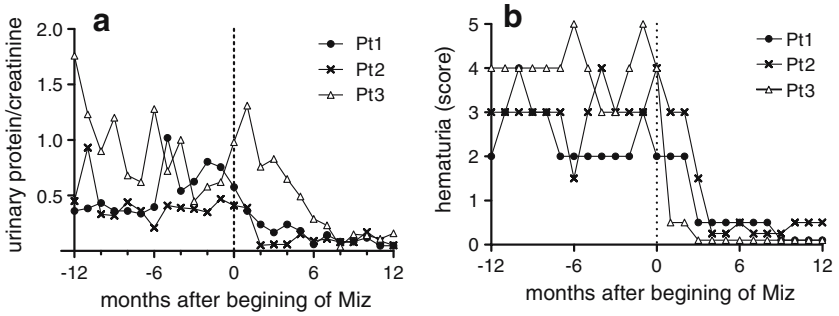

Fig. 1 Changes in the degree of proteinuria (**a**) and hematuria (**b**) before and after Miz treatment

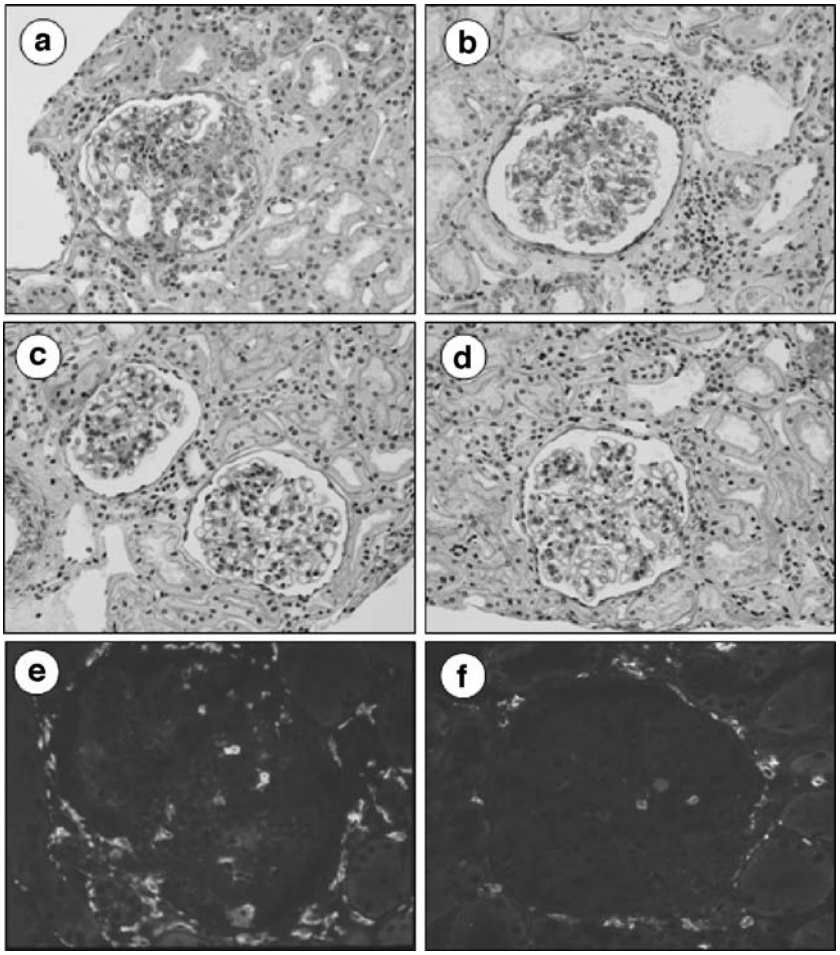

Fig. 2 Representative photographs of PAS staining from patient 1 at the second biopsy (**a**, **b**) showing marked glomerular cell proliferation with cellular crescents (**a**) and interstitial accumulation of leukocytes. The third biopsy from patient 1, following Miz treatment (**c**, **d**), shows an absence of active lesions. Immunofluorescence staining shows numerous Sn+ activated macrophages in the glomerulus and interstitium at the second biopsy of patient 1 (**e**), and the presence of Sn+ macrophages was significantly reduced at the third biopsy (**f**)

