Title

Dietary supplementation with eicosapentaenoic acid inhibits plasma cell differentiation and attenuates lupus autoimmunity

Article type

Original Research Article

Authors/Affiliations

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Ayaka Ito: aito@riem.nagoya-u.ac.jp Takayoshi Suganami: suganami@riem.nagoya-u.ac.jp **Figure S1** | Effect of EPA supplementation on body and tissue weights in lupus mice. Body and tissue weights. Four-week-old FVB/N mice were fed CE-2 diet supplemented with 5% (*wt/wt*) palmitate (Pal) or eicosapentaenoic acid (EPA) for 6 weeks. To induce lupus, the mice received imiquimod (IMQ) or vehicle (Veh) treatment 3 times a week during the last 4 weeks. Veh + Pal, n = 8; IMQ + Pal, n = 8; IMQ + EPA, n = 9. Data shown are mean value ± SEM of representative experiments at least three times independently. Statistical analysis was performed with Turkey's test. *p < 0.05; **p < 0.01; ns, not significant.

Figure S2 | Gating strategy used for immunophenotyping of spleens in **Figure 2**. Spleen from Veh-treated mice fed Pal-supplemented diet, IMQ-treated mice fed Pal-supplemented diet, and IMQ-treated mice fed EPA-supplemented diet were analyzed by flow cytometry.

Figure S3 | EPA does not affect $CD8^+$ T or myeloid cellurality in spleen of IMQ-induced lupus mice. Immunophenotyping of spleen from Veh-treated mice fed Pal-supplemented diet, IMQ-treated mice fed Pal-supplemented diet, and IMQ-treated mice fed EPA-supplemented diet by flow cytometry. Gating strategy is available in Figure S2. (A) Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of myeloid cells in total spleen cells. $CD11c^+MHCII^+$ dendritic cells (DC), $B220^+PDCA^+$ plasmacytoid DC (pDC), $CD11b^+Ly6G^-$ monocytes and $CD11b^+Ly6G^+$ neutrophils were analyzed. (B) Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of $CD8^+$ T cell subsets in total spleen cells. Total $CD3^+CD8^+$ T cells, $CD8^+CD62L^+CD44^-$ naïve T cells and $CD8^+CD62L^-CD44^+$ effector T cells (Teff) were analyzed. Veh + Pal, n = 8; IMQ + Pal, n = 8; IMQ + EPA, n = 8. Data shown are mean value \pm SEM of representative experiments at least three times independently. Statistical analysis was performed with Dunn's test (A: cell count of DC and Monocyte, B: Teff) or Tukey's test (if not otherwise stated). *p < 0.05; **p < 0.01; ns, not significant.

Figure S4 | Effect of EPA supplementation on body and tissue weights in lupus mice. (A) Gross morphology of spleen (left) and axillary lymph nodes (right). (B) Body and tissue weights. Eight week-old control C57BL/6J^{+/+} and C57BL/6J^{lpr/lpr} mice were fed CE-2 diet supplemented with 5% (*wt/wt*) Pal or EPA for 16 weeks. Lymph node panel indicates the weight of 2 axillary lymph nodes. B6^{+/+} + Pal, n = 9; B6^{lpr/lpr} + Pal, n = 7; B6^{lpr/lpr} + EPA, n = 8. Data shown are mean value ± SEM of representative experiments at least twice independently. Statistical analysis was performed with Turkey's test. *p < 0.05; **p < 0.01; ns, not significant.

Figure S5 | Gating strategy used for immunophenotyping of spleens in **Figure 4**. Spleen from B6^{+/+} mice fed Pal-supplemented diet, B6^{lpr/lpr} mice fed Pal-supplemented diet, and B6^{lpr/lpr} mice fed EPA-supplemented diet were analyzed by flow cytometry.

Figure S6 | EPA does not affect CD8⁺ T or myeloid cellurality in spleen of spontaneous lupus mice. Immunophenotyping of spleen from B6^{+/+} mice fed Pal-supplemented diet, B6^{*lpr/lpr*} mice fed Pal-supplemented diet, and B6^{*lpr/lpr*} mice fed EPA-supplemented diet by flow cytometry. Gating strategy is available in Figure S5. **(A)** Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of T cell subsets in total spleen cells. Total cells, CD62L⁺CD44⁻ naïve T cells and CD62L⁻CD44⁺ effector T cells (Teff) in CD4⁺ and CD8⁺ T cells were analyzed. **(B)** Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of myeloid cells in total spleen cells. CD11e⁺MHCII⁺ dendritic cells (DC), B220⁺PDCA⁺ plasmacytoid DC (pDC), CD11b⁺Ly6G⁻ monocytes and CD11b⁺Ly6G⁺ neutrophils were analyzed. B6^{+/+} + Pal, n = 9; B6^{*lpr/lpr*} + Pal, n = 7; B6^{*lpr/lpr*} + EPA, n = 8. Data shown are mean value ± SEM of representative experiments at least twice independently. Statistical analysis was performed with Dunn's test (A: Cell count of CD4 Total, Teff, CD8 naïve and Teff, B: population of pDC and Neutrophil, cell count of DC and pDC) or Tukey's test (if not otherwise stated). *p < 0.05; **p < 0.01; ns, not significant.

