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Serum Levels of Myeloid-Related Protein 8/14 (MRP8/14): Dynamics and Diagnostic Relevance after Kidney Transplantation

In a total of 723 sera from 52 kidney graft recipients and 23 organ donors the serum concentrations of MRP8/14 were retrospectively measured by an enzyme immunoassay in one run. The aim of this study was to find out quantitative changes of the MRP8/14 serum levels in connection with characterized events after kidney transplantation (KTx). In the pre-KTx sera tested the mean MRP8/14 level was 6.7 ± 3.5 µg/ml (reference value, 6.08 ± 2.22 µg/ml; upper normal level [x + 2s], 10.96 µg/ml). In 7 out of 52 recipients without intraoperative ATG prophylaxis the MRP8/14 level rose within the reference range to 10.2 ± 6.2 at the first post-KTx day. In contrast, the intraoperative T cell depletion by means of antilymphocytic antibodies caused a significant increase of the MRP8/14 level to 21.2 ± 12.5 µg/ml. In recipients with immediate (n=14) or delayed (n=11) graft function without any other complications all post-KTx values - except the post-KTx peak - were within the reference range. In 10 prednisolone-sensitive and 14 prednisolone-resistant rejection episodes no diagnostically usable changes of the MRP8/14 levels could be found except in one antibody-mediated accelerated rejection. Also, in 6 recipients with cytomegalovirus infection, the detection of pp65 positive peripheral blood cells was not associated with alterations of the MRP8/14 serum level. In contrast, significant elevations of the MRP8/14 levels were found in gram-positive bacteremia as well as in superinfected (CMV, soor) pneumocystis carinii pneumonias. In most cases the changes of MRP8/14 levels were comparable with those described for the lipopolysaccharide-binding protein, but in simultaneous determinations no correlation (R^2=0.067) could be found between the concentrations of both proteins at the same day. In contrast, the C-reactive protein rose in all kinds of inflammatory events, although to very different levels.

Key words:
kidney transplantation, MRP8/14, lipopolysaccharide-binding protein, CRP, CMV, pneumocystis carinii pneumonia, rejection

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Kaden J, May G, Strobelt V, Mauracher S, Zecher A, Wesslau C (2003) Serum Levels of Myeloid-Related Protein 8/14 (MRP8/14): Dynamics and Diagnostic Relevance after Kidney Transplantation. Tx Med 15: 31-39

Dynamik und diagnostische Bedeutung des MRP8/14-Serumspiegels nach Nierentransplantation

In einer retrospektiven Studie wurden 723 Seren von 52 Nierentransplantatempfängern und 23 Organspendern mittels Enzymimmunassay in einem Ansatz auf die Konzentration des von aktivierten Monozyten/Makrophagen und Granulozyten gebildeten MRP8/14-Komplexes untersucht. Ziel der Studie war die Erfas-

sung quantitativer Veränderungen des MRP8/14-Serumspiegels bei definierten Ereignissen nach Nierentransplantation (NTx). In den prä-NTx untersuchten Seren betrug der mittlere MRP8/14-Spiegel 6.7 ± 3,5 µg/ml (Normalbereich: 6.08 ± 2.22 µg/ml; obere Normgrenze [x+2s]: 10.96 µg/ml). Bei 7 der 52 Transplantatempfänger ohne intraoperative ATG-Prophylaxe stieg der MRP8/14-Spiegel am 1. postoperativen Tag auf 10,2 ± 6,2 µg/ml an, blieb dabei im Normbereich. Die intraoperative T-Zelldepletion mittels antilymphozytärer Antikörper war hingegen mit einem signifikanten Anstieg des MRP8/14-Spiegels auf 21,2 ± 12,5 µg/ml verbunden. In der postoperativen Periode lagen sowohl bei Patienten mit Transplantatsofortfunktion (n = 14) als auch mit verzögerter Funktionsaufnahme des Transplantates (n = 11) alle MRP8/14-Werte - bis auf den postoperativen Anstieg - innerhalb des Referenzbereiches. Im Zusammenhang mit 10 Prednisolon-sensitiven und 14 Prednisolon-resistenten Rejektionskrisen traten - mit Ausnahme einer akzelerierten Rejektion - keine diagnostisch verwertbaren Änderungen des MRP8/14-Spiegels auf. Auch im Zusammenhang mit dem Nachweis CMV-pp65-positiver Zellen im peripheren Blut blieben die MRP8/14-Spiegel im Referenzbereich. Signifikante MRP8/14-Anstiege wurden hingegen im Zusammenhang mit Bakteriämien sowie bei superinfizierten Pneumozystis carinii-Pneumonien (CMV, Soor) gefunden. Die beobachteten MRP8/14-Konzentrationsänderungen sind in ihrer Dynamik vielfach denen des Lipopolysaccharid-bindenden Proteins (LBP) vergleichbar, obwohl eine Korrelation zwischen beiden Proteinen bei Parallelbestimmung nicht nachgewiesen werden konnte (R^2 = 0,067). Das C-reaktive Protein reagierte hingegen, wenn auch mit sehr unterschiedlichen Konzentrationserhöhungen, auf alle entzündlichen Ereignisse.

