Aus der Klinik für Rheumatologie und Klinische Immunologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Ubiquitin proteasome system and myopathies

zur Erlangung des akademischen Grades
Doctor rerum medicinalium (Dr. rer. medic.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Khetam Ghannam

Aus Kuwait

Datum der Promotion: 04.09.2015

Gutachter/in:

N.N.

Inhaltsverzeichnis

Zusammenfassung **2**
Abstract **4**
Eidesstattliche Versicherung/Anteilserklärung **5**
Auszug aus der Journal Summary List **6**
Publikation **12**
Upregulation of immunoproteasome subunits in myositis indicates active inflammation with involvement of antigen presenting cells, CD8 T-cells and IFNγ 12
Lebenslauf **25**
Publikationsliste: **28**
Danksagung **29**

Zusammenfassung

Ziel

Idiopathische inflammatorische Myopathien (IIM) sind gekennzeichnet durch die Infiltration von Immunzellen in den Muskel und eine erhöhte MHC-I-Expression. Dies lässt auf eine erhöhte Antigenpräsentation und Beteiligung des Proteasom-Systems schließen. Um die Rolle von Immunoproteasomen bei der Myositis zu ergründen, wurden einzelne Zelltypen sowie Muskelgewebe betrachtet und nach möglichen Auslösern der Immunantwort gesucht.

Methoden

Die Expression von konstitutiven (PSMB5, -6, -7) und korrespondierenden Immunoproteasom-Untereinheiten (PSMB8, -9, -10) wurde mittels real-time RT-PCR in Muskelbiopsien und sortierten peripheren Blutzellen von Patienten mit IIM im Vergleich zu nicht-inflammatorischen Myopathien (NIM) und gesunden Spendern (HD) analysiert. Die Proteinanalyse in Muskelbiopsien wurde über Western-Blot-Verfahren durchgeführt. Affymetrix HG-U133 Transkriptom Daten aus Biopsien von verschiedenen Muskelerkrankungen, Immunzelltypen und spezifisch stimulierten Monozyten dienten zur Validierung und Analyse der Co-Regulierung bzw. Co-Expression von Genen.

Ergebnisse

Die Real-time RT-PCR Untersuchung ergab eine signifikant erhöhte Expression der Immunoproteasom-Untereinheiten (PSMB8 / -9 / -10) in dendritischen Zellen (DC), Monozyten und CD8 + T-Zellen bei IIM. In Muskelbiopsien der IIM lagen die Messwerte für die Immunproteasom-Untereinheiten höher als in NIM und übertrafen auch die Spiegel aus den parallel erhobenen Blutproben. Die Proteine PSMB8 und -9 wurden nur in IIM aber nicht in NIM Muskelbiopsien gefunden. Die Nachuntersuchung von bereits publizierten Muskel-Transkriptomen von 78 Myositisbiopsien und 20 gesunden Kontrollgeweben bestätigte diese Ergebnisse und zeigte eine Beteiligung von Antigenprozessierung und -präsentation. Der Vergleich mit den Referenzprofilen aus sortierten Immunzellen und gesundem Muskel bestätigte die Hochregulation von PSMB8 und -9 in Myositisbiopsien über eine durch Zellinfiltration bedingte Veränderung hinaus. Diese Hochregulation korrelierte am stärksten mit der Expression von STAT1, IRF1 und IFNγ. Eine Erhöhung der T-Zell-spezifischen Transkripte in aktivem IIM Muskelgewebe wurde von einem Anstieg der DC und Monozyten Markergene begleitet und spiegelte damit die Zelltyp-spezifische Beteiligung aus dem peripheren Blut wider.

Schlussfolgerungen

Das Proteasom-System ist bei aktiver IIM aktiviert, wobei die Hochregulation von Immunoproteasomen auf eine Involvierung in Antigenprozessierung und -präsentation bei diesen Erkrankungen hinweist. Dies könnte einen therapeutischen Ansatzpunkt durch spezifische Hemmung von Immunoproteasomen darstellen.

Abstract

Objective: In idiopathic inflammatory myopathies (IIM) infiltration of immune cells into muscle and upregulation of MHC-I expression implies increased antigen presentation and involvement of the proteasome system. To decipher the role of immunoproteasomes in myositis, we investigated individual cell types and muscle tissues and focused on possible immune triggers.
Methods: Expression of constitutive (PSMB5, -6, -7) and corresponding immunoproteasomal subunits (PSMB8, -9, -10) was analyzed by real-time RT-PCR in muscle biopsies and sorted peripheral blood cells of patients with IIM, non-inflammatory myopathies (NIM) and healthy donors (HD). Protein analysis in muscle biopsies was performed by western blot. Affymetrix HG-U133 platform derived transcriptome data from biopsies of different muscle diseases and from immune cell types as well as monocyte stimulation experiments were used for validation, coregulation and coexpression analyses.
Results: Real-time RT-PCR revealed significantly increased expression of immunoproteasomal subunits (PSMB8/-9/-10) in DC, monocytes and CD8+ T-cells in IIM. In muscle biopsies, the immunosubunits were elevated in IIM compared to NIM and exceeded levels of matched blood samples. Proteins of PSMB8 and -9 were found only in IIM but not NIM muscle biopsies. Reanalysis of 78 myositis and 20 healthy muscle transcriptomes confirmed these results and revealed involvement of the antigen processing and presentation pathway. Comparison with reference profiles of sorted immune cells and healthy muscle confirmed upregulation of PSMB8 and -9 in myositis biopsies beyond infiltration related changes. This upregulation correlated highest with STAT1, IRF1 and IFNγ expression. Elevation of T-cell specific transcripts in active IIM muscles was accompanied by increased expression of DC and monocyte marker genes and thus reflects the cell type specific involvement observed in peripheral blood.
Conclusions: Immunoproteasomes seem to indicate IIM activity and suggest that dominant involvement of antigen processing and presentation may qualify these diseases exemplarily for the evolving therapeutic concepts of immunoproteasome specific inhibition.

Eidesstattliche Versicherung/Anteilserklärung

„Ich, Khetam Ghannam, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: Ubiquitin proteasome system and myopathies selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.icmje.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.
Mein Anteil an der ausgewählten Publikation entspricht dem, der in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben ist.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

_____ _____

Datum Unterschrift

Ausführliche Anteilserklärung an der erfolgten Publikation

Khetam Ghannam, Lorena Martinez-Gamboa, Lydia Spengler, Sabine Krause, Biljana Smiljanovic, Marc Bonin, Salyan Bhattarai, Andreas Grützkau, Gerd-R. Burmester, Thomas Häupl, Eugen Feist, Upregulation of immunoproteasome subunits in myositis indicates active inflammation with involvement of antigen presenting cells, CD8 T-cells and IFNγ, PLoS One 9: e104048, 2014
Beitrag im Einzelnen: Konzipieren, Planen und Durchführen die Experimente, Analysieren die Daten und Schreiben das Manuskript.

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers/der betreuenden Hochschullehrerin

_____

Unterschrift des Doktoranden/der Doktorandin

_____

ISI Web of Knowledge℠

Journal Citation Reports®

WELCOME HELP

2013 JCR Science Edition

Journal Summary List

Journal Title Changes

Journals from: subject categories MULTIDISCIPLINARY SCIENCES VIEW CATEGORY SUMMARY LIST

Sorted by: Impact Factor SORT AGAIN

Journals 1 - 20 (of 55) [1 | 2 | 3] **Page 1 of 3**

MARK ALL UPDATE MARKED LIST

Ranking is based on your journal and sort selections.

Mark	Rank	Abbreviated Journal Title (linked to journal information)	ISSN	JCR Data						*Eigenfactor*® Metrics	
				Total Cites	Impact Factor	5-Year Impact Factor	Immediacy Index	Articles	Cited Half-life	*Eigenfactor*® Score	*Article Influence*® Score
☐	1	NATURE	0028-0836	590324	42.351	40.783	8.457	857	9.8	1.60419	22.110
☐	2	SCIENCE	0036-8075	537035	31.477	34.463	8.568	841	9.9	1.27630	17.644
☐	3	NAT COMMUN	2041-1723	17193	10.742	11.023	1.721	1591	1.9	0.12343	5.649
☐	4	P NATL ACAD SCI USA	0027-8424	565934	9.809	10.727	2.039	3901	8.2	1.50118	4.863
☐	5	SCI REP-UK	2045-2322	7118	5.078	5.078	0.823	2484	1.4	0.03624	2.168
☐	6	ANN NY ACAD SCI	0077-8923	46347	4.313	3.915	0.943	316	9.2	0.08392	1.353
☐	7	J R SOC INTERFACE	1742-5689	6525	3.856	4.875	1.034	292	3.9	0.02975	1.770
☐	8	PLOS ONE	1932-6203	226708	3.534	4.015	0.416	31496	2.5	1.16747	1.366
☐	9	PHILOS T R SOC A	1364-503X	12980	2.864	3.130	1.378	312	8.0	0.03427	1.386
☐	10	P JPN ACAD B-PHYS	0386-2208	970	2.562	2.914	0.406	32	4.4	0.00377	1.026
☐	11	P ROY SOC A-MATH PHY	1364-5021	16841	1.998	2.241	0.365	178	>10.0	0.01831	1.145
☐	12	NATURWISSENSCHAFTEN	0028-1042	6353	1.971	2.119	0.416	125	>10.0	0.00791	0.704
☐	13	SCI ENG ETHICS	1353-3452	650	1.516	1.377	0.209	91	6.3	0.00135	0.381
☐	14	CHINESE SCI BULL	1001-6538	9439	1.365	1.421	0.366	599	6.3	0.01811	0.360
☐	15	SCI AM	0036-8733	4946	1.328	1.686	0.315	124	>10.0	0.00560	0.745
☐	16	SCI WORLD J	1537-744X	3591	1.219	1.300	0.095	1502	2.9	0.01075	0.343
☐	17	P ROMANIAN ACAD A	1454-9069	173	1.115	0.644	0.172	64	1.9	0.00042	0.114
☐	18	J ROY SOC NEW ZEAL	0303-6758	544	1.077	1.596	0.059	17	>10.0	0.00057	0.602
☐	19	ISSUES SCI TECHNOL	0748-5492	253	1.059	0.804	0.290	31	6.4	0.00110	0.478
☐	20	S AFR J SCI	0038-2353	1764	1.031	0.966	0.191	68	>10.0	0.00194	0.325

MARK ALL UPDATE MARKED LIST

Journals 1 - 20 (of 55) [1 | 2 | 3] **Page 1 of 3**

ISI Web of Knowledge℠

Journal Citation Reports®

WELCOME HELP

2013 JCR Science Edition

Journal Summary List

Journal Title Changes

Journals from: subject categories MULTIDISCIPLINARY SCIENCES VIEW CATEGORY SUMMARY LIST

Sorted by: Impact Factor SORT AGAIN

Journals 21 - 40 (of 55) [1 | 2 | 3] **Page 2 of 3**

MARK ALL UPDATE MARKED LIST

Ranking is based on your journal and sort selections.

Mark	Rank	Abbreviated Journal Title (linked to journal information)	ISSN	JCR Data						*Eigenfactor*® Metrics	
				Total Cites	Impact Factor	5-Year Impact Factor	Immediacy Index	Articles	Cited Half-life	*Eigenfactor*® Score	*Article Influence*® Score
☐	21	COMPLEXITY	1076-2787	552	1.029	1.290	0.343	35	8.7	0.00115	0.473
☐	22	INT J BIFURCAT CHAOS	0218-1274	4328	1.017	1.052	0.195	246	8.3	0.00835	0.359
☐	23	SYMMETRY-BASEL	2073-8994	199	0.918	1.207	0.077	13	3.3	0.00161	0.585
☐	24	DISCRETE DYN NAT SOC	1026-0226	749	0.882	0.876	0.233	258	2.9	0.00212	0.199
☐	25	AN ACAD BRAS CIENC	0001-3765	1433	0.875	1.099	0.229	140	8.6	0.00234	0.314
☐	26	CURR SCI INDIA	0011-3891	7772	0.833	0.931	0.320	347	9.4	0.00774	0.260
☐	27	T ROY SOC SOUTH AUST	0372-1426	386	0.800	0.476	0.077	13	>10.0	0.00021	0.147
☐	28	ADV COMPLEX SYST	0219-5259	439	0.786	0.918	0.000	44	5.8	0.00151	0.353
☐	29	REND LINCEI-SCI FIS	2037-4631	191	0.757	0.924	0.192	52	3.6	0.00066	0.250
☐	30	MATH MODEL NAT PHENO	0973-5348	354	0.725	0.832	0.386	70	3.7	0.00255	0.466
☐	31	AM SCI	0003-0996	1859	0.643	0.874	0.200	45	>10.0	0.00157	0.452
☐	32	FRACTALS	0218-348X	647	0.632	0.647	0.000	24	>10.0	0.00102	0.303
☐	33	SAINS MALAYS	0126-6039	361	0.480	0.427	0.012	242	2.8	0.00082	0.064
☐	34	ACTA SCI-TECHNOL	1806-2563	132	0.458	0.431	0.090	100	3.1	0.00024	0.060
☐	35	CHIANG MAI J SCI	0125-2526	196	0.418	0.531	0.019	108	4.1	0.00043	0.088
☐	36	TECHNOL REV	1099-274X	292	0.383	0.444	0.022	46	>10.0	0.00072	0.238
☐	37	NEW SCI	0262-4079	733	0.379	0.251	0.098	224	7.9	0.00213	0.111
☐	38	INTERDISCIPL SCI REV	0308-0188	167	0.375	0.315	0.125	24	8.3	0.00025	0.122
☐	39	P EST ACAD SCI	1736-6046	367	0.373	0.457	0.034	29	9.3	0.00053	0.183
☐	40	ARAB J SCI ENG	1319-8025	494	0.367	0.410	0.083	324	4.6	0.00143	0.126

MARK ALL UPDATE MARKED LIST

Journals 21 - 40 (of 55) [1 | 2 | 3] **Page 2 of 3**

ISI Web of Knowledge℠

Journal Citation Reports®

WELCOME HELP

2013 JCR Science Edition

Journal Summary List

Journal Title Changes

Journals from: subject categories MULTIDISCIPLINARY SCIENCES VIEW CATEGORY SUMMARY LIST

Sorted by: Impact Factor SORT AGAIN

Journals 41 - 55 (of 55) [1 | 2 | 3] **Page 3 of 3**

MARK ALL UPDATE MARKED LIST

Ranking is based on your journal and sort selections.

