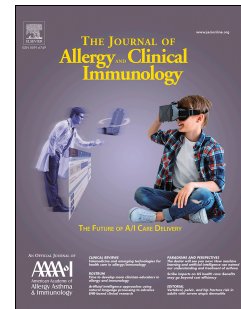


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Massive release of Th2 cytokines induced a cytokine storm during a severe mast cell activation event in an indolent systemic mastocytosis patient

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Abstract**Background:**

In subjects with systemic mastocytosis the number of mast cells is elevated manifold. These patients frequently suffer unpredictable and recurrent life-threatening mast cell activation (MCA) events.

Objective:

Our aim was to analyze the derangements of chemokine and cytokine concentrations during severe MCA attacks

Methods:

Samples from an indolent systemic mastocytosis patient were used for this study. A total of 41 chemokines and cytokines were simultaneously measured in triplicate and at multiple time points during two severe and two moderate MCA events. These were compared to 3 to 5 baseline samples, taken when clinical symptoms were not present.

Results:

During the severe MCA event, which required 2 days of treatment in the intensive care unit, peak CCL3, IL-1ra, IL-5, IL-6, IL-10, IL-13 and GM-CSF concentrations were statistically significantly elevated 29-, 99-, 44-, 280-, 93-, 7- and 6-fold above baseline respectively. A very similar pattern was observed during the second severe MCA event. In the moderate MCA event with PCR-proven Influenza A infection, the Th1-associated cytokines $\text{INF}\alpha$, $\text{INF}\gamma$ and $\text{TNF}\alpha$ were only statistically significantly elevated 5- to 7-fold above baseline. The correlation coefficients between highly elevated histamine and cytokine concentrations during the acute phase were >95% indicating the same cellular origin, possibly activated mast cells.

Conclusions:

One of the severe MCA events led to life-threatening symptoms over several days. During this event, the massive release of Th2 cytokines induced a hyperinflammatory state, fulfilling recently published criteria for cytokine release syndrome. Administration of IL-6 and IL-5 inhibiting biologicals might significantly shorten the acute phase of severe MCA events, likely offering significant clinical benefits to mastocytosis patients.

Conflicts of Interest

Thomas Boehm and Bernd Jilma are co-inventors of a patent regarding the use of recombinant human diamine oxidase in the treatment of conditions with excess histamine. Peter Valent received consultancy honoraria from Novartis, Blueprint, Pfizer and Celgene. The other authors declare they have no conflicts of interest.

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Key messages

- Massive release of Th2 cytokines induced a life-threatening cytokine release syndrome during mast cell activation in an indolent systemic mastocytosis patient
- Histamine as a possible driver of massive cytokine release with clinical improvement after rapid decrease of histamine concentrations, possibly mediated by endogenous diamine oxidase
- Approved anti-IL-6 and anti-IL-5 biopharmaceuticals like tocilizumab and mepolizumab, benralizumab or reslizumab might show significant clinical benefits during severe mast cell activation events.

Capsule summary: Extensive mast cell activation leads to massive release of Th2 cytokines inducing a life-threatening cytokine storm in an indolent systemic mastocytosis patient

Keywords: mast cell activation, mastocytosis, Th2 cytokines, cytokine storm, cytokine release syndrome

86 **Abbreviations:**

87	BL	=	Baseline
88	fSBL	=	Functional Small Bowel Length
89	GM-CSF	=	Granulocyte-Macrophage Colony-Stimulating Factor
90	IL	=	Interleukin
91	INF	=	Interferon
92	MC	=	Mast Cell
93	MCA(S)	=	Mast Cell Activation (Syndrome)
94	PDGF	=	Platelet-derived growth factor
95	SIRS	=	Systemic Inflammatory Response Syndrome
96	TNF	=	Tumour Necrosis Factor
97	TSLP	=	Thymic Stromal Lymphopoietin
98	VEGF	=	Vascular endothelial growth factor

99

Introduction

Primary mast cell activation syndrome (MCAS) or mastocytosis is an orphan disease with an incidence of approximately 1 in 10000.¹ The at least 2- to 10-fold elevated mast cell (MC) burden renders excessive MC activation (MCA) life-threatening.^{2,3} Many mastocytosis patients suffer recurrent MCA attacks.^{4,5} With the exception of the classical IgE/FcεRI-mediated anaphylaxis, which in many cases leads to a rapid uniphasic clinical course lasting less than an hour, we have limited knowledge regarding definite underlying MCA trigger mechanisms. Nevertheless, a second wave of clinical symptoms, or biphasic anaphylaxis, is seen in 10 to 20% of anaphylaxis cases and this is unlikely to be directly IgE/FcεRI-mediated. Protracted MCA in mastocytosis patients can last for a few days.^{6,7} Excessive histamine release might significantly contribute to clinical symptomatology during protracted MCA, but is unlikely to be the sole culprit considering the release of many other mediators during MC degranulation or activation.^{1,7,8}

Mast cells can release many chemokines and cytokines, although the authors caution that human in vivo data are missing in many cases.⁸ The cytokines IL-2, IL-6 and IL-10 were moderately elevated during classical anaphylaxis comparing moderate and severe reactions, as were tryptase and histamine, but the medium time from exposure to reaction onset was less than 10 minutes.⁹ The Th2 cytokines IL-5 and IL-13 were not increased. Pardanani (2013) showed that indolent systemic mastocytosis patients under non-event conditions had moderately elevated plasma concentrations of many cytokines compared to healthy controls, but this might simply reflect the increased MC burden.¹⁰ These data are, however, unlikely to be useful in helping us learn more about the molecular mechanisms driving protracted life-threatening MC activation and consequently finding new treatment options. A comprehensive characterization of the deranged chemokines and Th1/Th2-associated cytokines during the acute phase of protracted MCA events lasting many hours, or sometimes even days, has not

125 been published. This is true for both mastocytosis and general MCAS patients. This would be
126 necessary to propose new therapeutic targets. Currently, glucocorticoids, anti-histamines,
127 fluid substitution and other symptomatic treatment modalities such as fever or nausea
128 reduction are the only treatment options. These are frequently unsatisfactory.

129

130

Methods

Mastocytosis and mast cell activation events

This 43 year old Caucasian male with no concurrent comorbidities was diagnosed with KIT D816V positive indolent systemic mastocytosis in 2008. Bone marrow biopsy confirmed infiltrating MCs staining positive for c-kit, CD25 and tryptase. This patient suffers from recurrent MCA events once or twice per year. These events follow a similar course, but are of varying severity. During several events in the past an infectious cause was not diagnosed. Treatments used during the four events described herein included diphenhydramine, ranitidine, cetirizine, ondansetron, prednisolone and fluid substitution on admission and then as needed. A detailed description of the metabolome and lipidome derangements and medications administered for the most severe event, which occurred in February 2018, was recently published.⁷ Briefly, 250 mg of prednisolone was administered on admission and 100 mg prednisolone was administered 7, 14 and 26 hours after admission. Diphenhydramine was infused on admission (60 mg) and after 7 (30 mg), 9 (30 mg), 18 (30 mg) and 26 (30 mg) hours. Ten mg cetirizine was administered 7 hours post admission. Ranitidine (50 mg) and ondansetron (8 mg) were administered on admission, and 14 and 26 hours thereafter. During the Influenza A PCR-positive event in February 2020 Tamiflu (150 mg) was administered daily for 5 days. Under non-event conditions, only the anti-histamine medications cetirizine and ranitidine are required for the patient to remain almost symptom-free. Baseline blood samples were obtained during the DAOMAST study, a non-interventional study based on questionnaires and blood draws which included 43 mastocytosis patients. The study is briefly described in Boehm (2019).¹¹ The visits used for baseline normalization occurred in April 2013, September 2013 and March 2014, and the events happened in November 2014 (N14), June 2015 (J15), February 2018 (F18) and February 2020 (F20). The patient provided informed consent and was included in the non-interventional anaphylaxis

clinical study (EC:1018/2015) which was approved by the Ethics Committee of the Medical University of Vienna. Normal blood pressure and heart rate data were recently published.⁷

Multiplex assay

All serum and plasma samples were thawed after freezing and storage at -80°C and tested on the same microtiter plate using the LKTM010 Human Immunotherapy Magnetic Luminex Performance Assay (R&D Systems, Bio-Techne, Abingdon, UK). After one thaw/freeze cycle, the same samples were tested again on the same microtiter plate using a custom-built Luminex 17-plex kit. The standards were tested in duplicate and all serum and plasma samples in triplicate. Fluorescence data were measured in a Luminex 200 analyzer and concentrations calculated in pg/ml using the xPONENT[®] software Version 3.1.

Other measurements

Histamine was measured with the state of the art histamine ELISA as described in Boehm (2019)¹¹, both for baseline concentrations and during the N14 and F18 events. For the J15 and F20 events the Histamine Dynamic kit was used (cisbio, Perkin Elmer, Austria; Vienna). Other clinical parameters were measured in the clinical chemistry laboratory of the Vienna General Hospital using accredited standard methods.

Statistical Analysis

A subject specific reference distribution for each chemokine/cytokine was calculated using the singlicate serum cytokine concentrations at Week 0, the mean of the serum and plasma cytokine concentrations at Week 24 and Week 48 of the DAOMAST study. During all three visits the patient was asymptomatic. In addition, the 72 hour plasma cytokine concentrations of the F18 attack were conservatively added. Most chemokines/cytokines returned to baseline

values 72 hours after admission during the F18 event, reflected in a mean and median ratio of the baseline values without the 72 hours F18 values to the 72 hours F18 values of 1.009 and 1.00 respectively (Online Repository Table E1). Some values such as CCL20 or IL-6 were still >3-fold elevated in the F18 72 hour sample compared to baseline, substantially increasing the variance and the resulting p-values (Online Repository Table E1). In total 2 singlicate (serum Week 8 and 72 hours F18) and 2 duplicate (mean of serum and plasma Week 24 and serum and plasma Week 48) baseline values were included to estimate the reference distribution. For 9 cytokine parameters, serum concentrations were significantly different to plasma concentrations (Online Repository Table E2) and in these cases only the plasma cytokine profiles at Week 24, Week 48 and the 72 hour sample of the F18 attack were used. The fluorescence of all individual serum or plasma samples was measured in triplicate and the respective mean used for baseline calculations.

