2011/10/01

第2回　機能油脂懇話会

脂肪酸由来の生理活性物質と炎症の制御

東京大学大学院薬学系研究科
JSTさきがけ「炎症の慢性化機構の解明と制御」

有田　誠

東京大学 THE UNIVERSITY OF TOKYO

さきがけ PRESTO

Lipid Mediators from Polyunsaturated Fatty Acids

Arachidonic acid (AA) → Prostaglandins, Leukotrienes, Lipoxins

C20:4 ω6

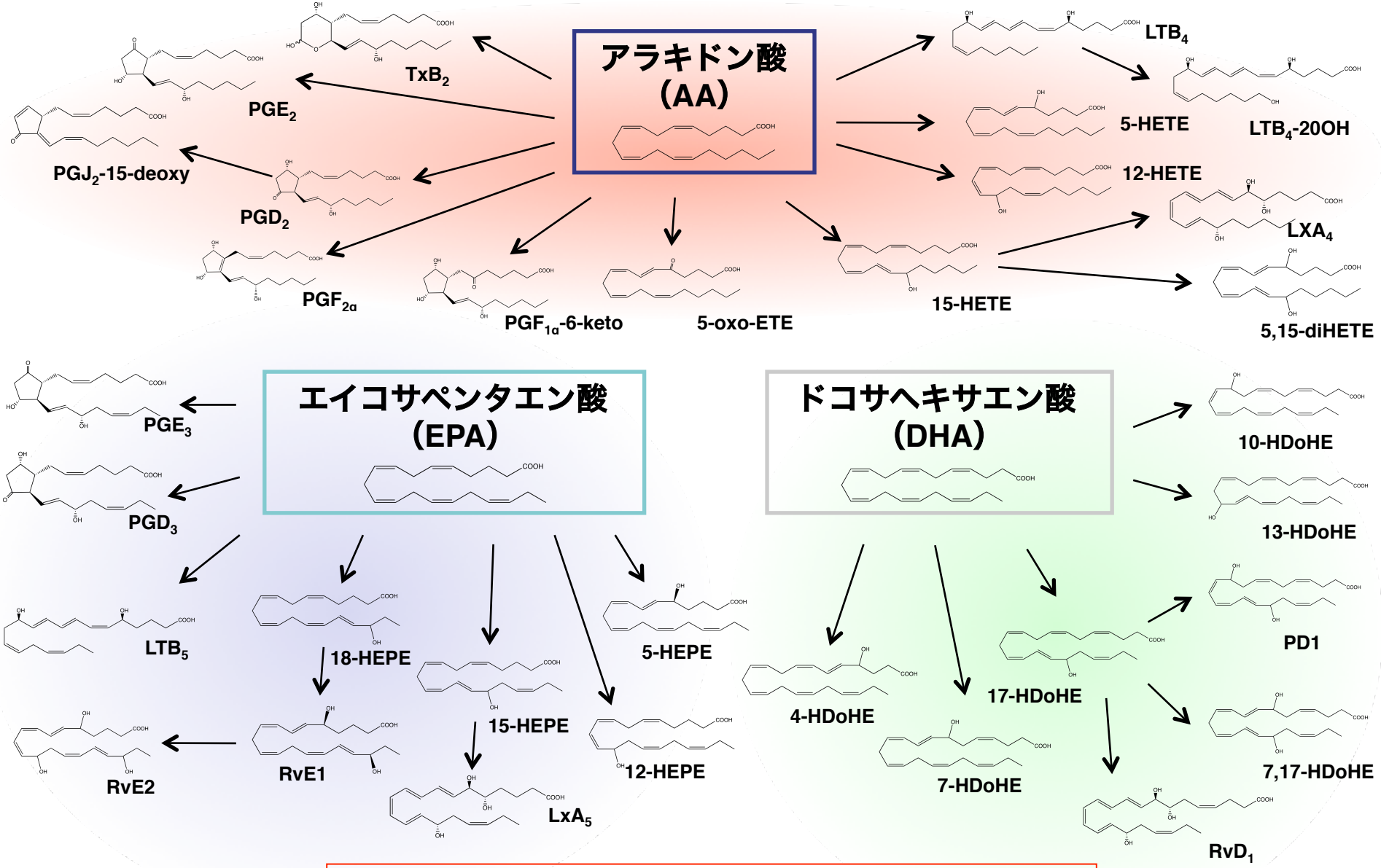
Eicosapentaenoic acid (EPA) → Resolvin Es

C20:5 ω3

Docosahexaenoic acid (DHA) → Resolvin Ds, Protectins

C22:6 ω3

脂肪酸代謝マップ



脂肪酸代謝フローの全体像を捉えることが重要

LC/MS/MSを用いた脂肪酸代謝物の包括的測定系

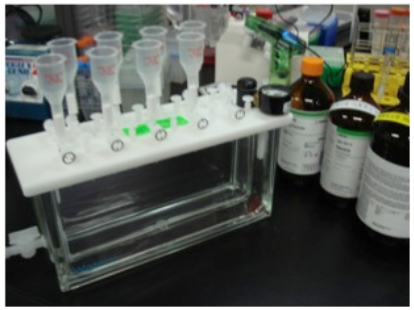
抽出

固相抽出カラム
Sep-Pak C18
(Waters)

サンプル

内部標準①

内部標準②



分離

UPLC（Waters）
C18カラム 1.7um
（1.0×150mm）

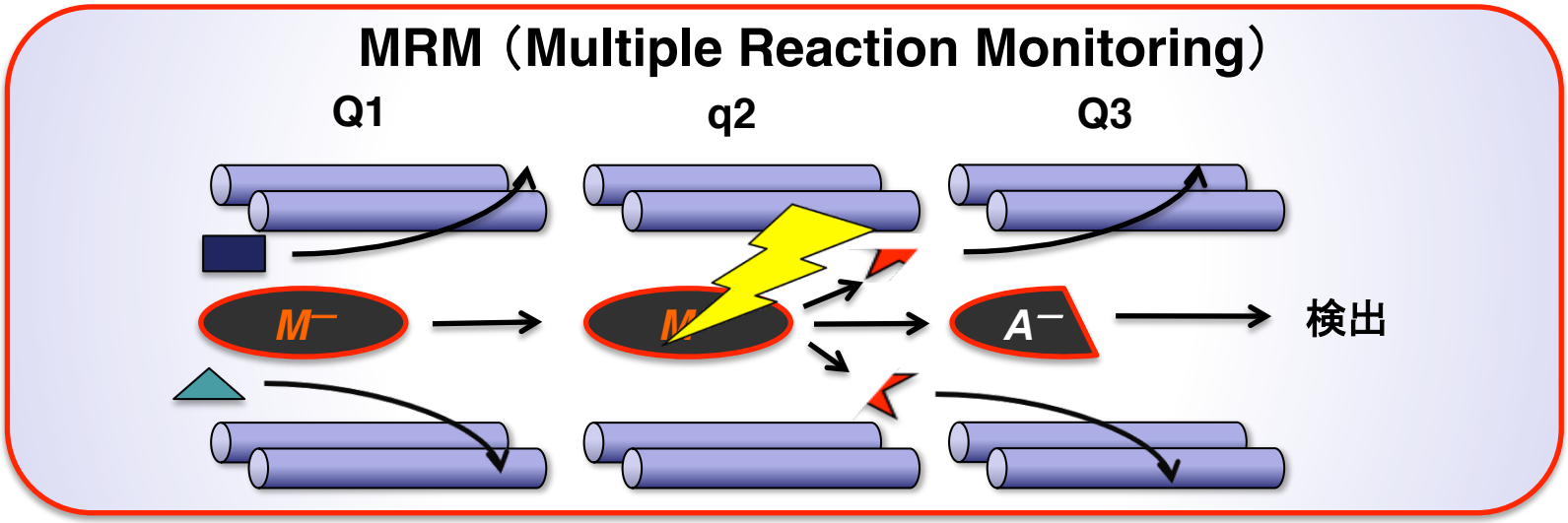
２液グラジェント
A液：0.1%酢酸水
B液：ACN/MeOH＝4/1

検出

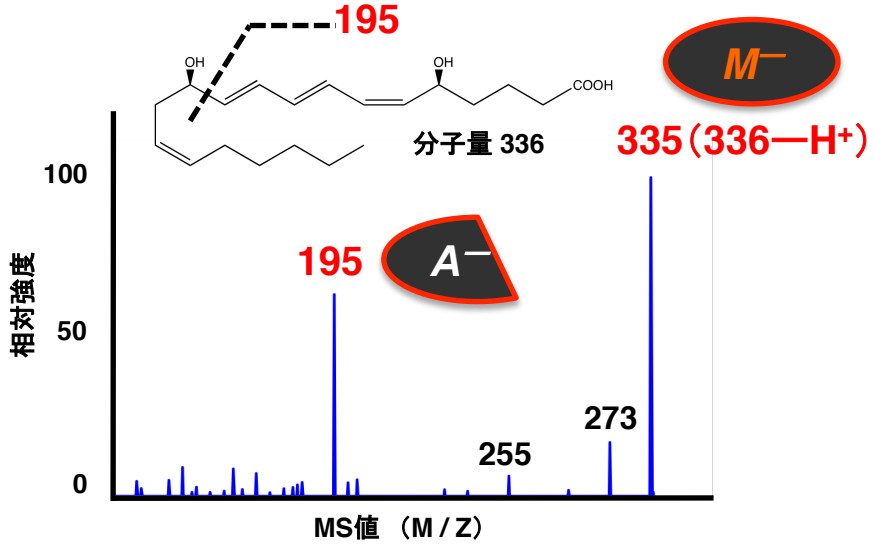
三連四重極型MS
QTRAP5500（AB SCIEX）

Multiple Reaction Monitoring
(MRM)

