

1 **Dexamethasone induces ω 3-derived immunoresolvents driving**
2 **resolution of allergic airway inflammation**

3
4 Katerina Pyrillou, PhD,^{a,b} Aikaterini-Dimitra Chairakaki, PhD,^a Constantin
5 Tamvakopoulos, PhD,^{b,†} and Evangelos Andreakos, PhD^{a,†}

6
7 ^aLaboratory of Immunobiology, Center for Clinical, Experimental Surgery and
8 Translational Research, Biomedical Research Foundation of the Academy of Athens,
9 11527 Athens, Greece

10 ^bDivision of Pharmacology-Pharmacotechnology, Center for Clinical, Experimental
11 Surgery and Translational Research, Biomedical Research Foundation of the Academy
12 of Athens, 11527 Athens, Greece

13
14 [†]Evangelos Andreakos and Constantin Tamvakopoulos contributed equally to this
15 work

16 **Reprints and correspondence:**

17 Evangelos Andreakos
18 Biomedical Research Foundation of the Academy of Athens
19 Soranou Efesiou 4, Athens, Greece
20 Tel: +30 210 6596 338

21 vandreakos@bioacademy.gr

23 Or
24 Constantin Tamvakopoulos
25 Soranou Efessiou Street, 4
26 11527 Athens, Greece
27 Tel. +30 210 6597475
28 ctamvakop@bioacademy.gr

29
30

31 **Funding Support:** This work was supported by core funding from the Hellenic Ministry
32 of Education, Research and Religious Affairs and the Hellenic Ministry of Health, and
33 by the research grants PREDICTA (Contract No 260895) from the European
34 Commission, MIDAS (Contract No MIS 377047) and RESOLVE-ASTHMA (Contract No
35 2601) from the Hellenic Ministry of Education, Research and Religious Affairs.

36

37 **Capsule Summary**

38 Dexamethasone induces the production of DHA-derived specialized proresolving lipids
39 and the resolution of allergic airway inflammation. This adds glucocorticosteroids to
40 the short list of drugs that are known to possess direct pro-resolving functions.

41

42 *To the Editor*

43 Resolution of inflammation has long been considered to be a passive process
44 that ensues in an inflammatory response following the dilution of pro-inflammatory
45 cues. However, this paradigm is shifting as it is becoming increasingly evident that
46 resolution is an active reaction that employs a complex molecular machinery that
47 orderly terminates inflammation. Among the multiple mechanisms involved,
48 specialized proresolving lipid mediators (SPMs) such as resolvins, protectins and
49 lipoxins have taken center stage¹. These are derived from ω 3 and ω 6 polyunsaturated
50 fatty acids through complex intra- and trans-cellular biosynthetic pathways, and
51 promote resolution of inflammation by their manifold and concerted functions
52 inhibiting leukocyte trafficking, dampening pro-inflammatory signaling, inducing
53 apoptosis, and promoting anti-inflammatory M2-like macrophage phenotypes
54 efferocytosis and tissue restitution¹.

55 Impaired induction of SPMs has been associated with defective resolution of
56 inflammation and chronic diseases including asthma. Thus, reduced Lipoxin A4 (LXA4)
57 levels have been detected in blood, bronchoalveolar lavage fluid (BAL), sputum and
58 exhaled breath condensates of severe asthma patients, correlating with disease
59 severity²⁻⁴. Moreover, impaired protectin D1 (PD1) production has been described in
60 asthma patients compared to healthy individuals^{5,6}. These observations are
61 functionally relevant since administration of synthetic forms of SPMs in animal models
62 of asthma have been notably effective in resolving inflammation⁷. Still, little is known
63 about the effect of known drugs in the production of proresolving lipids, hampering
64 the exploitation of this emerging new knowledge for therapy.