Schlüsselwörter:
Nierentransplantation, MRP8/14, Lipopolysaccharid-bindendes Protein, CRP, CMV, Pneumocystis carinii-Pneumonie, Rejektion

Introduction

In 1990 Mues et al. [1] reported on the accumulation of monocytes/macrophages in endomyocardial biopsies of patients suffering from myocarditis which could be stained with the monoclonal antibody 27E10. This antibody detects a differentiation antigen present on a subtype of monocytes/macrophages appearing in an early stage of inflammatory reaction [2]. Using the 27E10 antibody in affinity purification of the antigen from cellular extracts only two bands at 8 and 14 kDa were seen under reducing conditions which could be identified as MRP8 and MRP14 (rev. by [3]). Whilst MRP originally stands for **M**igration inhibition factor-**R**elated **P**roteins, their expression by myeloic/monocytic cells leads to the term **M**yeloid-**R**elated **P**roteins. After sequencing of MRP8 and 14 their identity with Calgranulin A and B [4], Calprotectin [5], the p8 and p14 proteins [6], the heavy and light chain of the L1 antigen [7], S100A8 and S100A9 [8] and the cystic fibrosis antigen (MRP8 [9, 10]) was noticed. These proteins belong to the S100 protein family [11, 12] which is implicated in Ca^{2+} dependent regulation of a variety of intracellular activities.

Already in 1989, Steinhoff et al. [13] described accumulations of $27E10^+$ macrophages in liver graft biopsies during acute rejections but also in infectious complications as virus hepatitis, cholangitis and sepsis. In 1994, Goebeler et al. [14] demonstrated for the first time differences in MRP8/14 complex assembly by renal graft infiltrating monocytes in situ. The MRP8/14 complex formation in monocytes was only found in acutely rejected grafts, but not in chronic graft rejection despite the expression of MRP8 and MRP14 monomers. In 1995, Burkhardt et al. [15] described massive infiltrations of kidney grafts with $MRP8/14^+$ cells as reliable, early sign of acute cellular rejection. The simultaneously measured MRP8/14 serum levels correlated with the number of $MRP8/14^+$ cells in immunohistology. Using a sandwich enzyme-linked immunosorbent assay Burkhardt et al. [16] achieved a 100% specificity and sensitivity for the diagnosis of rejection one day before antirejection therapy with a MRP8/14 serum level cutoff of 4.2 µg/ml (normal values: 0.6 ± 0.2 µg/ml). In urinary tract infections, delayed graft function (DGF) or cytomegalovirus (CMV) infections the MRP8/14 serum levels were below the cutoff. These data were reasons enough to check the MRP8/14 serum levels with respect to recognize ongoing acute rejections and to differentiate rejections from infections. In a first paper [17] we presented preliminary results in terms of individual courses showing elevated MRP8/14 serum levels after intraoperative high dose antilymphocyte globulin bolus as induction therapy, in nearly all cases of serious bacterial infections, in superinfected pneumocystis carinii pneumonias and in one case of humoral rejection. Now, we want to present the complete MRP8/14 data of 52 kidney graft recipients.

Material and Methods

Study Population

A total of 45 recipients (mean age, 43.8 ± 11.2 years; females, 16; males, 29) who underwent cadaveric kidney trans-

plantation [KTx] between April 1994 and February 1997 at the Kidney Transplant Centre in Berlin-Friedrichshain and received an intraoperative anti-T-lymphocyte globulin bolus (ATG-Fresenius) were included in this retrospective study. In addition, seven recipients (mean age 44.3 ± 14.8 years; females, 2; males, 5) who underwent cadaveric KTx between August 1989 and January 1990 served as a control group in order to study the influence of the surgical trauma without the concomitant intraoperative infusion of polyclonal antilymphocytic antibodies. From these recipients only the pre- and postoperative sera (n=14) were analyzed. Beside this, 23 sera from the appropriate graft donors obtained shortly before explantation were available for studying.

Thus, a total of 723 serum samples (686 from kidney graft recipients with ATG, 14 from recipients without ATG and 23 from kidney graft donors) kept at -20 °C were retrospectively analyzed in one run.

Reference values were obtained from 991 healthy volunteers (female 498, male 493) by the test kit manufacturer.

Study Design

Concentrations of MRP8/14 were measured retrospectively in serum samples from recipients with the following well-characterised postoperative courses or complications:

A. Immediate post-KTx graft function without any complication within the first 3 weeks (n=14).
B. Delayed graft function [DGF] without any other complication within the first 3 weeks (n=11).
C. Influence of surgical trauma with (n=17) or without (n=7) intraoperative antilymphocyte bolus infusion (comparison of pre-KTx and day 1 post-KTx serum levels).
D. Steroid-sensitive rejection episodes without signs of concomitant infections (n=10).
E. Steroid-resistant rejections without signs of concomitant infections (n=14).
F. Cytomegalovirus-infections (n=6).
G. Patients with bacteremia (n=5) or other major infections (eg pneumonia, purulent extravasate, heavily infected grafts).

Immunosuppression

Basic immunosuppression for all recipients in this study consisted of azathioprine (AZA), corticosteroids and ciclosporine. Details of our immunosuppressive regimen have already been published (18, 19). Briefly, the majority of patients received 4 mg/kg body weight (bw) AZA in their dialysis unit immediately before being called to the transplant centre. Since February 1990 [20] all recipients have received the Friedrichshain variant of antilymphocyte globulin induction therapy consisting of an intraoperative high-dose single ATG bolus (ATG-Fresenius, Gräfelfing, Germany, 9 mg/kg bw) in the operating room before completion of anastomoses (ie, the removal of clamps) via a central venous catheter. To avoid a cytokine release syndrome, 500 mg methylprednisolone [MP] were given about 60 min pre-ATG. Post-KTx the recipients received 40 mg MP for 7 days, subsequently switching to 35 mg prednisolone for 14 days tapering to 10-15 mg/day. Oral ciclosporine was started within 24 hours of surgery. During the first postoperative week a maintenance ciclosporine level of 100 ng/ml and thereafter of 200 ng/ml was given. AZA was restarted after surgery at a dose of 1 mg/kg per os and maintained as long as the leukocyte count was above 4 Gpt/l.

