Ultrapure Filter does not Confer Short-Term Benefits over Two Reverse Osmosis Systems in Chronic Hemodialysis Patients

Ayelet Grupper MD, Moshe Shashar MD, Talia Weinstein MD PhD, Orit Kliuk Ben Bassat MD, Shoni Levy MD, Idit F. Schwartz MD, Avital Angel MD, Aharon Baruch MD, Avishay Grupper MD, Gil Chernin MD and Doron Schwartz MD

Department of Nephrology, Tel Aviv Sourasky Medical Center, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ABSTRACT: **Background:** Dialysate purity contributes to the inflammatory response that afflicts hemodialysis patients.

Objectives: To compare the clinical and laboratory effects of using ultrapure water produced by a water treatment system including two reverse osmosis (RO) units in series, with a system that also includes an ultrapure filter (UPF).

Methods: We performed a retrospective study in 193 hemodialysis patients during two periods: period A (no UPF, 6 months) and period B (same patients, with addition of UPF, 18 months), and a historical cohort of patients treated in the same dialysis unit 2 years earlier, which served as a control group.

Results: Mean C-reactive protein, serum albumin and systolic blood pressure worsened in period B compared to period A and in the controls.

Conclusions: A double RO system to produce ultrapure water is not inferior to the use of ultrapure filters.

IMAJ 2019; 21: 5–9

KEY WORDS: hemodialysis, reverse osmosis (RO), ultrapure filters (UPF), ultrapure water, water treatment system

For Editorial see page 50

During a hemodialysis session the patient's blood comes into contact with120–160 L of dialysate through the dialysis membrane. Consequently, the purity of the dialysate for contaminants such as bacterial endotoxin from the municipal water supply and bacterial biofilm in the dialysis water storage and distribution system might contribute, in part, to the triggering of systemic inflammatory responses that afflict hemodialysis patients [1]. In recent years, the recommended maximal limit of bacterial and endotoxin contamination in dialysate has been reduced. The currently recommended ultrapure dialysate, according to the Association for the Advancement of Medical Instrumentation, introduced a bacterial count limit of less than 0.1 colony forming unit (CFU)/ml and an endotoxin level of less than 0.03 EU/ml [2]. The use of an ultrapure dialysate results in a reduction in endotoxin levels and is reported to reduce inflammatory responses in hemodialysis patients, reflected in lower plasma levels of C-reactive protein (CRP), interleukin-6, and β2-microglobulin levels [3-6]. Improvement in the purity of hemodialysis water was reported to affect some of the acute and long-term complications of dialysis, including pyrogenic reactions, the malnutrition-inflammation-atherosclerosis (MIA) syndrome, dialysis-related amyloidosis, erythropoietin hyporesponsiveness, and accelerated cardiovascular morbidity [5,7-10]. Although clinical practice guidelines worldwide support the regular use of ultrapure dialysate for hemodialysis [13-12], this additional step is costly, limiting its widespread adoption.

The water treatment system in our in-hospital hemodialysis unit consists of two separate reverse osmosis (RO) systems in a row. This dual system itself provides ultrapure quality water. Nevertheless, to ensure water quality while using high-flux dialyzers, we decided to add an additional ultrapure filter (UPF) in each dialysis machine as a part of routine care.

The aim of the current study was to determine whether the addition of a UPF to dialysis machines in the setting of two RO systems in a row impacts clinical parameters as well as laboratory markers of inflammation, nutrition and anemia in our chronic hemodialysis patients.

Previous studies compared the value of adding UPF to a regular water-purifying system. To our knowledge, this is the first study to analyze the value of adding a UPF to a treatment system composed of two RO in series.

PATIENTS AND METHODS

STUDY DESIGN

This is a retrospective analysis of clinical and laboratory parameters retrieved in two-time periods, defined as: 6 months prior to (period A), and 18 months following (period B) the addition of an ultrapure filter to all dialysis machines in our unit, which utilized two RO systems. A comparison was made with a control group consisting of a historical cohort of patients treated in the same unit 2 years earlier.

PATIENT CHARACTERISTICS

Included in this study were 193 chronic hemodialysis patients from a single outpatient dialysis unit in the Tel Aviv Sourasky Medical Center who had been on a chronic hemodialysis regimen when ultrapure filters were introduced. Inclusion criteria were age above 18 years old, hemodialysis treatment for at least 6 months prior to and one month following implementation of the new filter. We excluded 21 patients with active or chronic infection, inflammatory disease, or active malignancy. Eleven patients who died prior to filter implementation, or less than one month in period B, were also excluded.