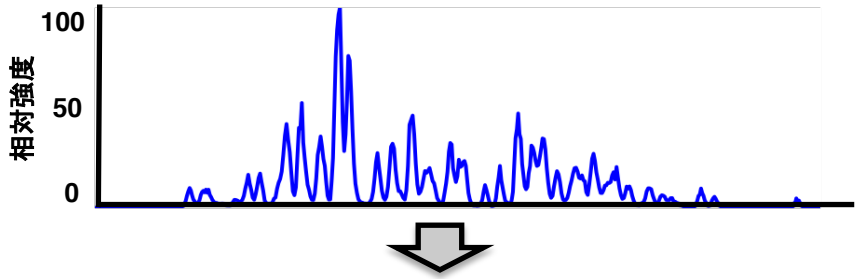
MRMシステムによる脂肪酸代謝物の測定



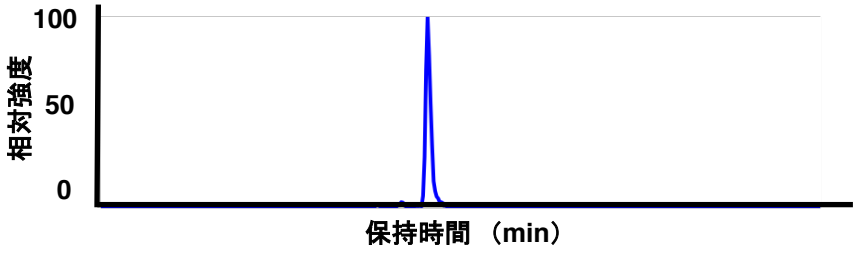
ロイコトリエンB$_4$のMS/MSスペクトル



トータルイオンクロマトグラム（TIC）
様々な脂肪酸代謝物のmixture



MRM ロイコトリエンB$_4$（335/195）



MRM総チャネル数　275チャネル

測定化合物総数　204種類

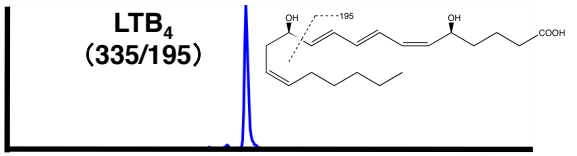
AA代謝物	47 種類
EPA代謝物	55 種類
DHA代謝物	56 種類
DPA代謝物	45 種類
その他	1 種類
内部標準	6 種類

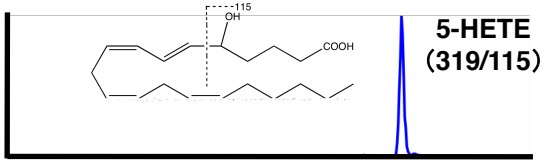
酸化脂肪酸 MRMチャネル

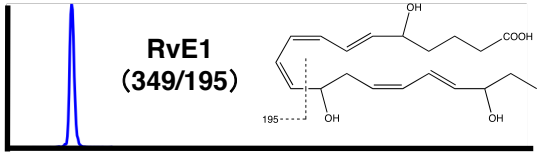
Name	Q1	Q3	RT
001_PGE2	351.098	271.2	15.20
002_PGD2	350.933	271.1	15.50
003_PGD2-1	350.933	189.1	15.50
004_15-keto-PGE2	349.019	235.0	15.70
005_PGE2-2OH	366.945	287.2	6.78
006_PGE2-2OH	366.945	189.1	6.78
007_LXA4	351.143	217.0	16.10
008_LXB4	351.120	221.1	15.30
009_PGF2a	353.137	193.2	14.90
010_PGF1a-6keto	369.187	163.0	12.60
011_TXB2	368.997	195.1	14.20
012_LTB4	335.111	195.0	19.10
013_LTB4-20OH	351.026	195.1	12.90
014_PGJ2-15-deoxy	315.272	271.1	21.40
015_LTD4	495.085	177.1	17.40
016_LTE4	438.081	333.2	18.10
017_LTE4	438.081	351.1	18.10
018_5-HETE	319.010	114.9	25.00
019_5,6-EET	319.084	191.1	27.30
020_5,6-DHT	337.100	115.1	22.80
021_8-HETE	319.106	154.9	24.10
022_8,9-EET	319.102	154.9	26.80
023_8,9-DHT	337.100	127.1	22.30
024_9-HETE	319.079	123.1	24.61
025_11-HETE	319.055	167.0	23.62
026_12-HETE	319.107	179.1	24.00
027_11,12-EET	319.105	167.0	26.50
028_11,12-DHT	337.100	167.1	22.00
029_15-HETE	319.157	218.9	23.00
030_14,15-EET	319.095	219.2	25.60
031_14,15-DHT	337.100	207.1	21.10
032_16-HETE	319.111	233.1	22.20
033_16-HETE	319.111	189.0	22.20
034_17-HETE	319.106	247.1	21.90
035_17-HETE	319.106	203.2	21.90
036_18-HETE	319.102	261.1	21.80
037_18-HETE	319.102	217.1	21.80
038_19-HETE	319.098	275.1	21.40
039_19-HETE	319.098	231.1	21.40
040_20-HETE	319.106	289.1	21.10
041_AA+O	319.018	257.2	—
042_5,6-diHETE-1	335.234	219.0	21.40
043_5,6-diHETE-2	335.235	219.0	21.80
044_5,6-diHETE-3	335.236	219.0	22.20
045_5,6-diHETE-1	335.234	115.0	21.40
046_5,15-diHETE	335.111	201.1	18.70
047_8,15-diHETE	334.966	234.9	18.30
048_AA+2O	335.234	273.1	—
049_AA+3O	351.091	289.1	—
050_5-oxo-ETE	317.041	203.2	26.40
051_12-oxo-ETE	317.041	203.2	24.30
052_15-oxo-ETE	317.040	113.2	23.50