A total of five recipients were switched from AZA to mycophenolate mofetil in the postoperative course at days 4, 5, 20, 280, 306 and 598 and three recipients were switched from ciclosporine to FK 506 at days 30, 32 and 38.

Rejection

For the diagnosis of rejection, the following clinical and laboratory signs were decisive: enlargement and tenderness of the graft, an increase in serum creatinine, concomitant changes in blood urea nitrogen, oliguria, albuminuria, immunoglobulinuria, sonographic changes and immunoactivation in fine-needle aspiration cytology [21]. The treatment consisted of 5 mg/kg bw MP for 5 consecutive days. Biopsy proven MP resistant rejections were tried to reverse by polyclonal antilymphocyte globulin using a dose-by-T-cell protocol (aspired values: 50-150 T- cells/µl). The relative number of T cells ($CD3^+$) was determined by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany) using IQP monoclonal antibodies (IQ Products, Groningen, The Netherlands). OKT3 (2.5 mg for 10 days; CILAG, Sulzbach, Germany) was given as rescue therapy or primarily in cases of humoral/ vascular rejections.

Cytomegalovirus (CMV) Infection

All donors and recipients were screened pre-KTx for CMV-specific IgG antibodies. In the CMV-seropositive donor/CMV-seronegative recipient combination, all recipients received CMV immunoglobulins prophylactically (Cytotect, Biotest, Dreieich, Germany) at postoperative days 1 (2 ml/kg), 18 (2 ml/kg) and 35 (1 ml/kg).

Since July 1995, all recipients were screened postKTx for CMV-pp65-antigen at least once a week using the commercially available CINAkit (Argene Biosoft, distributed by VIVA, Hürth/Köln, Germany). Details of test peformance and results have already been published [22].

Post-KTx serological diagnosis of CMV infection was done by the detection of CMV-specific IgM and/or IgG antibodies (IMx CMV IgM and IgG, Abbott, Wiesbaden, Germany).

The treatment of CMV disease depended on the severity of the clinical symptoms and included the application of human immunoglobulins with a high content of CMV-specific antibodies (2mg/kg bw) and/or ganciclovire (up to the disappearance of pp65) as well as the cessation or dose reduction of AZA.

Bacterial Infections

Blood culture tests from patients with signs of systemic infections were performed at several different times. All blood cultures were observed for at least seven days.

The detection of pneumocystis carinii infections was done in bronchoalveolar lavage by means of indirect immunofluorescence.

Detection of Myeloid-related Protein 8/14 (MRP8/14) in Serum

Serum samples for MRP8/14 assay came from our serum bank, containing all sera taken before transplantation and three times a week thereafter (always between 7.00 and 8.00 a.m.) up to discharge and also after re-hospitalization. MRP8/14 levels were measured in one run by a commercially available assay (MRP8/14 ELISA, Bühlmann Laboratories AG, CH-4123 Allschwil 1, Switzerland) according to the manufacturers guidelines.

Detection of C-reactive Protein (CRP) in Serum

The CRP serum levels were routineously determined by nephelometry (BN 100, Behringwerke AG, Marburg, Germany). For statistical analysis the CRP levels <5 mg/l were considered to be 1 mg/l.

Detection of Lipopolysaccharide-binding Protein (LBP) in Serum

LBP were measured by a commercially available immunoassay (ImmuliteLBP®, DPC, Los Angeles, USA) according to the manufacturers guidelines. Details of our results have already been published [23, 24].

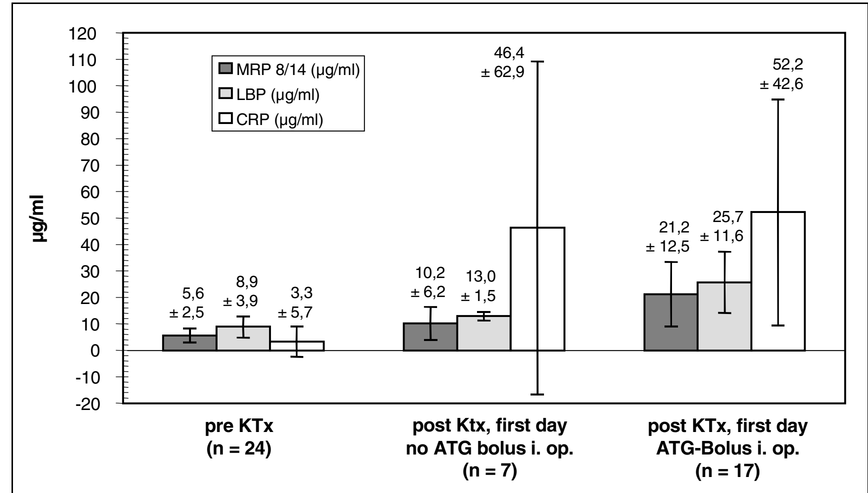

Fig. 1: MRP8/14, LBP and CRP serum levels in kidney graft recipients pre and post transplantation without and with intraoperative T cell depletion

