IL-17 induced inflammation modulates mPGES-1/PPAR-γ pathways in monocytes/macrophages

Running title: IL-17A and mPGES-1/PPAR-γ pathway

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49**ABSTRACT**

50**Background and Purpose:** Recent biochemical and pharmacological studies have reported that in
51several tissues and cell types, microsomal prostaglandin E_2 synthase (mPGES) and peroxisome
52proliferator-activated receptor-γ (PPAR-γ) expression are modulated by a variety of inflammatory
53factors and stimuli Considering that very little is known about the biological effects promoted by
54IL-17 in the context of mPGES-1/PPAR-γ modulation, we sought to investigate the contribution of
55this unique cytokine on these integrated pathways during the onset of inflammation.

56**Experimental Approach:** We evaluated PF 9184 (mPGES-1 antagonist) and Troglitazone (PPAR-
57γ agonist) activity utilising integrated *in vitro* and *in vivo* approaches. The dorsal air pouch model
58was employed, and inflammatory infiltrates were analysed by flow cytometry. Locally produced
59cyto-chemokines and prostaglandins were assessed using ELISA assays. Western blots were also
60employed to determine the activity of various enzymes involved in downstream signalling
61pathways.

62**Key Results:** PF 9184 and Troglitazone, in a time and dose-dependent manner, were shown to
63significantly modulate leukocyte infiltration, myeloperoxidase activity, and the expression of COX-
642/mPGES-1, NF-κB/IκB-α and mPGDS-1/PPAR-γ induced by IL-17. Moreover, both compounds
65were found to modulate prostaglandins (PGE_2, PGD_2, and PGJ_2) production, the expression of
66different pro-inflammatory cyto-chemokines and the recruitment of inflammatory monocytes in
67response to IL-17.

68**Conclusions and Implications:** Collectively, the data presented suggests that IL-17 may constitute
69a specific modulator of inflammatory monocytes during later phases of the inflammatory response.
70Therefore, the results of this study show, for the first time, that IL-17/mPGES-1/PPAR-γ "axis"
71could represent a potential therapeutic target for inflammatory-based and immune-mediated
72diseases.

73**Keywords:** IL-17A, Inflammation, mPGES-1, PGE_2, PPAR-γ.

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94**1. INTRODUCTION**

95Inflammation is a complex defence mechanism characterised by leukocyte extravasation from the 96vasculature to local tissue damage resulting from injurious and noxious agents/stimuli (Serhan, 972014). Neutrophils dominate the initial influx of leukocytes followed by monocytes and 98macrophages. The recruitment of inflammatory monocytes is correlated with a transient increase of 99pro-inflammatory mediators including cytokines, chemokines, prostaglandins (PGs), and 100leukotrienes (LTs) (D'Acquisto et al., 2010; Perretti et al., 2017). Indeed, inappropriate 101monocyte/macrophage survival or overactivation perpetuate inflammatory pathways and strengthen 102disease activity/duration (Zhou et al., 2009). Therefore, regulating the function of 103monocytes/macrophages during inflammation is critical to promote resolution and healing. PGs and 104LTs are potent bioactive lipid mediators involved not only in the onset of inflammation but also in 105numerous homeostatic functions (Funk, 2001). Their biosynthesis is initialized by cyclooxygenases 106(COXs) isoenzymes (COX-1 and COX-2) that convert arachidonic acid to PGH$_2$ (Hawkey, 1999) 107and subsequently isomerized, by three different PGE$_2$ synthases (situated downstream of COXs in 108the prostaglandin synthesis pathway), to PGE$_2$ (Koeberle et al., 2015). The cytosolic prostaglandin 109E$_2$ synthase (cPGES) and the microsomal prostaglandin E_2 synthase (mPGES)-2 are constitutive 110enzymes, whereas mPGES-1 is an inducible isoform (Samuelsson et al., 2007). PGE$_2$ production 111can also be indirectly modulated by the alternative pathway of peroxisome proliferator-activated 112receptor-γ (PPAR-γ), a member of the nuclear receptor superfamily of ligand-dependent 113transcription factors activated by 15-deoxy-Δ12,14-prostaglandin J$_2$ (15d-PGJ$_2$) (Cuzzocrea et al., 1142002). Anti-inflammatory effects related to PPAR-γ activation have previously been demonstrated 115in several studies (Ricote et al., 1998; Tsai et al., 2010) through its ability to increase the expression 116of nuclear factor kappa-B inhibitor α (IκB-α), an endogenous inhibitor which interferes with the 117activation of p65 nuclear factor kappa-B (NF-κB) (Korbecki et al., 2019), via its natural ligand 15d-118PGJ$_2$. Although a large body of work has been carried out to elucidate the biological function of

119PPAR-γ activation, this nuclear receptor's role remains poorly defined in the context of monocytes/
120macrophages activation via the arachidonic pathway.