Mark	Rank	Abbreviated Journal Title (linked to journal information)	ISSN	JCR Data						*Eigenfactor*® Metrics	
				Total Cites	Impact Factor	5-Year Impact Factor	Immediacy Index	Articles	Cited Half-life	*Eigenfactor*® Score	*Article Influence*® Score
☐	41	SCIENTIST	0890-3670	215	0.351	0.195	0.303	33	8.3	0.00040	0.089
☐	42	SCIENCEASIA	1513-1874	278	0.347	0.541	0.037	107	5.4	0.00061	0.119
☐	43	MAEJO INT J SCI TECH	1905-7873	106	0.329	0.450	0.000	51	4.1	0.00037	0.104
☐	44	J HOPKINS APL TECH D	0270-5214	188	0.315	0.245	0.032	31	>10.0	0.00012	0.048
☐	45	DEFENCE SCI J	0011-748X	357	0.310	0.492	0.013	76	5.9	0.00068	0.110
☐	46	ENDEAVOUR	0160-9327	418	0.261	0.278	0.080	25	>10.0	0.00038	0.180
☐	47	NATL ACAD SCI LETT	0250-541X	177	0.240	0.207	0.060	84	7.3	0.00023	0.044
☐	48	CR ACAD BULG SCI	1310-1331	359	0.198	0.165	0.077	234	5.1	0.00051	0.027
☐	49	P NATL A SCI INDIA A	0369-8203	72	0.179	0.142	0.000	51		0.00011	0.029
☐	50	HER RUSS ACAD SCI+	1019-3316	207	0.170	0.297	0.054	56	8.2	0.00046	0.113
☐	51	FRONT LIFE SCI	2155-3769	5	0.167	0.167	0.000	22		0.00001	0.023
☐	52	J NATL SCI FOUND SRI	1391-4588	68	0.143	0.196	0.000	39		0.00017	0.054
☐	53	R&D MAG	0746-9179	20	0.134	0.072	0.026	39		0.00006	0.021
☐	54	KUWAIT J SCI ENG	1024-8684	97	0.093	0.114		0		0.00011	0.031
☐	55	ANTHROPOLOGIST	0972-0073	65	0.051		0.062	81		0.00007	

MARK ALL UPDATE MARKED LIST

Journals 41 - 55 (of 55) [1 | 2 | 3] **Page 3 of 3**

ISI Web of Knowledge℠

Journal Citation Reports®

WELCOME HELP RETURN TO LIST PREVIOUS JOURNAL NEXT JOURNAL

2013 JCR Science Edition

Journal: PLoS One

Mark	Journal Title	ISSN	Total Cites	Impact Factor	5-Year Impact Factor	Immediacy Index	Citable Items	Cited Half-life	Citing Half-life
☐	PLOS ONE	1932-6203	226708	3.534	4.015	0.416	31496	2.5	7.4

Cited Journal Citing Journal Source Data Journal Self Cites

CITED JOURNAL DATA CITING JOURNAL DATA IMPACT FACTOR TREND RELATED JOURNALS

Journal Information

Full Journal Title: PLoS One
ISO Abbrev. Title: PLoS One
JCR Abbrev. Title: PLOS ONE
ISSN: 1932-6203
Issues/Year: 0
Language: ENGLISH
Journal Country/Territory: UNITED STATES
Publisher: PUBLIC LIBRARY SCIENCE
Publisher Address: 1160 BATTERY STREET, STE 100, SAN FRANCISCO, CA 94111
Subject Categories: MULTIDISCIPLINARY SCIENCES SCOPE NOTE

VIEW JOURNAL SUMMARY LIST VIEW CATEGORY DATA

Journal Rank in Categories: JOURNAL RANKING

***Eigenfactor*® Metrics**
***Eigenfactor*® Score**
1.16747
***Article Influence*® Score**
1.366

Journal Impact Factor

Cites in 2013 to items published in:	2012 = 67956		Number of items published in:	2012 = 23447	
	2011 = 63607			2011 = 13782	
	Sum: 131563			Sum: 37229	
Calculation:	Cites to recent items	131563	= **3.534**		
	Number of recent items	37229			

5-Year Journal Impact Factor

Cites in {2013} to items published in:	2012 = 67956		Number of items published in:	2012 = 23447	
	2011 = 63607			2011 = 13782	
	2010 = 35009			2010 = 6724	
	2009 = 23135			2009 = 4403	
	2008 = 15342			2008 = 2717	
	Sum: 205049			Sum: 51073	
Calculation:	Cites to recent items	205049	= **4.015**		
	Number of recent items	51073			

Journal Self Cites

The tables show the contribution of the journal's self cites to its impact factor. This information is also represented in the cited journal graph.

Total Cites	226708
Cites to Years Used in Impact Factor Calculation	131563
Impact Factor	3.534

Self Cites	29805 (13% of 226708)
Self Cites to Years Used in Impact Factor Calculation	17872 (13% of 131563)
Impact Factor without Self Cites	3.054

Journal Immediacy Index

Cites in 2013 to items published in 2013 = 13099
Number of items published in 2013 = 31496
Calculation: Cites to current items 13099 = **0.416**
Number of current items 31496

Journal Cited Half-Life

The cited half-life for the journal is the median age of its items cited in the current JCR year. Half of the citations to the journal are to items published within the cited half-life.
Cited Half-Life: 2.5 years

Breakdown of the citations ***to the journal*** by the cumulative percent of 2013 cites to items published in the following years:

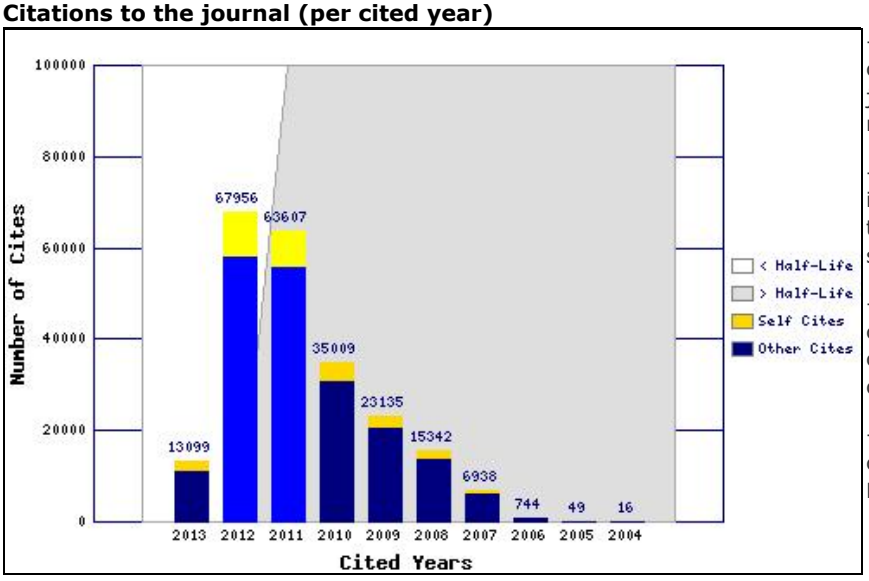
Cited Year	**2013**	**2012**	**2011**	**2010**	**2009**	**2008**	**2007**	**2006**	**2005**	**2004**	**2003-all**
# Cites from 2013	13099	67956	63607	35009	23135	15342	6938	744	49	16	813
Cumulative %	5.78	35.75	63.81	79.25	89.46	96.22	99.28	99.61	99.63	99.64	100

Cited Half-Life Calculations:
The cited half-life calculation finds the number of publication years from the current JCR year that account for 50% of citations received by the journal. Read help for more information on the calculation.

Cited Journal Graph

Click here for Cited Journal data table

This graph shows the distribution by cited year of citations to items published in the journal PLOS ONE.
Citations to the journal (per cited year)



- The white/grey division indicates the cited half-life (if < 10.0). Half of the journal's cited items were published more recently than the cited half-life.

- The top (gold) portion of each column indicates Journal Self Citations: citations to items in the journal from items in the same journal.

- The bottom (blue) portion of each column indicates Non-Self Citations: citations to the journal from items in other journals.

- The two lighter columns indicate citations used to calculate the Impact Factor (always the 2nd and 3rd columns).

Journal Citing Half-Life

The citing half-life for the journal is the median age of the items the journal cited in the current JCR year. Half of the citations in the journal are to items published within the citing half-life.
Citing Half-Life: 7.4 years

Breakdown of the citations ***from the journal*** by the cumulative percent of 2013 cites to items published in the following years:

Cited Year	**2013**	**2012**	**2011**	**2010**	**2009**	**2008**	**2007**	**2006**	**2005**	**2004**	**2003-all**
# Cites from 2013	27622	114437	131387	125797	116797	106256	97771	87900	80247	72796	547928
Cumulative %	1.83	9.41	18.12	26.46	34.20	41.24	47.72	53.55	58.86	63.69	100

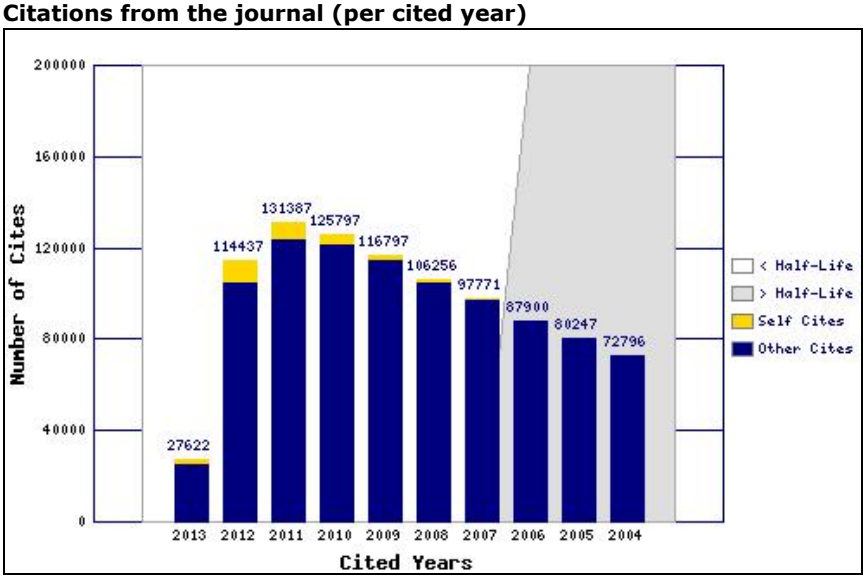
Citing Half-Life Calculations:
The citing half-life calculation finds the number of publication years from the current JCR year that account for 50% of citations in the journal. Read help for more information on the calculation.

Citing Journal Graph

Click here for Citing Journal data table

This graph shows the distribution by cited year of citations from current-year items in the journal PLOS ONE.

Citations from the journal (per cited year)



- The white/grey division indicates the citing half-life (if < 10.0). Half of the citations from the journal's current items are to items published more recently than the citing half-life.

- The top (gold) portion of each column indicates Journal Self-Citations: citations from items in the journal to items in the same journal.

- The bottom (blue) portion of each column indicates Non-Self Citations: citations from the journal to items in other journals.