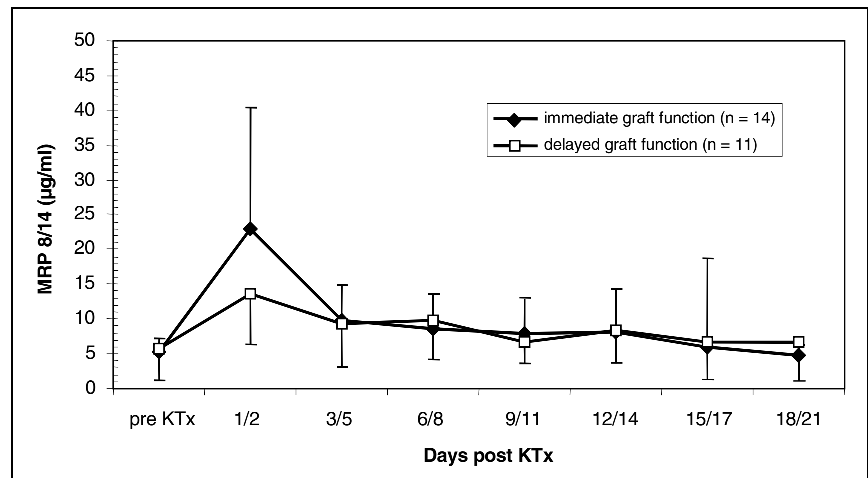

Fig. 2: Pre- and post-transplant dynamics of MRP8/14 serum levels in 25 recipients with immediate or delayed kidney graft function

Results

Reference Values

In a total of 991 serum samples from healthy blood donors (498 females and 493 males) the normal MRP8/14 levels were 6.08 ± 2.44 µg/ml (mean ± 1 standard deviation [SD]) with a normal range from 1.2 to 10.96 µg/ml (mean ± 2SD) [25]. There were no significant sex or age differences.

MRP8/14, LBP and CRP Levels Immediately Pre- and Post Transplantation

Fig. 1 shows the impact of the surgical trauma and the ATG bolus on the post-KTx MRP8/14 serum levels. Immediately pre-KTx in all sera tested (n=24) the MRP8/14 concentrations were within the normal range. The mean MRP8/14 concentration was determined to be 5.6 µg/ml. A total of 7 out of these 24 recipients received no intraoperative ATG bolus. At post-KTx day 1 the mean MRP8/14 concentration in these recipients was slightly increased (10.2 µg/ml; $0.1>p>0.05$) but still within the normal range. In kidney graft recipients prophylactically treated with an intraoperative ATG bolus the mean MRP8/14 serum level was determined to be 21.2 µg/ml at post-KTx day 1 (sera from 17 recipients were available for testing). This concentration was significantly higher than the pre-KTx value ($p < 0.01$). As shown in fig. 1, the LBP serum levels had comparable post-KTx increases. In contrast, CRP showed the highest post-KTx levels independently of the ATG prophylaxis. Considering the mean pre-KTx levels as 100%, the post-KTx levels increased in the non-ATG group to 182.1% for MRP8/14, to 146.1% for LBP and to 1406% for CRP and in the ATG group to 378.6% for MRP8/14, to 288.8% for LBP and to 1581.8% for CRP.

MRP8/14 Serum Levels in Dependence on the Post-Transplant Kidney Function

Fig. 2 shows the behaviour of pre- and post-KTx MRP8/14 serum levels in renal graft recipients with immediate (n=14) or delayed graft function (n=11) without any complication during the post-KTx weeks 1 to 3. Apart from a post-KTx MRP8/14 peak at day 1-2

(see fig. 1) in both groups, all other pre- or posttransplant levels (mean values) were within the normal range. There was no difference in the MRP8/14 post-KTx dynamics between the two different courses analyzed.

MRP8/14, LBP and CRP Serum Levels in Steroid-sensitive Rejections

A total of 10 recipients with well-functioning grafts experiencing steroid-sensitive rejection crises without any signs of a concomitant infection were studied. The mean serum creatinine level rose in the pre-rejection week from 155 to 202µmol/l. After a peak of 224 µmol/l at the beginning of therapy (5 x 5 mg/kg MP), the creatinine level started to decline. As shown in fig. 3, during this 3-week-period the MRP8/14 levels were slightly increased (with high standard deviations) during the pre-rejection period and the MP therapy (between 11.8 and 14.3 µg/ml), but returned to normal in the immediate post-therapy period (7.2 to 7.6 µg/ml).
The LBP level did not change at any time. In contrast, the CRP level slightly increased in the pre-rejection period up to 15 µg/ml, but fell toward zero immediately after beginning the MP therapy.