121Recently, several biochemical and pharmacological studies demonstrated a molecular interaction
122between COXs and PGES isoenzymes, which results in preferential functional coupling activity.
123Specifically, mPGES-2 was shown to utilize COX-1 to generate PGH_2 in contrast to mPGES-1
124which uses COX-2 (Samuelsson et al., 2007). Moreover, studies with PGES deficient mice have
125shown that induced PGE_2 synthesis is largely and preferentially dependent on mPGES-1 enzyme
126(Inada et al., 2006). Consistently, the upregulation of mPGES-1 expression and the involvement of
127COX-2/mPGES-1/PGE_2 cascade in terms of PGs production has been extensively reported in
128pathological settings in which PGE_2 is implicated, such as fever, pain and inflammatory-based
129diseases (Uematsu et al., 2002; Trebino et al., 2003). In line with these observations, it has been
130reported that in several tissues and different cell types including fibroblasts, osteoblasts,
131chondrocytes and osteoarthritic cartilage, mPGES-1 expression is enhanced by a variety of
132inflammatory factors including lipopolysaccharides (LPS), interleukin-(IL−)1β, tumor necrosis
133factor-α (TNF−α) and IL-17A (commonly known as IL-17), the latter of which was used in this
134study (Stichtenoth et al., 2001; Li et al., 2005).

135In this context IL-17, has received much attention as a significant driver of autoimmune and
136autoinflammatory conditions (Maione, 2016; Maione et al., 2020; Raucci et al., 2020) This cytokine
137is mainly produced by T-helper (Th)-17 lymphocytes, but it also released by natural killer T (NKT)
138cells, macrophages, neutrophils, monocytes, $CD8^+$ T cells, γδ T cells and innate lymphoid cells
139(Onishi & Gaffen, 2010). We have previously demonstrated that IL-17 sustains chronic
140inflammation and tissue remodelling rather than initiating it, through its ability to prime monocytes/
141macrophages towards an inflammatory phenotype (Maione et al., 2009; Maione et al., 2011;
142Maione, 2016). Considering that very little is known about the biological effects promoted by IL-17
143both *in vitro* and *in vivo* in the context of mPGES-1/PPAR-γ modulation, here we sought to
144investigate and characterize the role of IL-17 on these pathways during an ongoing inflammatory
145response.

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147**2. METHODS**

148**2.1 Materials**: PGE_2 Elisa kit, proteome profiler mouse cytokine array kit and recombinant mouse
149IL-17 were purchased from R&D System (Milan, Italy). PF 9184 and Troglitazone were purchased
150from Tocris (Milan, Italy), whereas FACS buffer and all conjugated antibodies from BioLegend
151(London, UK). PGD_2 Elisa kit and the primary antibodies for western blot analysis were obtained
152from Elabscience (Milan, Italy) whereas the HRP-conjugated IgG secondary antibodies from Dako
153(Copenhagen, Denmark). 15d-PGJ_2 Elisa kit was purchased from Abcam (Cambridge, UK). Unless
154otherwise stated, all the other reagents were from BioCell (Milan, Italy).

155**2.2 Murine cell isolation and culture:** Mouse macrophage cell line (J774A.1, ATCC® TIB-67™)
156was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine
157serum (FBS) (ATCC® 30-2020™) to a final concentration of 10%. Cells were seeded in petri
158culture dishes (100 × 20 mm, Falcon®) at a density of $5×10^5$ cells/dish and allowed growing for 24
159h. The medium was then replaced, and cells were treated for 4 and 24 h with recombinant mouse
160IL-17 (0.5-500 ng/ml). In another set of experiments, cells were treated with IL-17 (50 ng/ml) in
161presence or absence of PF 9184 (50 μM) or Troglitazone (50 μM) according to previous *in vitro*
162studies (Li et al., 2005). Following incubations for 4 and 24 h, cells were collected with a cell

163scraper and pellets were lysed at 4°C for 10 min with a buffer containing 1g/100 ml Triton X-100, 5
164mM EDTA in PBS (pH 7.4) containing protease inhibitors. After centrifugation at 14000×g for 10
165min at 4°C, the supernatant was collected and stored at -80°C for future western blot analysis.
166Protein concentration was determined by the Bio-Rad protein assay (Bio-Rad, Milan, Italy).

167**2.3 Animals:** Experimental procedures were carried out in 8-12-week-old male CD1 mice in
168compliance with the international and national law and policies and approved by Italian Ministry of
169Health. Animal studies were performed in compliance with the ARRIVE guidelines and with the
170recommendations made by the British Journal of Pharmacology (EU Directive 2010/63/EU for
171animal experiments, ARRIVE guidelines, and the Basel declaration including the 3Rs concept;
172Kilkenny et al., 2010; McGrath & Lilley, 2015). Mice, purchased from Charles River (Milan, Italy),
173were housed with *ad libitum* access to food and water and maintained on a 12 h light/dark cycle.
174Experimental study groups were randomized and blinded. All procedures were carried out to
175minimize the number of animals used (n=7 per group) and their suffering.

176**2.4 Air Pouch:** Dorsal air pouches were induced by injection of 2.5 ml of air on day 0 and day 3 as
177previously described (Maione et al., 2018a). On day 6, mice received the following treatments: i)
178Control (CTRL) 0.25 ml of 0.5% carboxymethyl cellulose (CMC); ii) IL-17 (1 μg) in 0.25 ml of
1790.5% CMC; iii) IL-17 (1 μg) in 0.25 ml of 0.5% CMC co-administrated with PF 9184 (1-9
180μg/pouch); iv) IL-17 (1 μg) in 0.25 ml of 0.5% CMC co-administrated with Troglitazone (1-9
181μg/pouch) accordingly to previous studies (Li et al., 2005; Maione et al., 2009). Mice were
182sacrificed after 4 and 24 h from the injection and air pouches washed thoroughly with 2 ml of PBS
183containing 50 U/ml heparin and 3 mM EDTA. Lavage fluids were centrifuged at 220×g for 10 min
184at 4°C to separate the exudates from the recruited cells. Inflammatory exudates were collected and
185measured to evaluate the level of inflammatory cyto-chemokines, whereas cell pellet subjected to
186western blot and FACS analysis as described below. Cell number was determined by TC20
187automated cell counter (Bio-Rad, Milan, Italy) using Bio-Rad's automated cell counter uses
188disposable slides, TC20 trypan blue dye (0.4% trypan blue dye w/v in 0.81% sodium chloride and
1890.06% potassium phosphate dibasic solution) and a CCD camera to count cells based on the
190analyses of capture images (Maione et al., 2018a; Bellavita et al., 2020).