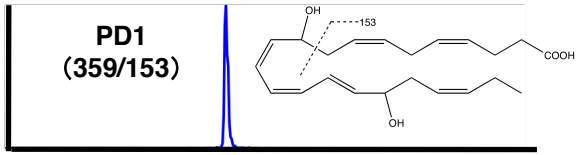
Name	Q1	Q3	RT
053_PGE3	349.013	233.2	14.10
054_PGE3	349.013	189.2	14.10
055_PGD3	349.112	188.9	14.40
056_LXA5	349.032	115.1	15.00
057_LXA5	349.010	215.0	15.00
058_LXB5	349.100	221.1	14.00
059_PGF3a	351.100	191.2	14.90
060_PGF1a-delta17-6-keto	367.100	163.0	12.60
061_TXB3	367.100	195.1	14.20
062_PGJ3-15-deoxy	313.100	269.1	21.40
063_LTD5	493.100	177.1	17.40
064_LTE5	436.100	349.1	18.10
065_5-HEPE	317.011	114.9	22.40
066_8-HEPE	316.988	155.0	21.80
067_9-HEPE	316.962	149.1	22.10
068_9-HEPE	316.962	123.1	22.10
069_11-HEPE	317.096	167.0	21.50
070_11-HEPE	317.096	195.0	21.50
071_12-HEPE	317.127	179.0	21.80
072_12-HEPE	317.052	135.0	21.80
073_15-HEPE	316.961	219.1	21.40
074_15-HEPE	316.961	175.1	21.40
075_14,15-EpETE	316.997	207.2	23.70
076_14,15-diHETE	335.258	207.1	18.00
077_18-HEPE	316.992	259.1	21.00
078_18-HEPE	317.139	215.2	21.00
079_17,18-EpETE	317.090	259.1	23.10
080_17,18-diHETE	335.258	247.2	18.70
081_17,18-diHETE	335.258	203.2	18.70
082_20-HEPE	317.046	287.0	20.72
083_EPA+O	317.044	255.2	—
084_LTB5	332.946	195.1	17.70
085_RvE2	333.108	115.1	17.09
086_6,18-diHEPE	333.100	205.1	18.10
087_8,18-diHEPE	332.962	155.0	16.70
088_8,18-diHEPE	332.962	159.1	16.70
089_9,18-diHEPE	333.100	139.1	18.20
090_11,18-diHEPE-1	333.100	167.1	16.83
091_11,18-diHEPE-2	333.100	167.1	17.11
092_11,18-diHEPE-3	333.100	167.1	18.14
093_11,18-diHEPE-1	333.098	123.0	16.83
094_11,18-diHEPE-2	333.098	123.0	17.11
095_11,18-diHEPE-3	333.098	123.0	18.14
096_12,18-diHEPE	332.940	179.1	16.96
097_12,18-diHEPE	332.940	163.1	16.96
098_15,18-diHEPE	333.100	219.1	17.70
099_17,18-diHEPE-1	333.104	201.0	18.10
100_17,18-diHEPE-2	333.105	201.0	18.60
101_17,18-diHEPE-1	333.104	245.1	18.10
102_17,18-diHEPE-2	333.105	245.1	18.60
103_17,18-diHEPE-1	333.104	213.1	18.10
104_17,18-diHEPE-2	333.105	213.1	18.60

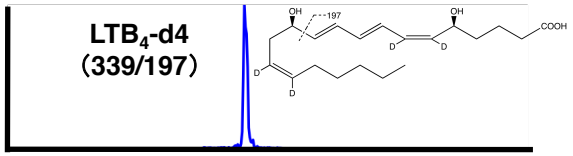
Name	Q1	Q3	RT
139_4-HDoHE	343.102	100.9	25.40
140_7-HDoHE	343.063	141.0	24.20
141_8-HDoHE	343.089	189.2	24.40
142_10-HDoHE	343.068	153.1	23.50
143_11-HDoHE	342.981	121.1	23.90
144_11-HDoHE	342.981	149.0	23.90
145_13-HDoHE	343.136	193.0	23.20
146_14-HDoHE	342.998	205.1	23.40
147_14-HDoHE	342.998	161.1	23.40
148_16-HDoHE	343.091	233.2	23.00
149_17-HDoHE	342.977	245.1	23.10
150_17-HDoHE	342.977	201.1	23.10
151_16,17-EpDPE	342.954	233.1	25.80
152_20-HDoHE	342.960	241.1	22.60
153_19,20-EpDPE	343.106	241.1	25.10
154_19,20-diHDoPE	361.044	229.2	20.00
155_21-HDoHE	343.077	255.1	22.10
156_22-HDoHE	342.960	313.1	22.50
157_DHA+O	343.094	281.2	—
158_4,14-diHDoHE	359.075	101.0	19.80
159_4,14-diHDoHE	359.107	187.1	19.80
160_7,14-diHDoHE	359.118	112.9	19.19
161_7,14-diHDoHE	359.118	177.0	19.19
162_7,17-diHDoHE	359.103	199.0	18.70
163_10,17-diHDoHE	359.107	153.0	18.60
164_4,20-diHDoHE	359.098	101.0	19.00
165_5,20-diHDoHE	359.100	113.1	19.10
166_7,20-diHDoHE	359.100	217.1	19.10
167_8,20-diHDoHE	359.100	125.2	19.20
168_10,20-diHDoHE	359.098	159.0	18.08
169_10,20-diHDoHE	359.035	239.3	18.08
170_11,20-diHDoHE	359.100	165.1	19.10
171_13,20-diHDoHE-1	358.913	193.1	17.90
172_13,20-diHDoHE-2	358.913	193.1	18.18
173_13,20-diHDoHE-3	358.913	193.1	18.51
174_14,20-diHDoHE	359.035	233.1	18.27
175_14,20-diHDoHE	359.032	205.0	18.27
176_19,20-diHDoHE-1	358.983	227.2	19.51
177_19,20-diHDoHE-2	358.983	227.2	19.97
178_20,22-diHDoHE	359.100	241.1	19.00
179_14,21-diHDoHE	359.047	253.1	17.60
180_4hy-19,20-EpDPE	359.080	100.9	20.27
181_5hy-19,20-EpDPE	359.080	245.1	20.27
182_7hy-19,20-EpDPE	359.091	141.0	19.60
183_8hy-19,20-EpDPE	359.128	125.2	19.66
184_10hy-19,20-EpDPE	359.109	153.0	19.36
185_11hy-19,20-EpDPE	359.100	165.2	19.42
186_11hy-19,20-EpDPE	359.100	193.0	19.42
187_13hy-19,20-EpDPE	359.092	193.0	19.34
188_14hy-19,20-EpDPE	359.104	205.0	19.42
189_14hy-19,20-EpDPE	359.104	161.2	19.42
190_16hy-19,20-EpDPE	359.108	233.2	18.38