MRP8/14, LBP and CRP Serum Levels in Steroid-resistant Rejections

In 14 recipients with steroid-resistant rejections the mean creatinine concentration rose in the pre-OKT3/ATG week from 285 µmol/l to 415 µmol/l in spite of the pre-treatment with 5 x 5 mg/kg bw MP. This was the prerequisite for biopsy and starting antibody therapy. As shown in fig. 4, the MRP8/14 levels were hardly influenced by both rejection and antibody therapy.
The LBP level did only slightly (but not significantly) increase from 10.0 µg/ml (day -2/-1 pre OKT3/ATG) to 13 µg/ml (peak at day 1 to 5 during OKT3/ATG).
Thus, the post-KTx MRP8/14 and LBP elevations (fig.1) seen after the intraoperative ATG bolus could not be found after infusion of 'normal' OKT3 (2.5 mg/d) or ATG doses (eg 3 mg/kg bw ATG Fresenius/d).
The CRP levels rose in a typical and already 1981 and 1982 described manner during the pre-rejection period with a

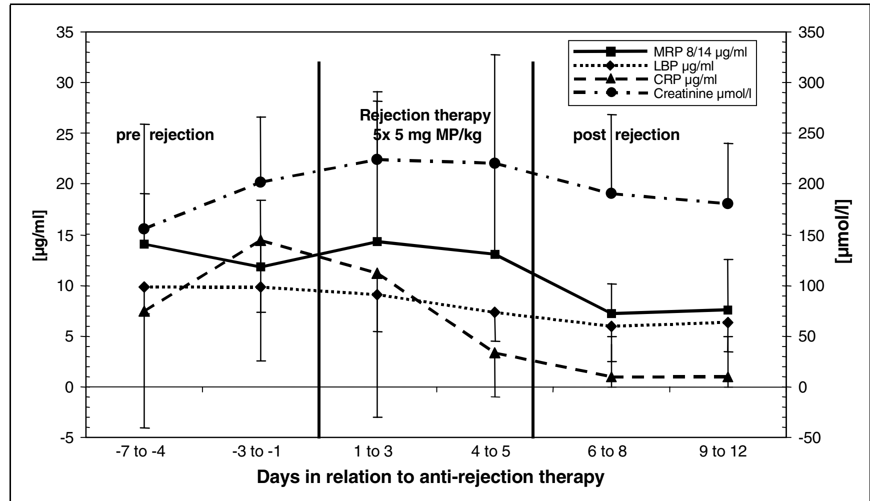

Fig. 3: MRP8/14, LBP, CRP and creatinine serums levels in connection with steroid sensitive rejections treated with methylprednisolone

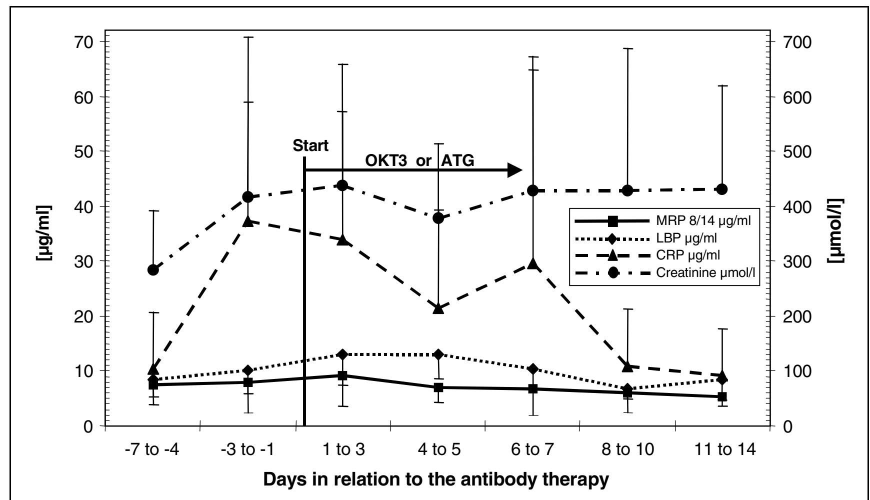

Fig. 4: MRP8/14, LBP, CRP and creatinine serum levels in connection with steroid resistant rejections treated with OKT3 or ATG

tendency to normalize under a successful therapy [26, 27]. In table 1, the data are listed in detail.

MRP8/14 Serum Levels in Non-Rejectors and Rejectors

Because Burkhardt et al. [16] found a significantly increased serum levels of MRP8/14 for several days during the first 2 weeks for the acute rejection group, we compared the MRP8/14 levels of rejectors (n=19) and non-rejectors (n=25) during the first three postoperative weeks. The fig. 5 clearly shows no significant differences of the mean MRP8/14 levels between the two groups during this time. Thus, in our hands the MRP8/14 levels were not predictive for rejections.

MRP8/14, LBP and CRP Serum Level in Relation to Bacteremia

Fig. 6 shows an indisputable association between bacteremia and the elevation of CRP (from <5 to 106 µg/ml), LBP (from 11.9 to 32.1 µg/ml) and MRP8/14 (from 9.5 to 21.7 µg/ml) serum concentrations. At the point of the first positive blood culture the MRP8/14 concentrations in these 5 recipients were 35.7/10.9/21.0/6.6/12.2 µg/ml, the LBP levels were 18.8/ 24.8/34.1/39.5/43.3 µg/ml and the CRP levels were 167.9/134.7/123.1/43.1/64

Tab. 1: Serum levels of MRP8/14, LBP, CRP and creatinine in association with steroid-resistant rejections (n = 11)

	MRP8/14 µg/ml	LBP µg/ml	CRP µg/ml	Creatinine µmol/l
Pre OKT3/ATG				
days -7 bis -4	7.4 ± 3.6	8.3 ± 2.9	10.3 ±10.3	285 ± 106
days –3 bis –1	7.9 ± 5.5	10.0 ± 4.1	37.3 ± 33.5	415 ± 175
During and post OKT3/ATG				
days 1 – 3	9.1 ± 5.5	13.0 ± 5.7	33.8 ± 32.2	437 ± 136
days 4 – 5	7.0 ± 2.8	13.0 ± 4.3	21.3 ± 18.1	377 ± 146
days 6 – 7	6.8 ± 4.8	10.4 ± 3.8	29.6 ± 35.3	428 ± 243
days 8 – 10	6.0 ± 3.4	6.7 ± 1.8	10.9 ± 10.3	428 ± 259
days 11 – 14	5.3 ± 1.6	8.4 ± 3.6	9.1 ± 8.5	430 ± 189