191**2.5 Myeloperoxidase assay:** Leukocytes myeloperoxidase (MPO) activity was assessed by
192measuring the H_2O_2−dependent oxidation of 3,3′,5,5′-tetramethylbenzidine as previously reported
193(Maione et al., 2009). Cellular lysate, from air pouch experiments, were homogenized in a solution
194composed of hexadecyltrimethylammonium bromide (0.5% w/v) in 50 mM sodium phosphate
195buffer at pH 5.4. After homogenization, samples were centrifuged at 1008×g for 10 min and the
196supernatant used for the assay. Aliquots of 20 μl were incubated with 160 μl of 3,3′,5,5′ -
197tetramethylbenzidine and 20 μl of H_2O_2 (in 80 mM phosphate buffer, pH 5.4) in 96−well plates.
198Plates were incubated for 5 min at room temperature (RT), and OD was read at 620 nm using a
199plate-reader (Biorad, Italy). Assay was performed in duplicates and normalized for protein content.

200**2.6 Elisa and ElisaSpot assay:** Enzyme-linked immunosorbent assays for PGE_2, PGD_2 and 15d-
201PGJ_2 were carried out on pouch inflammatory exudates. Briefly, 100 μl of samples, diluted
202standards, quality controls and dilution buffer (blank) were added to a pre-coated plate with
203monoclonal anti-PGE_2, PGD_2 or 15d-PGJ_2 for 2 h. After washing, 100 μl of biotin labelled antibody
204was added for 1 h. The plate was washed and 100 μl of streptavidin-HRP conjugate was added and
205the plate was incubated for a further 30 min period in the dark. The addition of 100 μl of the
206substrate and stop solution represented the last steps before the reading of absorbance (measured at
207450 and 405 nm for PGE_2, PGD_2 and 15d-PGJ_2 respectively) on a microplate reader. Antigen levels

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208in the samples were determined using a standard curve and expressed as pg/pouch (Raucci et al., 2092019). For cyto-chemokines protein array equal volumes (1.5 ml) of pouch inflammatory fluids in 210all described experimental conditions were incubated with the pre-coated proteome profiler array 211membranes according to the manufacturer's instructions. Dot plots were detected by using the 212enhanced chemiluminescence detection kit and Image Quant 400 GE Healthcare software (GE 213Healthcare, Italy) and successively quantified using GS 800 imaging densitometer software (Biorad, 214Italy) as previously described (Cristiano et al., 2019).

215**2.7 Flow Cytometry:** Cells collected from the pouch cavities, at 4 and 24 h, were first washed with 216PBS and then re-suspended in FACS buffer (PBS containing 1% FCS and 0.02% NaN_2) containing 217CD16/CD32 FcγIIR blocking antibody (clone 93; eBioscience, London, UK) for 30 min at 4°C. 218Thereafter, cells were labelled for 30 min at 4°C with the following conjugated antibodies (all from 219BioLegend, London, UK): CD45 (1:100; clone 30-F11), LY6C (1:100; clone HK1.4), LY6G 220(1:100; clone 1A8), CD115 (1:100; clone AFS98), CD11b (1:100; clone M1/70), F4/80 (1:100; 221clone BM8), CD206 (1:100; clone C068C2), prior to analysis by FACS calibre using CellQuest 222software (Becton Dickinson, Franklin Lakes, NJ). Neutrophils, macrophage and 223resident/inflammatory monocytes were defined according to the flow cytometry procedure 224previously described (Maione et al., 2018a; Kapellos et al., 2019; Podaru et al., 2019). At least 225$1\times10^4$ cells were analysed per sample, and determination of positive and negative populations was 226performed based on the staining obtained with related IgG isotypes. Flow cytometry was performed 227on BriCyte E6 flow cytometer (Mindray Bio-Medical Electronics, Nanshan, China) using MRFlow 228and FlowJo software operation (Raucci et al., 2019b)

229**2.8 Western blot analysis:** Whole cellular pellets homogenates (50 µg of protein) from *in vitro* and 230*in vivo* experiments were subjected to SDS-PAGE (10 and 12% gel) using standard protocols as 231previously described (Maione et al., 2018b). The proteins were transferred to nitrocellulose 232membrane (0.2 µm nitrocellulose membrane, Trans-Blot® TurboTM, Transfer Pack, Bio-Rad 233Laboratories, Hercules, CA, USA) in transfer buffer (25 mM Tris-HCl pH 7.4 containing 192 mM 234glycine and 20% v/v methanol) at 400 mA for 2 h. The membranes were saturated by incubation for 2352 h with non-fat dry milk (5% wt/v) in PBS supplemented with 0.1% (v/v) Tween 20 (PBS-T) for 2 236h at RT and then incubated with 1:1000 dilution of primary antibodies over-night at 4°C such as: 237mouse monoclonal anti-actin (E-AB-20094), mouse monoclonal anti-COX-2 (E-AB-27666), mouse 238monoclonal anti-IκB-α (4814), rabbit polyclonal anti-IL-17 Receptor (E-AB-63080), mouse 239monoclonal anti-NFκB (MAB3026), rabbit polyclonal anti-mPGDS-1 (TA301420), rabbit 240polyclonal anti-mPGES-1 (E-AB-32563), rabbit polyclonal anti-PPAR-γ (NBP2-22106), and then 241washed 3 times with PBS-T. In all cases, blots were then incubated with a 1:3000 dilution of related 242horseradish peroxidase-conjugated secondary antibody for 2 h at RT and finally washed 3 times 243with PBS-T. Protein bands were detected by using the enhanced chemiluminescence method 244(ClarityTM Western ECL Substrate, Bio-Rad Laboratories, Hercules, CA, USA) and Image Quant 245400 GE Healthcare software (GE Healthcare, Italy). Finally, protein bands were quantified using the 246GS 800 imaging densitometer software (Biorad, Italy) and normalized with respective actin.