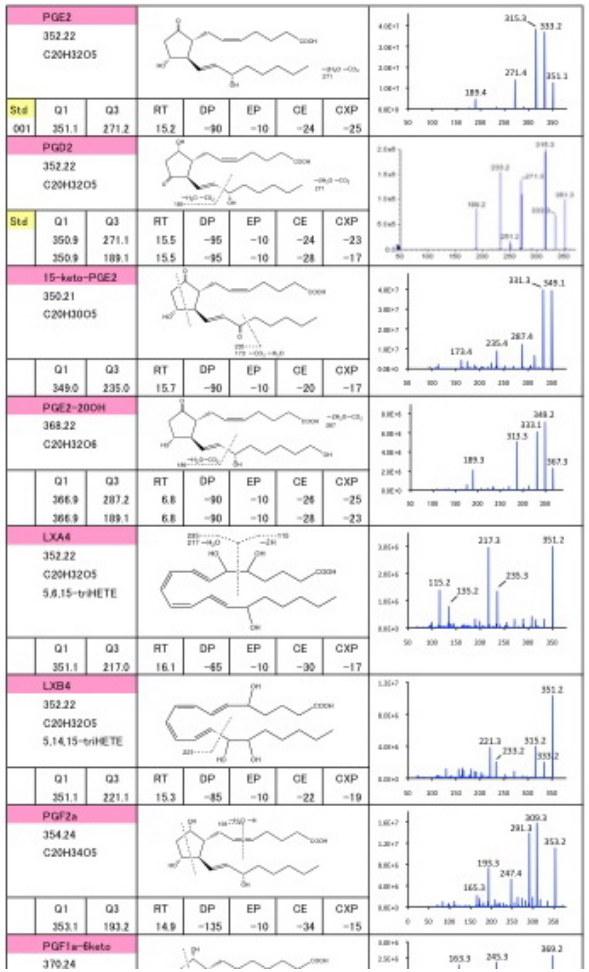




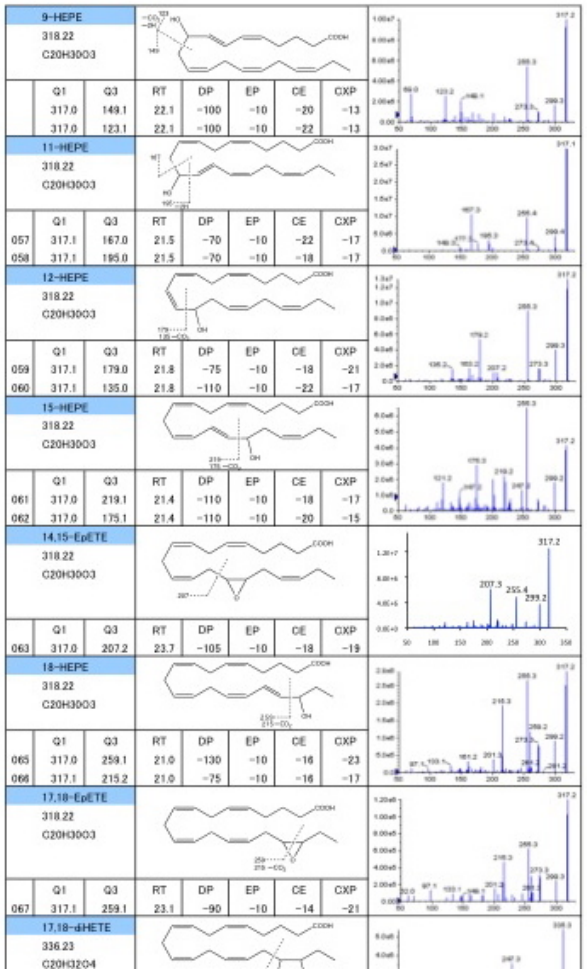


LC-MS/MS data and MRM conditions of PUFA metabolites

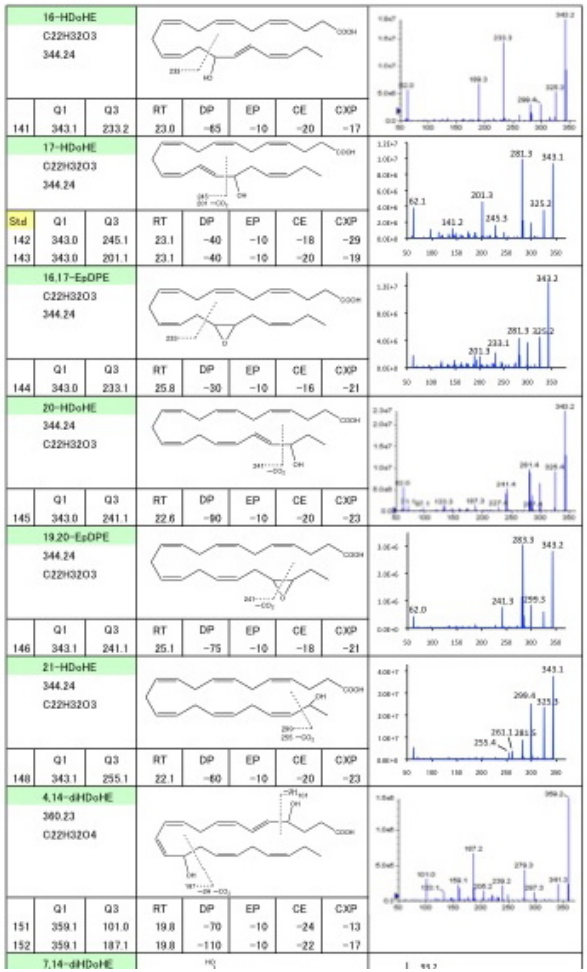
ArA-derived



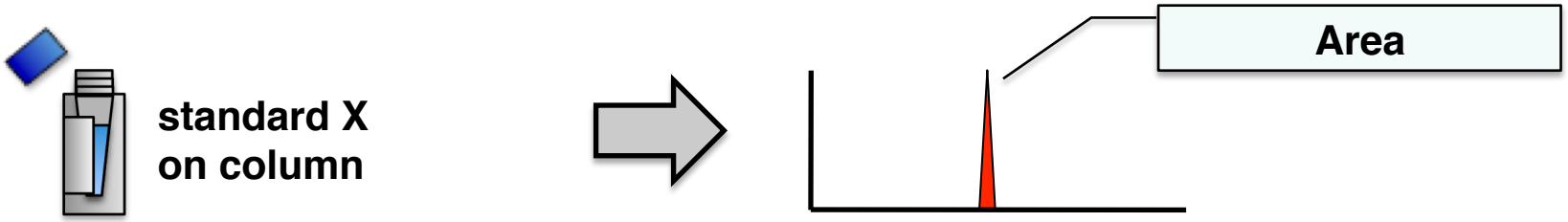
EPA-derived

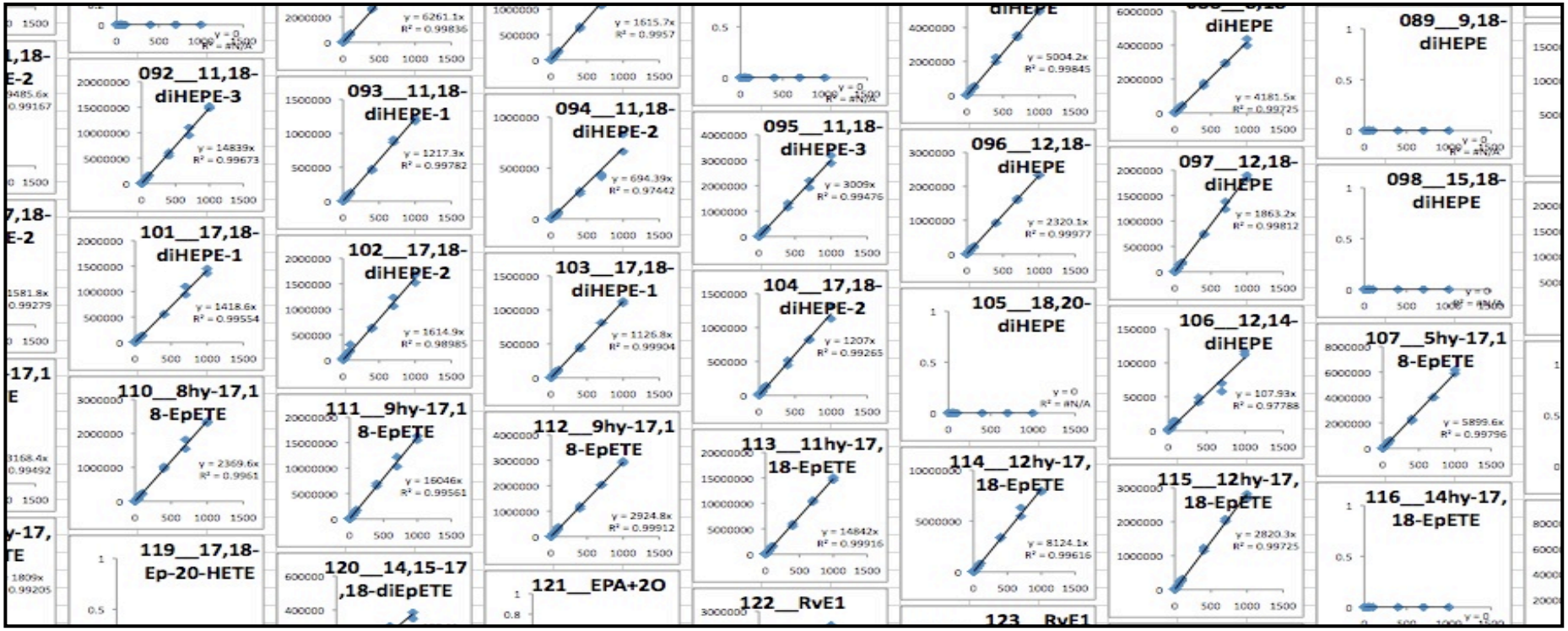


DHA-derived



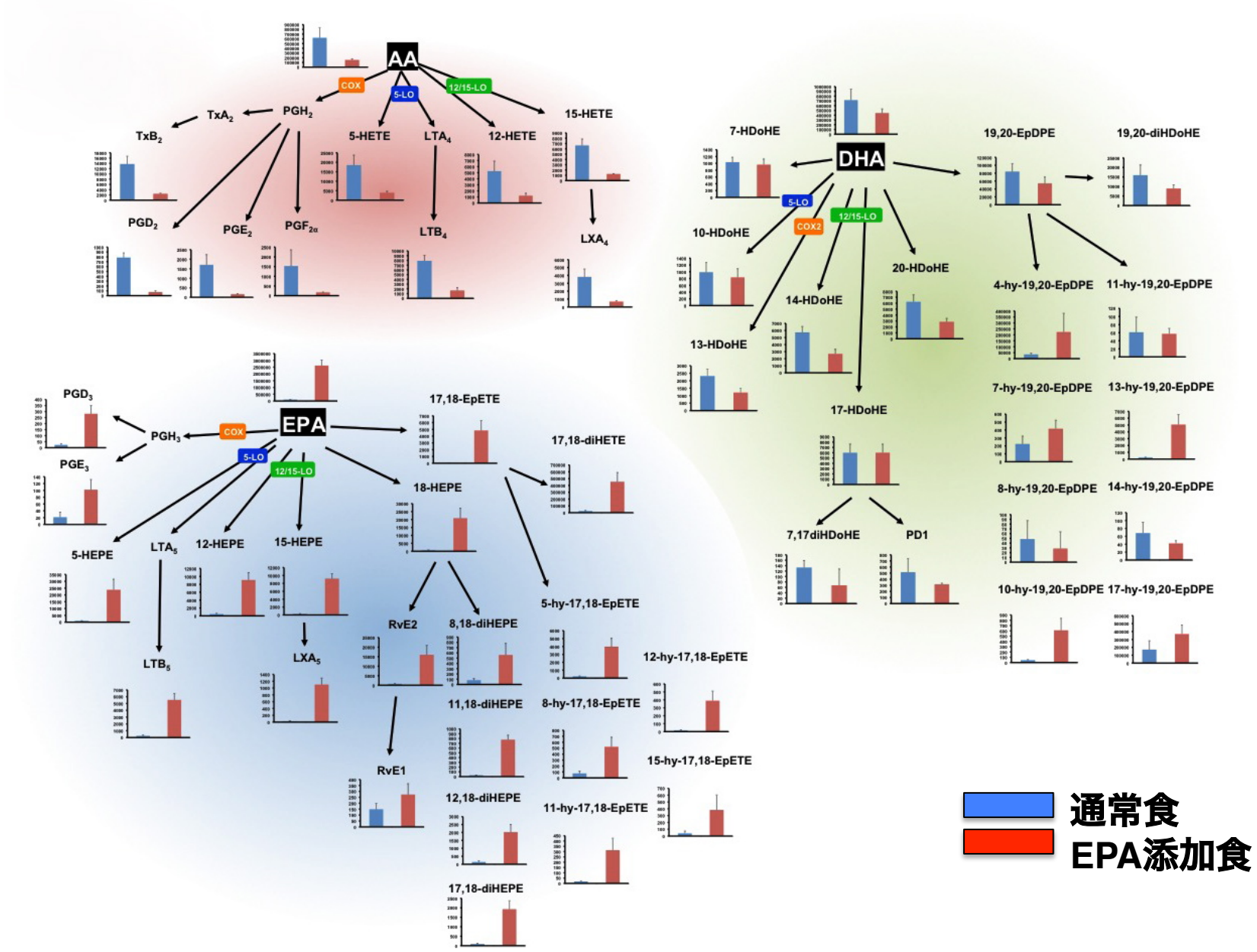