For each chemokine/cytokine we assumed that the values are approximately normally distributed on a natural logarithmic scale. The means and standard deviations of the respective reference distribution were calculated from the sample means and standard deviations of the reference measurements. If all reference values were below the detection limit, and this applies to the following 11 chemokines/cytokines: CCL3, $\text{INF}\alpha$, $\text{INF}\gamma$, IL-12, IL-15, IL-1 β , IL-33, CCL7, IL-17E/IL-25, TSLP and IL-5, both mean and standard deviation were estimated as one half of the detection limit. This is a conservative estimate because among all possible distributions with values between 0 and the detection limit, the distribution with one half of the values at 0 and one half at the limit has the largest variability and corresponding mean and standard deviation of one half of the detection limit. For example, if the detection limit is 4 pg/ml, the assumption is that half of the measurements have a value of 0 and the other half 4 pg/ml with mean and standard deviation of 2 pg/ml.

205 Duplicate measurements for all samples during the events were averaged as applicable before
206 being used in the statistical analysis. For each measurement during the events the null
207 hypothesis that the respective value belongs to the reference distribution was tested by an
208 outlier t-test. The t-test takes into account statistical uncertainty in the estimated standard
209 deviation. In case the standard deviation was conservatively assumed as one half of the
210 detection limit, an outlier z-test was used since no further uncertainty in the standard deviation
211 needs to be accounted for. The resulting p-values within an attack were adjusted by the
212 Benjamini-Hochberg method to account for testing multiple values within an event. Upper
213 and lower reference bounds were calculated using the 95% prediction intervals for the
214 assumed log-normal reference distribution.

Results

This patient with indolent systemic mastocytosis frequently suffers from MC activation events leading to hospital admission. We measured 41 chemokines, cytokines, growth factors and other parameters during 2 severe and 2 moderate MCA events. The major clinical characteristics of these MCA events are summarized in Table 1. The MCA events F18 and N14 fulfilled at least two systemic inflammatory response syndrome (SIRS) criteria. The F20 MCA event seems unique because it might have been triggered by PCR-proven Influenza A infection. An infection was not diagnosed during the other three events. The J15 event was moderate, with increased heart rate and gastro-intestinal symptoms but minimal fever, no skin involvement and minimal mean arterial pressure decrease. The F20 and J15 events do not fulfill the SIRS criteria.

All baseline values of the 41 measured parameters are shown in the Online Repository Fig E3. For 9 parameters with a significant difference between plasma and serum levels we used the mean plasma concentration of the 24 and 48 week baseline samples for presentation and mean fold above baseline calculations (Online Repository Table E2). For the other 32 parameters the mean of the five baseline values was used for presentation and normalization. Parameter concentrations were not corrected for the 20 to 30% hemodilution (1.25 to 1.43 multiplication factor) during the acute phase of the F18 MCA event (Online Repository Fig E2). The concentrations of the following 18 cytokine and chemokines compared to baseline values did not show any significant p-values during any of the four MCA events: CCL7, CCL8, CCL17, CCL20, CCL22, CXCL8, IL-1 α , IL-1 β , IL-2, IL-4, IL-12, IL-15, IL-17A, IL-17E/IL-25, Granzyme B, CD40L, bFGF and PDGF-AA.

The peak parameter concentrations, including the baseline values and the peak fold-changes compared to baseline of all chemokines, cytokines and other parameters increasing or decreasing more than 3-fold for all four MCA events are shown in Table 2.

In Table 3 we attempt to define cytokines and chemokines associated with severity by normalizing the baseline-normalized peak fold changes a second time using the peak-fold changes of the moderate non-SIRS MCA event J15 with a cut-off of 5-fold in at least one MCA event. The chemokines CCL2 and CCL3 and the cytokines IL-1ra, IL-5, IL-6, IL-10 and IL-13 were 8.6-, 29-, 23-, 44-, 95-, 6.5- and 6.3-fold elevated respectively during the severe F18 event after normalization to the moderate J15 event. A similar pattern is seen with the severe N14 MCA event, although it is of lower magnitude. Several other chemokines and cytokines are more than 5-fold elevated (Table 3). CCL22, IL-3, PDGF-AA and VEGF-A are 15-, 141-, 22- and 38-fold reduced respectively in the most severe MCA event F18 compared to J15. The Th1-associated cytokines IL-2, INF γ and TNF α are 6- to 7-fold elevated in the F20 event compared to the other three MCA events.

The time courses of relative chemokine, Th1/2-associated cytokine, growth factor and other parameter concentration changes after normalization to baseline values for the most severe F18 MCA event are shown in Fig 1. The corresponding p-values are listed in the figure legend and a time course of p-values for selected parameters (IL-1ra, IL-5, IL-6, IL-10, IL-11, IL-13, CCL3 and GM-CSF) are presented in the Online Repository Fig E2. Histamine data were added to indicate that starting at 3 hours post hospital admission the degree of MCA might have increased, reflected in ascending histamine concentrations with a peak of approximately 30 ng/ml (270 nM)) at 7 to 9 hours after admission. During the same time window tryptase concentrations increased 3.4 fold from 95 to 327 ng/ml. Mean baseline tryptase concentrations were 94 ng/ml. Symptoms started 12 hours prior to admission with fatigue and multiple vomiting episodes. The initially increasing histamine concentrations which were

followed by a rapid drop were clearly reflected in several clinical parameters including glucose, lactate, aPTT and leukocytes. Additionally, changes in heart rate were noted (Table 1).⁷ Several chemokines demonstrated a similar pattern of an increase followed by a flattening or even a decrease at the histamine peak (Fig 1). The prototypical Th1 cytokines such as IL-1 α , IL-1 β , IL-12, IL-15, INF α , INF γ and TNF α minimally changed compared to baseline values (Fig 1C and D). In contrast, Th2 cytokines, some growth factors and other parameters showed at times dramatic increases/decreases during the acute phase of the F18 event (Fig 1E to G). Interleukin-1ra, IL-5, IL-6 and IL-10 increased more than 50-fold above baseline values during the acute phase. Interleukin-13 also increased more than 20-fold using the first time point after admission as the starting concentration (Fig 1F). The growth factors IL-3, VEGF-A and PDGF-AA showed 18-, 28- and 24-fold decreases respectively during the peak phase (Fig 1H). For IL-3 and VEGF-A the peak p-values without and with Benjamini-Hochberg correction are 0.02/0.063 and 0.029/0.048 respectively. For PDGF-AA the p-values are likely not significant, because the 72 hour time point used for statistical analysis is 7-fold below the other baseline values, strongly increasing the variance (Online Repository Table E1). Most chemokine and cytokine concentrations returned to baseline values 72 hours after admission with a few remaining minimally (< 4-fold) increased or decreased (Fig 1).

Additional chemokine data with minimal changes compared to baseline are shown in the Online Repository Fig E4.

Histamine concentrations are statistically significantly correlated and show high correlation coefficients with many parameters during the severe MCA event F18, indicating the same cellular origin, possibly activated mast cells (Online Repository Table E3). During the acute phase, the plasma concentrations of IL-6, IL-10 and IL-1ra increased 89- (p-value <0.01), 20- (<0.01) and 31-fold (0.027) of the baseline values per hour respectively (Online Repository Table E4 and Fig E5).

A similar analysis using chemokine and cytokine data from two additional MCA events without diagnosis of an underlying infection is presented in Fig 2. The corresponding p-values are listed in the figure legend. The patterns of cytokine elevation are comparable between the severe F18 and N14 events, but the magnitude is reduced in the N14 event. Notable differences to the F18 event are a 9- and 17-fold elevation of CCL20 and IL-3 (Benjamini-Hochberg corrected p-value 0.012) respectively. The J15 MCA event was a moderate MCA attack not fulfilling SIRS criteria and this is also reflected in the low fold-above baseline values of e.g. IL-6 or IL-1ra (Fig 2B). Additional chemokine and Th1-associated cytokine changes during the N14 and J15 MCA event are shown in the Online Repository Fig E6.

During the fourth MCA attack the chemokine/cytokine alterations are different, likely because of PCR-proven Influenza A infection. The peak fold-changes always measured at admission compared to baseline are presented in Table 2. The time line, which includes 6 time points covering 11 hours post admission, is shown in the Online Repository Fig E7. In this event some chemokines and Th1-associated cytokines are clearly elevated compared to the other three events (Table 2). The patient suffered more than 10 vomiting episodes at home prior to admission, and thus it is likely that the peak chemokine and cytokine changes already occurred at home. This event does not fulfill the SIRS criteria (Table 1) and the magnitude of, for example, IL-6 or IL-1ra changes was much lower compared to the severe F18 and N14 events (Table 2). Additional chemokine, Th1- and Th2-associated cytokine data from this event are shown in the Online Repository Fig E8 and Fig E9.

Figure 3 provides a summary of the time courses of some major metabolic, lipidomic and chemokine/cytokine derangements during the severe F18 event in relation to histamine, diamine oxidase and tryptase concentrations and the functional small bowel length (fSBL). The fSBL reflects the metabolic health of the jejunal and ileal epithelial cells.⁷ The main component for calculating the fSBL is the citrulline concentration, which is only synthesized

312 by gastrointestinal epithelial cells and strongly decreased during the acute phase of the F18
313 event.⁷

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Discussion:

The acute phase of the severe MCA F18 event required 2 days of treatment on the intensive care unit. During this time, the functional health of the small bowel epithelial cells, reflected in the synthesis capabilities of citrulline and ornithine, was reduced almost 20-fold to 6% of normal.⁷ This metabolic shut-down, likely caused by massive local mast cell activation followed by hypoperfusion and hypoxia, might be considered to be organ dysfunction or even organ failure. At the same time, plasma lactate concentrations were highly raised to at least 7 mM. Proinflammatory cytokines such as IL-5, IL-6, IL-10 or IL-13 were greatly increased during the acute phase.