247**2.9 Statistical analysis:** In this study, statistical analysis complies with the recommendations of the 248British Journal of Pharmacology on experimental design and analysis in pharmacology (Curtis et 249al., 2018) and data sharing and presentation in preclinical pharmacology (Alexander et al., 2018; 250George et al., 2017). All data are presented as means ± S.D. and were analysed using Student's t-251test (two groups) or one-way ANOVA followed by Bonferroni's for multiple comparison test (more 252than two groups). GraphPad Prism 8.0 software (San Diego, CA, USA) was used for analysis.

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253Differences between means were considered statistically significant when P≤0.05 was achieved. 254Sample size was chosen to ensure alpha 0.05 and power 0.8. Animal weight was used for 255randomization and group allocation. No animals were excluded from the analysis.

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257**3. RESULTS**

258**3.1 PF 9184 and Troglitazone, in a time- and dose-dependent manner, revert leukocytes** 259**accumulation and activation at site of inflammation:** We knew from our previous studies 260(Maione et al., 2009; Maione et al., 2018a) that single administration of IL-17 (1 μg/pouch) into a 2616-day old air pouch causes a transient infiltration of leukocytes by 4 h, which peaks at 24 h and then 262declines by 48 h. To test the potential dose-responsive effect of an mPGES-1 antagonist and a 263PPAR-γ agonist at this local site of inflammation, we administered PF 9184 (PF, 1-9 μg/pouch) and 264Troglitazone (TRO, 1-9 μg/pouch) concomitantly with IL-17. All mice were sacrificed at 4, 24 and 26548 h time point. Consistent with our previous findings, 4 and 24 h after IL-17 administration mice 266showed significant differences in the number of inflammatory leukocytes compared to vehicle-267treated (Fig. 1A-B). No significant differences were observed at 48 h (Fig. 1C). Interestingly, mice 268receiving PF at a dose of 9 μg/pouch showed a marked decrease (~40%) in inflammatory infiltrates, 269compared to IL-17-treated mice, at both 4 and 24 h (Fig. 1A-B). A similar anti-inflammatory profile 270was observed after TRO administration (9 μg/pouch), with a marked reduction in infiltrating 271leukocytes at 4 and 24 h (Fig. 1A-B). No biological effects were observed at 48 h (Fig. 1C). Based 272on these results, we selected the most active PF and TRO (9 μg/pouch) dose for all subsequent 273analyses. The level of myeloperoxidase (MPO, Fig. 1D-F), a peroxidase enzyme most abundantly 274expressed in polymorphonuclear leukocytes, and different prostaglandins such as PGE_2, PGD_2 and 27515d-PGJ_2, were measured in pouch fluid. Administration with IL-17 correlated with increased 276levels of MPO (Fig. 1D-E) and PGE_2 (Fig. 1G-H) at both 4 and 24 h. Conversely a reduction in 277PGD_2 (Fig. 1I-J) and 15d-PGJ_2 (Fig. 1K-L) at both 4 and 24 h was observed. When co-injected in 278the presence of PF and TRO the opposite profile was observed with a marked reduction in MPO 279(Fig. 1E) activity and PGE_2 (Fig. 1G,H), and reversal in PGD_2 (Fig. 1I,J) and 15d-PGJ_2 levels (Fig. 2801K,L). Collectively these data indicate time- and dose-dependent protective effects of a selective 281mPGES-1 antagonist and a PPAR-γ agonist on inflammatory cell recruitment and activation in 282response to IL-17 induced inflammation.

283**3.2 mPGES-1 and PPAR-γ enzymatic pathway modulation on the onset of ongoing** 284**inflammation**: Previous studies have shown in various tissues and cell types, both COX-2 and 285mPGES-1 expression is enhanced in response to a range of inflammatory mediators including LPS, 286IL−1β, TNF−α, and IL-17 (Raucci et al., 2019a). We, therefore, carried out western blot analysis on 287recruited cells and found that COX-2 (Fig. 2B) and mPGES-1 (Fig. 2H) were upregulated following 288IL-17-induced inflammation and were both significantly reduced at 4 and, in particular, 24 h after 289PF and TRO treatment. Moreover, we found that IL-17 receptor (IL-17R) expression (increased in 290IL-17-treated animals compared to CTRL group at 24 h) were not influenced by either PF or TRO 291administration (Fig. 2A). We also observed a substantial increase in NF-κB expression in mice 292treated with IL-17 (Fig. 2C). The natural inhibitor of NF-κB (IκB-α) was also measured and the 293converse was observed in mice treated with PF or TRO at 4, and 24 h (Fig. 2F). Notably, mPGDS-1 294and PPAR-γ expression were weakly present in IL-17 group, whereas it significantly increased in 295PF (only at 24 h) and TRO-treated mice (at both 4 and 24 h), confirming the hypothesis that 296activation of an alternative molecular pathway following thiazolidinedione administration (Fig. 2972D,G respectively). Densitometric values (at the bottom of the Fig. 2) are expressed as OD Ratio

298with actin (Fig. 2E) for both 4 and 24 h. Uncropped and original western blots are presented in
299Supplementary Figure 1-8.