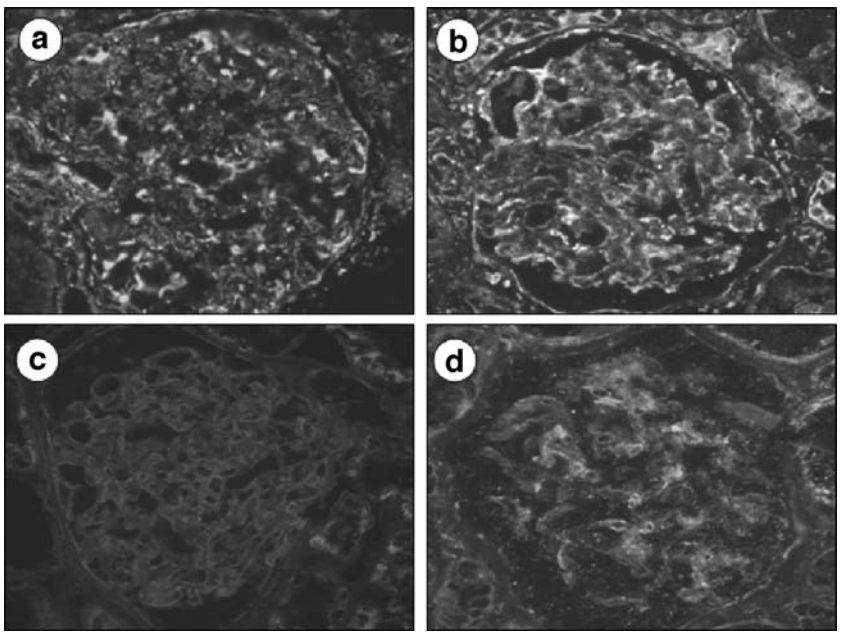

Fig. 3 Immunofluorescence staining shows ubiquitous glomerular expression of the 14-3-3ε protein in normal kidney (**a**) and in a patient with IgAN (**b**). In contrast, HSP60 is absent in normal glomeruli (**c**), but is clearly expressed within inflamed glomeruli in a patient with IgAN (**d**)

respectively, after beginning their Miz treatment. A marked improvement in renal histology was evident following Miz treatment (Fig. 2c,d, Table 1).

Macrophages and Miz binding proteins

Analysis of the second protocol biopsy in the three steroid-resistant patients showed substantially elevated numbers of Sn+ macrophages in both glomeruli and the interstitium (Fig. 2c,d). The third biopsy, after the commencement of Miz treatment, showed a substantial reduction in the number of glomerular and interstitial macrophages, including Sn+ activated macrophages (Table 1).

Immunofluorescence staining for Miz binding proteins was performed. As shown in Fig. 3, the 14-3-3ε protein is ubiquitously expressed in the glomeruli of normal human kidney and in IgAN. In contrast, HSP60 is absent from normal glomeruli but is expressed in IgAN (Fig. 3).

Discussion

We report here on three patients with steroid-resistant pediatric IgAN who were successfully treated with Miz beginning more than 3 years after the commencement of steroid therapy. Previous studies have shown the efficacy of Miz treatment in IgAN when used in combination with steroids and anti-coagulants [4–6]. However, those studies used Miz in a combination therapy from the beginning of treatment. In contrast, in our study, our findings argue that Miz is also effective in the treatment of patients with chronic steroid-resistant IgAN.

Our previous studies have suggested that Sn-expressing activated macrophages play an important role in the progression of various types of glomerulonephritis, including IgAN [7, 8]. In this study, Miz treatment significantly reduced glomerular and interstitial lesions and significantly reduced the number of activated Sn+ macrophages in both compartments. Miz has been reported to act as a selective inhibitor of inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, which results in suppression of T and B cell proliferation [10]. The ability of Miz treatment to reduce the number of activated macrophages in patients with IgAN could be due to an indirect effect of suppressing T cell-mediated recruitment and activation of macrophages. Alternatively, this could be due to a direct effect of the drug on macrophage function, a possibility supported by experimental studies showing that Miz treatment can ameliorate interstitial fibrosis by preventing macrophage accumulation [11, 12], and that Miz can exert direct effects on macrophage function in vitro [13].

It has recently been reported that 14-3-3 proteins, which are Miz-binding proteins, interact with the glucocorticoid receptor, and that the interaction may enhance the transcriptional activity of the receptor, suggesting a steroid-sparing effect of Miz [14]. Miz has also been reported to bind to HSP 60 [15]. We found that 14-3-3 protein and HSP60 are expressed by glomerular cells of patients with IgAN, suggesting that Miz may act directly on intrinsic renal cells. The potential mechanism whereby Miz may ameliorate glomerular injury via these binding proteins has not been elucidated; however, it has been reported that HSP60 is induced in experimental kidney disease and aggravates renal injury as an immunologic danger signal [16]. Together with our finding that HSP60 is expressed only in inflamed glomeruli, it may be that Miz inhibits signal transduction mechanisms that lead to glomerular injury. Thus, Miz may protect glomeruli against progressive damage in IgAN by effects on intrinsic renal cells and on cells of the immune system (macrophages and T cells), together with enhancing steroid responsiveness.

It is noteworthy that Miz causes far fewer complications in patients than azathioprine does, and it does not appear to cause significant myelosuppression or nephrotoxicity [10]. Since long-term, high-dose, steroid therapy results in severe side effects, Miz might be useful to reduce the dose of steroid without reducing the efficacy of treatment.