Journal Source Data

	Citable items			
	Articles	**Reviews**	**Combined**	**Other items**
Number in JCR year 2013 (A)	31227	269	31496	2
Number of references (B)	1492677	16227	1508904	
Ratio (B/A)	47.8	60.3	47.9	0.0

OPEN ACCESS Freely available online

PLOS ONE

Upregulation of Immunoproteasome Subunits in Myositis Indicates Active Inflammation with Involvement of Antigen Presenting Cells, CD8 T-Cells and IFNγ

Khetam Ghannam[1], Lorena Martinez-Gamboa[1], Lydia Spengler[1], Sabine Krause[2], Biljana Smiljanovic[1], Marc Bonin[1], Salyan Bhattarai[1], Andreas Grützkau[3], Gerd-R. Burmester[1], Thomas Häupl[1]*[9], Eugen Feist[1][9]

1 Department of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany, **2** Friedrich Baur Institute, Ludwig Maximilians University, Munich, Germany, **3** German Arthritis Research Center, Berlin, Germany

Abstract

Objective: In idiopathic inflammatory myopathies (IIM) infiltration of immune cells into muscle and upregulation of MHC-I expression implies increased antigen presentation and involvement of the proteasome system. To decipher the role of immunoproteasomes in myositis, we investigated individual cell types and muscle tissues and focused on possible immune triggers.

Methods: Expression of constitutive (PSMB5, -6, -7) and corresponding immunoproteasomal subunits (PSMB8, -9, -10) was analyzed by real-time RT-PCR in muscle biopsies and sorted peripheral blood cells of patients with IIM, non-inflammatory myopathies (NIM) and healthy donors (HD). Protein analysis in muscle biopsies was performed by western blot. Affymetrix HG-U133 platform derived transcriptome data from biopsies of different muscle diseases and from immune cell types as well as monocyte stimulation experiments were used for validation, coregulation and coexpression analyses.

Results: Real-time RT-PCR revealed significantly increased expression of immunoproteasomal subunits (PSMB8/-9/-10) in DC, monocytes and CD8+ T-cells in IIM. In muscle biopsies, the immunosubunits were elevated in IIM compared to NIM and exceeded levels of matched blood samples. Proteins of PSMB8 and -9 were found only in IIM but not NIM muscle biopsies. Reanalysis of 78 myositis and 20 healthy muscle transcriptomes confirmed these results and revealed involvement of the antigen processing and presentation pathway. Comparison with reference profiles of sorted immune cells and healthy muscle confirmed upregulation of PSMB8 and -9 in myositis biopsies beyond infiltration related changes. This upregulation correlated highest with STAT1, IRF1 and IFNγ expression. Elevation of T-cell specific transcripts in active IIM muscles was accompanied by increased expression of DC and monocyte marker genes and thus reflects the cell type specific involvement observed in peripheral blood.

Conclusions: Immunoproteasomes seem to indicate IIM activity and suggest that dominant involvement of antigen processing and presentation may qualify these diseases exemplarily for the evolving therapeutic concepts of immunoproteasome specific inhibition.

Citation: Ghannam K, Martinez-Gamboa L, Spengler L, Krause S, Smiljanovic B, et al. (2014) Upregulation of Immunoproteasome Subunits in Myositis Indicates Active Inflammation with Involvement of Antigen Presenting Cells, CD8 T-Cells and IFNγ. PLoS ONE 9(8): e104048. doi:10.1371/journal.pone.0104048

Editor: Frederick Miller, National Institutes of Health, United States of America

Received March 3, 2014; **Accepted** July 6, 2014; **Published** August 6, 2014

Funding: German Research Foundation grant DFG FE470/3-1; German Research Foundation grant DFG GRK1631 (MyoGrad); EU IMI grant BeTheCure, contract no 115142-2; European Science Foundation, grant EUMYONET. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: thomas.haeupl@charite.de

[9] These authors contributed equally to this work.

Introduction

Idiopathic inflammatory myopathies (IIM) are a heterogenous group of muscle diseases characterized by inflammatory infiltrates in the skeletal muscle. Three major diseases are defined: dermatomyositis (DM), polymyositis (PM), and inclusion body myositis (IBM). The main autoimmune response in DM resembles a microangiopathy affecting skin and muscle tissue. In polymyositis and inclusion body myositis, cytotoxic CD8+ T cells invade muscle fibers. All of the invaded fibers and numerous non-invaded fibers express increased amounts of major histocompatibility complex class I (MHC-I) molecules [1]. In fact, the presence of MHC-I/CD8+ complex is considered as a characteristic immunopathological marker of PM and IBM [2]. Continuous upregulation of expression of MHC class I molecules in muscle fibers is thought to induce an endoplasmic reticulum stress response with accumulation of misfolded glycoproteins and activation of nuclear factor kappa B (NF-κB). As a consequence, MHC-I/CD8+ complexes may form and perpetuate an autoinflammatory response [3].

The ubiquitin-proteasome system (UPS) is a 26S, non-lysosomal, multicatalytic, and multisubunit complex involved in the ubiquitin-dependent, selective intracellular degradation of proteins [4]. In this way, the proteasome plays a central role in the activation of transcription factors such as NF-κB [5]. Furthermore, it is involved in the generation of peptides presented by the MHC-I to the antigen receptors of cytotoxic T cells [6,7], and thus, is involved in the regulation of the inflammatory response. Many studies suggest that the proteasome participates in muscle fiber degradation in various physiological and pathological conditions and may therefore also play an important role in myositis [8,9].

The 26S proteasome is composed of a proteolytically active core, namely the 20S proteasome, and one or two 19S regulator complexes. The 20S or constitutive proteasome is a cylindrical particle that consists of four rings, each composed of seven different subunits. The outer two rings are formed by seven alpha-type subunits (PSMA1–PSMA7), while the inner two rings contain seven beta-type subunits (PSMB1–PSMB7) [6]. The proteolytically active sites are limited to three constitutive beta subunits, proteasome subunit beta type 5-PSMB5, proteasome subunit beta type 6-PSMB6 and proteasome subunit beta type 7-PSMB7.[10]. Importantly, under the influence of the pro-inflammatory cytokine IFNγ, the structure and the catalytic properties of the constitutive proteasome are modified by substitution of the catalytic subunits PSMB5, PSMB6 and PSMB7 with three catalytic immunosubunits proteasome subunit beta type 8-PSMB8, proteasome subunit beta type 9-PSMB9 and proteasome subunit beta type 10-PSMB10 respectively, leading to the formation of the so-called immunoproteasome. This process is considered to strongly influence the production of peptides for antigen presentation by MHC class I as well as the immune response [11–13]. As IFNγ is also secreted in IIM [14], the immunoproteasomal system may also contribute to pathomechanisms in myositis. Recently, mutations in human were detected in proteasome subunit PSMB8, which cause joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome in addition to other autoinflammatory syndromes [15–18]. Impaired immunoproteasome assembly and decreased proteolytic activity have been confirmed in some of these diseases. On the other hand, elevated levels of circulating proteasomes as well as autoantibodies against several proteasomal subunits have been detected in patients with autoimmune myositis and other autoimmune disorders [19,20]. Moreover, it has also been shown that anti-proteasome autoantibodies derived from patients with connective tissue diseases, including a patient with polymyositis, were capable of blocking the stimulation of the catalytic proteasome core complex by the proteasome activator PA28, consisting of the subunits alpha (PSME1) and beta (PSME2) [21]. Additionally, an upregulation of some proteasomal genes has been shown in PBMCs of patients with systemic autoimmune disorders including patients with PM [22].

Based on the existing but so far indirect assumption of an involvement of the proteasome system in the pathogenesis of IIM, we investigated the expression of all catalytic proteasome subunits in inflammatory and non-inflammatory myopathies in order to search for an activation of the 20S core complex in patients with autoimmune myositis. Results were validated and comprehensively screened in a large panel of disease related as well as cell type and stimulation specific transcriptome data sets in order to confirm our results and to identify induction mechanisms and the regulatory network for the immune proteasome subunits.

Materials and Methods

Ethics Statement

The study was performed in accordance with the 1964 Declaration of Helsinki and approved by the "Charité University Medicine ethics committee I of Charité Campus Mitte" and patients provided written consent to participate in the study.

Patients and healthy donors

Expression of proteasome subunits was investigated in 17 patients with idiopathic inflammatory myopathies (IIM), including polymyositis (PM, n = 5), dermatomyositis (DM, n = 5), and overlap-syndromes with myositis (OM, n = 7) (8 male, 9 female, mean age 54.3 years, age range 22–72 years). Patients were selected based on typical clinical symptoms, laboratory and/or muscle biopsy findings, which were indicative for the different groups of myopathies. Patients with PM and DM fulfilled the classification criteria according to Bohan and Peter [23,24]. For details of diagnosis and clinical parameters see supplementary table S1. Autoantibody screening was performed according to diagnostic standards including ANA in indirect immunofluorescence on HEp2 cells. Depending on the result of the ANA pattern, further differentiation of the antibody reactivity was performed for detection of ENA using ELISA. Furthermore, a profile of myositis specific autoantibodies including anti-Jo1-, anti-SRP-, anti-Mi2, anti-PM/Scl and anti-U1RNPantibodies as well as anti-proteasomal antibodies was analyzed in each suspected case of myositis. Controls included 7 patients with different non-inflammatory myopathies (NIM, 4 male, 3 female, mean age 48.7 years, age range 35–59 years). Time and patient matched samples of muscle biopsies and blood were taken from 14 patients. Control samples from 15 healthy donors (HD, 2 male, 13 female, mean age 45 years, age range 27–56 years without clinical signs of disease, no clinical signs of muscular weakness, no medication) included only blood and no muscle biopsies. All patients were diagnosed at the Department of Rheumatology and Clinical Immunology, Charité – University Medicine Berlin and informed consent was obtained from all subjects.

Isolation of peripheral blood mononuclear cells (PBMCs) and cellular subsets

PBMCs were collected by Ficoll density gradient centrifugation (Biochrom, Germany) and divided into two fractions. One was used for separation of dendritic cells (DCs) by Magnetic Cell Separation (MACS) using the Blood Dendritic Cell Isolation Kit-II (Miltenyi Biotec, Germany), the other for isolation of T lymphocytes (CD4+, CD8+), B lymphocytes (CD19+), and monocytes (CD14+) by fluorescence activated cell sorting (FACS) with a FACS DiVa Flow Cytometer (BD, Germany).

Collection of muscle biopsies

Muscle tissues were stabilized in RNA later (Qiagen, Germany) to avoid RNA degradation and were stored at −70°C. For RNA isolation, biopsies were ground with a pestle and mortar in the presence of liquid N_2 to protect RNA against degradation. Disrupted samples were then added to lysis buffer containing β-mercaptoethanol.

RNA isolation, reverse transcription into cDNA and real-time reverse transcriptase-polymerase chain reaction (real time RT-PCR)

RNA was isolated from muscle tissues and blood cells using the NucleoSpin RNA/Protein kit (Macherey-Nagel, Germany). First-