3003.3 PF 9184 and Troglitazone selectively modulate the recruitment of inflammatory
301monocytes: We went on to further characterize the phenotype of recruited cells at different time
302points. Flow cytometry was employed to determine neutrophil and monocyte/macrophage subsets.
303Specifically, to identify potential differences in leukocytes sub-populations total cells were gated,
304(Fig. 3A, 4A, and 4N, *gate* R1) followed by single cells (Fig. 3B, 4B, and 4O, *gate* R2). CD45 (pan
305leukocyte/immune cell marker; Fig. 3C) and CD11b (myeloid marker; Fig. 4C, P) were selected
306($^{+ve}$, *gate* R3). Neutrophils were identified as CD45^{+ve}/Ly6G^{Hi}/Ly6C^{Hi} as shown both at 4 (Fig. 3D-
307G) and 24 h (Fig. 3I-L). Monocytes and macrophages were further delineated based upon a range of
308markers. CD11b^{+ve} cells were plotted for Ly6C and CD115 at 4 (Fig. 4D-G) and 24 h (Fig. 4I-L) to
309distinguish CD11b^{+ve}/CD115^{+ve}/Ly6C^{lo} patrolling monocytes (*gate* R4) from
310CD11b^{+ve}/CD115^{+ve}/Ly6C^{hi} inflammatory monocytes (*gate* R5) (Raucci et al., 2019a; Kapellos et al.,
3112019; de Brito et al., 2019), and for CD206 and F4/80 (Fig. 4Q-T and 4V-Y at 4 and 24 h
312respectively) to identify CD11b^{+ve}/F480^{+ve}/CD206^{+ve} reparative macrophages (Podaru et al., 2019).
313Our results show that in IL-17-injected mice, at 4 h, the majority of cells recovered were neutrophils
314(Fig. 3H) which were largely replaced by inflammatory monocytes (Fig. 4M) and lower proportion
315of reparative macrophages (Fig. 4Z) at 24 h. Interestingly, both PF and TRO treatments
316significantly inhibited this selective and time-dependent recruitment of neutrophils and
317inflammatory monocytes (Fig. 3H,M and 4H,M). Moreover, TRO administration at 24 h maintained
318similar levels of reparative macrophages as the control group (Fig. 4Z). In all experimental
319conditions, no significant differences were found in resident monocyte recruitment (*gate* R4).
320Reported values were strengthened by a low percentage of positive cells found in the staining for
321the isotype control antibodies (Supporting Information 1). These results suggest that PF and TRO
322treatment significantly disrupts the recruitment of inflammatory cells in the first phase of the
323response and that TRO selectively promotes resolution in the subsequent reparative phase.

3243.4 Co-administration of PF 9184 and Troglitazone with IL-17 into the air pouch decreases
325the release of cyto-chemokines in the inflammatory fluids: To gain some insights into other
326possible differences in the inflammatory response caused by co-administration of an mPGES-1
327antagonist or a PPAR-γ agonist with a IL-17 inflammatory stimulus, we used an unbiased approach
328(pre-made protein array) based on profiling cytokines and chemokines present in the inflammatory
329fluids. As shown in Figure 5, the pouch fluid obtained from IL-17 administered mice showed a
330large increase, at 4 (Fig. 5A-D) and, in particular, 24 h (Fig. 5F-I), of cyto-chemokines profile
331compared to vehicle (CTRL group). When comparing pouch fluids from PF and TRO treated
332groups with IL-17 (alone), we observed a selective decrease in a range of mediators (Fig. 5A-D and
333Fig. 5F-I at 4 and 24 h respectively). Densitometric analysis revealed that the PF treated group had
334a specific modulation, at 4 h (Fig. 5E), in the following factors: B lymphocyte chemoattractant
335(BLC), soluble intercellular adhesion molecular-1 (sICAM-1), IL-16, interferon γ-induced protein-
33610 (IP-10), keratinocyte chemoattractant (KC), macrophage colony-stimulating factor (MCSF),
337junctional epithelium (JE), monocytes chemoattractant protein-5 (MCP-5), monokine induced by
338interferon γ (MIG), macrophage inflammatory proteins (MIPs), metallopeptidase inhibitor-1
339(TIMP-1) and triggering receptor expressed on myeloid cells-1 (TREM-1) compared to IL-17 group
340(Fig. 5E). This profile was confirmed at the 24 h time-point (Fig. 5J) in addition to complement
341component 5a (C5a), interferon-γ (INF-γ), IL-1α, IL-1β, IL-7, interferon-inducible T-cell α
342chemoattractant (ITAC) and stromal cell-derived factor-1 (SDF-1) (Fig. 5J). A similar inhibitory
343profile was found in TRO injected mice, but surprisingly we observed a more prominent

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344modulation of BLC, C5a, IL-17, IP-10, SDF-1 and TIMP-1 at 4 h (Fig. 5E) and of BLC, sICAM-1, 345IL-16, IL-17, JE, MCP-5 and MIG at 24 h time-point (Fig. 5J). Δ increase/decrease of cito-346chemokines modulation in all experimental conditions are reported in Supporting Information 2.