Table S1. List of resources used in this study

Fluorescein (FTC) AffiniPure Goat Anti-Mouse IgG (H+L)Jackson ImmunoResearchCat#115-095-003 RRID : AB 2338589Fluorescein-Conjugated Goat IgG Fraction To Mouse Complement C3MP BiomedicalsCat#SKU 0855500PE/Cy7 anti-mouse CD138 (Syndccan-1) AntibodyBioLegend281-2Cat#142513 RRID : AB 2562197APC anti-mouse/human CD45R/B220 AntibodyBioLegendRA3-6B2Cat#103211 RRID : AB 312996BrilliantViolet510TM BioLegendRA3-6B2Cat#103247 RRID : AB 2561394PE anti-mouse/humanCD45R/B220 AntibodyBioLegendSA367H8Cat#152607 RRID : AB 2632903PerCP/Cyanine5.5 anti-mouse CD23 AntibodyBioLegendB3B4Cat#103247 RRID : AB 2632903PerCP/Cyanine5.5 anti-mouse CD21/CD35 AntibodyBioLegendB3B4Cat#101617 RRID : AB 2562923APC/Cy7 anti-mouse CD21/CD35 AntibodyBioLegend145-2C11Cat#103047 RRID : AB 2562923APC/Cy7 anti-mouse CD3eBioLegend145-5C11Cat#100308 RRID : AB 2564587PE/Cy7 Anti-mouse/human GL7 Antigen AntibodyBioLegend145-5C11Cat#100308 RRID : AB 312673BrilliantViolet 510TM anti-mouse BioLegendBioLegendRA3-6B2Cat#10301 RRID : AB 312673BrilliantViolet 510TM anti-mouseBioLegendIM7Cat#10301 RRID : AB 312067PE/Cy7 AntibodyBioLegendIM7Cat#10301 RRID : AB 313004APC Cy7 Anti-mouse/humanBioLegendMEL-14Cat#107635 RRID : AB 313004APC/Cy7 APC	Antibody	Source	Clone	Identifier
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APC/Cv7_anti-mouse/human_CD11h Cat#101225	APC/Cy7 anti-mouse/human CD11b	D' I 1		
Antibody BioLegend M1/70 RRID : AB_830641	-	BioLegend	M1//0	

TruStainFcX TM (anti-mouseCD16/32)Antibody	BioLegend	93	Cat#101320 RRID : AB 1574975
PE/Cy7 anti-mouse CD279 (PD-1)	BioLegend	29F.1A12	Cat#135215 RRID : AB 10696422
PE anti-mouse Foxp3	TONBO Biosciences	3G3	Cat#50-5773-U025
APC anti-mouse CD25	BioLegend	3C7	Cat#101909 RRID : AB 961208
Alexa Fluor® 647 anti-mouse Blimp-1 Antibody	BioLegend	5E7	Cat#150003 RRID : AB 2565617
PerCP/cyanine5.5 anti-mouse CD8a Antibody	BioLegend	53-6.7	Cat#100733 RRID : AB_2075239
Cy3 Peanut Agglutinin (PNA)	Vector laboratories		Cat#CL-1073-1
Purified anti-mouse CD40 Antibody	BioLegend	HM40-3	Cat#102902 RRID : AB_312945

Chemical/Peptide/Recombinant protein	Source	Identifier
DAPI (used for nuclear staining)	BioLegend	Cat#422801
Collagenase Type IV	Worthington	Cat#CLS4
DNase I	Roche Diagnostics	Cat#11284932001
R848 Resiquimod	AdipoGen	Cat#AG-CR1-3582
LPS from E. coli O111:B4	Sigma-Aldrich	Cat#L4391-1MG
Recombinant murine IL4	Pepro Tech	Cat#214-14
Recombinant murine IFNy	Pepro Tech	Cat#315-05
Palmitic acid BioXtra, ≥99%	Sigma-Aldrich	Cat#P5585
cis-5,8,11,14,17-Eicosapentaenoic acid sodium salt ≥99% (capillary GC)	Sigma-Aldrich	Cat#E6627-25MG
BESELNA cream 5%	Mochida Pharmaceutical	Cat#87629
Foxp3/Transcription Factor Staining Buffer Kit	TONBO Biosciences	Cat#TNB-0607-KIT
Laurdan	Cayman Chemical	Cat#19706