Several potent chemokines like CCL2, CCL3, CCL4 and CXCL10, which are associated with published cytokine release syndrome events, were also elevated in the F18 MCA event.^{12,13} CCL3 is a strong mast cell activator which also activates endothelial cells and recruits monocytes and neutrophils after intradermal injection in healthy volunteers.¹⁴⁻¹⁶ Although IL-1ra and IL-10 are strongly elevated during the MCA event, their counterregulatory response to proinflammatory cytokines seems insufficient, similar to published cytokine storm data.¹² Alternatively, IL-10 might be Th1 suppressing but Th2 proinflammatory via the activation of mucosal mast cells and the production of IL-5.¹⁷ The F18 event clearly fulfills the SIRS criteria. Consequently, the MCA F18 event might be considered as Th2-associated cytokine release syndrome, because the three criteria of the latest definition of a cytokine storm are fulfilled.¹²

The massive release of Th2-associated cytokines, hypothetically released from activated mast cells^{8,18-20}, is clearly different to the previously published cytokine release syndromes, where the cytokine drivers are IL-6 and Th1-biased cytokines such as $\text{INF}\gamma$, IL-1 β or TNF.¹² These Th1 cytokines did not change at all during the three MCA events which were not related to a diagnosed infection. In the F20 event with PCR-proven Influenza A infection, Th1 cytokines

were clearly, but nonetheless only moderately elevated. It is tempting to speculate that the Th1 cytokines might have suppressed the development of a Th2 cytokine release syndrome by their downregulation of Th2 responses.

Plasma histamine concentrations reached 30 ng/ml (normal levels are below 1 ng/ml) and might have contributed not only to generalized flushing, hypotension and heart rate increase, but also to the hyperinflammatory state. The local interstitial histamine concentrations during mast cell activation might be as high as 1000 μ M (see Online Repository for calculations), possibly higher in mastocytosis patients.²¹ At these locally highly elevated concentrations histamine is strongly proinflammatory, inducing neutrophil, basophil and eosinophil migration, activation and degranulation, increasing vascular permeability and causing or enhancing GM-CSF, IL-5, IL-6, IL-10 and IL-13 release.²¹⁻²⁴ Histamine could be a key driver for the duration and severity of protracted MCA attacks. The patient's clinical condition during the F18 attack improved significantly after histamine dropped rapidly, namely 5-fold, in the few hours after the peak. The more than 150-fold elevated diamine oxidase concentrations may have contributed to this.^{7,11} Clinical improvement was demonstrated by rapidly decreasing lactate and glucose concentrations, as well as decreasing activated partial thromboplastin time. The patient's heart rate decreased and mean arterial pressure values increased.⁷

What are the possible consequences for treatment? Tocilizumab, an antibody against the IL-6 receptor, was recently approved for the treatment of chimeric antigen receptor (CAR) T cells-induced cytokine storms. The peak plasma IL-6 concentration during the F18 severe MCA event was 1100 pg/ml and 280-fold elevated compared to baseline values. Considering the proinflammatory role of IL-6 and the strong positive correlations between mortality and elevated IL-6 concentrations²⁵⁻²⁷, life-threatening MCA symptoms might be reduced by using tocilizumab or other anti-IL-6 biologicals. During life-threatening MCA events, the risk-benefit ratio would appear to be biased towards benefit considering the side effect profile of

366 tocilizumab demonstrated in several thousand rheumatoid arthritis patients.^{26,28} The
367 proinflammatory Th2-biased cytokine IL-5 was 44-fold elevated compared to baseline or the
368 mild J15 event and therefore might significantly contribute to the symptomatology during
369 severe MC activation. Mepolizumab, benralizumab and reslizumab are anti-IL-5/IL-5 receptor
370 antibodies approved for the treatment of severe asthma and hypereosinophilic diseases.²⁹
371 Efficient blockade of IL-5 might mitigate clinical symptom development during protracted
372 MCA. Considering the derangements of IL-3, IL-5 and GM-CSF during the severe MCA
373 attacks, the monoclonal antibody CSL311, which targets the common β chain of the
374 corresponding cytokine receptors, might be an interesting therapeutic option.³⁰
375 Blocking the Th2-associated cytokine IL-13 with tralokinumab, recently approved for the
376 treatment of atopic dermatitis and possibly with clinical benefits for asthma patients, might
377 also be considered.³¹ The similarities between increased chemokine and cytokine
378 concentrations during pulmonary antigen challenge and asthma and the MCA events in this
379 patient are striking, although this mastocytosis patient does not appear to show bronchospasm
380 during attacks.^{32,33}
381 A limitation of this study is that these data are derived from one indolent systemic
382 mastocytosis patient, albeit covering four independent MCA events. Nevertheless, increases
383 in IL-5, IL-6, IL-10, and IL-13 have also been noted during uniphasic classical anaphylaxis in
384 a study comparing healthy subjects and people who have suffered severe reactions.⁹
385 Additionally, Pardanani (2013) published differences between baseline values of indolent
386 systemic mastocytosis patients and healthy controls.¹⁰ Our data might be a reflection of the
387 massive activation of the highly abundant mast cells in mastocytosis patients reaching a
388 threshold of histamine and several cytokines, which consequently induced a
389 hyperinflammatory state for hours to days. Therefore, we believe that characterization of
390 chemokines and cytokines during an appropriate time window in other mastocytosis and
391 possibly in secondary or tertiary MCAS patients will generate comparable data.

The baseline data drawn during hospital visits a few years prior to the major F18 MCA event might not be considered suitable for use, because chemokines and cytokines may not remain stable during prolonged storage. If stability during storage at -80°C were an issue, it would be difficult to explain why 18 of the 41 parameters did not change at all, and why 23 parameters returned to baseline after 72 hours, despite strongly increasing or decreasing during the acute phase. If the baseline chemokines and cytokines were degraded during storage, the baseline-normalized chemokine and cytokines concentrations should start at highly elevated levels, but we did not see this. The steep slopes during the acute phase of the F18 event also strongly suggest that we measured massive activation and degranulation of mast cells and likely other activated and migrated inflammatory cells.

The administered anti-histamines diphenhydramine, cetirizine and ranitidine might have suppressed the Th2 polarizing effect of histamine including histamine-mediated IL-6 expression.^{35,36} Consequently, without anti-histamine treatment the strongly elevated Th2 cytokines might have been even higher. Glucocorticoids strongly suppress chemokine and cytokine expression independently of a Th1 or Th2 bias and therefore the administered prednisolone could have suppressed the highly elevated chemokines and cytokines.³⁷ Fold-changes could have been more pronounced without glucocorticoid administration. It is not clear whether exogenous glucocorticoids had enough time to exert their anti-inflammatory effects within the few hours following admission as their main mechanism of action is modulation of transcription. Nevertheless, the anti-inflammatory cytokines IL-1ra and IL-10 might have been increased by glucocorticoid treatment.

In conclusion, we provide strong evidence that severe, life-threatening MCA events are orchestrated by the release of several potent proinflammatory chemokines and prototypical Th2 cytokines. The strong correlations of histamine and cytokine concentrations and clinical symptoms with a clear clinical improvement after histamine concentrations dropped significantly after the peak suggest using recombinant human diamine oxidase to rapidly

remove histamine and anti-IL-6 and anti-IL-5 biologicals to mitigate the severity of acute MCA events.^{7,34} Double-blind randomized controlled studies during severe acute MCA attacks will be almost impossible to perform in mastocytosis or MCAS patients and recombinant human diamine oxidase is not available, but IL-5 and IL-6 blocking reagents have been approved and might show significant clinical benefits in severe life-threatening Th2 cytokine-driven mast cell activation events.

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We greatly appreciate the participation of this patient in the DAOMAST and multiple times in the Anaphylaxis study, the latter partially under very stressful circumstances. This study would not have been possible without this exceptional attitude. We thank Karin Petroczi for help in performing the multiplex assay. We are indebted to Sarah Ely for the final polish in the proper usage of the English language.

Author Contributions

TB conceived the idea, designed the experiments, performed data analysis and interpretation, prepared the figures and wrote the manuscript. RR performed statistical analyses. JM and MW performed multiplex measurements and analysis. PV provided clinical data during regular control visits and critically read the manuscript. BJ is the principal investigator in the DAOMAST and Anaphylaxis studies and contributed to the writing of the manuscript. All authors read, revised and approved the final version of the manuscript.