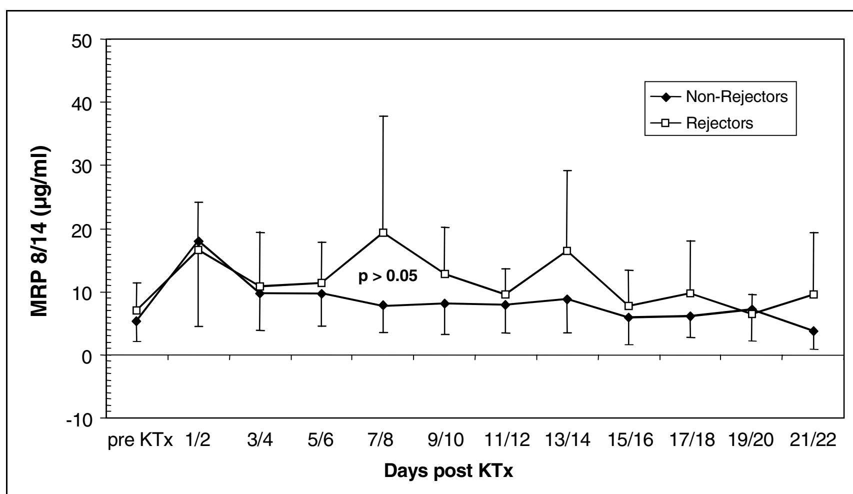

Fig. 5: MRP8/14 serum levels in non-rejectors and rejectors during the postoperative three weeks

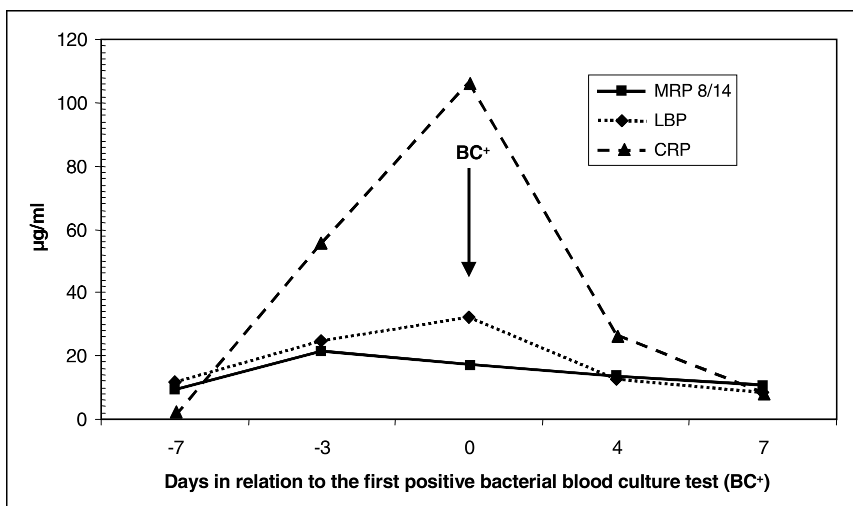

Fig. 6: MRP8/14, LBP and CRP serum levels in five patients with bacteremia

µg/ml. At that time the following bacteria could be identified: staphylococcus aureus (3x), plasmakoagulase-negative staphylococcus (1x), grampositive coccus (1x). Under an efficient antibiotic regimen the MRP8/14, LBP and CRP serum level declined and at day 7 after initiating of antibiotics all elevated protein values were within the normal range again. Table 2 shows the mean values of all three proteins one week before und one week after the detection of bacteremia.

MRP8/14 and LBP Serum Levels in Relation to CMV Antigenemia

Fig. 7 demonstrates both MRP8/14 and LBP serum levels (mean ± 1 SD) in relation to the first detection of the CMV antigen pp65 in peripheral blood polymorphonuclear cells. In order to compare the 6 individual courses the laboratory data were arranged according to the first day of pp65 detection (post-KTx days 15/17/22/23/27/29; x ± s = 22.1 ± 5.4). In fig. 7, the day 1 is the first day of pp65 detection in all recipients. This fig. clearly shows that CMV antigenemia (without concomitant bacterial superinfection) was not associated with elevations of neither MRP8/14 nor LBP levels. Also all CRP values (not shown) were below the cutoff (>5 µg/ml).

MRP8/14 and LBP Serum Levels in Kidney Graft Donors

As already reported [17], the serum levels of both proteins in 23 organ donors (MRP8/14, x±1SD=45±36.8 µg/ml; LBP, x±1SD=32.7±18.4µg/ml) were significantly increased in comparison to the normal values. But, the statistical analysis of the MRP8/14 and LBP concentrations which were measured in the same sera did not show any correlation (R^2=0.067). The individual data are listed in table 3.