Linear standard curves for the quantitative analyses





Linear range: 1pg～1000pg on column

脂質メタボローム解析の具体例

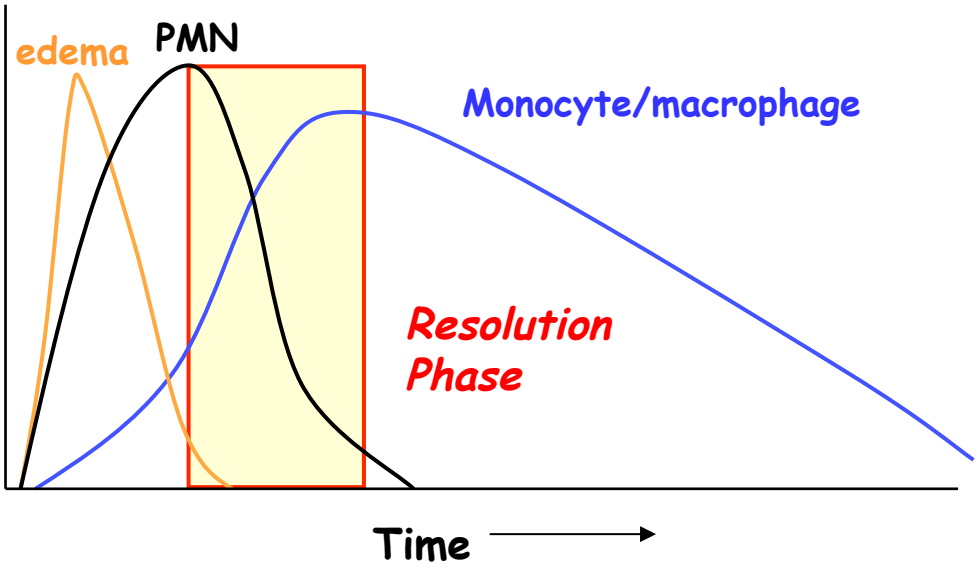


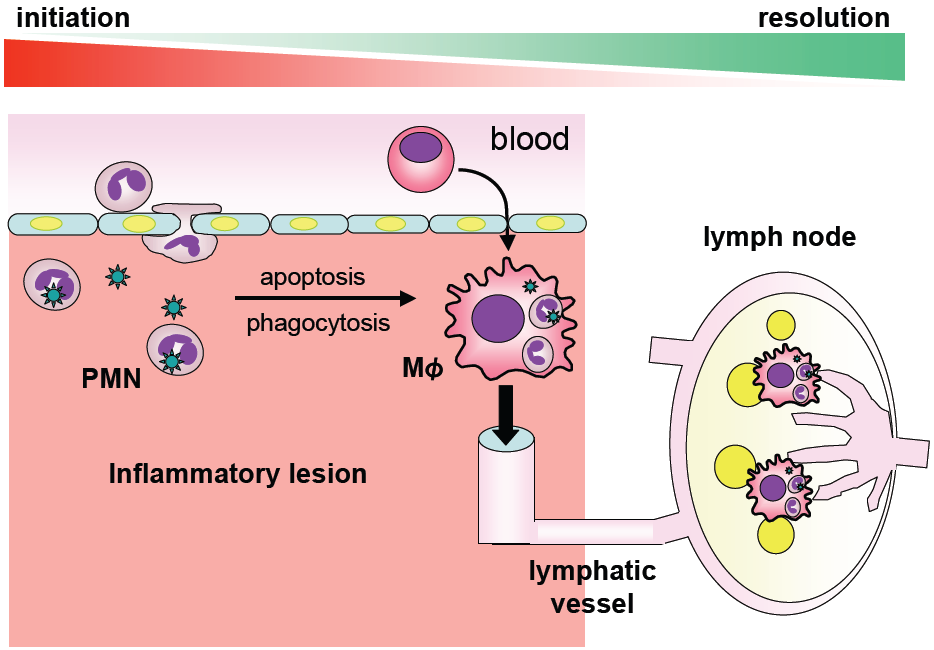
メタボロミクスを用いた

1．炎症の収束機構の解析

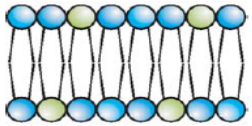
2．n-3系脂肪酸由来の抗炎症性代謝物の新規探索

炎症反応の開始(initiation)と収束(resolution)