References

1. Valent P, Akin C, Bonadonna P, Hartmann K, Brockow K, Niedoszytko M, et al. Proposed diagnostic algorithm for patients with suspected mast cell activation syndrome. *J Allergy Clin Immunol Pract*. 2019;7:1125-1133.e1. doi: 10.1016/j.jaip.2019.01.006.
2. Desborough JP, Taylor I, Hattersley A, Garden A, Wolff A, Bloom SR, et al. Massive histamine release in a patient with systemic mastocytosis. *Br J Anaesth*. 1990;65:833-6. doi: 10.1093/bja/65.6.833.
3. Wimazal F, Geissler P, Shnawa P, Sperr WR, Valent P. Severe life-threatening or disabling anaphylaxis in patients with systemic mastocytosis: a single-center experience. *Int Arch Allergy Immunol*. 2012;157:399-405. doi: 10.1159/000329218.
4. Hermine O, Lortholary O, Leventhal PS, Catteau A, Soppelsa F, Baude C, et al. Case-control cohort study of patients' perceptions of disability in mastocytosis. *PLoS One*. 2008;3:e2266. doi: 10.1371/journal.pone.0002266.
5. Jennings S, Russell N, Jennings B, Slee V, Sterling L, Castells M, et al. The Mastocytosis Society survey on mast cell disorders: patient experiences and perceptions. *J Allergy Clin Immunol Pract*. 2014;2:70-6. doi: 10.1016/j.jaip.2013.09.004.
6. Murali MR, Castells MC, Song JY, Dudzinski DM, Hasserjian RP. Case records of the Massachusetts General Hospital. Case 9-2011. A 37-year-old man with flushing and hypotension. *N Engl J Med*. 2011;364:1155-65. doi: 10.1056/NEJMcpc1013929.
7. Boehm T, Ristl R, Joseph S, Petroczi K, Klavins K, Valent P, et al. Metabolome and lipidome derangements during a severe mast cell activation event in a patient with indolent systemic mastocytosis. *J Allergy Clin Immunol*. 2021;S0091-6749(21)00607-2. doi: 10.1016/j.jaci.2021.03.043.
8. Mukai K, Tsai M, Saito H, Galli SJ. Mast cells as sources of cytokines, chemokines, and growth factors. *Immunol Rev*. 2018;282:121-150. doi: 10.1111/imr.12634.
9. Stone SF, Cotterell C, Isbister GK, Holdgate A, Brown SG. Emergency Department Anaphylaxis Investigators. Elevated serum cytokines during human anaphylaxis: Identification of potential mediators of acute allergic reactions. *J Allergy Clin Immunol*. 2009;124:786-92.e4. doi: 10.1016/j.jaci.2009.07.055.
10. Pardanani A, Finke C, Abdelrahman RA, Lasho TL, Hanson CA, Tefferi A. Increased circulating IL-2R α (CD25) predicts poor outcome in both indolent and aggressive forms of mastocytosis: a comprehensive cytokine-phenotype study. *Leukemia*. 2013;27:1430-3. doi: 10.1038/leu.2013.11.

- 474 **11.** Boehm T, Reiter B, Ristl R, Petroczi K, Sperr W, Stimpfl T, et al. Massive release of the
 475 histamine-degrading enzyme diamine oxidase during severe anaphylaxis in mastocytosis
 476 patients. *Allergy*. 2019;74:583-593. doi: 10.1111/all.13663.
- 477 **12.** Fajgenbaum DC, June CH. Cytokine Storm. *N Engl J Med*. 2020;383:2255-2273. doi:
 478 10.1056/NEJMra2026131.
- 479 **13.** Medoff BD, Sauty A, Tager AM, Maclean JA, Smith RN, Mathew A, et al. IFN-gamma-
 480 inducible protein 10 (CXCL10) contributes to airway hyperreactivity and airway
 481 inflammation in a mouse model of asthma. *J Immunol*. 2002;168:5278-86. doi:
 482 10.4049/jimmunol.168.10.5278.
- 483 **14.** Alam R, Forsythe PA, Stafford S, Lett-Brown MA, Grant JA. Macrophage inflammatory
 484 protein-1 alpha activates basophils and mast cells. *J Exp Med*. 1992;176:781-6. doi:
 485 10.1084/jem.176.3.781.
- 486 **15.** Lee SC, Brummet ME, Shahabuddin S, Woodworth TG, Georas SN, Leiferman KM, et al.
 487 Cutaneous injection of human subjects with macrophage inflammatory protein-1 alpha
 488 induces significant recruitment of neutrophils and monocytes. *J Immunol*. 2000;164:3392-
 489 401. doi: 10.4049/jimmunol.164.6.3392.
- 490 **16.** Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. *Cytokine*
 491 *Growth Factor Rev*. 2002;13:455-81. doi: 10.1016/s1359-6101(02)00045-x.
- 492 **17.** Polukort SH, Rovatti J, Carlson L, Thompson C, Ser-Dolansky J, Kinney SR, et al. IL-10
 493 enhances IgE-mediated mast cell responses and is essential for the development of
 494 experimental food allergy in IL-10-deficient mice. *J Immunol*. 2016;196:4865-76. doi:
 495 10.4049/jimmunol.1600066.
- 496 **18.** Bressler RB, Lesko J, Jones ML, Wasserman M, Dickason RR, Huston MM, et al.
 497 Production of IL-5 and granulocyte-macrophage colony-stimulating factor by naive
 498 human mast cells activated by high-affinity IgE receptor ligation. *J Allergy Clin Immunol*.
 499 1997;99:508-14. doi: 10.1016/s0091-6749(97)70078-2.
- 500 **19.** Hagaman DD, Okayama Y, D'Ambrosio C, Prussin C, Gilfillan AM, Metcalfe DD.
 501 Secretion of interleukin-1 receptor antagonist from human mast cells after
 502 immunoglobulin E-mediated activation and after segmental antigen challenge. *Am J*
 503 *Respir Cell Mol Biol*. 2001;25:685-91. doi: 10.1165/ajrcmb.25.6.4541.
- 504 **20.** Metcalfe DD. Mast cells and mastocytosis. *Blood*. 2008;112:946-56. doi: 10.1182/blood-
 505 2007-11-078097.
- 506 **21.** Packard KA, Khan MM. Effects of histamine on Th1/Th2 cytokine balance. *Int*
 507 *Immunopharmacol*. 2003;3:909-20. doi: 10.1016/S1567-5769(02)00235-7.

- 508 **22.** Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in
 509 allergic inflammation: the search for new antihistamines. *Nat Rev Drug Discov.*
 510 2008;7:41-53. doi: 10.1038/nrd2465.
- 511 **23.** Jemima EA, Prema A, Thangam EB. Functional characterization of histamine H4 receptor
 512 on human mast cells. *Mol Immunol* 2014;62:19-28. doi: 10.1016/j.molimm.2014.05.007.
- 513 **24.** Wechsler JB, Szabo A, Hsu CL, Krier-Burris RA, Schroeder HA, Wang MY, et al.
 514 Histamine drives severity of innate inflammation via histamine 4 receptor in murine
 515 experimental colitis. *Mucosal Immunol.* 2018;11:861-870. doi: 10.1038/mi.2017.121.
- 516 **25.** Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR, et al.
 517 Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the
 518 Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch Intern Med.*
 519 2007;167:1655-63. doi: 10.1001/archinte.167.15.1655.
- 520 **26.** Kang S, Tanaka T, Narazaki M, Kishimoto T. Targeting interleukin-6 signaling in clinic.
 521 *Immunity.* 2019;50:1007-1023. doi: 10.1016/j.immuni.2019.03.026.
- 522 **27.** Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al.
 523 An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med.*
 524 2020;26:1636-1643. doi: 10.1038/s41591-020-1051-9.
- 525 **28.** Koike T, Harigai M, Inokuma S, Ishiguro N, Ryu J, Takeuchi T, et al. Effectiveness and
 526 safety of tocilizumab: postmarketing surveillance of 7901 patients with rheumatoid
 527 arthritis in Japan. *J Rheumatol.* 2014;41:15-23. doi: 10.3899/jrheum.130466.
- 528 **29.** Dougan M, Dranoff G, Dougan SK. GM-CSF, IL-3, and IL-5 Family of Cytokines:
 529 Regulators of Inflammation. *Immunity.* 2019;50:796-811. doi:
 530 10.1016/j.immuni.2019.03.022.
- 531 **30.** Panousis C, Dhagat U, Edwards KM, Rayzman V, Hardy MP, Braley H, et al. CSL311, a
 532 novel, potent, therapeutic monoclonal antibody for the treatment of diseases mediated by
 533 the common β chain of the IL-3, GM-CSF and IL-5 receptors. *MAbs.* 2016;8:436-53. doi:
 534 10.1080/19420862.2015.1119352.
- 535 **31.** Marone G, Granata F, Pucino V, Pecoraro A, Heffler E, Loffredo S, et al. The intriguing
 536 role of interleukin 13 in the pathophysiology of asthma. *Front Pharmacol.* 2019;10:1387.
 537 doi: 10.3389/fphar.2019.01387.
- 538 **32.** Holgate ST, Bodey KS, Janezic A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES,
 539 MIP-1 alpha, and MCP-1 into asthmatic airways following endobronchial allergen
 540 challenge. *Am J Respir Crit Care Med.* 1997;156:1377-83. doi:
 541 10.1164/ajrccm.156.5.9610064.

- 542 **33.** Busse WW, Lemanske RF Jr. Asthma. *N Engl J Med*. 2001;344:350-62. doi:
543 10.1056/NEJM200102013440507.
- 544 **34.** Gludovacz E, Schuetzenberger K, Resch M, Tillmann K, Petroczi K, Vondra S, et al.
545 Heparin-binding motif mutations of human diamine oxidase allow the development of a
546 first-in-class histamine-degrading biopharmaceutical. *Elife*. 2021;10:e68542. doi:
547 10.7554/eLife.68542.
- 548 **35.** Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine
549 production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin*
550 *Invest*. 2001;108(12):1865-73. doi: 10.1172/JCI13930.
- 551 **36.** Desai P, Thurmond RL. Histamine H₄ receptor activation enhances LPS-induced IL-6
552 production in mast cells via ERK and PI3K activation. *Eur J Immunol*. 2011;41(6):1764-
553 73. doi: 10.1002/eji.201040932.
- 554 **37.** Barnes PJ. Corticosteroids: the drugs to beat. *Eur J Pharmacol*. 2006;533(1-3):2-14. doi:
555 10.1016/j.ejphar.2005.12.052.

FIG 1. Time course of chemokine and Th1/Th2 cytokine changes during the severe MCA event F18.

The concentrations of 41 chemokines and cytokines were measured in 7 samples (5 in duplicate) during the MCA event and normalized to 3 to 5 baseline (BL) samples; The means of triplicate determinations of the singlicate and duplicate samples are shown; Histamine (HIS) concentrations have recently been published⁷ and are shown as white triangles with a dotted line as a reference line; The y-axes represent fold-changes compared to BL on a log2 scale; **A**, and **B**, Selected chemokines; The peak Benjamini-Hochberg corrected (BH_corr) p-values of the parameters from left to right are: 0.022, <0.001 (CCL3), 0.045 (A) and not significant (NS), NS, 0.039 (B) ; **C**, and **D**, Th1-associated cytokines; All BH_corr p-values are NS; **E**, and **F**, Th2-associated chemokines; The peak BH_corr p-values of the parameters from left to right are: <0.01, NS, 0.011, <0.01(E) and <0.001, <0.01, <0.01, 0.019, NS (F); **G**, and **H**, Growth factors and other parameters; Only the peak BH_corr p-values for GM-CSF (<0.01) and VEGF (0.048) are significant; Reverse fold changes reflect a decrease in concentrations compared to baseline; The time course of p-values of selected chemokines are presented in the Online Repository Fig E1; Gran B = Granzyme B; Perio = Periostin.