3473.5 **Protective effect of PF 9184 and Troglitazone on murine isolated, and IL-17-stimulated,** 348**macrophage:** To further confirm the validity of our *in vivo* findings, we carried out *in vitro* studies 349using a murine macrophage cell line J774. Stimulation of macrophages with increasing 350concentrations of IL-17 (0.5-500 ng/ml) induced a significant increase in IL-17R expression at both 3514 and 24 h (Fig. 6A). Notably, pre-treatment of IL-17 (50 ng/ml)-stimulated macrophages with PF 352or TRO (50 μM) reverted the expression of COX-2 and mPGES-1 at 4 and 24 h (Fig. 6B). 353Moreover, PPAR-γ expression was weakly present in IL-17 group, whereas it significantly 354increased in PF (only at 24 h) and TRO-treated mice (at both 4 and 24 h), further strengthening our 355hypothesis of activation of an alternative pathway following thiazolidinedione administration (Fig. 3566B). Densitometric values (at the bottom of the Fig. 6A, B) are expressed as OD Ratio with actin 357for both 4 and 24 h. Original western blots are presented in Supplementary Fig 9-14.

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3594. DISCUSSION AND CONCLUSIONS

360The integration of inflammatory signals is paramount to controlling the intensity and duration of the 361immune response. Eicosanoids, particularly PGE_2, are critical molecules in the initiation of 362inflammation and transition from innate to acquired immune responses. mPGES-1, an integral 363membrane enzyme functionally coupled to COX-2, whose regulated expression controls PGE_2 364levels at sites of inflammation (Park et al., 2006; Kalinski, 2012), has pleiotropic effects on many 365cells of the immune system, influencing both the innate and acquired immune responses 366(Sreeramkumar et al., 2012; Sreeramkumar et al., 2016). PGE_2 can promote the influx and 367activation of neutrophils, macrophages and mast cells (Westman et al., 2004; Kojima et al., 2008), 368but can also suppress NKT cytolytic and granulocyte functions (Jakobsson et al., 1999). *In vitro* 369evidence from several groups have shown that the induction of mPGES-1 is increased in response 370to the pro-inflammatory cytokines IL-1β, TNF, or LPS and that its expression, in certain immune-371mediated inflammatory diseases (IMIDs), maybe upregulated by a wide range of stimuli 372(Korotkova et al., 2008). Li and colleagues (Li et al., 2005) have shown that expression and 373regulation of microsomal prostaglandin E synthase-1 and peroxisome proliferator-activated 374receptor-gamma in human osteoarthritic cartilage and chondrocytes are regulated by pro-375inflammatory stimuli such as IL-1α and TNF-α and that the concomitant presence of IL-17 376displayed a synergistic effect reversed by troglitazone or exogenous PGE_2. These data suggest that 377mPGES-1 may prove to be an interesting therapeutic target for controlling PGE_2 synthesis in certain 378IMIDs such as rheumatoid arthritis (RA), psoriatic arthritis (PsA) and spondylarthritis (SpA) 379(Jouzeau et al., 2008; Navarini et al., 2020).

380Self-regulation of the COXs pathway does not consist solely of positive feedback but also involves 381mechanisms that inhibit the inflammatory response. At the centre of the COXs auto-inhibitory 382pathway, are PPARs. Activation of inflammatory responses causes an increase in the expression of 383PPAR-α and a decrease in PPAR-γ (Kapoor et al., 2007; García-Alonso et al., 2013). PPARs are 384nuclear receptors activated by oxidised and nitrated fatty acid derivatives and cyclopentenone 385prostaglandins (PGA_2 and 15d-PGJ_2) during the inflammatory response. Other activators include 386nonsteroidal anti-inflammatory drugs (NSAID), fatty acids, especially polyunsaturated fatty acid 387(PUFA) (arachidonic acid, ALA, EPA, and DHA) and thiazolidinedione derivates (Yousefnia et al.,

3882018). The primary function of PPARs during the inflammatory reaction is to promote the 389inactivation of NF-κB (by direct binding of p65 NF-κB) or the proteolytic degradation of p65 NF-390κB. PPARs also cause an increase in the expression of IκB-α, SIRT1, and PTEN, which interfere 391with the activation and function of NF-κB in inflammatory reactions (Thommesen et al., 1998). 392From a pathological point of view, it should also be taken into account that IL-1α, TNF-α, IL-17 393and PGE$_2$, which are involved in the pathogenesis and progression of certain IMIDS, also 394downregulate PPAR-γ expression in a dose- and time-dependent manner (Afif et al., 2007; 395Korbecki et al., 2014). Several lines of evidences suggest that PPAR-γ activation may have 396therapeutic benefits in RA, PsA and possibly other chronic articular diseases (Murakami, 2011; 397Korbecki et al., 2019).