65 Here, we have investigated the role of dexamethasone (DXM), a ~~prototype~~
66 prototypical glucocorticosteroid, in the generation of SPMs and the resolution of
67 inflammation. We employed an established model of allergic airway disease based on
68 ovalbumin (OVA) sensitization and challenge (**Fig E1, A**) which triggers a Th2-driven
69 inflammatory response in the airways peaking at day 1 post-challenge and gradually
70 resolving thereafter (**Fig E1, B-D**)⁷. This is accompanied by the production of DHA-
71 derived protectins such as PD1 and PDX, and their intermediate metabolite 17-HDHA,
72 in the lung which starts at day 1 and peaks at day 4 post-challenge (**Fig E1, E**), in line
73 with their proresolving activity. By contrast, vehicle control (PBS) challenge does not
74 trigger any SPMs (**Fig E1, E**). Prostaglandins PGD2 and PGE2 which are required for the
75 activation of SPM pathways⁸ are also induced, while the EPA-derived metabolite RvE1
76 is not detectable (data not shown). The induction of SPMs is topical as none of these
77 mediators is up-regulated in serum (**Fig E2**).

78 Using this setting, we administered DXM therapeutically, at days 1, 2 and 3
79 post-challenge, and assessed its effects (**Fig 1, A**). Surprisingly, we observed rapid and
80 robust activation of the DHA•17-HDHA pathway within 6h of DXM administration (day
81 1 post-challenge), resulting in over ~~103~~-fold higher concentrations of PD1 and PDX
82 compared to naïve animals whereas vehicle (PBS)-treated controls exhibited no
83 difference (**Fig 1, B**). This may even be an underestimation as in naïve and most
84 vehicle-control treated animals PD1 and PDX are completely undetectable but an
85 arbitrary value of half the limit of quantification was still used for fold calculations.
86 RvE1 and LXA4 were not detected while PGD2 and PGE2, although present, were not
87 differentially altered (data not shown). This early boost in SPM production was not
88 anymore detectable 24h or 72h after DXM administration (day 2 or 4 post-challenge),

89 as levels in vehicle-control treated animals were also increased, while at 144h (day 7
90 post-challenge) higher, yet not significant, levels of PD1 and PDX were observed in the
91 control group (**Fig 1, B**). These findings demonstrate that DXM acutely induces the
92 mobilization of the DHA•17-HDHA•PD1/PDX pathway during allergic airway disease
93 in mice.

94 We then examined whether the ability of DXM to up-regulate PD1 and PDX
95 could also modulate resolution of allergic airway disease. DXM was unable to generate
96 a notable anti-inflammatory or proresolving effect within 6h of treatment, a very short
97 time interval (**Fig 1, C-D**). However, at 24h (day 2 post-challenge) and onwards DXM
98 reduced total leukocyte and eosinophil numbers in the BAL more rapidly than vehicle
99 control, and decreased OVA-specific IL-5 and IL-13 levels in mediastinal lymph nodes
100 (MLNs). Accordingly, DXM shortened the resolution interval (R_i), the time interval
101 from the peak of inflammation at day 1 post-challenge to the 50% reduction point, by
102 2 days, a very significant effect for this model (**Fig 1, E-F**). To confirm that DXM
103 accelerates the resolution of inflammation through the induction of DHA-derived
104 SPMs, rather than other effects on the immune system, we employed 12,15-
105 lipoxygenase deficient (*Alox15^{-/-}*) mice which cannot metabolize DHA to downstream
106 bioactive mediators such as protectins (**Fig 2, A-B**). Abrogation of PD1 and PDX
107 generation completely reversed DXM-mediated resolution of allergic airway
108 inflammation including total leukocyte and eosinophil infiltration in the BAL, and Th2
109 cytokine production in MLNs (**Fig 2, C-D and E3, A-B**). Although this does not exclude
110 the involvement of other bioactive lipids requiring 12,15-lipoxygenase for their
111 generation, the potent proresolving activity of synthetic PD1 administration in similar

112 settings^{5, 7} indicates that at least PD1 and its functionally similar isomer PDX are
113 principally involved.

114 In conclusion, our study describes a novel function of DXM, a ~~prototype~~
115 prototypical glucocorticosteroid, in the activation of SPM networks. It reveals that
116 DXM enhances the ~~catabolism~~-metabolism of DHA to its bioactive derivatives PD1 and
117 PDX. This effect is rapid, inducing at 103-fold higher levels than ~~background~~-vehicle
118 within 6h of treatment, providing an early boost to the process of resolution, markedly
119 reducing both the duration and magnitude of inflammation. This effect is also
120 transient as no differences between DXM and vehicle-treated animals were observed
121 later on, possibly reflecting the induction of counter-regulatory pathways suppressing
122 SPM networks, and lack of need for additional SPMs in an effectively resolving
123 situation. Notably, the effect of DXM is specific for the D-series SPMs since there was
124 no observed mobilization of other proresolving pathways such as lipoxin A4 or E-series
125 resolvins nor PGD2 and PGE2.