FIG 2. Characterization of cytokine, chemokine and growth factor changes during the severe N14 and moderate J15 MCA events.

The concentrations of 41 chemokines and cytokines were measured in 2 samples from the N14 and J15 MCA events and compared to 3 to 5 baseline (BL) samples; The N14 samples were collected on the day of admission 4 hours apart. The J15 samples were collected on admission and on the second day 17 hours later. The means of triplicate determinations are shown; The y-axes represent fold-changes compared to BL; **A**, Selected chemokines and histamine (HIS); For N14 the peak Benjamini-Hochberg corrected (BH_corr) p-values of the parameters from left to right are: <0.01 (CCL3), NS, 0.045, NS, 0.028; For J15 only the p-value for CXCL11 is significant (0.024); **B**, and **C**, Selected Th2-associated chemokines; In Panel B for the N14 event the peak BH_corr p-values of the parameters from left to right are: <0.01, 0.016, <0.01, <0.001 and <0.01; In Panel B for the J15 event only IL-10 (0.023) and IL-11 (<0.001) show significant peak BH_corr p-values; In Panel C for the N14 event only the BH_corr p-values for IL-33 (0.033) and Perio (0.03) are significant and for the J15 event only for TSLP (0.049) and Perio (0.046); **D**, Growth factors and other parameters; For the N14 event only the BH_corr p-values of IL-3 (0.012) and GM-CSF (<0.01) are significant and for the J15 event it is also only IL-3 (0.03) and GM-CSF (<0.01) which show significant BH_corr p-values; Perio = Periostin.

FIG 3. Time course of major chemokine/cytokine derangements in relation to histamine and tryptase concentrations during the severe MCA event F18. Histamine (HIS), tryptase (TRYP), diamine oxidase (DAO) and lysophosphatidylcholine (LPC) concentrations and the functional small bowel length (fSBL) data have recently been published.⁷ For LPCs the mean of 11 LPCs comprising 96% of the LPCs in plasma have been used. The 5 LPC species with at least 3 unsaturated bonds, and therefore weak autotaxin substrates, have been excluded. Only chemokines and cytokines increasing more than 5-fold compared to baseline (BL) values have been selected; All x-axes data have been normalized to BL values. The y-axes represent fold-changes compared to BL on a log2 scale; **A**, HIS (filled squares), TRYP (white circles), DAO (black circles); LPC (white squares) and fSBL (white triangle); **B**, HIS (black squares), CCL3 (black circles); CXCL10 (white circles), IL-1ra (black diamonds), IL-10 (white diamonds), IL-5 (white triangles), IL-13 (black triangles), IL-6 (white squares) and GM-CSF (crosses).

606 **TABLE 1. Major clinical characteristics of the four MCA events.**

F18		SIRS Y/N
Temperature	Many hours > 38°C; >40°C during acute phase	Y
Heart rate	130 bpm during acute phase; peak 180 bpm; normal mean (SD) 68 (7.8)	Y
WBC	15000 G/L during acute phase	Y
Resp. rate	Mean (SD) 19.8 (2.92) using 8 values averaged every 15 min	Y/N
(Glucose	8.9 to 13.9 mM during acute phase	Y)
MAP	Between 50 and 70 for many hours; normal mean (SD) 91 (5.9)*	3.5
General	Multiple vomiting events, nausea, weak, fSBL** 6% at symptom peak	SIRS
Lactate	At least 7 mM during acute phase, rapidly decreasing after peak	YES
ICU	48 hours ICU treatment; external cooling device (CoolGuard)	
Skin	Generalized erythematous rash	
N14		
Temperature	Several hours >39°C	Y
Heart rate	Consistently > 110 bpm; normal mean (SD) 68 (7.8)	Y
WBC	6300 G/L at admission; 8010 G/L on Day 2	N
Resp. rate	No data	nd
(Glucose	7.7 mM at admission; 10.6 mM 5 hours later; 6.4 mM next day	Y)
MAP	Between 50 and 70 during the first 7 hours post admission	2.0
General	Two days of fever at home, headache, nausea, urticaria	SIRS
	Multiple vomiting events, weak	YES
Skin	Generalized erythematous rash	
F20		
Temperature	38.1°C on admission, decreased after admission	Y/N
Heart rate	> 100 bpm; normal mean (SD) 68 (7.8)	Y
WBC	6140 G/L at admission; 7000 after 24 and 4200 after 38 hours	N
Resp. rate	No data	nd
(Glucose	9.1 mM at admission; 10.1 mM 24 hours later	Y)
MAP	Blood pressure recorded several times as normal and stable	1.5
Infection	Influenza A PCR positive; treated with 150 mg daily Tamiflu for 5 days	SIRS
General	Vomited approximately 10 times at home prior to admission at 04.00 AM	NO
	Patient took rescue medication before admission: 25 mg prednisolone; 50 mg diphenhydramine; 10 mg cetirizine	
Skin	Generalized erythematous rash	
J15		
Temperature	<38°C during the 2 day hospital stay	N
Heart rate	112 bpm on admission; normal mean (SD) 68 (7.8)	Y
WBC	8320 G/L on admission; 8580; 8170; 7510 during the first 24 hours	N
Resp. rate	< 20 breaths per minute	N
(Glucose	6.9 mM on admission; next day 5.7 mM	N)
MAP	Between 70 and 80; normal mean (SD) 91 (5.9)	1.0
General	Weak, no fever, rhinitis, nausea	SIRS
Skin	No rash and no urticaria	NO

*Systolic/diastolic mean (SD) 124/75 (7.7; 5.4) 24 measurements without symptoms; **fSBL =

functional small bowel length; SIRS (systemic inflammatory response syndrome) criteria:

Temperature < 36°C, >38°C; heart rate > 90 bpm; white blood cells (WBC) < 4000, >12000 G/L;

610 respiratory rate > 20 breaths per min; (glucose > 7.7 mM); SD = standard deviation; MAP = mean
611 arterial pressure; ICU = intensive care units; bpm = beats per minute.

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TABLE 2. Mean baseline with peak concentrations (pg/ml) and corresponding peak fold-changes during the four MCA events.

	Mean BL (pg/ml)	Peak concentrations (pg/ml)				Peak fold-changes compared to BL				p-values
		F18	N14	J15	F20	F18	N14	J15	F20	
CCL2	170	<u>536</u>	118	63	147	<u>3.2</u>	0.69	0.37	0.86	0.022
CCL3	2.5	<u>72</u>	25	2.5	2.5	<u>29</u>	10	1	1	<0.001
CCL4	130	<u>462</u>	224	156	270	<u>3.6</u>	1.7	1.2	2.1	0.045
CCL8	46	17	131	99	<u>178</u>	0.36	2.9	2.2	<u>3.9</u>	NS
CCL17	266	297	368	259	<u>1174</u>	1.1	1.4	1	<u>4.4</u>	NS
CCL20	19	143	<u>176</u>	99	51	7.6	9.4	5.3	2.7	NS
CCL22	179	<u>11</u>	125	173	<u>216</u>	<u>0.06</u>	0.7	1	<u>1.2</u>	NS
CXCL2	457	1521	1648	598	<u>2401</u>	3.3	3.6	1.3	<u>5.3</u>	NS
CXCL8	2	4.9	1.8	5.2	<u>6.4</u>	2.4	0.9	2.5	<u>3.2</u>	NS
CXCL10	50	<u>545</u>	334	269	374	<u>11</u>	6.7	5.4	7.5	0.039
CXCL11	24	26	98	<u>113</u>	104	1.1	4.1	<u>4.7</u>	4.4	0.016
IL-2	2.1	0.9	2	1.6	<u>10</u>	0.42	0.95	0.77	<u>4.9</u>	NS
INF α	2.1	2.5	2.1	2.1	<u>10</u>	1.2	1	1	<u>4.9</u>	0.028
IFN γ	2.7	3.3	2.7	2.7	<u>17</u>	1.2	1	1	<u>6.1</u>	0.013
TNF α	1.1	1.3	0.4	1.1	<u>7.5</u>	1.2	0.4	1	<u>7</u>	<0.01
sCD40L	420	1445	902	2306	<u>2941</u>	3.4	2.1	5.5	<u>7</u>	NS
IL-1ra	217	<u>21422</u>	10057	937	1965	<u>99</u>	46	4.3	9	<0.01
IL-4	0.3	0.5	0.4	0.3	1.7	1.6	1.3	0.8	5.4	NS
IL-5	1.2	<u>52</u>	10	1.2	1.2	<u>44</u>	8.2	1	1	<0.001
IL-6	3.9	<u>1104</u>	222	12	34	<u>280</u>	56	2.9	8.7	0.011
IL-10	30	<u>2821</u>	795	431	631	<u>93</u>	26	14	21	<0.01
IL-11	70	343	<u>1093</u>	820	407	4.9	<u>16</u>	12	5.8	<0.001
IL-13	34	<u>221</u>	66	35	114	<u>6.6</u>	2	1	3.4	<0.01
IL-33	1.9	7.6	1.8	1.9	<u>12</u>	4.1	1	1	<u>6.2</u>	0.016
GM-CSF	11	<u>69</u>	42	40	46	<u>6</u>	3.7	3.5	4.1	<0.01
bFGF	0.5	5.6	3.6	4	<u>9.9</u>	10	6.7	7.3	<u>18</u>	NS