398IL-17 is an archetype molecule for the entire family of IL-17 cytokines. Currently believed to be 399produced by a specific subset of CD4$^+$ T cells, named Th17 cells, but also by many innate cell 400components (D'Acquisto et al., 2010), this cytokine is functionally located at the interface of innate 401and adaptive immunity (Maione, 2016). Specifically, IL-17 ability to induce the release of a range 402of cyto-chemokines and growth factors, has led to its emergence as an essential co-ordinator of 403local inflammatory reactions due to its ability to modulate neutrophil and monocyte accumulation in 404inflamed tissues. Furthermore, there is growing evidence that suggests targeting IL-17 signalling 405may prove useful in a variety of inflammatory-based diseases including RA, OA, asthma, Crohn's 406disease (CD), psoriatic like-disease and PsA. Here, using a well-established preclinical model of 407ongoing inflammation, the dorsal air pouch (Maione et al., 2009), we tested the hypothesis that pre-408treatment with an mPGES-1 antagonist or PPAR-γ agonist could retard the process of IL-17-409induced inflammation. We confirmed the pro-inflammatory action (at 4 and 24 h) of IL-17, but also 410found a novel protective role for PF and TRO, as exemplified by a reduction in both PMN 411(polymorphonuclear leukocytes) recruitment (at both 4 and in particular 24 h), MPO activity and 412modulation of crucial lipid mediators strictly related to mPGES-1 and PPAR-γ enzymatic activity 413(PGE$_2$, PGD$_2$, and PGJ$_2$). From a mechanistic basis, we demonstrated that IL-17 increased in a time-414dependent manner, the levels of its receptor (IL-17R), COX-2, mPGES-1, and NF-κB and 415decreased mPGDS-1, PPAR-γ and IκB-α expression, in pouch-recruited leukocytes. Moreover, co-416administration with PF and TRO was shown to significantly revert PMN recruitment, MPO activity, 417and reduce the level of lipid mediators and, most importantly, to modulate mPGES-1, mPGDS-1, 418PPAR-γ and NF-κB/IκB-α expression, leaving unchanged IL-17R expression. Another exciting 419aspect of our study is that we provide the first reported evidence of an indirect, co-ordinated 420functional regulation in both neutrophils and monocytes by mPGES-1 and PPAR-γ. This is most 421likely related to the presence of complex mechanisms regulating COX-2/mPGES-1 *in vivo* which 422go onto to impact PPAR-γ activity (Maione et al., 2020).

423This hypothesis is consistent with previous studies showing that pre-adipocytes stably transfected 424with either COX-1 or COX-2 had lower PPAR-γ expression (Chu et al., 2009) and that mice 425genetically deficient for mPGES-1 had basal elevations in PPAR-γ expression and transcriptional 426activity (Kapoor et al., 2007). The mechanisms by which the COX-2/mPGES-1/PGE$_2$ axis and the 427nuclear receptor PPAR-γ interact during the inflammatory process are not entirely delineated but 428may linked to PGE$_2$ ability (mainly produced by neutrophils at the early stage of inflammation) to 429decrease the amount of well-known lipid mediators (such as PGD$_2$ and PGJ$_2$) which are implicated 430in the induction of PPAR-γ (Maione et al., 2020). This biological event is interconnected with a 431cellular shift from neutrophils to monocytes as exemplified by the release of specific neutrophil 432(KC, C5a) and monocytes/macrophages (INF-γ, IL-16, IL-17, IP-10, JE, MIPs, TREM-1, ICAM, 433IL-1α/β, MCPs, MIG, TIMP-1) cyto-chemokines. It is well-established in the literature that i)

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434PPAR-γ acts as a negative regulator of macrophage activation (Ricote et al., 1998) due to its ability 435to control polarization of monocyte differentiation between pro-inflammatory (M1) and alternative 436anti-inflammatory (M2) macrophage phenotypes (Tontonoz & Spiegelman, 2008) and to reduce 437neutrophil migration to sites of injury (Napimoga et al., 2008); ii) neutrophils are a principle 438cellular source of mPGES-1, with activated M1, rather than alternatively activated M2 439macrophages, a secondary source (Posadas et al., 2000; Mosca et al., 2007). It is clear from this 440current study that the release of PGE_2 and PGD_2/PGJ_2 related to COX-2/mPGES-1 and 441PPAR-γ/mPGDS-1 expression follows the temporal shift from neutrophils to monocytes that are 442implicated in the potential resolution of inflammatory response. This was confirmed by our 443experiments performed in the presence of either PF or TRO's where both compounds were shown to 444revert the inflammatory response (Fig. 7). To further support this association between IL-17 and 445mPGEs-1/PPARγ expression and modulation, and in light of the detrimental role of macrophages 446and macrophage-derived cytokines in RA and SpA synovium (Kabala et al., 2020), we performed *in* 447*vitro* experiments where we used J774 cell line. Interestingly, we confirmed our *in vivo* results 448demonstrating a direct involvement of mPGES-1/PPAR-γ axis in IL-17 stimulated-macrophages.

449In conclusion, our results reveal a novel interaction between IL-17 and mPGES-1/PPAR-γ 450generated by macrophages/inflammatory monocytes during inflammation. Therefore, we believe 451that the IL-17/mPGES-1/PPAR-γ "axis" could represent a potential therapeutic target for 452inflammatory-based and immune-mediated diseases.

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454Author contributions

455FR, AS, GMC, MGR, AAM, MP and MGF performed the experiments. EP, VV, CI, FC, RS, NM, 456MA, AJI and FM performed data analysis and wrote the manuscript. FR, AJI and FM revised the 457final version of the manuscript for intellectual contents. All authors gave final approval to the 458publication.

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460Declaration of transparency and scientific rigour

461This Declaration acknowledges that this paper adheres to the principles for transparent reporting 462and scientific rigour of preclinical research as stated in the BJP guidelines for Design & Analysis, 463Immunoblotting, and Animal Experimentation, and as recommended by funding agencies, 464publishers and other organisations engaged with supporting research.