126 At present, very few pharmaceutical substances have a documented ability to
127 activate SPM networks. Aspirin and statins provoke conformational changes on
128 cyclooxygenases altering their enzymatic activity and leading to the production of
129 epimeric forms of lipoxins, resolvins and protectins, depending on the substrate⁹.
130 Resiquimod, a TLR7/8 agonist, induces in turn DHA-derived SPMs in human
131 macrophages and experimental settings of airway disease⁷. Synthetic forms of SPMs
132 such as RvE1 and LXA4 are also being explored although their short half-life and
133 chemical stability constitute major hurdles towards their clinical application. Our
134 study therefore adds DXM as a new agonist of resolution, expanding the short list of
135 clinically used drugs that are able to actively induce SPM pathways. It also provides

136 novel insight into the mechanism of action of DXM, a well-known and widely-used
137 drug, with important implications for glucocorticosteroid use in the clinic.

138

139 We acknowledge support of Ms M. I. Saridaki for technical assistance and Prof. Nikos
140 Papadopoulos and Charles Serhan for advice.

141

142 Katerina Pyrillou, PhD,^{a,b} Aikaterini-Dimitra Chairakaki, PhD,^a Constantin
143 Tamvakopoulos, PhD,^{b,†} and Evangelos Andreakos, PhD^{a,†}

144

145 From ^aLaboratory of Immunobiology, Center for Clinical, Experimental Surgery and
146 Translational Research, Biomedical Research Foundation of the Academy of Athens,
147 11527 Athens, Greece; ^bDivision of Pharmacology-Pharmacotechnology, Center for
148 Clinical, Experimental Surgery and Translational Research, Biomedical Research
149 Foundation of the Academy of Athens, 11527 Athens, Greece. E.mail:

150 vandreakos@bioacademy.gr

151

152 [†]Evangelos Andreakos and Constantin Tamvakopoulos contributed equally to this
153 work

154

155 **Author contributions:** KP, CT and EA conceived and designed the experiments; KP and
156 ADC performed the experiments; KP, ADC, CT and EA analyzed and interpreted the
157 data; KP and EA wrote the paper.

158

159 **Funding Support:** This work was supported by core funding from the Hellenic Ministry
160 of Education, Research and Religious Affairs and the Hellenic Ministry of Health, and
161 by the research grants PREDICTA (Contract No 260895) from the European
162 Commission, MIDAS (Contract No MIS 377047) and RESOLVE-ASTHMA (Contract No
163 2601) from the Hellenic Ministry of Education, Research and Religious Affairs.

164

165 **Disclosure of potential conflict of interest:** The authors declare no conflict of interest.

166

167

168 **References**

- 169 1. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology.
170 Nature 2014; 510:92-101.
- 171 2. Levy BD, Bonnans C, Silverman ES, Palmer LJ, Marigowda G, Israel E, et al.
172 Diminished lipoxin biosynthesis in severe asthma. Am J Respir Crit Care Med
173 2005; 172:824-30.
- 174 3. Kazani S, Planaguma A, Ono E, Bonini M, Zahid M, Marigowda G, et al. Exhaled
175 breath condensate eicosanoid levels associate with asthma and its severity. J
176 Allergy Clin Immunol 2013; 132:547-53.
- 177 4. Vachier I, Bonnans C, Chavis C, Farce M, Godard P, Bousquet J, et al. Severe
178 asthma is associated with a loss of LXA4, an endogenous anti-inflammatory
179 compound. J Allergy Clin Immunol 2005; 115:55-60.