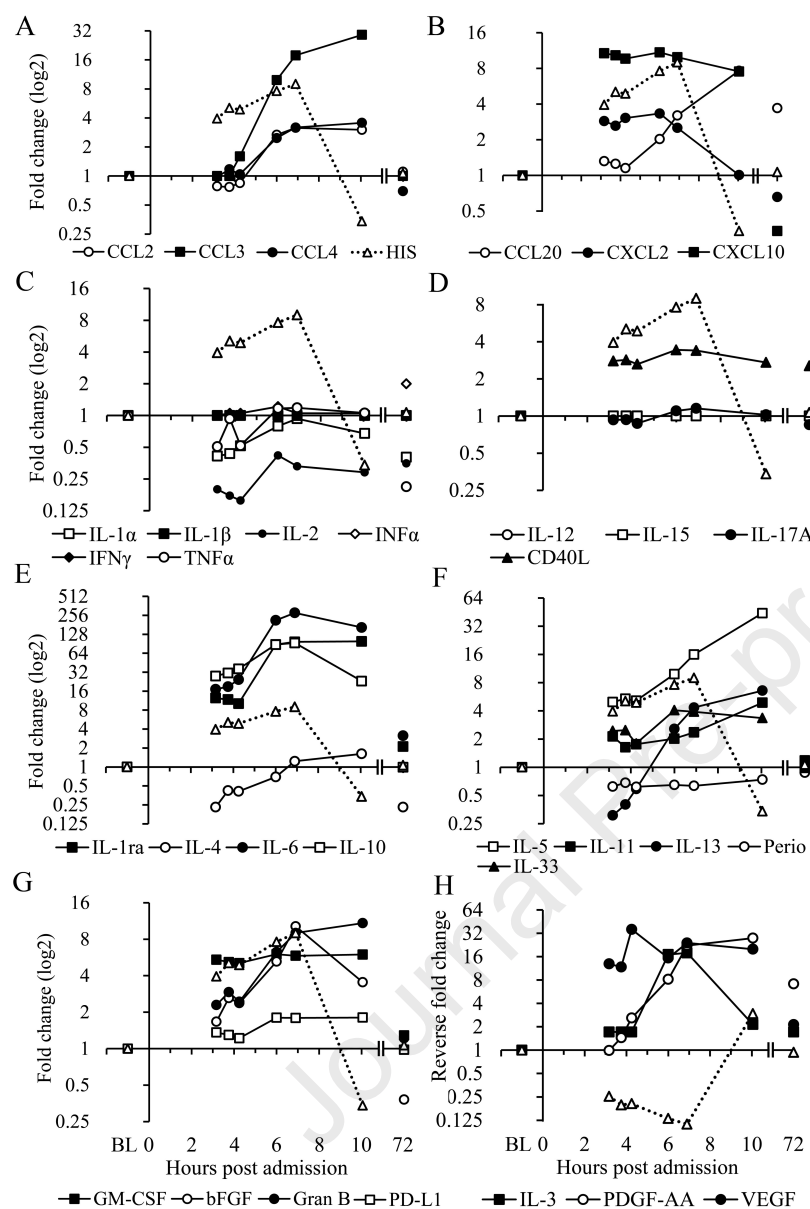
IL-3	26	<u>1.5</u>	462	205	70	<u>0.06</u>	18	7.9	2.7	0.012
PDGF-AA	520	<u>19</u>	498	410	2488	<u>0.04</u>	1	0.79	4.8	NS
VEGF-A	10	<u>0.4</u>	0.8	16	34	<u>0.04</u>	0.08	1.6	3.4	NS
Gran B	2.7	29	8.5	8.9	36	11	3.1	3.3	13	NS
PD-L1	53	96	66	56	177	1.8	1.2	1.1	3.3	0.023

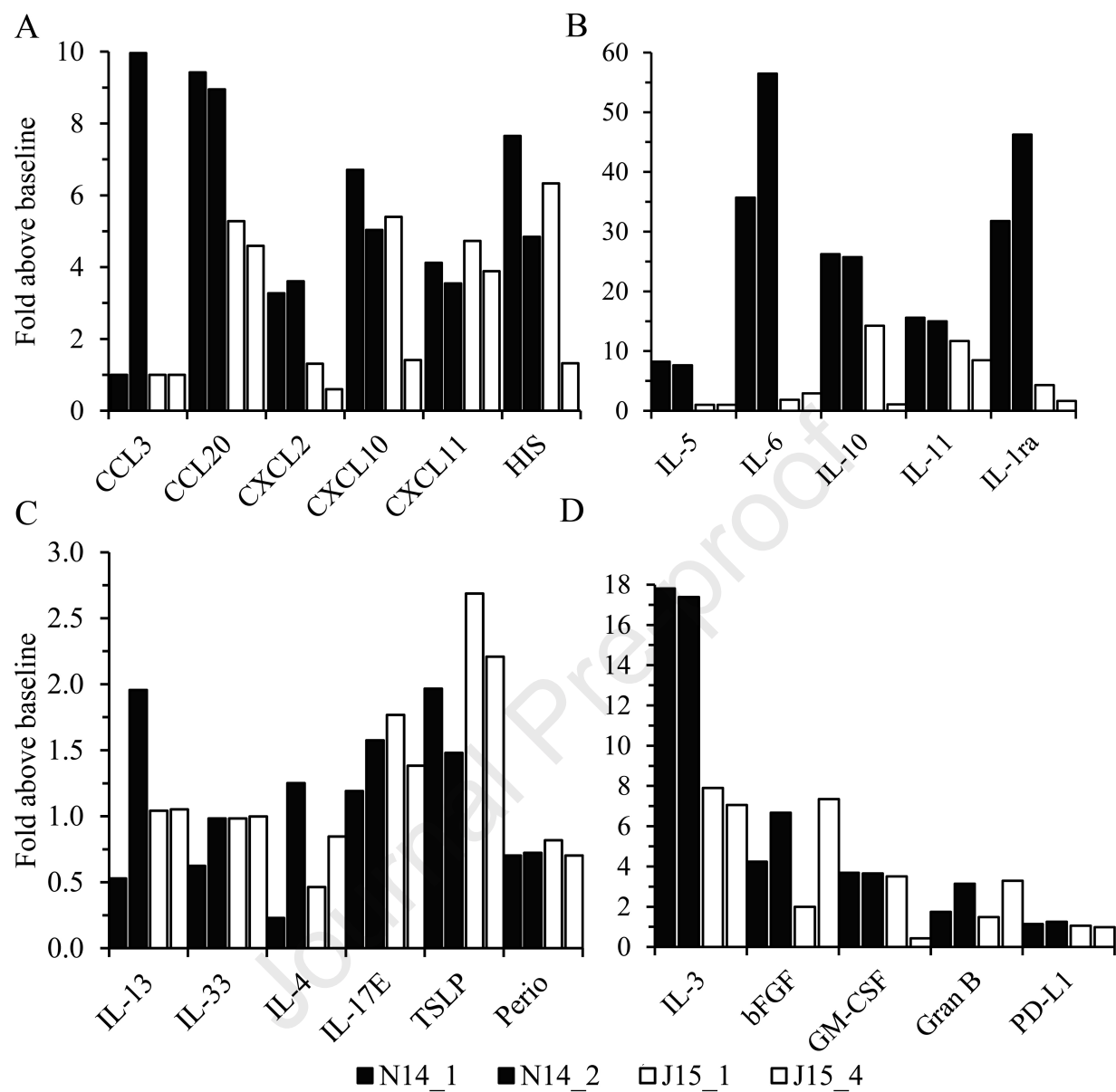
Parameters with fold changes below 3 above or below baseline in none of the four MCA events are not shown; These proteins include CCL7, CCL11, IL-1 α , IL-1 β , IL-12p70, IL-15, IL-17A, IL-17E, TSLP and Periostin; Cursive numbers in bold boxes represent parameter peak concentration decreasing during the severe MCA event F18; Peak concentrations and peak fold-increases from the four MCA attacks are written in bold and underlined and the Benjamini-Hochberg corrected (BH_corr) p-values for the peak concentrations have been added in the last column; CCL22, IL-3, PDGF-AA and VEGF-A are reduced maximally 16-, 18-, 28- and 24-fold respectively during the F18 event, but only the VEGF-AA reduction shows a significant BH_corr p-value of 0.048; Gran B = Granzyme B, BL = Baseline.

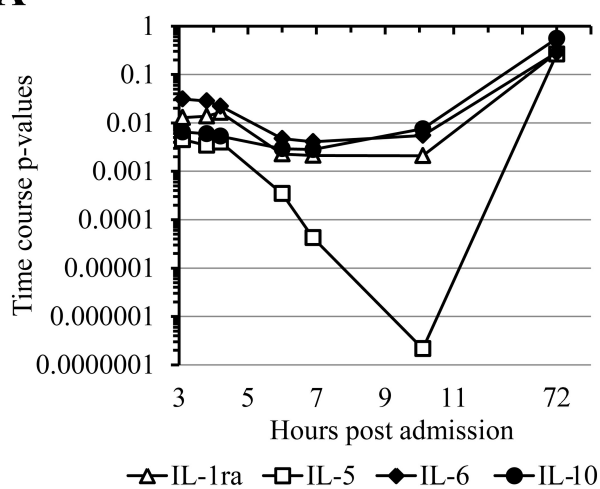
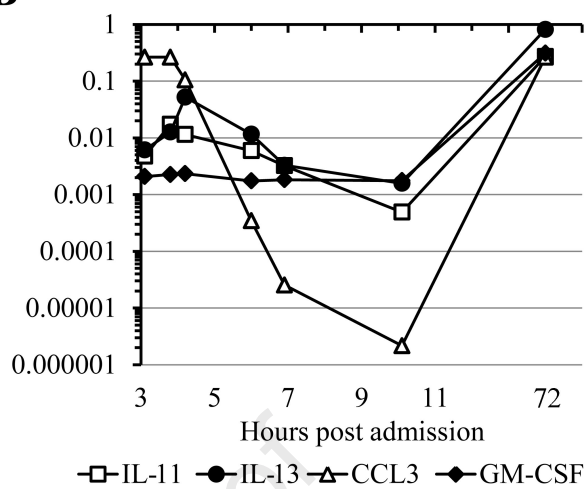
TABLE 3. Peak fold chemokine and cytokine increases/decreases after normalization to the moderate MCA event J15.