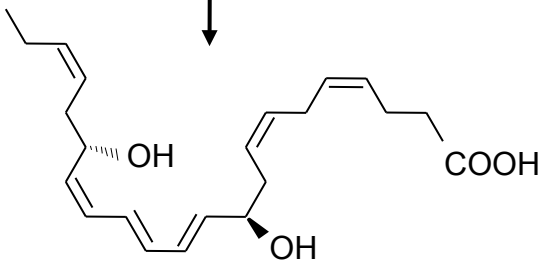


DHA由来の抗炎症性メディエーター：プロテクチンD1（PD1）



ドコサヘキサエン酸
（DHA; C22:6 n-3）

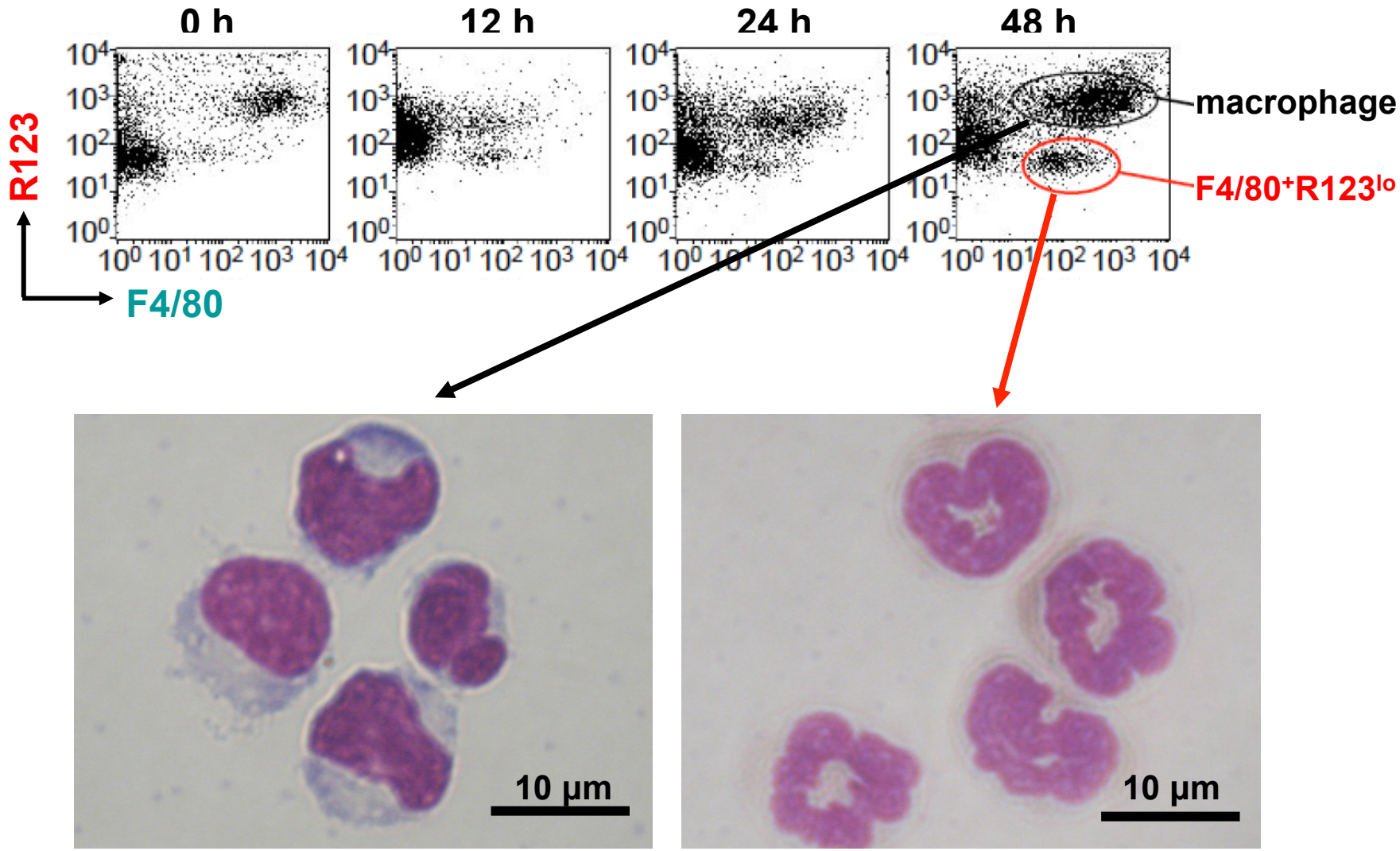
12/15-LOX



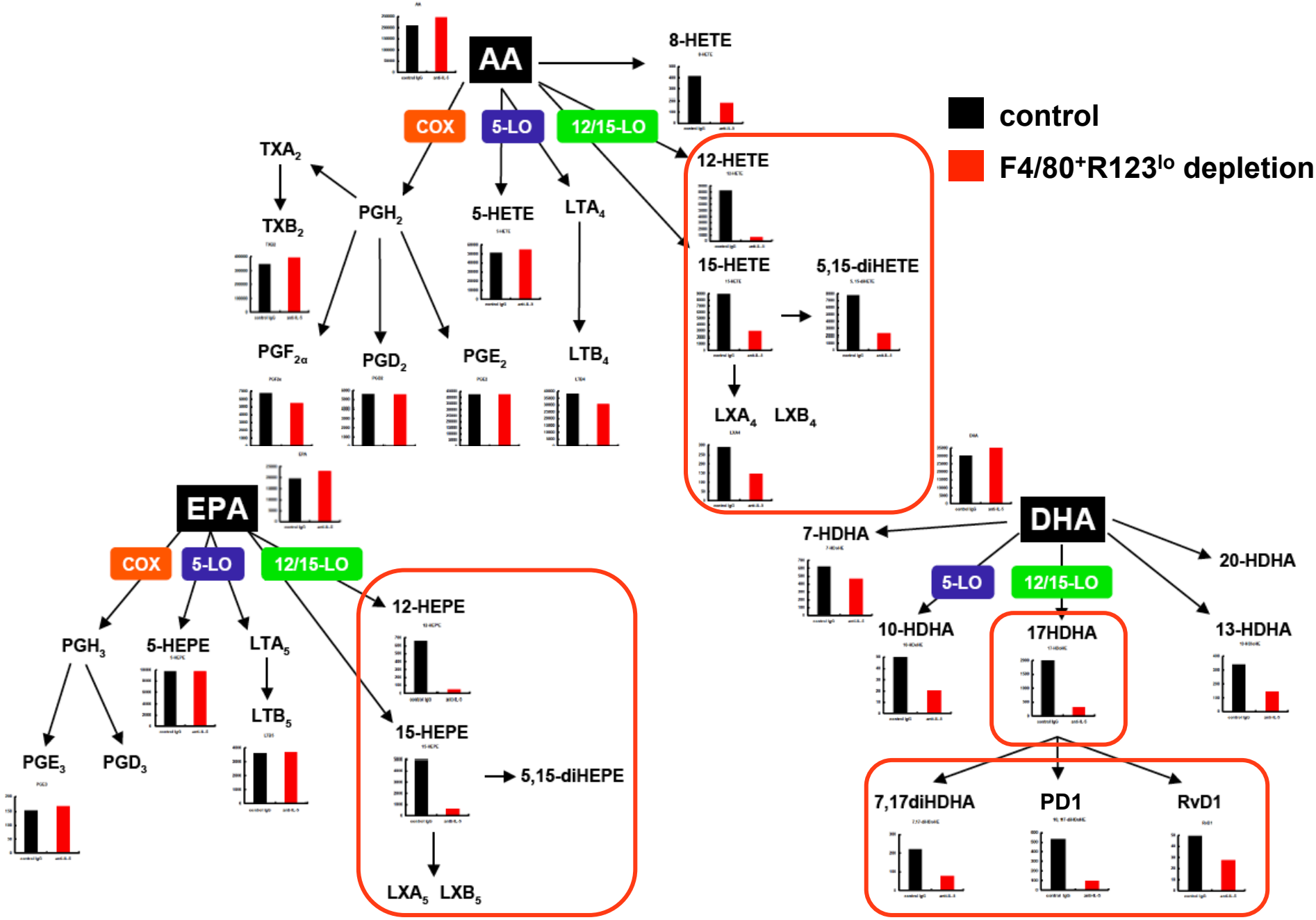
Protectin D1
(10,17*S*-diHDHA)

- 急性炎症（好中球浸潤）の抑制
- マクロファージの貪食能促進
- 神経細胞死の抑制
- 上皮組織（角膜）の修復促進
- インスリン抵抗性の改善

炎症収束期に出現するF4/80^{+}R123^{lo}細胞

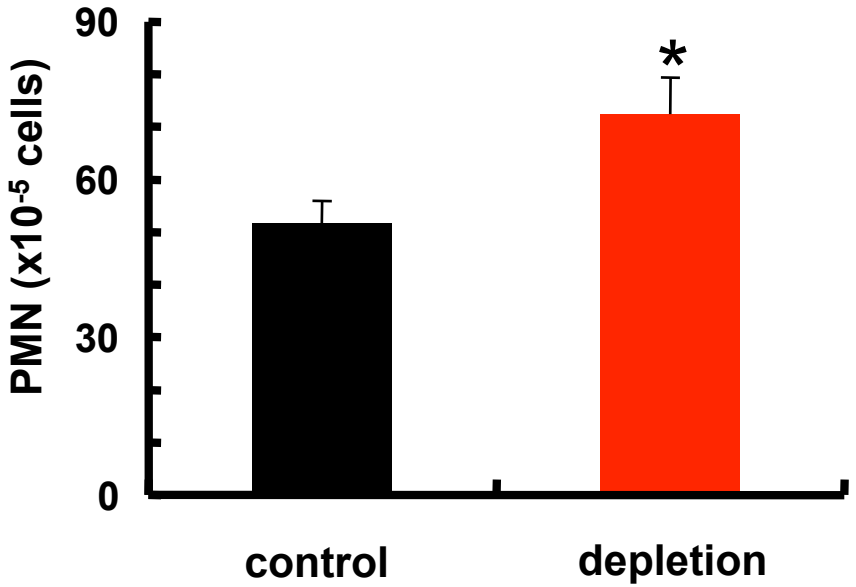


F4/80^{+}R123^{lo} depletionによる脂肪酸代謝物の変動



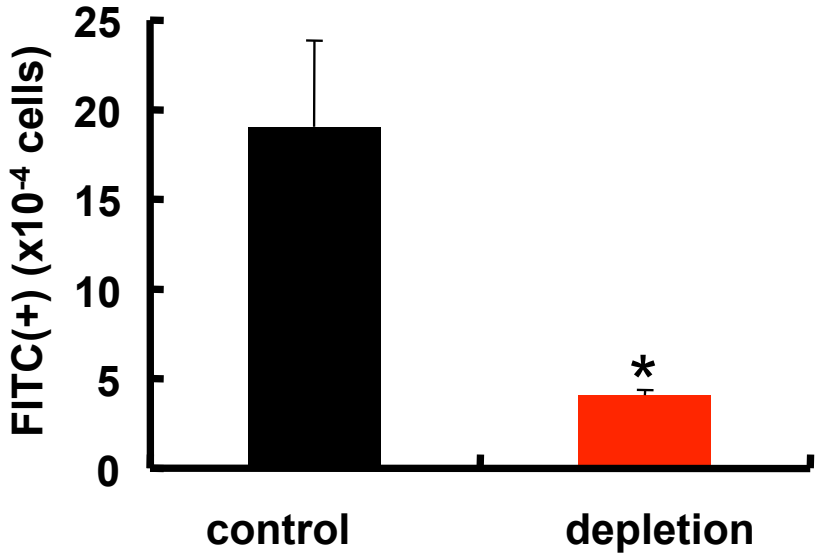
F4/80^{+}R123^{lo} depletionにより炎症収束が遅延する