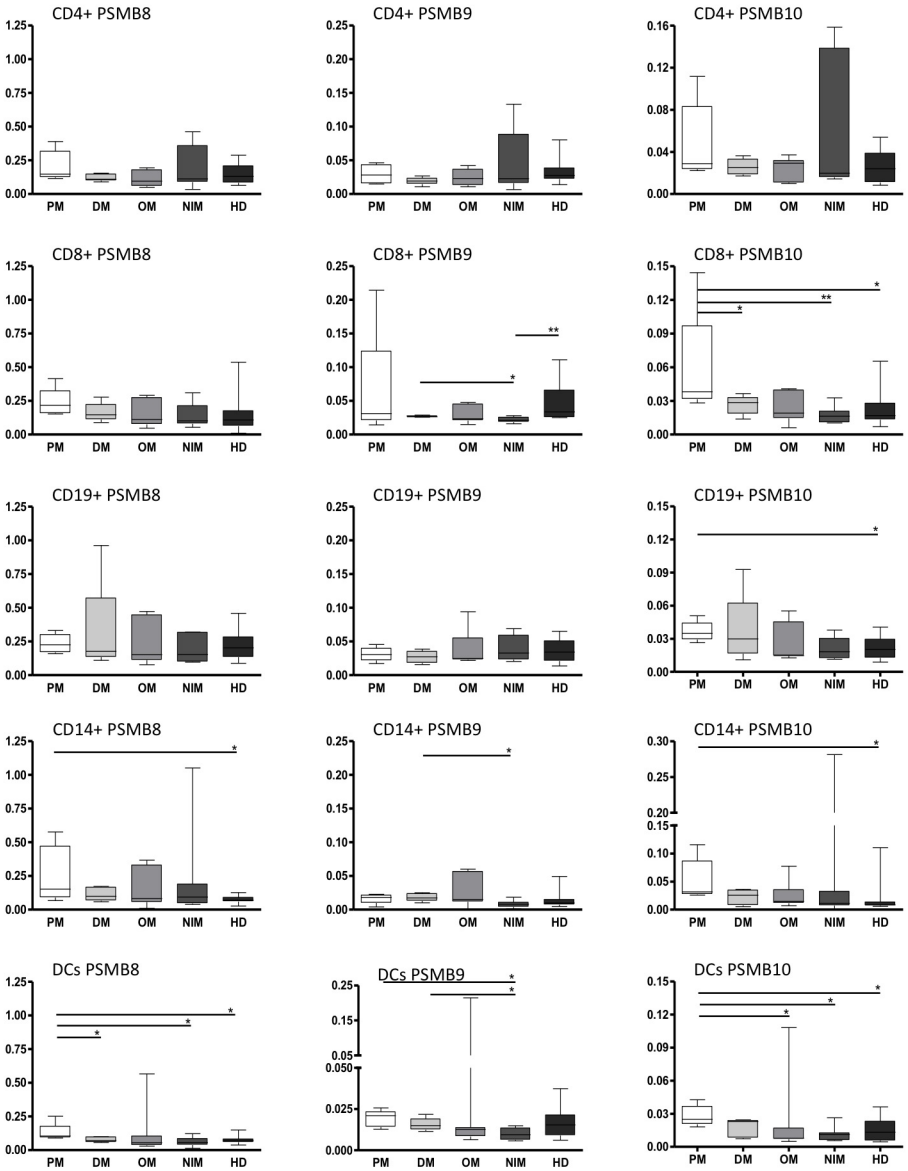


Figure 1. Expression of immunoproteasomal subunits in immune cells: Gene expression of immunoproteasomal subunits (PSMB8–10) in CD4+, CD8+, CD19+, CD14+ and DCs of patients with myopathies (PM, DM, OM, NIM) and controls (HD). Data are shown as relative expression normalized to beta actin. Box plots indicate percentiles 0, 25, 50, 75 and 100. Groups were compared by Mann-Whitney U test and statistical significance is indicated for $p<0.05$ (*) and $p<0.01$ (**). Significantly higher expression of PSMB8 was observed in CD14+ cells and DC of PM patients compared to HD or DM, NIM and HDs, respectively. PSMB9 was increased in CD8+ and CD14+ of DM and in DCs of PM and DM patients compared to NIM. PSMB10 was found increased in PM patients compared to DM, NIM and HD in CD8+, compared to HD in CD19+ and CD14+ cells and compared to OM, NIM, and HD in DCs.
doi:10.1371/journal.pone.0104048.g001

strand cDNA was synthesized using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Germany) according to the instructions of the manufacturer.

Forward and reverse primers of proteasome subunits PSMB5, PSMB6, PSMB7, PSMB8, PSMB9 and PSMB10 for real time RT-PCR were designed as described elsewhere [25].

Amplification reactions contained SYBR Green PCR Master Mix (Applied Biosystems), 200 nM forward and reverse primers for each gene and cDNA. Real-time PCR was performed in triplicates using the ABI prism 5700 Sequence Detection System (Applied Biosystems).

Relative expression of the target compared to the house keeping gene beta-actin was determined as $R = \frac{E^{C_{T_{house-keeping-gene}}}}{E^{C_{T_{target-gene}}}}$ [26], with E representing the amplification efficiency of the respective primer system. Mann-Whitney U-test was applied for group comparisons (figures 1, 2, 3).

Western blot analysis

Muscle biopsies were minced in the presence of radioimmunoprecipitation assay (RIPA) lysis buffer. PBMCs were used as positive control and treated with the same lysis buffer. 30 µg of total protein from tissue homogenate or cell lysate were loaded and fractionated by 15% sodium dodecyl sulfate polyacrylamide gel

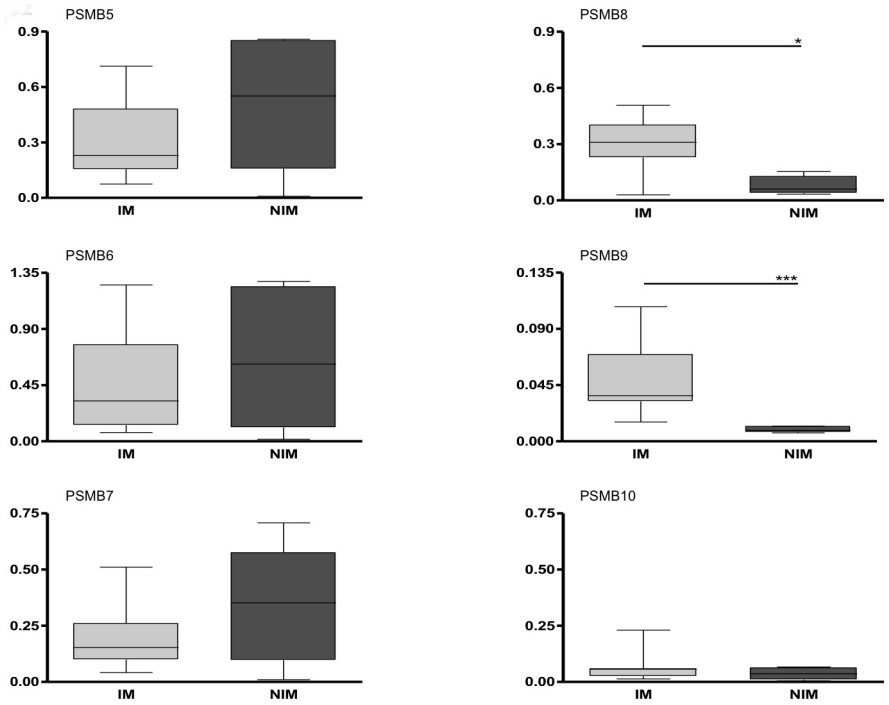


Figure 2. Expression of catalytic proteasomal subunits in muscle: Gene expression analysis of constitutive and immunoproteasomal subunits (PSMB5–10) in muscle biopsies of patients with inflammatory myopathies (IM) and patients with non inflammatory myopathies (NIM). Data are shown as relative expression normalized to beta actin. Box plots indicate percentiles 0, 25, 50, 75 and 100. Groups were compared by Mann-Whitney U test and statistical significance is indicated for $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***). Comparing to NIM, mean relative expression levels in IM revealed 4-fold for PSMB8 [0.302±0.139 and 0.075±0.041] and about 5-fold increase for PSMB9 [0.049±0.029 and 0.009±0.002] but less than 2-fold for PSMB10 [0.065±0.064 and 0.036±0.022].
doi:10.1371/journal.pone.0104048.g002

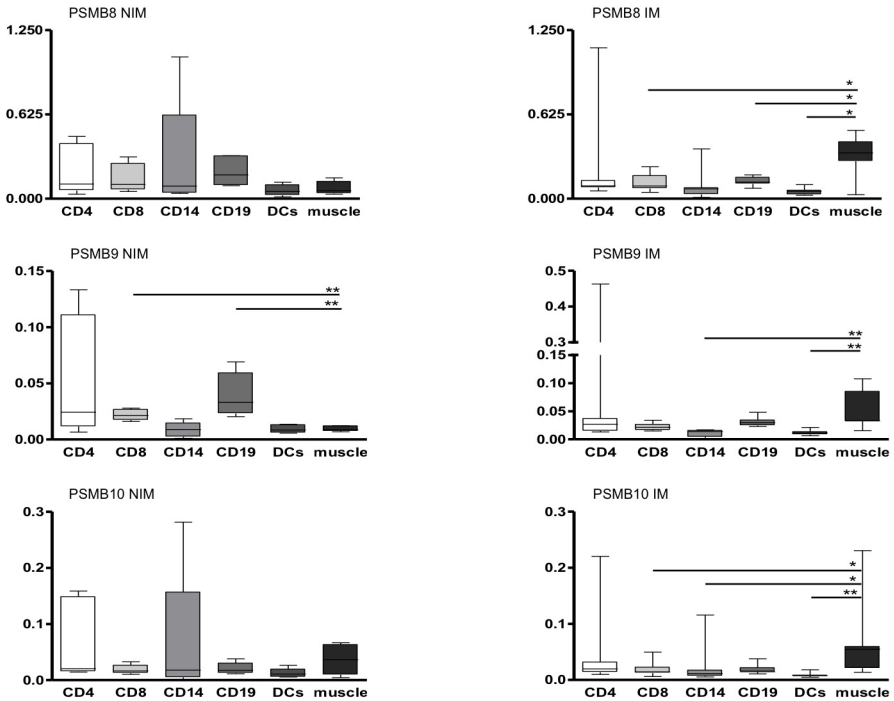


Figure 3. Comparison of immunoproteosomal subunit expression between paired samples from isolated cells and muscle tissue: Gene expression analysis of immunoproteasomal subunits (PSMB8–10) in muscle biopsies vs. CD4+, CD8+, CD14+, CD19+, and DCs from patients with inflammatory myopathies (IM) and patients with non-inflammatory myopathies (NIM). Data are shown as relative expression normalized to beta actin. Box plots indicate percentiles 0, 25, 50, 75 and 100. Groups were compared by Mann-Whitney U test and statistical significance is indicated for $p<0.05$ (*) and $p<0.01$ (**).
doi:10.1371/journal.pone.0104048.g003

electrophoresis. After transfer to a polyvinylidene fluoride (PVDF) membrane, the blots were incubated overnight at 4°C with monoclonal antibodies against proteasome PSMB8 or proteasome PSMB9, both diluted 1:1000 (Enzo Life Sciences, USA). Staining was performed with polyclonal rabbit anti mouse immunoglobulins conjugated with horseradish peroxidase (1:1000; Dako, Denmark) and visualised by enhanced electrochemiluminescence Pierce ECL Western Blotting Substrate (Thermo Scientific, USA) (figure 4). For quantification relative to a housekeeping gene, beta actin was detected using the same procedure and mouse anti-beta actin diluted 1:10,000 as primary antibody (Sigma, USA). Quantification on western blot images was performed with the intensity histogram function of Adobe Photoshop (Munich, Germany).

Microarray data and statistical analysis

For validation, transcriptome data of muscle biopsies were collected from the open access database Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). The selection included muscle tissue biopsies from different types of IIM, non-inflammatory myopathies, septic patients, volunteers after IL-6 infusion and healthy controls as well as purified dendritic cells from peripheral blood (GSE2044, GSE3112, GSE5370, GSE39454, GSE3307, GSE13205, GSE10685 and GSE23618; table S2). Own data included transcriptomes of stimulated monocytes (GSE38351) [27] and sorted immune cell populations (CD14+ monocytes, CD15+ granulocytes, CD4+ T-cells, CD8+ T-cells, CD19+ B-cells and CD56+ NK-cells, each n = 3; GSE58173) from peripheral blood of healthy donors, generated after RNA extraction, amplification and hybridization to Affymetrix GeneChip HG-U133 Plus 2.0 arrays according to standard protocols to minimize influence on gene expression by sorting [28]. All data were analysed in the BioRetis database (www.bioretis.de) using standard algorithms [29]. Selection of differentially expressed genes was performed by "default increased" filtering in BioRetis [29]. Probesets were ranked by an equally weighted sum-score for "SLR" and "frequency of increase" in disease compared to control (table S3).

Signals were quantile normalized for evaluation of cell infiltration and correlation analysis (figure 5). Percentage of immune cell infiltration was estimated using tissue and cell type specific marker probesets by comparing the reference transcriptomes of each individual cell type and healthy muscle tissue with each other (figure 6). To assess the maximum of infiltration related signal intensity for the immunoproteasome transcripts in each myositis biopsy a linear model of relationship between signal intensity and percentage of infiltration was applied. The highest expected signal (S_{exp}) related to infiltration was calculated as $S_{exp} = S_{max} \cdot P_{inf}$ with P_{inf} as the percentage of all non-muscle cell types (infiltrated cells) and S_{max} as the maximum signal observed in the reference transcriptomes of any of the purified immune cells from healthy donor. The median of log-transformed and z-normalized signals of all 1209 myositis related probesets was used for scoring and sorting of the samples (myositis score).