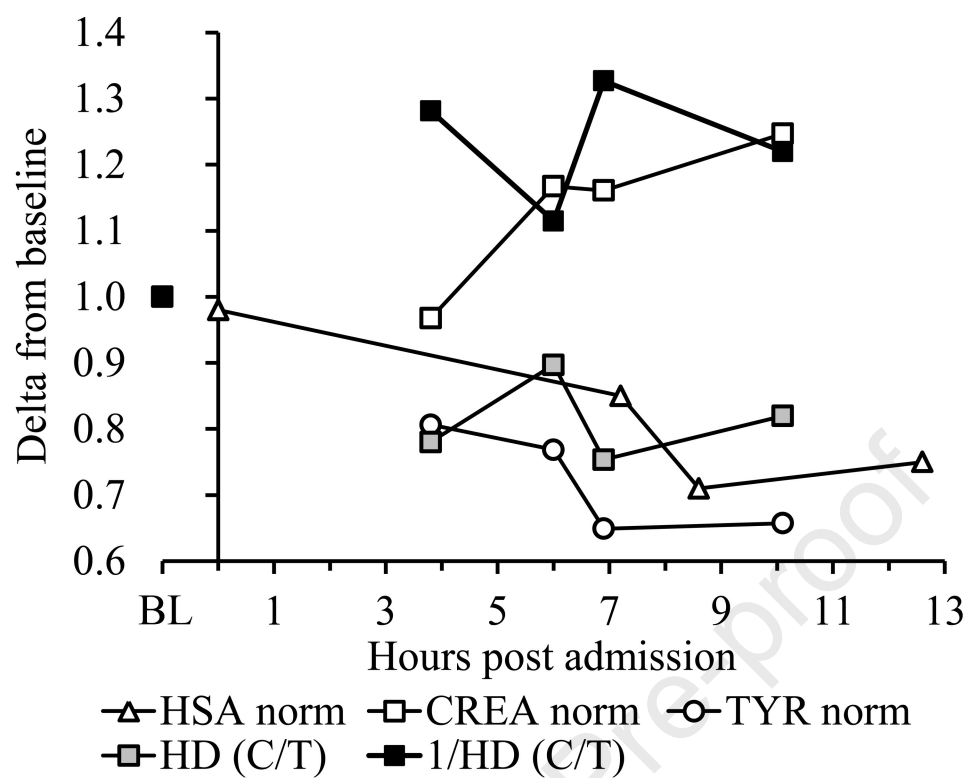
	F18/J15	N14/J15	J15/J15	F20/J15	Direction
CCL2	<u>8.6</u>	1.9	1.0	2.3	up
CCL3	<u>29</u>	10.0	1.0	1.0	
IL-1ra	<u>23</u>	11	1.0	2.1	
IL-4	1.9	1.5	1.0	<u>6.4</u>	
IL-5	<u>44</u>	8.2	1.0	1.0	
IL-6	<u>95</u>	19	1.0	2.9	
IL-10	<u>6.5</u>	1.8	1.0	1.5	
IL-13	<u>6.3</u>	1.9	1.0	3.2	
IL-33	4.1	1.0	1.0	<u>6.2</u>	
IL-2	0.5	1.2	1.0	<u>6.3</u>	
IFN γ	1.2	1.0	1.0	<u>6.1</u>	down
TNF α	1.2	0.4	1.0	<u>7.1</u>	
CCL22	<u>-15</u>	-1.4	1.0	-0.8	
IL-3	<u>-141</u>	-0.4	1.0	-2.9	
PDGF-AA	<u>-22</u>	-0.8	1.0	-0.16	
VEGF-A	<u>-38</u>	-21	1.0	-0.5	

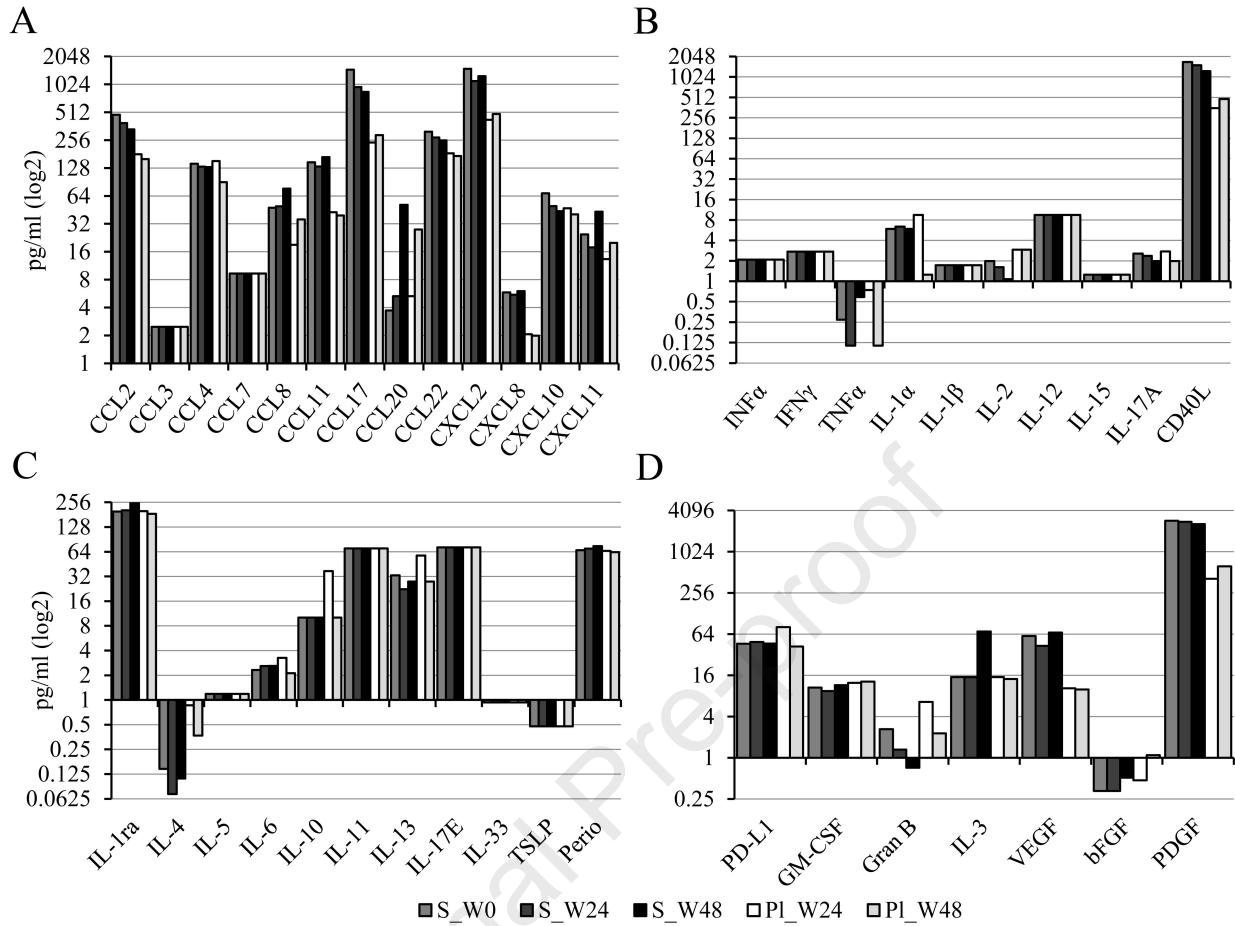
Parameters are only included if they show an at least 5-fold peak increase or decrease compared to the moderate MCA event J15; Decreased parameter concentrations are converted to reverse fold as indicated by a minus sign; The highest peak fold increases or decreases are written in bold font and underlined.