Discussion

MRP8 and 14 belong to the S100 family of calcium-binding proteins associated with myeloid cell differentiation (therefore the name **M**yeloid-**R**elated **P**roteins). These proteins are co-expressed and associate to form noncovalent heterodimers in a Ca^{2+} dependent manner [28]. The antimicrobial activity of this complex is maybe one of its major function (therefore the name calprotectin, proposed by Steinbakk et al. [5]). Phagocytes expressing these proteins were described in a variety of inflammatory conditions including rheumatoid arthritis, bowel and lung

Tab. 2: MRP8/14, LBP und CRP serum levels in relation to the detection of bacteremia (grampositive cocci) at the first time (day 0)

	day –7	day –3	day 0	day +4	day +7
MRP8/14 (µg/ml)	9.5	21.7	17.3	13.7	10.7
LBP (µg/ml)	11.9	24.6	32.1	12.6	8.2
CRP (µg/ml)	1.0 (<5.0)	55.5	106.6	26.4	8.4

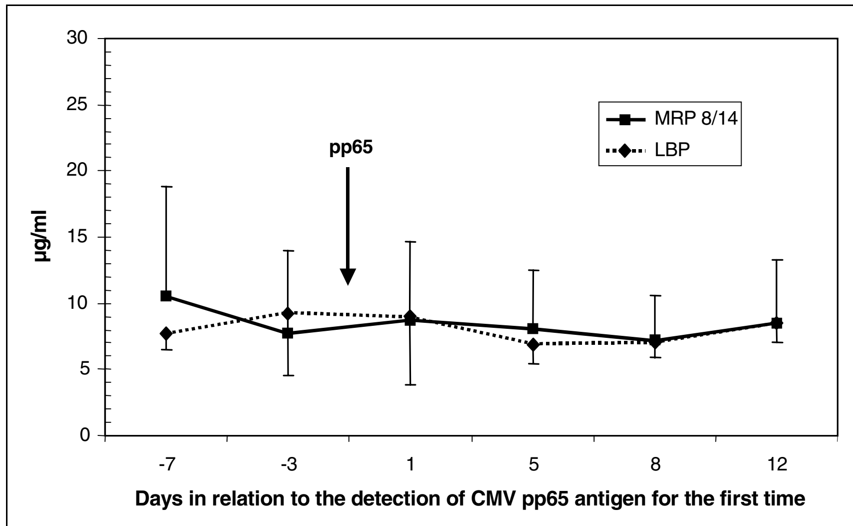

Fig. 7: MRP8/14 and LBP serum levels in kidney graft recipients with cytomegalovirus infection indicated by pp65 antigenemia

Tab. 3: MRP8/14 and LBP serum levels in sera from 23 kidney graft donors

Registration number	LBP [µg/ml]	MRP8/14 [µg/ml]	Graft function postNTx
1727	12.1	99.10	immediate
1745	42.9	94.11	immediate
1753	54.8	35.40	immediate
1755	12.1	22.94	immediate
1764	23.8	99.00	immediate
1765	14.6	148.90	immediate
1767	29.2	57.32	immediate
1770	26.5	55.24	nonfunctioning
1772	14.1	8.28	immediate
1774	55.6	23.15	immediate
1781	26.9	19.66	immediate
1782	40.1	31.08	immediate
1786	48.8	8.40	immediate
1796	61.3	45.06	delayed
1805	32.2	48.71	immediate
1820	16.3	75.18	immediate
1822	59.9	37.61	delayed
1827	70.7	13.11	immediate
1830	25.1	41.82	immediate
1839	15.4	5.50	delayed
1862	18.0	42.13	immediate
1912	11.9	8.76	delayed
1915	40.7	13.77	immediate
Mean (x)	32.74	44.96	
± 1 SD	18.41	36.77	

diseases and allograft rejection [14, 29]. The surface expression is a rapid response to an increase of intracellular Ca^{2+} levels. During the transendothelial migration of the phagocytes the preformed MRP8/14 complex is secreted in the extracellular space (e.g. serum) thus offering the possibility of its quantification [3, 30, 31]. Thus, their release in certain conditions could be of some value in the differential diagnosis of inflammatory reactions.

For quantification ELISAs are commercially available, but already with respect to the normal values there are significant differences between these tests. Burkhardt et al. [16] using the MRP8/14 ELISA from BMA Biomedicals (Augst, Switzerland) found normal values of 0.6 ± 0.2 µg/ml (n = 18 healthy control subjects). In the manufacturers manual the normal range for this test is indicated to be 0.5 - 3 µg/ml. In our MRP8/14 ELISA (Bühlmann Laboratories, Allschwill, Switzerland) the normal values are ten times higher (6.08 ± 2,22 µg/ml; normal range, 1.2 ± 10.96 µg/ml). The different normal ranges measured in healthy subject and published up to 1997 were already reviewed by Johne et al. [32]. Therefore it is not possible to compare the absolute values, but it should be possible to compare the diagnostic significance.

More recently, Madland et al. [33] reported on a significant correlation between calprotectin and CRP (r=0.67) in patients with rheumatoid arthritis and confirmed the results that this protein is a good measure of disease activity and joint inflammation [34, 35, 36). Thus, MRP8/14 may be considered as a very sensitive inflammation marker.