Results

Dendritic and monocytic cells of the peripheral blood are the leading cell type for immunoproteasome upregulation in myositis

Relative quantification of proteasomal subunit expression by real time RT-PCR revealed a myositis related significant increase of at least one of the immunoproteasomal subunits PSMB8, PSMB9 and/or PSMB10 in all investigated cellular subsets except CD4+ (figure 1). DCs were the leading subset with differences in all immunoproteasomal subunits followed by monocytes, CD8+

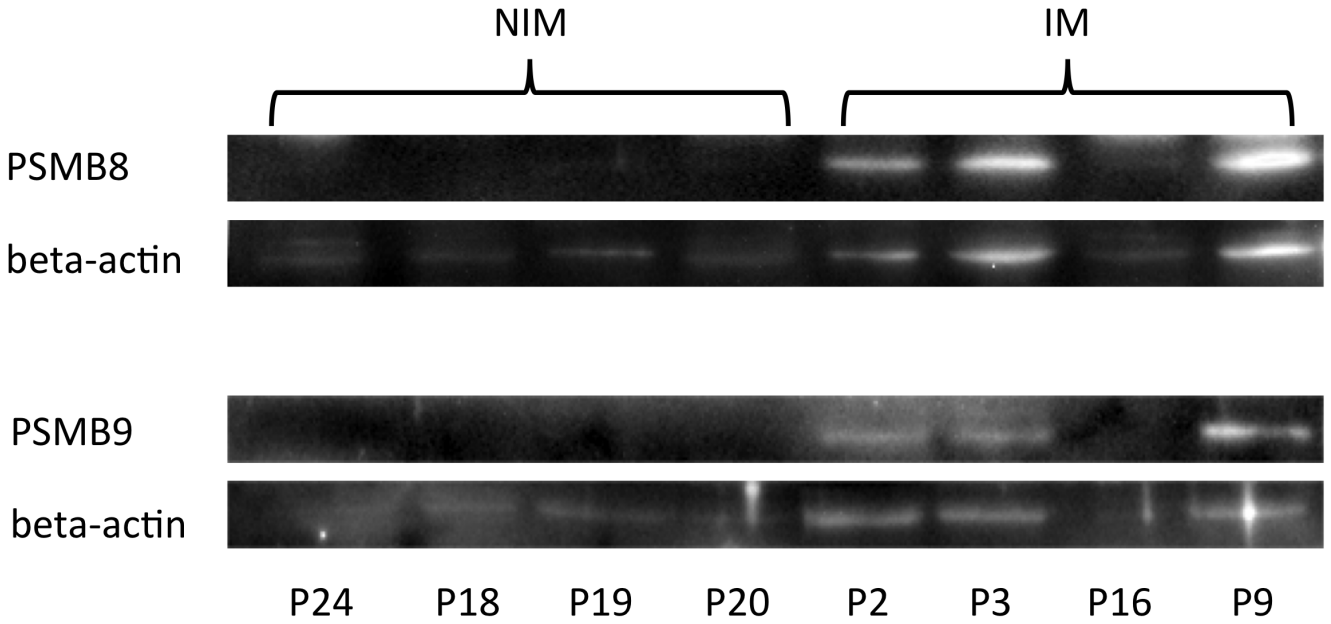


Figure 4. Protein expression of the immunoproteasome subunits PSMB8 and PSMB9 relative to beta actin in IM and NIM. The protein expression of the two subunits was analyzed by western blot in muscle biopsies from four non-inflammatory myopathies (NIM) and four inflammatory myopathies (IM). Intensity of chemiluminescent signals for PSMB8 and PSMB9 was normalized to corresponding signals of the housekeeping protein beta actin, which was detected in a second staining procedure on the same membrane. Only IM samples revealed PSMB8 and -9 protein expression.
doi:10.1371/journal.pone.0104048.g004

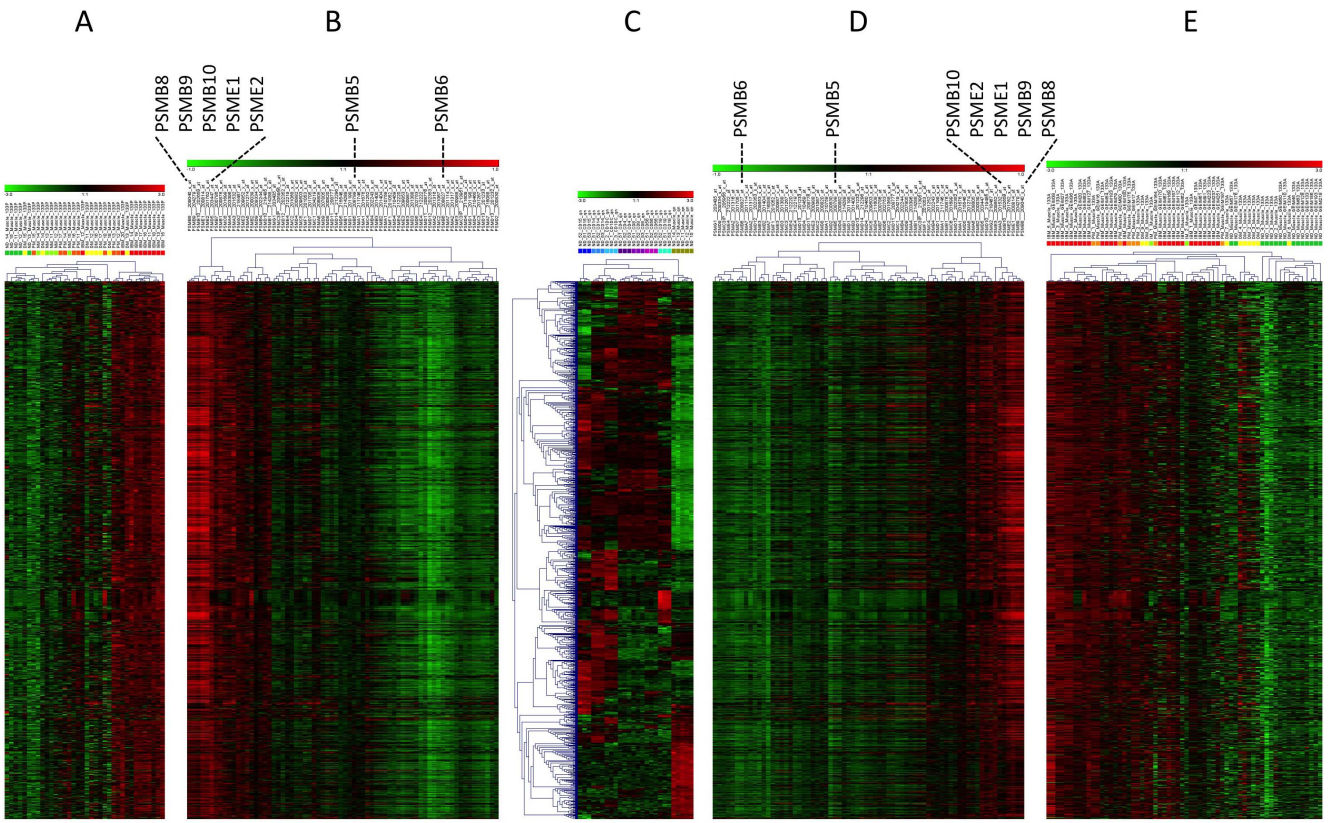


Figure 5. Correlation of proteasome subunit expression with myositis signatures and overlap with immune cell infiltration: Expression of all proteasome units were investigated in transcriptome data of muscle biopsies referenced in table S2 and were correlated with genes increased in myositis muscle tissue. The heatmap of the probeset signal intensities (A) is opposed to the heatmap of the gene by gene correlation matrix (B) in 133P array experiments. Identical analysis in 133A arrays are shown in D and E. Expression of the 1209 probesets in reference transcriptomes of purified immune cells from the blood of healthy donors served as basis for clustering by probesets and cell types (C). Identification of cell type specific transcriptional patterns in the 1209 probesets demonstrates the extent of overlap of these probesets with immune cell related transcripts. Myositis samples include IBM (red), PM (orange), DM (yellow), NM (necrotizing myopathy)/IM (inflammatory myopathy) (light green) and HD (green). Immune cell types include neutrophils (dark blue), monocytes (blue), dendritic cells (light blue), CD4+ T-cells (dark violet), CD8+ T-cells (violet), NK-cells (light violet), B-cells (cyan), and healthy muscle (moss-green). A detailed graph with all probesets labelled is presented in supplemental material (figure S4).
doi:10.1371/journal.pone.0104048.g005

and finally CD19+ lymphocytes. Differences were most prominent in PM and to a lower extent in DM patients when compared to non-inflammatory myopathies (NIM) and healthy donors (HD). Differences between immunoproteasomal and corresponding constitutive subunits were especially found between PSMB8 and PSMB5 in all investigated peripheral blood cell types of all patients (figure S1) and HD (data not shown).

Immunoproteasomal subunit expression is higher in IIM than NIM muscle biopsies and indicates a substitution of constitutive subunits

In all patients with inflammatory myopathies (IM) including PM, DM and OM, immunoproteasomal subunits PSMB8 and PSMB9 were significantly increased when compared to NIM patients (figure 2). In contrast, PSMB10 was less increased in IM patients compared to NIM and not statistically significant. The ratios between immunoproteasomal and corresponding constitutive subunits (PSMB8/PSMB5, PSMB9/PSMB6 and PSMB10/PSMB7) in IM [1.79, 0.35 and 0.42, respectively] compared to non-inflammatory biopsies [0.94, 0.1 and 0.18, respectively] indicate an imbalance and suggest substitution of constitutive by immunoproteasomal subunits in patients with IIM.

Immunoproteasomal subunit expression in IIM is higher in muscle compared to donor matched immune cells of peripheral blood

In paired samples of blood and tissue from 7 IM and 6 NIM patients collected at the same time, real time RT-PCR revealed significant increase of immunoproteasomal subunits in muscle compared to blood cells only in IM. In contrast, in NIM, expression of immunoproteasomal subunits was higher in most blood cell types compared to muscle tissue (figure 3). Constitutive subunits were higher expressed in all muscle biopsies from IM and NIM when compared to any cell type of the blood (data not shown).

Regulation of PSMB8 and PSMB9 protein in myopathy patients

To confirm that the elevated transcriptional activity is also translated into protein, PSMB8 and PSMB9 proteins were investigated in muscle tissue of representative patients with

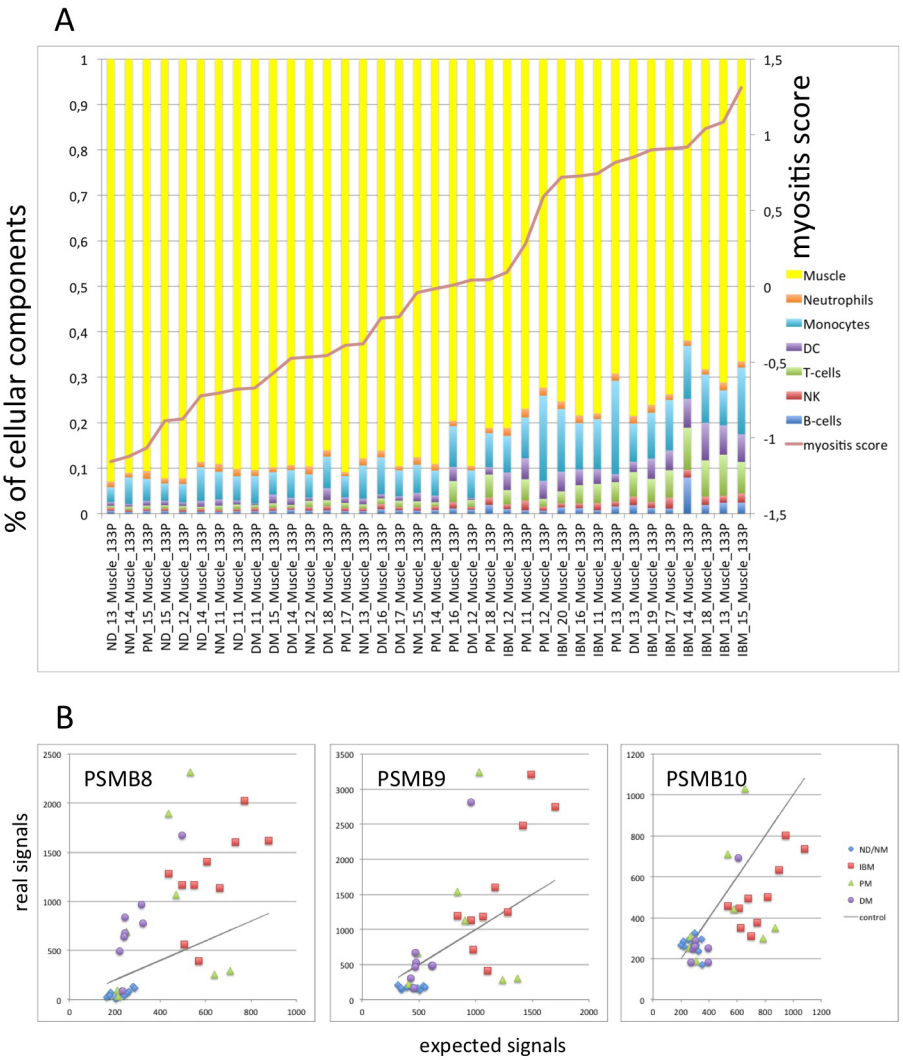


Figure 6. Quantification of cellular infiltration and confirmation of PSMB8/-9 gene activation in myositis muscle samples: Transcriptome data referenced in table S2 were re-investigated. A) Cell type infiltration was quantified based on the expression of cell specific marker transcripts (figure S5). Signal ratios between muscle biopsy and purified cell type were calculated for each cell type specific transcriptional markers. Taking the median of the ratios in each cell specific marker set and scaling their sum to 100% revealed an estimate of the cellular composition. Increase of monocyte, dendritic cell and T-cell transcription patterns corresponds to the molecular myositis score. (ND = normal donor; NM = necrotizing myopathy). B) The maximum of infiltration related signal intensity was calculated as described in materials and methods. Comparing these expected intensities (x-axis) with real intensities (y-axis) in each sample, PSMB8 and PSMB9 are higher expressed than expected in the majority of IBM and several of the PM muscle. In DM, PSMB8 expression also exceeds the expected signal intensity, although on a lower level. One out of the 8 DM samples (DM_13) showed consistently a pattern similar to the highly inflamed IBM samples. All other conditions including controls remained below the expected intensity. For PSMB10, the increased signal intensity in several samples of IBM seems to correspond to the level of infiltration.

doi:10.1371/journal.pone.0104048.g006

dermatomyositis (P2 and P3), polymyositis (P9), overlap syndrom (P16) and non-inflammatory muscle diseases (P18, P19, P20, P24). Western blot analysis revealed equal or higher protein detection intensities of PSMB8 compared to actin in the patients with inflammatory muscle diseases and slightly weaker intensities for PSMB9. In all samples investigated from non-inflammatory diseases both immunoproteasomal subunits were not visible as a distinct band (figure 4). Intensities determined for PSMB8/-9 relative to actin were also significantly lower in NIM than in IIM. Correlation between immunoproteasomal protein and RNA transcript expression, both as relative quantities compared to actin protein or transcript, revealed high correlation coefficients of $R = 0.90$ for PSMB8 and $R = 0.80$ for PSMB9.