PMN numbers in exudates



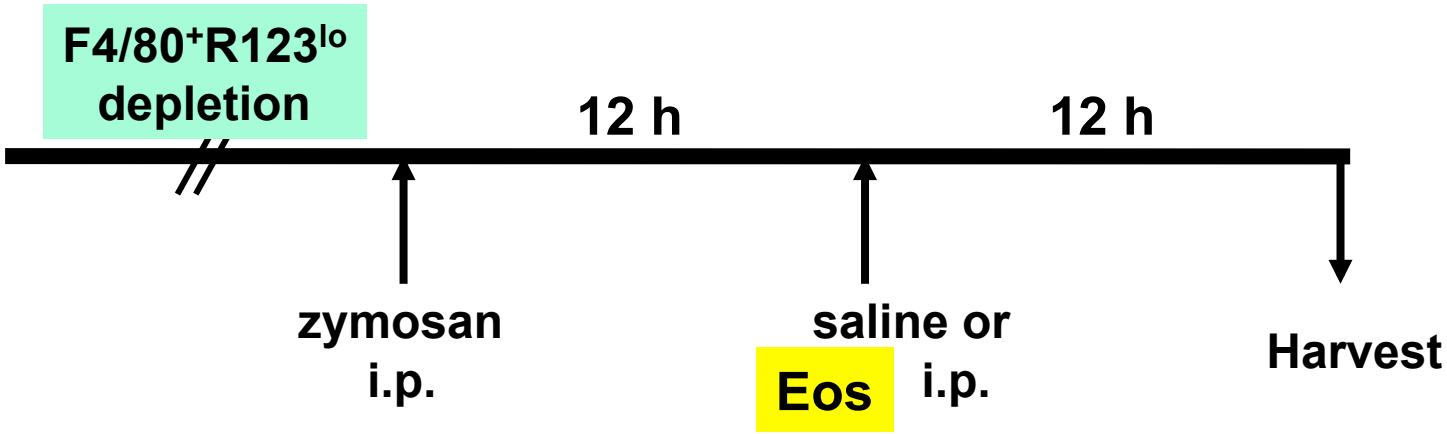
*p < 0.05, n = 4-6, mean ± s.e.m

Leukocytes carrying zymosan in parathymic lymph node



*p < 0.05 n = 5-6, mean ± s.e.m

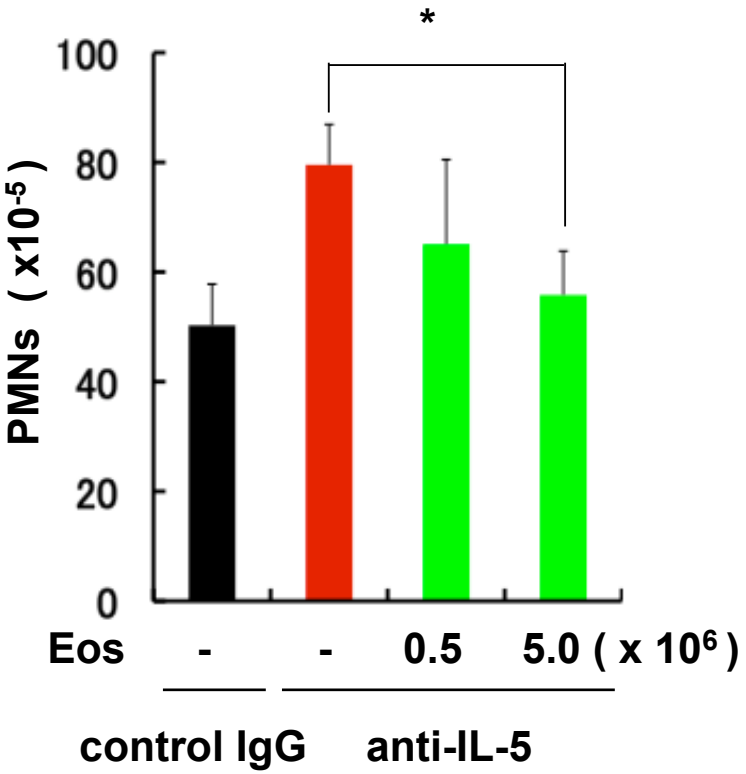
F4/80^{+}R123^{lo} depletionによる炎症収束の遅延は好酸球を外から補うことで再び回復するか？