For the control group, we retrieved data of chronic hemodialysis patients from the same unit who were treated for at least 3 months before July 2008 (2 years prior to initiation of the study), and follow-up was available for a minimum of 7 months, with the same inclusion criteria as in the study group. Sixty-one patients (35.7%) from the control group were also included in the study group. Water quality measurements in the control group were similar to the study group (data not shown).

Data retrieved from medical files included demographic characteristics, medical history, medication list, dialysis prescription and dialysis parameters, and laboratory results. The study was approved by the Institutional Review Board (registration number 0568-10-TLV).

HEMODIALYSIS TREATMENT CHARACTERISTICS

Regular hemodialysis was performed with volumetrically controlled ultrafiltration machines (AK-200S, GAMBRO, USA). Each treatment session lasted 3–5 hours, with blood flow rates of 250–350 ml/min and dialysate flow rates of 500 ml/min. Patients received a single-use biocompatible synthetic high-flux or high-efficiency membrane (ELISIO 19,21H, NIPRO, Japan)

Ultrapure dialysate was achieved by operating two RO units in a row. During January 2011, we added a UPF (U 8000S GAMBRO) within the hydraulic pathway of all dialysis machines, which was replaced every 100 treatments. Samples of standard dialysis fluid were obtained from pre-specified locations (storage tank, distribution pipes at the entry to the unit, and post-UPF water), cultured, and analyzed for determinations of endotoxin concentration once a month.

LABORATORY TESTS AND DIALYSIS PARAMETERS

Blood samples were drawn on a routine basis once a month for complete blood count, serum calcium, phosphor, albumin, and high-resolution CRP. Parathyroid hormone (PTH), ferritin, iron and transferrin levels were measured every 3 months. Transferrin saturation was calculated as the ratio of serum iron and transferrin. Machine-calculated KT/V and blood pressure values were retrieved from the first hemodialysis treatment each month. For each patient, parameters were adjusted to the period (months) on dialysis and age.

CHRONIC MEDICAL TREATMENT

Doses of intravenous iron and erythropoiesis-stimulating agent (ESA) were determined by the nephrologists on service for each patient in order to adhere to the following optimal levels: hemoglobin 10–12 g/L, ferritin 200–600 and transferrin saturation of 20–50%.

STATISTICAL METHODS

Continuous variables were evaluated for normal distribution using a histogram and described as median and interquartile range (IQR), and categorical variables were described as frequency and percentage.

Variables that were measured once before and after the change were compared using the McNemar test (categorical variables) or Wilcoxon Signed Rank test (continuous variables). Univariate linear mixed model was used to evaluate the mean of the continuous variables that were measured several times before and after the change. Mean and standard error (SE) were reported. The Multivariate Linear Mixed Model was used to evaluate difference between pre-and post-implementation of UPF while controlling for potential confounders (age, gender, KT/V, and time on dialysis). The Kaplan-Meier curve was used to describe mortality during follow-up. Median survival time was reported.

One-way repeated measure ANOVA tests were used to evaluate difference in the study and control groups, and trends over time. A P value < 0.05 was considered statistically significant. All statistical tests were two-tailed. SPSS was used for all statistical analyses (IBM crop. Released 2013. IBM SPSS statistics for Windows, version 22.0. Armonk, NY: IBM Corp.).

RESULTS

The study enrolled 193 chronic hemodialysis patients, none of whom were lost to follow-up during the study period. Subjects' characteristics, outlined in Table 1, revealed that most of the patients were males, and nephrosclerosis and/or diabetic nephropathy were the leading etiologies for end-stage renal disease (ESRD). Sixty-six percent had an atrioventricular (AV) access (A-V fistula or a synthetic graft) and 59% used high-flux dialyzers.

During the study period an AV fistula was created in five patients who were dialyzed through a tunneled catheter, while three patients switched from AV access to a venous tunneled catheter. There was no significant difference in hemodialysis access or type of dialyzers between period A, B and the control group.

The median survival time was 35.1 months during the follow-up period. The mortality rate was 18.6% per year, which was comparable to the mortality rate in our institution 2 years previously (control group). Dialysis adequacy depicted as online KT/V did not differ between period A and B (1.22 0.25 vs. 1.21 ± 0.21 P = 0.89, respectively).