Transcriptome data for validation and pathophysiologic classification of defined transcripts

To validate these results and to characterize the importance of immunoproteasomes in myositis compared to other molecular mechanisms, we investigated open access transcriptome data of muscle biopsies from patients with IIM and NIM as well as from healthy donors. Transcriptomes from different types of immune cells and stimulation experiments with IFN, TNF and LPS were selected to address functional interpretation (table S2).

Immunoproteasomal subunits PSMB8/-9 are leading candidates in the pathophysiology of IIM muscle inflammation. Muscle transcriptomes from patients with IBM, PM and DM were compared to healthy controls and revealed 1209 probesets equal to 927 genes, which were upregulated in at least one of the myositis diseases (table S3) as a molecular correlate of disease activity. PSMB8 ranked at position 9 and PSMB9 at position 58 out of the 927 genes when scored for magnitude and consistency of increase. Based on these 1209 probesets, IIM samples did not cluster by disease specific patterns but overall intensity of molecular changes. High molecular activity was most frequent in IBM followed by PM and DM muscle biopsies. This was observed independently for both 133A and 133P data sets (figure 5A, 5E, S2). Of all known proteasomal subunits, PSMB8/-9 as well as their activator subunits PSME1 and -2 [30] correlated best with the 1209 probesets while most of the constitutively expressed subunits including PSMB5 and -6 were inversely correlated. This was again independently observed in 133A and 133P datasets (figure 5B and 5D). Characterizing the panel of 1209 probesets with DAVID (http://david.abcc.ncifcrf.gov/) revealed that almost each gene annotated to the antigen processing and presentation pathways of MHC-I and II was included (figure S3).

Transcription of PSMB8/-9 is actively up-regulated in IIM muscle tissue. Investigating gene expression for the 1209 probesets in reference signatures of healthy muscle and immune cell populations uncovers that the majority is also part of the physiologic expression in immune cells and is sufficiently specific to correctly cluster the immune cell profiles (figure 5 C tree of clustered samples). Thus, these transcripts could be unregulated but passively transported by cell infiltration into muscle or also upregulated by additional gene activation (figure 5). Such active upregulation seems to occur for transcripts specific for healthy muscle (bottom of figure 5 C), which were increased in myositis. A magnification of figure 5 with detailed description of genes is provided in figure S4.

Compared to immune cells, there is no relevant expression of immunoproteasomes in healthy muscle. Selection of optimized cell type specific marker transcripts (figure S5) disclosed quantitatively the cell type specific transcriptional activities in the muscle biopsies, which were besides muscle especially related to T-cells, monocytes and dendritic cells and corresponded to the overall molecular change in myositis muscle transcriptomes (myositis score; figure 6 A). Comparing real expression levels of immunoproteasome subunits in myositis with intensities expected from immune cell infiltration demonstrated that PSMB8/-9 were actively upregulated in patients with IBM, PM and DM, especially when high expression levels not only of the immunoproteasomal subunits but also of genes differentially expressed in inflammatory myopathies compared to healthy muscle were observed and thus were indicative for high molecular disease activity (figure 6 B). PSMB10 expression, however, matched with expected intensities, which indicated no additional upregulation.

Transcription factors STAT1 and IRF1 correlate with upregulation of PSMB-8 and -9 expression in IIM and in IFN stimulated monocytes. Regulatory mechanisms for immunoproteasomes in myositis were searched by correlation of transcription factors defined by GO:0003700 with PSMB8/-9 in all muscle biopsy samples. This revealed STAT1, IRF1, TRIM22 and IRF9 as potential regulators, which were predominantly increased in IBM, but also present in many PM and several DM samples. PSMB5/-6 were not or inversely correlated. In different healthy donor immune cell types and healthy muscle, correlation between PSMB8/-9 and these transcription factors was much weaker. However, monocytes stimulated with TNF, LPS, IFNα2 or IFNγ revealed highest correlation between PSMB8/-9 and STAT1 followed by IRF1 (table S4). This was related to IFNα2 and IFNγ. In contrast, TNF suppressed both transcription factors and PSMB8/-9, while LPS induced both transcription factors but suppressed PSMB8/-9 expression (table S4).

In IIM, IFNγ but not type-1 IFN correlate with PSMB8/-9 expression and T-cell markers including CD8. Although both types of interferons may induce PSMB8/-9 [27], in myositis muscle transcriptomes only IFNγ was significantly increased with dominance in IBM (74% increased change call, 4.9-fold increased) and correlated with PSMB8/-9. These analyses were separately performed with 133P and 133A data sets and independently confirmed each other (figure 7). To trace the cellular origin, signals of IFNγ in myositis were correlated with all cell type specific marker transcripts and the median of all correlation coefficients per cell type was calculated. IFNγ expression correlated best with T-cells (133P: $R = 0.78$; 133A: $R = 0.62$) and CD8 (133P: $R = 0.80$; 133A: $R = 0.69$) followed by dendritic cells (133P: $R = 0.66$; 133P: $R = 0.57$) in both, 133A and 133P datasets. NK-cell association was also high in 133P ($R = 0.54$) but much lower in 133A datasets ($R = 0.17$).

Immunoproteasome activation is specific for IIM but not non-inflammatory myopathies. Reanalysis of muscle transcriptomes from 12 different muscle diseases (GSE3307) with non-inflammatory myopathies except from juvenile dermatomyositis (JDM) revealed upregulation of PSMB8/-9 only in samples from JDM and in one out of four samples from limb girdle muscular dystrophy 2I (LGMD2I). Furthermore, muscle biopsies from healthy donors after IL-6 infusion (GSE10685) or patients with sepsis induced multiple organ failure (GSE13205) did not reveal any increase of the immunoproteasome expression, indicating that expression of PSMB8/-9 in muscle is specific for IIM.

Discussion

With this first comprehensive analysis of muscle biopsies and blood derived immune cells in myositis on the catalytic subunits of the proteasome, we could demonstrate a central role of immunoproteasomes in the inflammatory process of IIM including IBM, PM and DM on transcriptional as well as protein expression level. We identified PSMB8/-9 upregulation only in combination with infiltration of antigen presenting cells and associated with the expression of IFNγ and CD8 accompanied by T-cell infiltration in biopsies of inflamed muscle. This perfectly integrates into current concepts of IIM pathophysiology and extends these towards a strong involvement of mechanisms and modulations of antigen processing.

IIM are of unknown aetiology and present with characteristic upregulation of MHC-class I and II molecules [1,31], which was also detectable in the myositis transcriptome data. Antigen presentation is affected by replacement of constitutive with immunoproteasomal subunits [13]. In this study, we observed that physiologic expression of the immunoproteasome subunits in healthy controls was not only restricted to professional antigen presenting cells but found in all subtypes of peripheral blood immune cells. Healthy muscle tissue in contrast expressed only constitutive subunits while immunoproteasome expression was negligible. Immunoproteasomal processing is especially linked to the MHC-I pathway [13]. Thus, it is important that in our investigation transcription was highly correlated with protein production of immunopreteasomes and also associated with the histological finding of MHC-I protein upregulation in muscle tissue of the analyzed IIM samples.

A

Affy_ID	Gene_Symbol	133P PSMB8___209040_s_at	133P PSMB9___204279_at	133P PSMB10___202659_at	133P PSMB5___208799_at	133P PSMB6___208827_at	133P PSMB7___200786_at	133A PSMB8___209040_s_at	133A PSMB9___204279_at	133A PSMB10___202659_at	133A PSMB5___208799_at	133A PSMB6___208827_at	133A PSMB7___200786_at
207932_at	IFNA8	-0,20	-0,20		-0,11	0,09	-0,35						-0,16
208375_at	IFNA1	-0,28	-0,29	-0,32			-0,29	-0,18	-0,11	-0,21	0,19	0,22	0,10
211338_at	IFNA2	-0,27	-0,20	-0,14	0,14	0,09	-0,46	-0,15	-0,12		0,17	0,08	0,15
211145_x_at	IFNA21	-0,23	-0,21	-0,17	-0,17	0,28	-0,27	-0,17	-0,24	-0,16	0,13	0,08	0,16
214569_at	IFNA5	-0,27	-0,29	-0,15	0,23	0,48	-0,26	-0,47	-0,35	-0,46	0,17	0,28	0,08
208261_x_at	IFNA10	-0,34	-0,30	-0,33	-0,13	0,27	-0,25	-0,38	-0,29	-0,45	0,16	-0,12	0,26
208448_x_at	IFNA16	-0,33	-0,27	-0,28		0,14	-0,40	-0,31	-0,17	-0,29		0,07	0,25
208182_x_at	IFNA14	-0,08	-0,14	-0,15	0,36	0,42	-0,07	-0,32	-0,20	-0,22	0,18	-0,09	0,19
208548_at	IFNA6		-0,12	-0,09	0,18	0,33	-0,08	-0,30	-0,15	-0,32		0,16	0,30
208259_x_at	IFNA7	-0,19	-0,19	-0,15	-0,20	0,31	-0,15	-0,46	-0,30	-0,38		0,10	0,23
207964_x_at	IFNA4	-0,11	-0,14	-0,19		0,41		-0,23	-0,14	-0,23	-0,08	-0,17	
211405_x_at	IFNA17	-0,13	-0,15	-0,20	0,28	0,45	-0,07	-0,27	-0,22	-0,24		0,11	
208344_x_at	IFNA1 /// IFNA13	-0,07	-0,09	-0,11		0,32	-0,15	-0,15	-0,12	-0,19	-0,06	-0,14	-0,08
208173_at	IFNB1	-0,20	-0,26	-0,29	-0,08	0,11	-0,15	-0,18	-0,13	-0,25	-0,10	0,09	
210354_at	IFNG	0,82	0,91	0,84	-0,14	-0,36		0,59	0,70	0,58	-0,26	-0,19	-0,07
204191_at	IFNAR1	-0,34	-0,23		0,12	0,28	-0,27	-0,15	-0,08	-0,25	-0,07		0,08
204785_x_at	IFNAR2	0,37	0,43	0,56	0,08	-0,10	0,15	-0,17		-0,25	-0,39	-0,17	-0,07
204786_s_at	IFNAR2	0,34	0,33	0,25		-0,29	0,39	0,31	0,26	0,12	0,07	-0,38	
201642_at	IFNGR2		0,11	0,27	-0,09	-0,17	-0,42	0,36	0,48	0,24	-0,53	-0,16	
202727_s_at	IFNGR1	0,09	0,12	0,09		-0,42	0,26	0,34	0,31	0,14	-0,26	-0,51	
211676_s_at	IFNGR1	0,08			-0,10	-0,20	-0,15	0,12	0,14		-0,60	-0,15	0,12