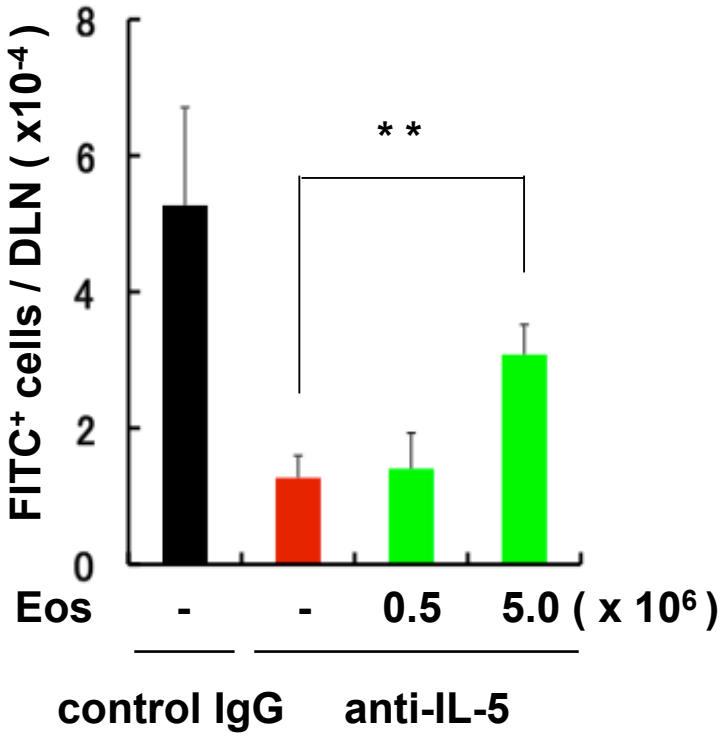
Eos（好酸球）：IL-5トランスジェニックマウスより単離

F4/80^{+}R123^{lo} depletionによる炎症収束の遅延は好酸球を外から補なうことで回復する

PMN numbers in exudates

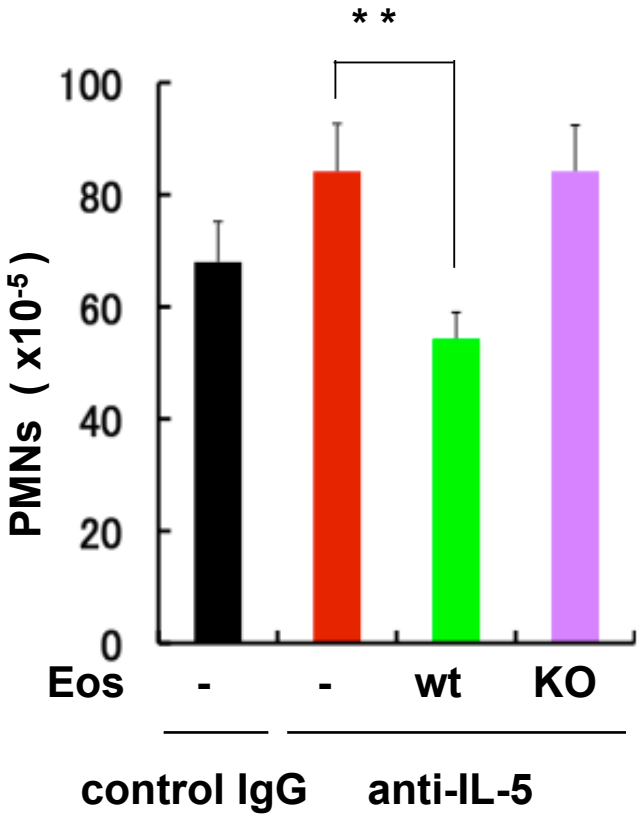


Leukocytes carrying zymosan in parathymic lymph node

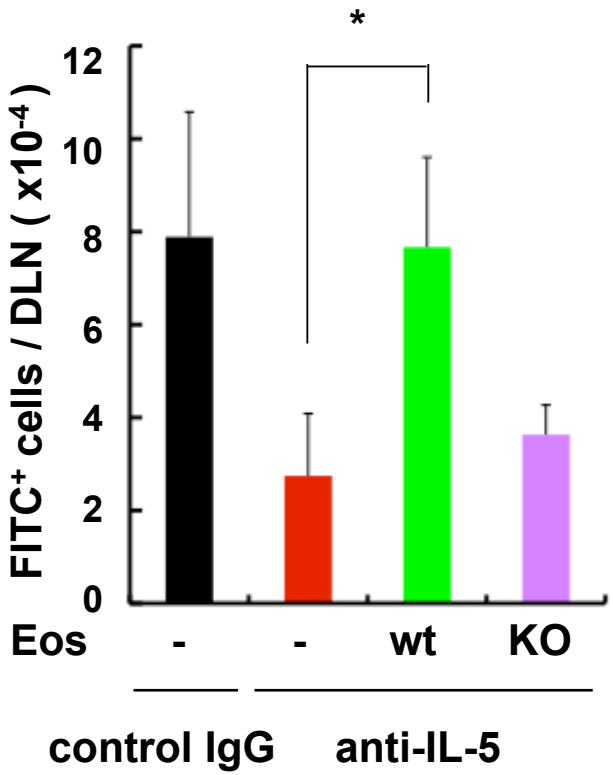


好酸球の炎症収束を促進する機能は 12/15-LOXを欠損した好酸球では認められない

PMN numbers in exudates



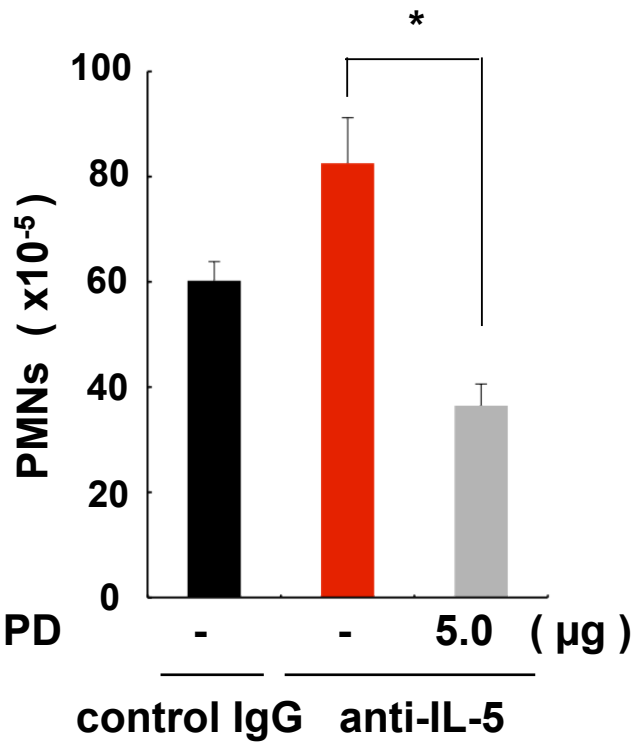
Leukocytes carrying zymosan in parathymic lymph node



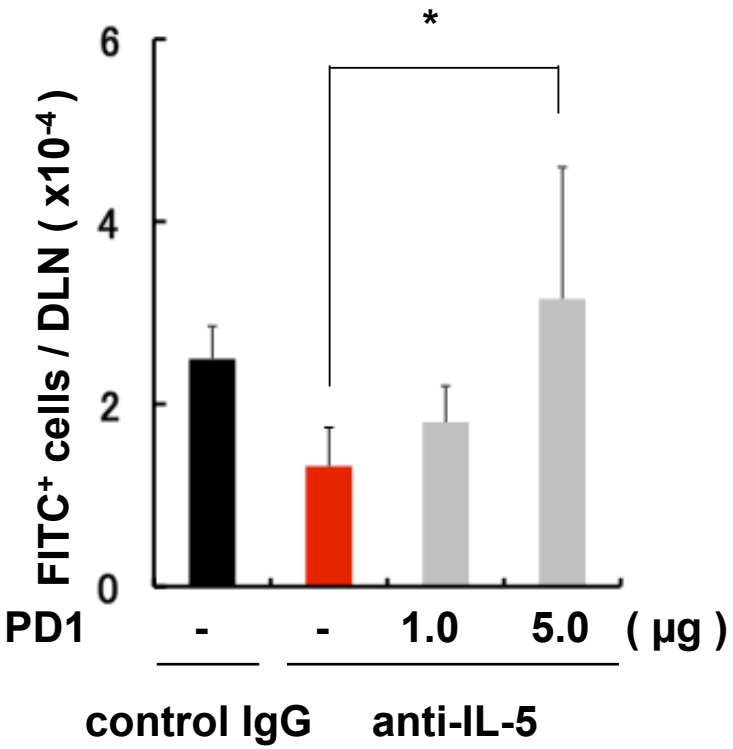
wt: wild type eosinophils
KO: 12/15-LOX-deficient eosinophils

F4/80^{+}R123^{lo} depletionによる炎症収束の遅延は 12/15-LOX産物Protectin D1の投与で回復する

PMN numbers in exudates

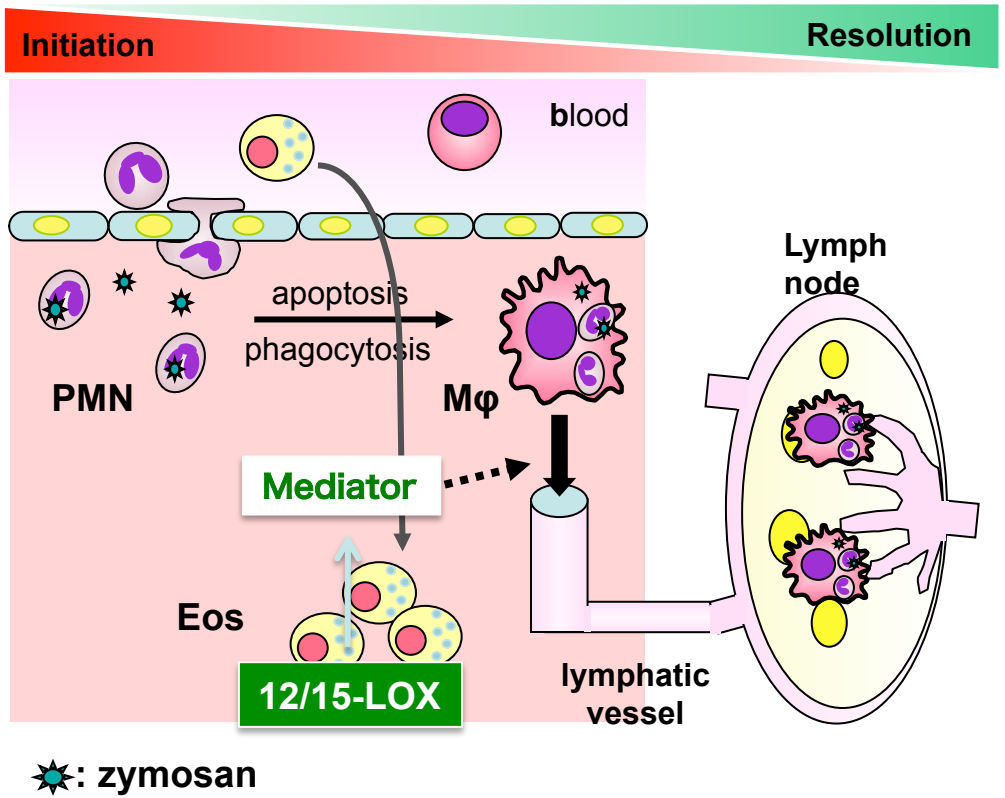


Leukocytes carrying zymosan in parathymic lymph node



PD1: Protectin D1 *i.p.* injection

炎症収束における好酸球の役割



FASEB J. 25、561-568 (2011)