ELISA	Source	Identifier
Mouse Anti-Nuclear Antibodies (ANA) Total Ig	Alpha Daiagnostic	Cat#5210
ELISA Kit	International	
Mouse BAFF/BLyS/TNFSF13B Immunoassay	R&D Systems	Cat#MBLYS0
LBIS Mouse Anti-dsDNA ELISA Kit	FUJIFILM Wako Shibayagi	Cat#AKRDD-061
Mouse Anti-Histones Ig's ELISA Kit, 96 tests,	Alpha Daiagnostic	Cat#5610

Quantitative	International	
Mouse IgG Uncoated ELISA Kit	Invitrogen	Cat#88-50400-22
Mouse IgM Uncoated ELISA Kit	Invitrogen	Cat#88-50470-22
Mouse IL-12 p70 ELISA Kit	Invitrogen	Cat#BMS6004
Mouse IL-6 Quantikine ELISA Kit	R&D Systems	Cat#M6000B
Mouse TNF-alpha Quantikine ELISA Kit	R&D Systems	Cat#MTA00B

Cell separation reagent	Source	Identifier
FcR Blocking Reagent, mouse	Miltenyi Biotec	Cat#130-092-575
B cell Isolation Kit II	Miltenyi Biotec	Cat#130-090-862
Pan B cell Isolation kit II	Miltenyi Biotec	Cat#130-104-443
CD11c microbeads Ultrapure	Miltenyi Biotec	Cat#130-108-338

Animal/Cell	Source	Identifier
Mouse: C57BL/6J+/+ females 8 weeks of age	Japan SLC, Inc	N/A
Mouse: C57BL/6Jlpr/lpr females 8 weeks of age	Japan SLC, Inc	N/A
Mouse: FVB/N females 4 weeks of age	CLEA Japan, Inc	N/A
Ba/F3-CL1	RIKEN BRC	Cat#RBC4474
Reporter cell: B16-Blue IFN-α/β cells	Invivogen	Cat#bb-ifnt1

Software	Source	Identifier
FlowJo software	BD Biosciene	https://www.flowjo.com/
SimFCS software	Laboratory for Fluorescence Dynamics	lfd.uci.edu/globals/
Prism9	GraphPad	https://www.graphpad.com/

Table S2. List of primers used to detect mRNAs

Name	Fw/Rv	Sequence
36b4	Fw	5'-GGCCCTGCACTCTCGCTTTC-3'
	Rv	5'-TGCCAGGACGCGCTTGT-3'
Baff	Fw	5'-AACAGACGCGCTTTCCAG-3'
	Rv	5'-AGGAGGAGCTGAGAGGTCTACAT-3'
Prdm1	Fw	5'-TTCTCTTGGAAAAACGTGTGGG-3'
	Rv	5'-GGAGCCGGAGCTAGACTTG-3'
Cd79b	Fw	5'-TGTTGGAATCTGCAAATGGA-3'
	Rv	5'-TAGGCTTTGGGTGATCCTTG-3'
Ifnb	Fw	5'-TGGAGCAGCTGAATGGAAAG-3'
	Rv	5'-ATCTCTTGGATGGCAAAGGC-3'
<i>Il6</i>	Fw	5'-GCTACCAAACTGGATATAATCAGGA-3'
	Rv	5'-CCAGGTAGCTATGGTACTCCAGAA-3'
Il12b	Fw	5'-ACATCTACCGAAGTCCAATGCA-3'
	Rv	5'-GGAATTGTAATAGCGATCCTGAGC-3'
Irf4	Fw	5'-ACCACTGGCAAGGCCCAT-3'
	Rv	5'-GCAGTCTGAGAGCGCCAAG-3'
Tnfa	Fw	5'-ACCCTCACACTCAGATCATCTTC-3'
	Rv	5'-TGGTGGTTTGCTACGACGT-3'
Xbp1	Fw	5'-GAGTCCGCAGCAGGTG-3'
	Rv	5'-GTGTCAGAGTCCATGGGA-3'

Product	Fish meal-free diet (No. 012, CREA Japan)		Fish meal- supplemer 5% fatt (wt/	nted with y acid
	kcal/100g	kcal%	kcal/100g	kcal%
Protein	80	23	76	21
Carbohydrate	222	65	212	58
Fat	39	11	80	22
Total	341	100	367	100

Table S3. Composition of experimental diets