B

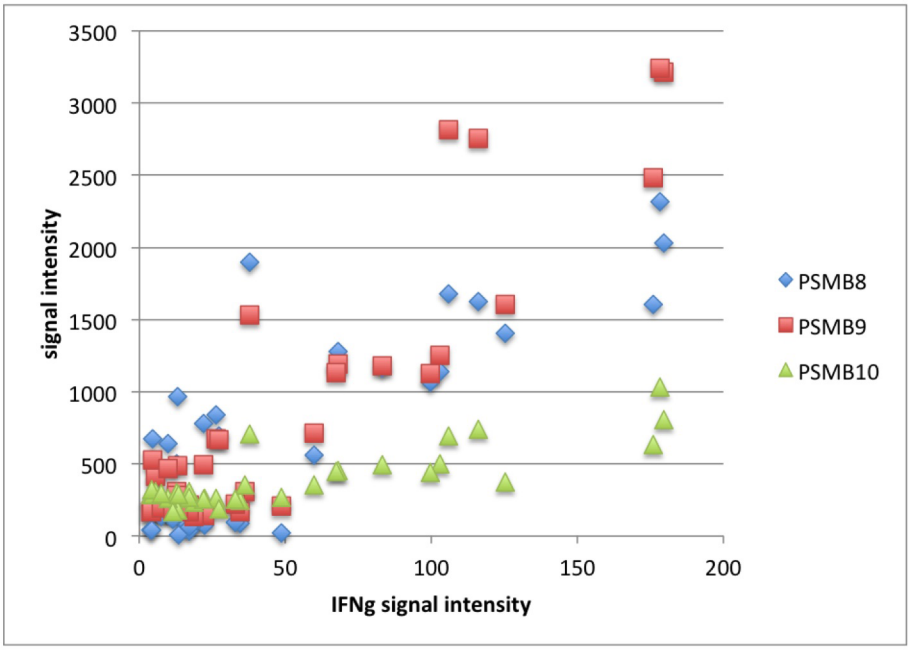


Figure 7. Correlation of immunoproteasome with IFN and IFNR expression in myositis muscle tissue: Transcriptome data referenced in table S2 were re-investigated. A) Correlation analysis was performed for 133P and 133A datasets independently. Only IFNγ revealed high correlation coefficients with PSMB8, PSMB9 and PSMB10. The corresponding constitutively expressed subunits PSMB5-7 were not or even negatively correlated. B) Comparing the association of IFNγ with PSMB8, -9 and -10 for each sample individually, the increase was much higher for PSMB8/-9 compared to PSMB10 as described before (figure 6).
doi:10.1371/journal.pone.0104048.g007

Our reanalysis of the myositis transcriptomes revealed that molecular changes of active inflammation were mainly associated with an increase of immune cell transcriptome signatures and interestingly independent from clinical diagnosis. Immunoproteasome subunit expression in IIM was upregulated especially in DC and monocytes of the peripheral blood but also in muscle, where PSMB8/-9 correlated with the molecular changes of inflammation and thus infiltration of immune cells. The increase of PSMB8/-9 expression in IIM was higher than the level expected by infiltration of immune cells and was also higher in total biopsy material with an immune cells fraction below 40% compared to any of the purified (100%) donor matched immune cells from peripheral blood. This clearly indicates that these immunoproteasomal

transcripts must be upregulated in one or more of the cell types of inflamed muscle.

Recently, transcriptomes obtained from type I and II IFN stimulated monocytes [27] revealed similar expression patterns of immunoproteasomal subunits with upregulation of PSMB8/-9 but not -10, suggesting the involvement of type I and/or type II IFN triggers on PSMB8/-9 regulation in inflamed muscle tissues. IFNγ was reported to induce MHC-II and enhance MHC-I expression in affected muscles of PM and DM patients [32]. Recently, an IFNα signature and influence is discussed especially in DM but also in PM [33]. In contrast, a role of IFNγ triggering is assumed especially in IBM [34]. In this study, we could identify in the myositis transcriptomes only IFNγ but not IFNα as a predominant trigger for PSMB8/-9, which also correlated with the expression of STAT1 and IRF-1. Both transcription factors were also reported to mediate IFNγ induced PSMB9 expression in murine macrophages [35]. Interestingly, IFNγ production by T-cells was reported to depend on immunoproteasomes [36]. Especially in IBM the link of MHC-I with immunoproteasomal units suggests an important role for antigen processing via PSMB8 and -9 dependent mechanisms. This may be induced by misfolded protein from fiber degradation or suspected retroviral or viral triggers in this type of myositis [37] and thereby contribute to CD8+ T-cell triggering, expansion and IFNγ production.

Thus, immunoproteasomes and antigen processing seem to be pivotal in molecular pathomechanisms of myositis and may serve particularly as biomarker of myositis activity. This is further supported by the lack of immunoproteasome activation in our reanalysis of muscle transcriptomes from non-inflammatory myopathies [38], patients with severe inflammatory syndroms like sepsis [39] or conditions related to high IL-6 levels in the circulation [40].

This important association of immunoproteasome upregulation with myositis activity raises the discussion, whether these diseases qualify as a model for therapeutic targeting of immunoproteasomes. Inhibitors are currently developed and tested with controversial effects [41,42]. Application of the PSMB8 selective inhibitor PR-957 in experimental arthritis or colitis could reduce cytokine production and attenuate disease activity [43,44]. On the other hand, the recently described mutations c.224C>T (p.Thr75Met) [15], G201V [16], G197V [17] and c.405C>A [18] in PSMB8 were all associated with decreased subunit activity and different inflammatory syndroms, suggesting that immunoproteasome suppression may cause additional effects depending on dosage and cell type involvement. Studies in PSMB8/-9 deficienct mice suggested that inflammation induced immunoproteasome expression in tissue may also prevent CD8+ T-cell mediated autoimmunity [45].

Knowing that immunoproteasomes modulate antigen processing with effects on MHC-I peptide presentation [46], intracellular protein homeostasis [47] and CD8 T-cell responses [48] especially in mixed proteasomes [49], immunoproteasome inhibition may be a double-edged sword. Influencing antigen processing and presentation may reduce CD8+ T-cell triggering through MHC-I but may also increase toxicity by accumulating misfolded proteins.

In summary, our results support the hypothesis that the proteasome system is activated and contributes to a perpetuating crosstalk between antigen-presenting cells and T-cells via immunoproteasome generated peptides and IFNγ. Assuming altered autoantigen processing as driving mechanism, suppression of the immunoproteasome could be a promising therapeutic concept. Therefore, further studies are needed that focus on antigens and peptides, which are specifically processed by immunoproteasomes in IIM as well as on mechanisms of suppressing or modulating antigen processing and presentation.

Supporting Information

Figure S1 High expression levels of PSMB8 compared to PSMB5 in all isolated cells: Gene expression of constitutive (PSMB5-7) and immunoproteasomal subunits (PSMB8-10) in CD4+, CD8+, CD19+, CD14+, DCs and muscles of all patients. Data are shown as relative expression normalized to beta actin. Box plots indicate percentiles 0, 25, 50, 75 and 100.
(TIF)

Figure S2 Differences in upregulation of myositis related genes between IBM, PM, DM, NM and IM: All 1209 probesets were sorted by a sum-score for magnitude and frequency of increase in myositis. The heat map presents each disease group by the mean values of the signal intensity in all samples of the group. Combined scoring according to analysis on the 133A and 133P platform demonstrates that the strongest increase is observed in IBM followed by PM and DM, while IM and NM were closest to healthy control. This pattern was observed in 133A samples as well as in 133P samples independently of combined scoring (A and B) or scoring based on each individual platform (C and D).
(TIF)

Figure S3 Identification of genes involved in MHC-I and MHC-II antigen processing and presentation pathways: The 1209 probesets upregulated in myositis were uploaded into the DAVID database (http://david.abcc.ncifcrf.gov/) for functional annotation. All genes highlighted with a red star are included in the 1209 probesets.
(TIF)

Figure S4 This is the corresponding image to figure 5 in the manuscript. It lists all gene names and is provided as an additional jpg-file "Figure_S4" for further magnification (http://www.charite-bioinformatik.de/supplementary_data/immunoproteasomes/04_Sj9CPykssy0xPLMnMz0vMAfGjzOLNLU_Figure_S4.jpg).
(TIF)

Figure S5 Cell type specific transcripts and corresponding changes of gene expression in myositis: Cell type specific transcripts were determined from transcriptomes of monocytes, neutrophils, CD1+ dendritic cells, T-cells, B-cells, NK-cells and muscle tissue by filtering for cell type specific transcripts with signal level >2000 in the population of interest, < 200 in all other populations and a fold change of >20 if possible. In the heatmap on the right side, there is some overlapping expression in the different types of phagocytic cells and in the different lymphocyte populations. CD4+ and CD8+ T-cells do not allow the establishment of a transcript pattern that will distinguish them from other cell types and at the same time will differentiate between these two T-cell subpopulations. In the heatmap on the left side, all myositis transcriptomes were mapped to these marker panels and samples were sorted by intensity of change in the 1209 "myositis genes". This was performed using the median of log-transformed and z-normalized signals of all 1209 probesets for each sample as a score (myositis score). Sorting myositis samples from the lowest score on the left side (predominantly normal donor samples) to the highest score on the right side (predominantly IBM samples), there is an increase especially of transcripts related to monocytes, dendritic cells and T-cells corresponding to the severity of myositis with a corresponding decrease of muscle specific transcripts. (Figure S5 is also provided as an additional separate jpg-file for further magnification: http://www.charite-

bioinformatik.de/supplementary_data/immunoproteasomes/yMDI2dDbwsPIJdDBwNDNwCjLzDgowsDIEK_Figure_S5.jpg).
(TIF)

Table S1 Clinical data of patients with DM, PM, OM and NIM.
(XLS)

Table S2 Collection of transcriptome data from the Gene Expression Omnibus repository: These transcriptome data were used for analysis of the role of immunoproteasomes in inflammatory and non-inflammatory muscle diseases compared to other genes differentially expressed in myositis.
(XLS)

Table S3 Probesets and genes identified as upregulated in IBM, PM and/or DM with signal intensities and molecular scores: Datasets of GSE2044, GSE3112, and GSE39454 were used to identify molecular changes in IBM, PM and DM compared to healthy muscle biopsies. Data generated with the different platforms HG-U133A (133A) and HG-U133Plus 2.0 (133P) were analysed separately to avoid technical bias. Each disease entity was compared to healthy controls. Selection of differentially expressed probesets was based on the frequency of change in pairwise comparisons between arrays from two different groups, on signal log ratio (SLR), on t-test statistics and on cut-off for absolute signal intensities combined to a default filtering as provided in BioRetis. Probesets, which were upregulated in the same disease in both platforms, were selected and combined from all diseases to a total of 1209 probesets/927 genes. To score these probesets by dominance of increase, the frequency of change call for all pairwise comparisons and the SLR were z-normalized across all selected probesets and then scaled to the maximum of "1". The sum of both normalized values was used for ranking, thus identifying genes with the best sum-score for "highly increased" and "most frequently increased" in disease compared to control in the top ranks. These probesets were sorted by a sum-score for magnitude and frequency of increase. See file "Table_S3.xls" accessible via: http://www.charite-bioinformatik.de/supplementary_data/immunoproteasomes/Tcx8DAP8zJzMDIKSjY3M_R2cDA3YCQ_nD9_Table_S3.xls. 1. sheet "probesets" with the list of the 1209 probesets sorted by the composed score of SLR and change call. 2. sheet "genes" with the list of the 927 genes sorted by the composed score of SLR and change call. 3. sheet "PSM 133P" with signals for all proteasomal units of the 133P arrays. 4. sheet "PSM 133A" with signals for all proteasomal units of the 133A arrays. Data of all microarrys and group comparisons are available in BioRetis (www.bioretis.com).
(XLS)

Table S4 Correlation of PSMB8 and PSMB9 expression in myositis with transcriptions factors: Correlation coefficients on the basis of signal values from myositis muscle biopsies (columns A1–A4), immune cells (B1–B2) and cytokine stimulated monocytes (C1–C2) are presented in combination with the z-normalized signal values of purified immune cells (columns D1–D21), healthy muscle profiles (E1–E5), cytokine stimulated monocytes (F1–F22), muscle biopsies based on 133P arrays (G1–G36) and 133A arrays (H1–H62); (see file Table_S4.xls download link: http://www.charite-bioinformatik.de/supplementary_data/immunoproteasomes/KFQlWFwAVoDPCrACAxzA0UDfzyM_N1W_ID_Table_S4.xls).
(XLS)

Acknowledgments

We thank Toralf Kaiser and Jenny Kirsch from the Deutsches Rheuma-Forschungszentrum, Flow cytometry Core Facility in Berlin for technical assistance in cell sorting. The authors have no commercial or other associations to disclose.

Author Contributions

Conceived and designed the experiments: EF KG TH G-RB SK. Performed the experiments: KG LM-G BS MB SB LS AG. Analyzed the data: TH KG MB SB BS EF LM-G. Contributed reagents/materials/analysis tools: EF TH AG BS MB SB LS SK. Wrote the paper: KG TH EF G-RB LM-G SK BS AG SB MB LS.