Table 1. Patients' characteristics: comparison between the study group and similar patients treated 2 years prior to initiation of the study (controls)

Variables, mean ± SD	Study group N=193	Control group N=171	*P* value
Age (years)	66.8 ± 14.9	67.2 ± 12.4	0.78
Mean time on chronic HD (months)	40.7 ± 22.1	37.9 ± 24	0.24
Gender, female N (%)	60 (31.1%)	58 (33.9%)	0.58
Cause for ESRD: N (%)			0.69
Diabetic nephropathy	65 (33.7%)	72 (36.2%)	
Nephrosclerosis	35 (18.1%)	30 (17.5%)	
Glomerulonephritis	25 (13%)	19 (11.1%)	
Polycystic disease	8 (4.1%)	11 (6.4%)	
Urologic	21 (10.9%)	20 (11.7%)	
Other /unknown	39 (20.2%)	19 (11.1%)	
Medical active conditions: N (%)			0.51
Atherosclerosis	74 (38.8%)	68 (39.7%)	
Chronic viral infection	17 (8.8%)	11 (6.4%)	
Heart failure	26 (13.5%)	21 (12.2%)	
Hemodialysis access: N (%)			0.44
Tunneled catheter	66 (34.2%)	66 (38.5%)	
Arteriovenous access	127 (65.8%)	105 (61.4%)	
Hemodialysis filter type: N (%)			0.24
High-flux	113 (58.5%)	89 (52%)	
High-efficiency	80 (41.5%)	82 (48%)	

HD = hemodialysis, ESRD = end-stage renal disease

Bacterial and endotoxin measurements of the purified water throughout our water distribution system are shown in Table 2. All measurements were in accordance with the definition of ultrapure dialysis fluid. No significant differences were found between the purity of the dialysis water in the locations that were sampled (storage tank, distribution pipes, and post-ultrapure filter water pre-dialyzer), before and after UPF implementation. A comparison between clinical and biochemical data between period A and B is summarized in Table 3.

Mean hemoglobin levels were significantly higher in period A vs. B after adjustment for age, gender, ESA dose, iron sucrose dose, KT/V and hemodialysis (HD) access (mean difference 0.32 g/dl, 95% confidence interval [95%CI] 0.17–0.46, $P < 0.001$).

While transferrin saturation, iron sucrose and ESA dose were comparable during the follow-up period, ferritin levels were significantly higher in period A vs. B. Stratification to iron sucrose dosage revealed that this difference was only significant in patients treated with a maximum dose of 100 mg iron sucrose per month.

Mean high-resolution CRP level in period B was higher compared to period A. After adjustment for age, gender, KT/V and HD access, the mean difference was 3.7 mg/L (95%CI 0.9–0.6, $P = 0.01$). In general, patients with tunneled catheter had a higher mean CRP than patients with AV access (14.5 vs. 12.9 mg/L, $P = 0.045$). The increase in CRP during period B was noticed only in patients with AV access and not in patients with tunneled catheter. Subgroup analysis on patients dialyzed with high-flux dialyzers revealed significantly higher CRP levels in period B compared to A, while in patients on high-efficiency dialyzers the difference did not reach statistical significance. Each year added to patients' time on hemodialysis was associated with an increase of 0.02 mg/L in serum CRP throughout both the study and control periods ($P = 0.043$).

A statistically significant decline in mean estimated serum albumin was observed in period B compared to A and in both subgroups of patients stratified by dialyzer type [Table 3], after adjustment for age, gender, KT/V and HD access. The mean difference was 0.5 g/L (95%CI 0.18-0.7, $P = 0.002$). In general, patients with AV access had higher serum albumin than patients with tunneled catheter (mean difference 2.1 g/L,

Table 2. Dialysate water measures (mean ± SD of 3 months measurements) of endotoxin levels and dialysate cultures along the water treatment system: comparison between period A and B

	Period A		Period B		*P* value	
	Culture (CFU/ml)	Endotoxin	Culture (CFU/ml)	Endotoxin	Culture	Endotoxin
Storage tank	‹ 0.1	0.011 ± 0.007	‹ 0.1	0.012 ± 0.008	NA	0.9
Distribution pipes	‹ 0.1	0.014 ± 0.009	‹ 0.1	0.014 ± 0.009	NA	0.75
Post-ultrapure filter water	‹ 0.1	0.012 ± 0.009	‹ 0.1	0.013 ± 0.006	NA	0.91

CFU = colony-forming unit

Table 3. Laboratory and clinical parameters of patients included in the study: comparison between period A and B