Therefore, this marker could be of great value also in the field of organ transplantation, because both rejections and infections induce inflammatory changes. Some of them (e.g. CRP) are already used within the diagnostic procedure, but the limitation is the unspecificity regarding to the underlying causes of inflammation. From that point of view the results of Burkhardt et al. [16] indicating a high sensitivity and specificity in the early recognition of rejections were very encouraging, particularly as all MRP8/14 levels during urinary tract infections, DGF or CMV infections were below the cutoff for rejections (4.2 µg/ml). Unfortunately no results were available about MRP8/14 levels during serious infections. There-

fore, we want to find out the impact of certain conditions (surgical trauma, intraoperative ATG prophylaxis, immediate and delayed graft function, steroid-sensitive and -resistant rejections, viral and bacterial infections) on the MRP8/14 serum concentration.
At the first time we compared the dynamics of MRP8/14 levels with those of CRP and LBP. More recently, we were able to report on the possibility to differentiate rejections, viral infections and bacterial infections by LBP. Only systemic non-viral infections and massive lymphocytolysis were associated with elevated LBP serum levels [23, 24].
In contrast to the already reported elevated serum levels in 23 organ donors (MRP8/14, x ± 1SD = 45± 36.8 µg/ml; LBP, x ± 1SD = 32.7 ± 18.4 µg/ml, [17]), in all but five pretransplant sera (n = 52) the MRP8/14 serum levels were within the normal range (<x ± 2SD). The mean MRP8/14 pre-KTx value was determined to be 6.7 µg/ml (1SD = 3.5) and the mean LBP value [14] was 8.8 µg/ml (1SD = 3.5 µg/ml).
The surgical trauma was associated only with significantly elevated CRP levels (from 3.3±5.7 to 46.4±42.6 µg/ml) within 24 hours. This is in agreement with our data reported already in 1981 [26] which point out the role of CRP as 'classic' acute phase reactant. In contrast, the increases of LBP (from 8.9±3.9 to 13.0±1.5 µg/ml, $p < 0.05$) and MRP8/14 (from 5.6±2.5 to 10.2±6.2 µg/ml, $0.1>p>0.05$) took place within the normal range of these proteins. After thoracotomy, Garret et al. [37] decribed an increase of calprotectin concentration from 0.6 µg/ml (range, 0.4 to 1 µg/m) before operation to 6.1 µg/ml (range, 3.8 to 0.2 µg/ml) eight hours after the operation ($p<0.05$). In a cardiopulmonary bypass subgroup, the calprotectin concentration increased to a maximum of 11 µg/ml (range, 7.3 to 14.5 µg/ml) four hours postoperatively ($p<0.01$) indicating the influence of synthetic materials (artificial surfaces) on leukocyte activation.

The intraoperative T-cell depletion by means of antilymphocyte globulin, in order to progress in the field of peritransplant tolerance induction, did significantly elevate the serum levels of all three proteins examined within the first 24 hours. As already reported, this massive lymphocytolysis at the time of opening the anastomoses (declamping) was simultaneously associated with a strong elevation of interleukin 6, tumor necrosis factor alpha [38] and interleukin 10, but not interleukin 12 [39]. Thus, these data prove a non-infectious elevation of MRP8/14, LBP and CRP.
Beside this post-KTx peak, in all uncomplicated courses the MRP8/14 levels were within the normal range. We could not find out any differences between immediate or delayed graft function. A quite similar behavior was reported for LBP [24].
Interestingly, in recipients experiencing steroid-sensitive or -resistant rejection crises there were no diagnostic usable changes in the MRP8/14 serum levels. The only finding pointing to an association between MRP8/14 and rejection was a slightly elevated MRP8/14 serum level during the pre-rejection period (between 14.1±11.8 and 11.8±6.5, but without a sharp increase) which dropped down after methylprednisolone into the normal range. The only rejection with dramatic elevation of the MRP8/14 level was an accelerated humoral one at post-KTx day 5 to 6 leading to a stop of graft function and therapeutic infusion of antilymphocyte globulin [17].
In summary, our data do not confirm the findings of Burkhardt et al. [16]. In contrast to the systemic events like post-KTx lmphocytolysis by ATG or bacteremia which are accompanied with elevated MRP8/14 serum levels, the locally accumulated phagocytes within the acutely rejected kidney graft [14, 15] do maybe not release as much MRP8/14 into the serum as necessary for a diagnostic procedure. Also in patients with rheumatoid arthritis much intense increase in calprotectin concentration has been observed in the local inflammatory site (e.g. synovial fluid) than in blood [40, 41].
With respect to systemic viral and bacterial infections indicated by viremia (pp65, CMV) or bacteremia only in the five cases with bacteremia an MRP8/14 serum level increase was detectable. Comparing the three proteins studied the strongest increase was found in CRP followed by LBP and last MRP8/14. Under an efficient antibiotic regimen all increased protein concentrations declined and at day 7 all values were within the normal range again.
In summary, after kidney transplantation increased MRP8/14 serum levels were found immediately (within 24 hours) after intraoperative high dose antilymphocyte globulin bolus infusion (mean 21.2 µg/ml), in connection with bacteremia (mean 21.7 µg/ml), in two superinfected (one CMV, one soor) pneumocystis carinii-infections [17] and in one accelerated humoral rejection [17]. Thus, the quantification of MRP8/14 serum levels is only of limited diagnostic value after renal transplantation. In contrast, the acute phase protein CRP seems to be an essential monitoring parameter after KTx, whereas LBP supports the differentiation of rejections and bacterial infections at a high level.

Acknowledgement

The authors thank Dr. T. Jermann from the Bühlmann Laboratories AG, Allschwil, Switzerland, for the gift of MRP8/14 enzyme immunoassays and Dr. R. Dostatni from the Milenia Biotec GmbH, Bad Nauheim, Germany, for technical support.

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