References

1. Dalakas MC, Hohlfeld R (2003) Polymyositis and dermatomyositis. Lancet 362: 971–982.
2. Dalakas MC (2004) Inflammatory disorders of muscle: progress in polymyositis, dermatomyositis and inclusion body myositis. Curr Opin Neurol 17: 561–567.
3. Dalakas MC (2006) Mechanisms of disease: signaling pathways and immunobiology of inflammatory myopathies. Nat Clin Pract Rheumatol 2: 219–227.
4. Rechsteiner M, Hoffman L, Dubiel W (1993) The multicatalytic and 26S proteases. J Biol Chem 268: 6065–6068.
5. Muratani M, Tansey WP (2003) How the ubiquitin-proteasome system controls transcription. Nat Rev Mol Cell Biol 4: 192–201.
6. Coux O, Tanaka K, Goldberg AL (1996) Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem 65: 801–847.
7. Groettrup M, Soza A, Kuckelkorn U, Kloetzel PM (1996) Peptide antigen production by the proteasome: complexity provides efficiency. Immunol Today 17: 429–435.
8. Kumamoto T, Fujimoto S, Ito T, Horinouchi H, Ueyama H, et al. (2000) Proteasome expression in the skeletal muscles of patients with muscular dystrophy. Acta Neuropathol 100: 595–602.
9. Mitch WE, Goldberg AL (1996) Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. N Engl J Med 335: 1897–1905.
10. Groll M, Ditzel L, Lowe J, Stock D, Bochtler M, et al. (1997) Structure of 20S proteasome from yeast at 2.4 angstrom resolution. Nature 386: 463–471.
11. Aki M, Shimbara N, Takashina M, Akiyama K, Kagawa S, et al. (1994) Interferon-gamma induces different subunit organizations and functional diversity of proteasomes. J Biochem 115: 257–269.
12. Boes B, Hengel H, Ruppert T, Multhaup G, Koszinowski UH, et al. (1994) Interferon gamma stimulation modulates the proteolytic activity and cleavage site preference of 20S mouse proteasomes. J Exp Med 179: 901–909.
13. Kuckelkorn U, Frentzel S, Kraft R, Kostka S, Groettrup M, et al. (1995) Incorporation of major histocompatibility complex–encoded subunits LMP2 and LMP7 changes the quality of the 20S proteasome polypeptide processing products independent of interferon-gamma. Eur J Immunol 25: 2605–2611.
14. Lundberg IE, Nyberg P (1998) New developments in the role of cytokines and chemokines in inflammatory myopathies. Curr Opin Rheumatol 10: 521–529.
15. Agarwal AK, Xing C, DeMartino GN, Mizrachi D, Hernandez MD, et al. (2010) PSMB8 encoding the beta5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. Am J Hum Genet 87: 866–872.
16. Arima K, Kinoshita A, Mishima H, Kanazawa N, Kaneko T, et al. (2011) Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome. Proc Natl Acad Sci U S A 108: 14914–14919.
17. Kitamura A, Maekawa Y, Uehara H, Izumi K, Kawachi I, et al. (2011) A mutation in the immunoproteasome subunit PSMB8 causes autoinflammation and lipodystrophy in humans. J Clin Invest 121: 4150–4160.
18. Liu Y, Ramot Y, Torrelo A, Paller AS, Si N, et al. (2012) Mutations in proteasome subunit beta type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity. Arthritis Rheum 64: 895–907.
19. Egerer K, Kuckelkorn U, Rudolph PE, Ruckert JC, Dörner T, et al. (2002) Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune diseases. J Rheumatol 29: 2045–2052.
20. Feist E, Dörner T, Kuckelkorn U, Schmidtke G, Micheel B, et al. (1996) Proteasome alpha-type subunit C9 is a primary target of autoantibodies in sera of patients with myositis and systemic lupus erythematosus. J Exp Med 184: 1313–1318.
21. Brychcy M, Kuckelkorn U, Hausdorf G, Egerer K, Kloetzel PM, et al. (2006) Anti-20S proteasome autoantibodies inhibit proteasome stimulation by proteasome activator PA28. Arthritis Rheum 54: 2175–2183.
22. Krause S, Kuckelkorn U, Dörner T, Burmester GR, Feist E, et al. (2006) Immunoproteasome subunit LMP2 expression is deregulated in Sjogren's syndrome but not in other autoimmune disorders. Ann Rheum Dis 65: 1021–1027.

23. Bohan A, Peter JB (1975) Polymyositis and dermatomyositis (second of two parts). N Engl J Med 292: 403–407.
24. Bohan A, Peter JB (1975) Polymyositis and dermatomyositis (first of two parts). N Engl J Med 292: 344–347.
25. Morawietz L, Martinez-Gamboa L, Scheffler S, Hausdorf G, Dankof A, et al. (2009) Expression of proteasomal immunosubunit beta1i is dysregulated in inflammatory infiltrates of minor salivary glands in Sjogren's syndrome. J Rheumatol 36: 2694–2703.
26. Pfaffi MW (2001) A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res v.29(9): e45.
27. Smiljanovic B, Grün JR, Biesen R, Schulte-Wrede U, Baumgrass R, et al. (2012) The multifaceted balance of TNF-alpha and type I/II interferon responses in SLE and RA: how monocytes manage the impact of cytokines. J Mol Med (Berl) 90: 1295–1309.
28. Smiljanovic B, Grün JR, Steinbrich-Zollner M, Stuhlmüller B, Häupl T, et al. (2010) Defining TNF-alpha- and LPS-induced gene signatures in monocytes to unravel the complexity of peripheral blood transcriptomes in health and disease. J Mol Med (Berl) 88: 1065–1079.
29. Menssen A, Edinger G, Grün JR, Haase U, Baumgrass R, et al. (2009) SiPaGene: A new repository for instant online retrieval, sharing and meta-analyses of GeneChip expression data. BMC Genomics 10: 98.
30. Ahn JY, Tanahashi N, Akiyama K, Hisamatsu H, Noda C, et al. (1995) Primary structures of two homologous subunits of PA28, a gamma-interferon-inducible protein activator of the 20S proteasome. FEBS Lett 366: 37–42.
31. Jain A, Sharma MC, Sarkar C, Bhatia R, Singh S, et al. (2007) Major histocompatibility complex class I and II detection as a diagnostic tool in idiopathic inflammatory myopathies. Arch Pathol Lab Med 131: 1070–1076.
32. Kalovidouris AE (1992) The role of cytokines in polymyositis: interferon-gamma induces class II and enhances class I major histocompatibility complex antigen expression on cultured human muscle cells. J Lab Clin Med 120: 244–251.
33. Greenberg SA, Higgs BW, Morehouse C, Walsh RJ, Kong SW, et al. (2012) Relationship between disease activity and type 1 interferon- and other cytokine-inducible gene expression in blood in dermatomyositis and polymyositis. Genes Immun 13: 207–213.
34. Ivanidze J, Hoffmann R, Lochmuller H, Engel AG, Hohlfeld R, et al. (2011) Inclusion body myositis: laser microdissection reveals differential up-regulation of IFN-gamma signaling cascade in attacked versus nonattacked myofibers. Am J Pathol 179: 1347–1359.
35. Brucet M, Marques L, Sebastian C, Lloberas J, Celada A (2004) Regulation of murine Tap1 and Lmp2 genes in macrophages by interferon gamma is mediated by STAT1 and IRF-1. Genes Immun 5: 26–35.
36. Rockwell CE, Monaco JJ, Qureshi N (2012) A critical role for the inducible proteasomal subunits LMP7 and MECL1 in cytokine production by activated murine splenocytes. Pharmacology 89: 117–126.
37. Ferrer I, Martin B, Castano JG, Lucas JJ, Moreno D, et al. (2004) Proteasomal expression, induction of immunoproteasome subunits, and local MHC class I presentation in myofibrillar myopathy and inclusion body myositis. J Neuropathol Exp Neurol 63: 484–498.
38. Bakay M, Wang Z, Melcon G, Schiltz L, Xuan J, et al. (2006) Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of Rb-MyoD pathways in muscle regeneration. Brain 129: 996–1013.
39. Fredriksson K, Tjader I, Keller P, Petrovic N, Ahlman B, et al. (2008) Dysregulation of mitochondrial dynamics and the muscle transcriptome in ICU patients suffering from sepsis induced multiple organ failure. PLoS One 3: e3686.
40. Mortensen OH, Andersen K, Fischer C, Nielsen AR, Nielsen S, et al. (2008) Calprotectin is released from human skeletal muscle tissue during exercise. J Physiol 586: 3551–3562.
41. Huber EM, Basler M, Schwab R, Heinemeyer W, Kirk CJ, et al. (2012) Immuno- and constitutive proteasome crystal structures reveal differences in substrate and inhibitor specificity. Cell 148: 727–738.
42. Verbrugge SE, Assaraf YG, Dijkmans BA, Scheffer GL, Al M, et al. (2012) Inactivating PSMB5 mutations and P-glycoprotein (multidrug resistance-associated protein/ATP-binding cassette B1) mediate resistance to proteasome inhibitors: ex vivo efficacy of (immuno)proteasome inhibitors in mononuclear blood cells from patients with rheumatoid arthritis. J Pharmacol Exp Ther 341: 174–182.
43. Muchamuel T, Basler M, Aujay MA, Suzuki E, Kalim KW, et al. (2009) A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. Nat Med 15: 781–787.
44. Basler M, Dajee M, Moll C, Groettrup M, Kirk CJ (2010) Prevention of experimental colitis by a selective inhibitor of the immunoproteasome. J Immunol 185: 634–641.
45. Zaiss DM, Bekker CP, Grone A, Lie BA, Sijts AJ (2011) Proteasome immunosubunits protect against the development of CD8 T cell-mediated autoimmune diseases. J Immunol 187: 2302–2309.
46. Kloetzel PM, Ossendorp F (2004) Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. Curr Opin Immunol 16: 76–81.
47. Seifert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, et al. (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142: 613–624.
48. Chen W, Norbury CC, Cho Y, Yewdell JW, Bennink JR (2001) Immunoproteasomes shape immunodominance hierarchies of antiviral CD8(+) T cells at the levels of T cell repertoire and presentation of viral antigens. J Exp Med 193: 1319–1326.
49. Zanker D, Waithman J, Yewdell JW, Chen W (2013) Mixed Proteasomes Function To Increase Viral Peptide Diversity and Broaden Antiviral CD8+ T Cell Responses. J Immunol 191: 52–59.

Lebenslauf

"Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht."

Lebenslauf

"Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht."

Lebenslauf

"Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht."

Publikationsliste:

1. Ghannam K, Martinez-Gamboa L, Spengler L, Krause S, Smiljanovic B, et al. (2014) Upregulation of Immunoproteasome Subunits in Myositis Indicates Active Inflammation with Involvement of Antigen Presenting Cells, CD8 T-Cells and IFNgamma. PLoS One 9: e104048.
2. Martinez-Gamboa L, Lesemann K, Kuckelkorn U, Scheffler S, Ghannam K, et al. (2013) Gene expression of catalytic proteasome subunits and resistance toward proteasome inhibition of B lymphocytes from patients with primary sjogren syndrome. J Rheumatol 40: 663-673.

Danksagung

Ich möchte mich an dieser Stelle bei vielen Personen bedanken, die mich in dieser spannenden Phase meiner akademischen Laufbahn begleitet haben.

Großer Dank gebührt zu allererst meinem Doktorvater PD Dr. med. Eugen Feist für die freundliche Überlassung des hochinteressanten Themas. Ohne seine geduldige, stetige und hilfreiche Betreuung sowie Unterstützung wäre diese Arbeit nicht gelungen. Jede Phase dieser Arbeit wurde von ihm intensiv, professionell und warmherzig begleitet.

Mein besonderer Dank gilt auch Prof. Dr. med. Gerd R. Burmester für die Bereitstellung des Arbeitsplatzes. Seine wertvollen akademischen Ratschläge und seine Hilfe kamen mir in zahlreichen Angelegenheiten sehr zugute.

Für die unermüdliche und professionelle Einführung in Microarray Analysen danke ich PD Dr. med. Thomas Häupl ganz herzlich. Für seine konstruktiven Ideen, die zu hervorragenden Ergebnissen führten, gebührt ihm ganz besonderer Dank. Mit großem Engagement hat er jederzeit tatkräftig geholfen.

Mein Dank gilt Lorena Martinez-Gamboa. Sie hat mich während meiner ganzen Promotionsphase begleitet, stand mir immer mit Rat und Tat bei inhaltlichen sowie methodischen Fragen zu Seite und verstand es, mich in den richtigen Momenten zu motivieren und mir Mut zu machen. Vielen Dank!

Ein ganz besonderer Dank geht an meine Mitdoktoranden und Mitdoktorandinnen sowie alle Mitarbeiter und Mitarbeiterinnen des Instituts für die außerordentlich gute Zusammenarbeit.

Ich danke meinem Mann, der mir stets Mut zugesprochen und mich in meiner Arbeit bestärkt hat. Hätte er mir nicht den Rücken freigehalten, wäre meine Arbeit in dieser Form nicht möglich gewesen.

Und nicht zuletzt danke ich meinen Eltern und Brüdern, die in jeglicher Hinsicht die Grundsteine für meinen Weg gelegt haben.