Variables, mean ± SD	Period A	Period B	*P* value
Hemoglobin (g/dl)	11.4 ± 0.07	11.09 ± 0.03	‹ 0.001
Tunneled catheter	11.32 ± 0.05	11.15 ± 0.04	0.69
Arteriovenous access	11.46 ± 0.06	11.11 ± 0.05	0.81
High-efficiency dialyzer	11.45 ± 0.1	11.08 ± 0.07	0.8
High-flux dialyzer	11.38 ± 0.09	11.19 ± 0.08	0.59
Ferritin (ng/ml)	721.5 ± 204	675.5 ± 255	0.023
Low-dose iron sucrose	780 ± 246	659 ± 289	0.003
High-dose iron sucrose	692.3 ± 238	703.9 ± 186	0.76
Transferrin saturation (%)	24.75 ± 13.4	24.85 ± 16.9	0.61
CRP (mg/L)	12.5 ± 0.9	15.3 ± 0.5	0.012
Tunneled catheter	12.9 ± 0.9	15.6 ± 0.7	0.25
Arteriovenous access	12.1 ± 1	15.2 ± 0.9	0.016
High-efficiency dialyzer	12.7 ± 0.7	13.8 ± 0.9	0.52
High-flux dialyzer	13.0 ± 0.5	15.7 ± 0.6	0.04
Albumin (g/L)	38.2 ± 0.25	37.7 ± 0.19	0.022
Tunneled catheter	36.99 ± 0.23	35.7 ± 0.15	0.07
Arteriovenous access	38.79 ± 0.18	38.22 ± 0.2	0.013
High-efficiency dialyzer	37.3 ± 0.2	36.08 ± 0.18	0.009
High-flux dialyzer	38.71 ± 0.14	38.23 ± 0.25	0.04
Systolic blood pressure (mmHg)	138.3 ± 20.1	140.5 ± 23.4	0.007
Tunneled catheter	137.9 ± 22.7	135.3 ± 20.8	0.71
Arteriovenous access	139.5 ± 18.5	141.7 ± 23.7	0.04
High-efficiency dialyzer	137.03 ± 21.7	139.3 ± 21.9	0.68
High-flux dialyzer	138.9 ± 19.4	141.5 ± 23.1	0.06
Diastolic blood pressure (mmHg)	72.5 ± 12.1	17.05 ± 13.1	0.74
Tunneled catheter	71.4 ± 11	72.5 ± 12.6	0.73
Arteriovenous access	73 ± 10.9	72.1 ± 11.7	0.7
High-efficiency dialyzer	68.3 ± 10.7	70.3 ± 10.6	0.06
High-flux dialyzer	75.4 ± 11.2	74 ± 11.6	0.4

95%CI 1.6–2.5, $P < 0.001$). The decrease in serum albumin after implantation of the filter was significant only in patients with an arteriovenous access, as shown in Table 3. Each year added to patients' time on dialysis was associated with a decrease of 0.03 g/L in serum albumin throughout the study period as well as in the control group ($P = 0.002$)

No significant changes were observed in all other laboratory parameters, namely white blood cell counts, serum calcium, phosphorus, and PTH as outlined in Table 3. Systolic blood pressure was 4.07 (CI 1.2–7) mmHg higher in period B compared to A when adjusted for KT/V, age, access and antihypertensive medications ($P = 0.007$).