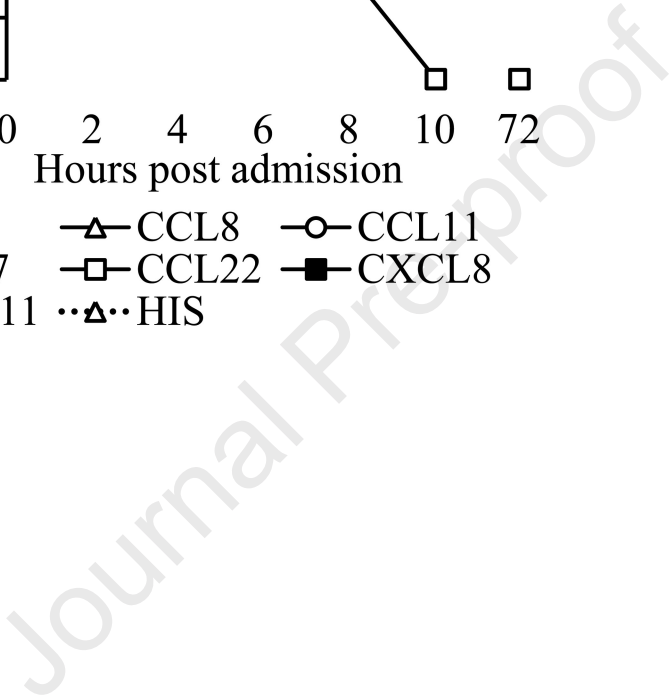


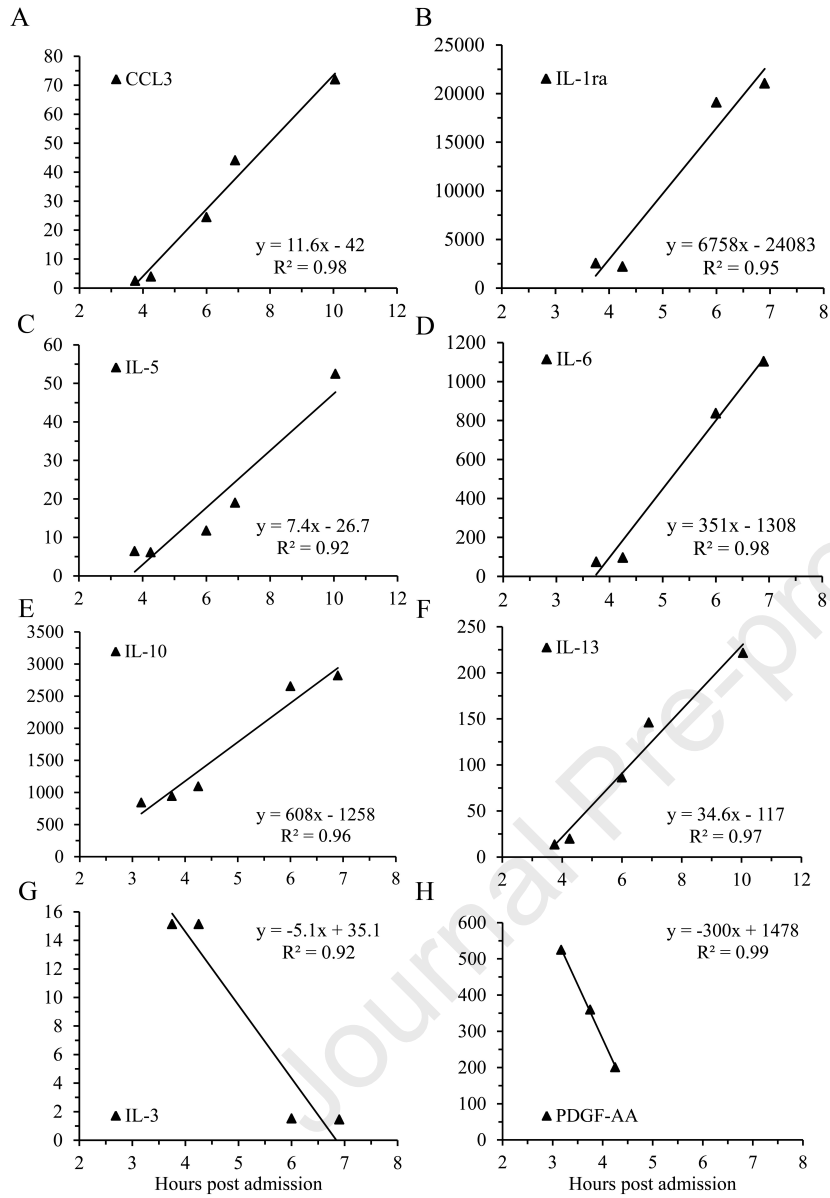


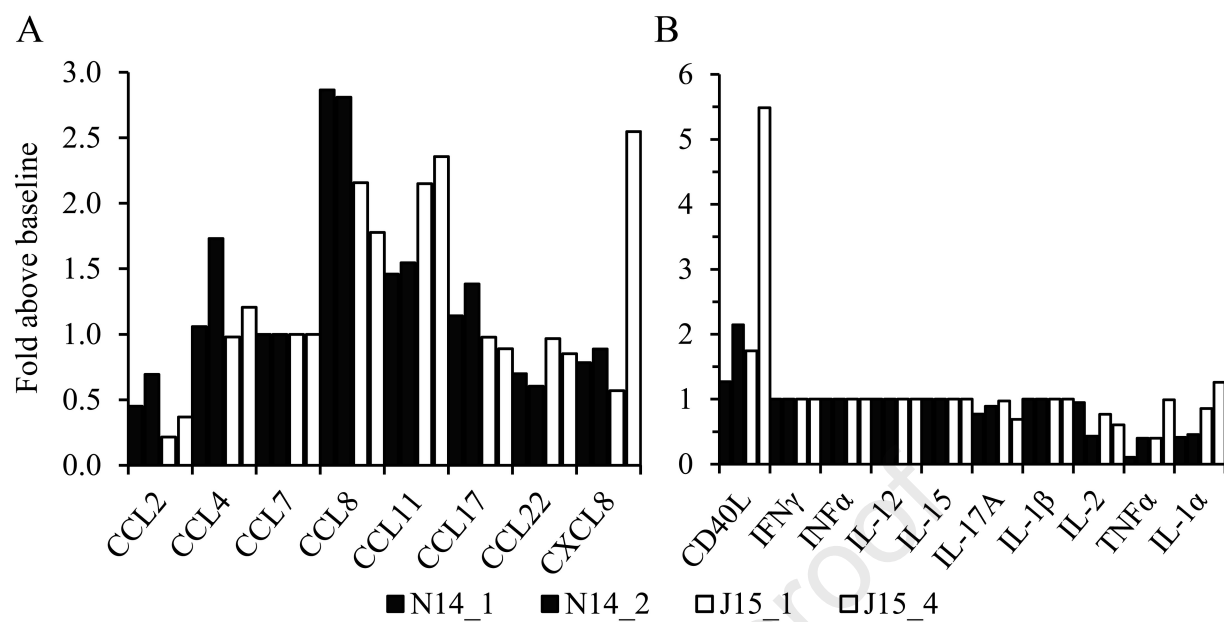
A**B**

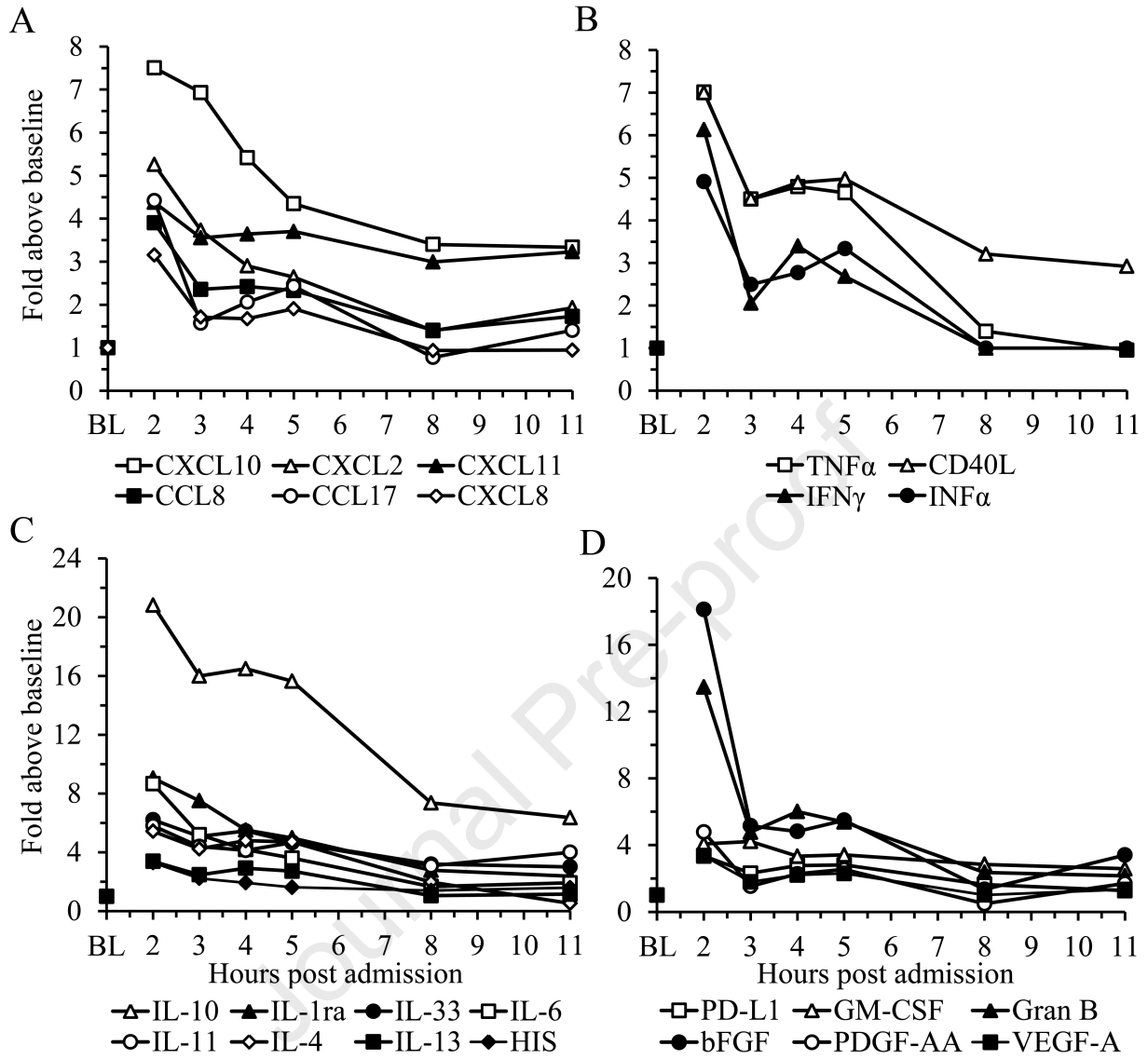




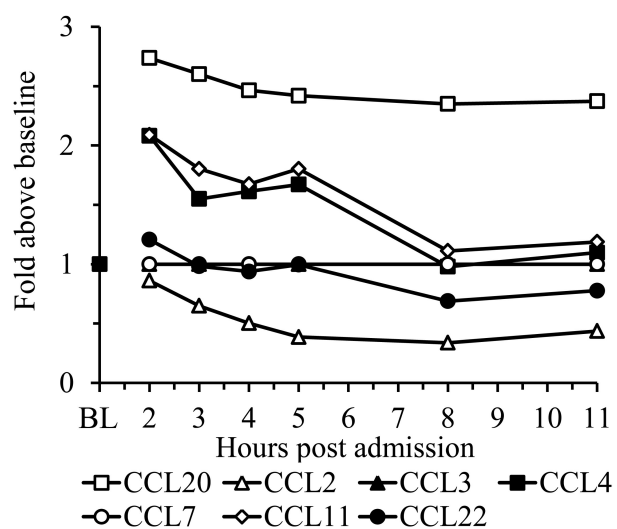








A



B