- 180 5. Levy BD, Kohli P, Gotlinger K, Haworth O, Hong S, Kazani S, et al. Protectin D1
181 is generated in asthma and dampens airway inflammation and
182 hyperresponsiveness. *J Immunol* 2007; 178:496-502.
- 183 6. Miyata J, Fukunaga K, Iwamoto R, Isobe Y, Niimi K, Takamiya R, et al.
184 Dysregulated synthesis of protectin D1 in eosinophils from patients with
185 severe asthma. *J Allergy Clin Immunol* 2013; 131:353-60 e1-2.
- 186 7. Koltsida O, Karamnov S, Pyrillou K, Vickery T, Chairakaki AD, Tamvakopoulos C,
187 et al. Toll-like receptor 7 stimulates production of specialized pro-resolving
188 lipid mediators and promotes resolution of airway inflammation. *EMBO Mol*
189 *Med* 2013; 5:762-75.
- 190 8. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class
191 switching during acute inflammation: signals in resolution. *Nat Immunol* 2001;
192 2:612-9.
- 193 9. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute
194 inflammation: impact of aspirin and statins. *Circ Res* 2010; 107:1170-84.

195

196

197 **Keywords**

198 Allergic asthma, allergic airway disease, anti-inflammation, resolution of
199 inflammation, glucocorticosteroids, dexamethasone, protectins, Alox15

200

201 **Abbreviations**

202 SPMs = specialized pro-resolving lipid mediators, DHA = docosahexaenoic acid, PUFAs
203 = polyunsaturated fatty acids, PD1 = protectin D1, PDX = protectin DX, LM = lipid
204 mediators, LXA4 = lipoxin A4, LTB4 = leukotriene B4, BAL = bronchoalveolar lavage
205 fluid, LOX = lipoxygenases, COX = cyclooxygenases, NSAIDs = non-steroidal anti-
206 inflammatory drugs, PGs = prostaglandins, LTs = leukotrienes, GCs =
207 glucocorticosteroids, GR = glucocorticoid receptor, DXM = dexamethasone, OVA =
208 ovalbumin, EXC4 = eoxin C4, RvE1 = resolvin E1, RvD1 = resolvin D1, 17-HDHA = 17-
209 hydroxy-docosahexaenoic acid, 7-HDHA = 7-hydroxy-docosahexaenoic acid, EPA =
210 eicosapentaenoic acid, LC-MS/MS = liquid chromatography tandem mass
211 spectrometry, R_i = resolution interval, IL = interleukin

212 **Figure Legends**

213 **Figure 1.** Effect of DXM on the biosynthesis of DHA-derived proresolving lipid
214 mediators and the resolution of allergic airway inflammation. (A) Schematic of the
215 experimental protocol. DXM treatment is depicted in red. (B) Tissue lipidomic profiling
216 over a time-course of 7 days post-challenge showing early activation of the DHA-17-
217 HDHA-PD1/PDX pathway triggered by DXM. Data are expressed as fold induction
218 relative to naïve non-inflamed mice. (C) Total leukocyte, eosinophil and lymphocyte
219 numbers in BAL. (D) T_H2 cytokine responses in mediastinal lymph nodes. (E) Resolution
220 curves for total leukocyte and eosinophil numbers in BAL. (F) Calculated resolution
221 intervals (R_i) based on total leukocyte and eosinophil resolution curves. Data
222 represent mean±SEM of n=9-23 mice/group pooled from at least three independent
223 experiments. In fold calculations half the limit of quantification (LOQ) was used for
224 samples beyond detection. ***P<0.001, **P<0.01 and *P<0.05 compared to PBS-
225 treated controls.

226

227 **Figure 2.** Dependence of DXM pro-resolving capacity on the activation of the DHA-17-
228 HDHA-PD1/PDX pathway. (A) Schematic of the experimental protocol employing wild
229 type (WT) and Alox15-deficient (*Alox15*^{-/-}) mice depicted in red. Data are expressed as
230 fold induction relative to naïve WT mice. (B) Tissue lipidomics analysis ~~in~~ of non-
231 treated *Alox15*^{-/-} mice compared to WT mice. (C) Effect of DXM administration on total
232 leukocyte, eosinophil and lymphocyte numbers in BAL at day 4 post-challenge. (D)
233 Effect of DXM administration on T_H2 cell responses at day 4 post-challenge. Data
234 represent mean±SEM of n=6-23 mice/group pooled from at least three independent

235 experiments. ~~BLQ: Below the Limit of Quantification.~~ *** $P < 0.001$, ** $P < 0.01$ and
236 * $P < 0.05$ compared to WT mice.