COMPARISON WITH CONTROL GROUP

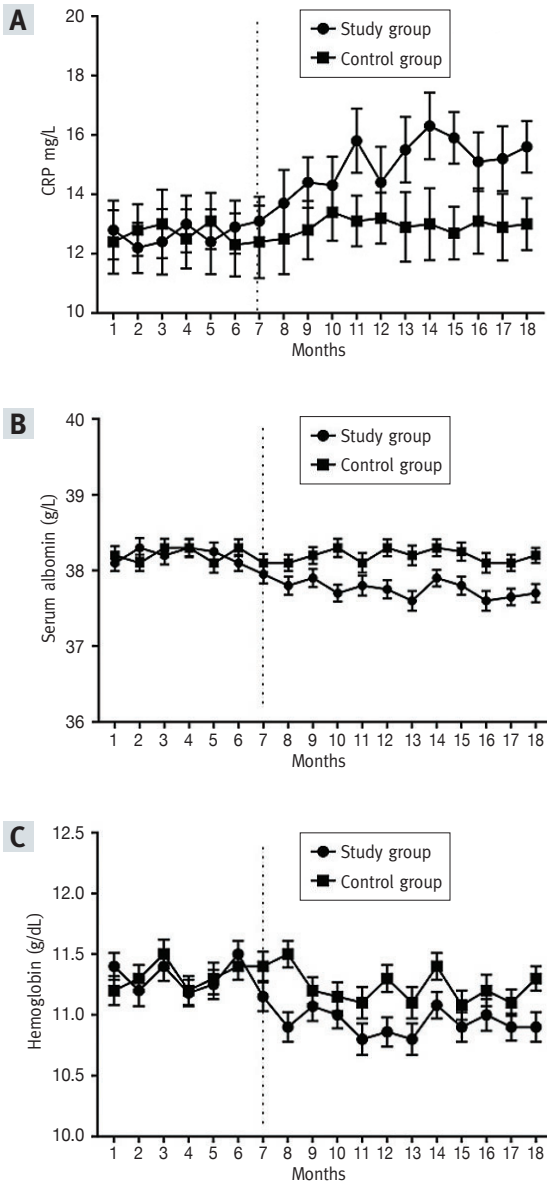
The mean CRP level in period A was not different from controls, and was 0.3 mg/L higher in period B ($P = 0.041$) [Figure 1A]. Mean serum albumin level was statistically lower in period B than both period A and the control group (0.32 g/L, $P = 0.037$) [Figure 1B]. Mean hemoglobin was not different between the study and control group [Figure 1C].

DISCUSSION

Previous studies have suggested that dialysis patients benefit from improving dialysate microbial purity by adding a UPF [3-6]. In the current study, we assessed the superiority of the aforementioned technique. We did not observe any favorable effect from adding a UPF filter to a dialysis water treatment system composed of two RO units. Furthermore, several parameters appeared to worsen following the addition of UPF: serum hemoglobin and serum albumin decreased while CRP levels increased. The decrease in serum albumin and CRP were apparent only in patients with AV access but not in those with a tunneled catheter. It is conceivable to assume that utilization of tunneled catheters results in a higher state of inflammation and, therefore, the impact of adding the UPF may not be detected. Whether the inflammatory burden of dialysis catheters provides immunity to other possible insults is a speculation that remains to be explored. In contrast, we also found that serum hemoglobin levels decreased in all patients. A possible explanation for this observation is the newer declining target levels of hemoglobin in dialysis patients recommended by the international guidelines.

If our findings imply that the observed increased inflammatory parameters following installation of UPF results from decreased water purity, it would be prudent to assume that patients treated with high-flux dialyzers would be more susceptible to the dialysate-related inflammatory burden. Indeed, mean CRP levels in period B increased significantly only in patients on high-flux dialyzers and not in those using other filters. Using UPF carries several significant problems. It requires maintenance by the dialysis staff which involves its replacement every 100 treatments. In addition, UPF needs to be flushed whenever the dialysis machine is on the off mode for a pre-specified time. In contrast, the addition of a RO unit requires no additional maintenance effort compared to a single unit. The cost of using two RO units is significantly lower than with the UPF. In our unit, we perform 30,000

Figure 1. Serial changes of mean ± SEM of **[A]** CRP, **[B]** serum albumin, and **[C]** hemoglobin. A monthly comparison between the study group and similar patients treated 2 years prior to initiation of the study (controls). The dashed line delineates between period A and B in the study group



[A] $P = 0.041$ (period B vs control), **[B]** $P = 0.037$ (period B vs control), **[C]** $P = 0.19$

dialysis treatments per year, which translates to 30,000 euros invested in UPF. This is far more expensive than the cost of an additional RO unit.

The main question posed by our findings is how the addition of UPF contributes to increased CRP and lower serum albumin and hemoglobin levels, all of which characterize the malnutrition-inflammation-atherosclerosis syndrome in hemodialysis patients. One can argue that UPF may carry a few hazards which may serve to explain these unexpected findings. UPFs retain bacteria and endotoxins very efficiently. However, when not in use they are subjected to biofilm formation. In contrast, RO units are in continuous use and therefore the risk of biofilm formation is reduced.

There is concern that monitoring of bacterial count and endotoxins once a month may fail to detect variations in water quality or fluctuant bacterial counts due to biofilms, which could be the unrevealed cause of our findings. Previous studies compared the value of adding UPF to a regular water-purifying system. To our knowledge, this is the first study to compare these two dialysis water-purifying methods.