In conclusion, Miz is a useful drug for treatment of pediatric IgAN. Although the precise mechanism by which Miz treatment suppresses IgAN is not yet clear, our findings suggest that inhibition of macrophage activation may be an important mechanism by which Miz suppresses steroid-resistant IgAN.

Acknowledgments This work was supported by Grant-Aids for Scientific Research (C: No.19791633 to Y.I.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, The Mother and Child Health Foundation, and the Study Group on IgA nephropathy, as well as Grant-Aids for Promotion of Niigata University Research Projects from Niigata University.

References

1. Kusumoto Y, Takebayashi S, Taguchi T, Harada T, Naito S (1987) Long-term prognosis and prognostic indices of IgA nephropathy in juvenile and adult Japanese. Clin Nephrol 28:118–124
2. Wyatt RJ, Kritchevsky SB, Woodford SY, Miller PM, Roy S 3rd, Holland NH, Jackson E, Bishof NA (1995) IgA nephropathy: long-term prognosis for pediatric patients. J Pediatr 127:913–919
3. Yoshikawa N, Ito H, Nakamura H (1988–1989) IgA nephropathy in children from Japan Clinical and pathological features. Child Nephrol Urol 9:191–199
4. Nagaoka R, Kaneko K, Ohtomo Y, Yamashiro Y (2002) Mizoribine treatment for childhood IgA nephropathy. Pediatr Int 44:217-223
5. Kawasaki Y, Suzuki J, Sakai N, Etoh S, Murai H, Nozawa R, Suzuki H (2004) Efficacy of prednisolone and mizoribine therapy for diffuse IgA nephropathy. Am J Nephrol 24:147-153

6. Kawasaki Y, Hosoya M, Suzuki J, Onishi N, Takahashi A, Isome M, Nozawa R, Suzuki H (2004) Efficacy of multidrug therapy combined with mizoribine in children with diffuse IgA nephropathy in comparison with multidrug therapy without mizoribine and with methylprednisolone pulse therapy. Am J Nephrol 24:576-581
7. Ikezumi Y, Suzuki T, Hayafuji S, Okubo S, Nikolic-Paterson DJ, Kawachi H, Shimizu F, Uchiyama M (2005) The sialoadhesin (CD169) expressing a macrophage subset in human proliferative glomerulonephritis. Nephrol Dial Transplant 20:2704-2713
8. Ikezumi Y, Suzuki T, Imai N, Ueno M, Narita I, Kawachi H, Shimizu F, Nikolic-Paterson DJ, Uchiyama M (2006) Histological differences in new-onset IgA nephropathy between children and adults. Nephrol Dial Transplant 21:3466-3474
9. Hamasaki T, Mori M, Kinoshita Y, Saeki T, Sakano T (1997) Mizoribine in steroid-dependent nephrotic syndrome of childhood. Pediatr Nephrol 11:625–627
10. Hughes SE, Gruber SA (1996) New immunosuppressive drugs in organ transplantation. J Clin Pharmacol 36:1081–1092
11. Sato N, Shiraiwa K, Kai K, Watanabe A, Ogawa S, Kobayashi Y, Yamagishi-Imai H, Utsunomiya Y, Mitarai T (2001) Mizoribine ameliorates the tubulointerstitial fibrosis of obstructive nephropathy. Nephron 89:177-185
12. Kikuchi Y, Imakiire T, Yamada M, Saigusa T, Hyodo T, Hyodo N, Suzuki S, Miura S (2005) Mizoribine reduces renal injury and macrophage infiltration in non-insulin-dependent diabetic rats. Nephrol Dial Transplant 20:1573-1581
13. Zhong B, Tajima M, Takahara H, Nochi H, Tamoto K, Tamura N, Kobayashi S, Tamura Y, Ikeda M, Akimoto T, Yoshino S, Hashimoto H (2005) Inhibitory effect of mizoribine on matrix metalloproteinase-1 production in synovial fibroblasts and THP-1 macrophages. Mod Rheumatol 15:264-268
14. Takahashi S, Wakui H, Gustafsson JA, Zilliacus J, Itoh H (2000) Functional interaction of the immunosuppressant mizoribine with the 14-3-3 protein. Biochem Biophys Res Commun 274:87–92
15. Itoh H, Komatsuda A, Wakui H, Miura AB, Tashima Y (1999) Mammalian HSP60 is a major target for an immunosuppressant mizoribine. J Biol Chem 274:35147-35151
16. Lang A, Benke D, Eitner F, Engel D, Ehrlich S, Breloer M, Hamilton-Williams E, Specht S, Hoerauf A, Floege J, von Bonin A, Kurts C (2005) Heat shock protein 60 is released in immune-mediated glomerulonephritis and aggravates disease: in vivo evidence for an immunologic danger signal. J Am Soc Nephrol 16:383-931