There were several limitations in our study, the most significant being the absence of an appropriate control group. In order to address this issue, we compared our data with those of a similar group of patients who were treated in the same unit 2 years prior to initiation of the current study and had similar baseline characteristics. This comparison validates that the differences between period A and B are not related to time on dialysis but to the different modes of water treatment techniques. In addition, since the follow-up period was relatively short, we were unable to study outcome parameters.

CONCLUSIONS

We found that using a double RO system to produce ultrapure water is not inferior to a more sophisticated and expensive system, based on UPF. The long-term consequences of our observations require further study.

Correspondence
Dr. A. Grupper
Dept. of Nephrology, Tel Aviv Sourasky Medical Center, 6 Weizmann St, Tel Aviv 64239, Israel
Phone: (972-3) 697-3443.
Fax: (972-3) 697-2495
email: ayeletg@tlvmc.gov.il

References

1. Bommer J, Jaber BL. Ultrapure dialysate: facts and myths. *Semin Dial* 2006; 19: 115-19.
2. Martin K, Laydet E, Canaud B. Design and technical adjustment of a water treatment system: 15 years of experience. *Adv Ren Replace Ther* 2003; 10: 122-32.
3. Sitter T, Bergner A, Schiffl H. Dialysate-related cytokine induction and response to recombinant human erythropoietin in haemodialysis patients. *Nephrol Dial Transplant* 2000; 15: 1207-11.
4. Schiffl H, Lang SM, Stratakis D, Fischer R. Effects of ultrapure dialysis fluid on nutritional status and inflammatory parameters. *Nephrol Dial Transplant* 2001; 16: 1863-9.
5. Hsu PY, Lin CL, Yu CC, et al. Ultrapure dialysate improves iron utilization and erythropoietin response in chronic hemodialysis patients. A prospective cross-over study. *J Nephrol* 2004; 17: 693-700.
6. Furuya R, Kumagai H, Takahashi M, Sano K, Hishida A. Ultrapure dialysate reduces plasma levels of beta 2-microglobulin and pentosidine in hemodialysis patients. *Blood Purif* 2005; 23: 311-16.
7. Ward RA. Ultrapure dialysate. *Semin Dial* 2004; 17: 489-97.
8. Masakane I. Review: clinical usefulness of ultrapure dialysate – recent evidence and perspectives. *Ther Apher Dial* 2006; 10: 348-54.
9. Lamas JM, Alonso M, Sastre F, Garcia-Trio G, Saavedra J, Palomares L. Ultrapure dialysate and inflammatory response in haemodialysis evaluated by darbepoetin requirements – a randomized study. *Nephrol Dial Transplant* 2006; 21: 2851-8.
10. Susantitaphong P, Riella C, Jaber BL. Effects of ultrapure dialysate on markers of inflammation, oxidative stress, nutrition and anemia parameters: a meta-analysis. *Nephrol Dial Transplant* 2013; 28: 438-46.
11. Section IV. Dialysis fluid purity. *Nephrol Dial Transplan*t 2002; 17 (S): 45-62.
12. Kawanishi H, Masakane I, Tomo T. The new standard of fluids for hemodialysis in Japan. *Blood Purif* 2009; 27 (Suppl 1): 5-10.

Capsule

DEL-1 promotes macrophage efferocytosis and clearance of inflammation

Resolution of inflammation is essential for tissue homeostasis and represents a promising approach to inflammatory disorders. **Kourtzelis** and colleagues found that developmental endothelial locus-1 (DEL-1), a secreted protein that inhibits leukocyte-endothelial adhesion and inflammation initiation, also functions as a non-redundant downstream effector in inflammation clearance. In human and mouse periodontitis, waning of inflammation was correlated with DEL-1 upregulation, whereas resolution of experimental periodontitis failed in DEL-1 deficiency. This concept was mechanistically substantiated in acute monosodium-urate-crystal-induced inflammation, where the pro-resolution function of DEL-1 was attributed to effective apoptotic neutrophil clearance (efferocytosis). DEL-1-mediated efferocytosis induced liver X receptor-dependent macrophage reprogramming to a pro-resolving phenotype and was required for optimal production of at least certain specific pro-resolving mediators. Experiments in transgenic mice with cell-specific overexpression of DEL-1 linked its anti-leukocyte-recruitment action to endothelial cell-derived DEL-1 and its efferocytic/pro-resolving action to macrophage-derived DEL-1. Thus, the compartmentalized expression of DEL-1 facilitates distinct homeostatic functions in an appropriate context that can be harnessed therapeutically.

Nature Immunol 2018; 20: 40

Eitan Israeli