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466REFERENCES

467Afif, H., Benderdour, M., Mfuna-Endam, L., Martel-Pelletier, J., Pelletier, J. P., Duval, N., Fahmi, 468H. (2007). Peroxisome proliferator-activated receptor gamma1 expression is diminished in human 469osteoarthritic cartilage and is downregulated by interleukin-1beta in articular chondrocytes. 470*Arthritis Res. Ther*, 9. R31. DOI: 10.1186/ar2151

471Alexander, S. P. H., Roberts, R. E., Broughton, B. R. S., Sobey, C. G., George, C. H., Stanford, S. 472C., … Ahluwalia, A. (2018). Goals and practicalities of immunoblotting and 473immunohistochemistry: A guide for submission to the British Journal of Pharmacology. *British 474Journal of Pharmacology,* 175, 407–411. DOI: 10.1111/bph.14112

21

22

475Bellavita, R., Raucci, F., Merlino, F., Piccolo, M., Ferraro, M. G., Irace, C., … Maione, F. (2020).
476Temporin L-derived peptide as a regulator of the acute inflammatory response in zymosan-induced
477peritonitis. *Biomed Pharmacother,* 123, 109788. DOI: 10.1016/j.biopha.2019.109788

478Chu, X., Xu, L., Nishimura, K., Jisaka, M., Nagaya, T., Shono, F., Yokota, K. (2009). Suppression
479of adipogenesis program in cultured preadipocytes transfected stably with cyclooxygenase
480isoforms. *Biochim. Biophys. Acta,* 1791, 273–280. DOI: 10.1016/j.bbalip.2009.01.022

481Cristiano, C., Volpicelli, F., Lippiello, P., Buono, B., Raucci, F., Piccolo, M., … Maione, F. (2019).
482Neutralization of IL-17 rescues amyloid-β-induced neuroinflammation and memory impairment.
483*Br. J. Pharmacol,* 176, 3544-3557. DOI: 10.1111/bph.14586

484Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., …
485Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: updated and
486simplified guidance for authors and peer reviewers. *Br. J. Pharmacol*, 175(7), 987-993. DOI:
48710.1111/bph.14153

488Cuzzocrea, S., Wayman, N. S., Mazzon, E., Dugo, L., Di Paola, R., Serraino, I., … Thiemermann,
489C. (2002). The cyclopentenone prostaglandin 15-deoxy-Delta(12,14)-prostaglandin J(2) attenuates
490the development of acute and chronic inflammation. *Molecular Pharmacology,* 61, 997-1007. DOI:
49110.1124/mol.61.5.997

492D'Acquisto, F., Maione, F., Pederzoli-Ribeil, M. (2010). From IL-15 to IL-33: the never-ending list
493of new players in inflammation. Is it time to forget the humble aspirin and move ahead? *Biochem.*
494*Pharmac,* 79, 525-534. DOI: 10.1016/j.bcp.2009.09.015

495de-Brito, N. M., da-Costa, H. C., Simões, R. L., Barja-Fidalgo, C. (2019). Lipoxin-Induced
496Phenotypic Changes in CD115 + LY6C hi Monocytes TAM Precursors Inhibits Tumor
497Development. *Front Oncol*, 9, 540. DOI: 10.3389/fonc.2019.00540

498Funk, C. D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science,* 294,
4991871-1875. DOI: 10.1126/science.294.5548.1871

500García-Alonso, V., López-Vicario, C., Titos, E., Morán-Salvador, E., González-Périz, A., Rius, B.,
501… Clària, J. (2013). Coordinate functional regulation between microsomal prostaglandin E
502synthase-1 (mPGES-1) and peroxisome proliferator-activated receptor γ (PPARγ) in the conversion
503of white to brown adipocytes. *J. Biol. Chem,* 288, 28230-28242. DOI: 10.1074/jbc.M113.468603

504George, C. H., Stanford, S. C., Alexander, S., Cirino, G., Docherty, J. R., Giembycz, M. A., …
505Ahluwalia, A. (2017). Updating the guidelines for data transparency in the British Journal of
506Pharmacology − data sharing and the use of scatter plots instead of bar charts. *British Journal of*
507*Pharmacology,* 174, 2801–2804. DOI: 10.1111/bph.13925

508Hawkey, C. J. (1999). COX-2 inhibitors. *Lancet,* 353, 307-314. DOI: 10.1016/s0140-
5096736(98)12154-2

510Inada, M., Matsumoto, C., Uematsu, S., Akira, S., Miyaura, C. (2006). Membrane-bound
511prostaglandin E synthase-1-mediated prostaglandin E2 production by osteoblast plays a critical role
512in lipopolysaccharide-induced bone loss associated with inflammation. *J. Immunol,* 177, 1879-
5131885. DOI: 10.4049/jimmunol.177.3.1879

23

24

514Jakobsson, P. J., Thorén, S., Morgenstern, R., Samuelsson, B. (1999). Identification of human 515prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a 516potential novel drug target. *Proc. Natl. Acad. Sci. U S A,* 96, 7220-7225. DOI: 51710.1073/pnas.96.13.7220

518Jouzeau, J. Y., Moulin, D., Koufany, M., Sebillaud, S., Bianchi, A., Netter P. (2008). 519Pathophysiological relevance of peroxisome proliferators activated receptors (PPAR) to joint 520diseases - the pro and con of agonists. *J. Soc. Biol,* 202, 289-312. DOI: 10.1051/jbio:2008034

521Kabala, P. A., Malvar-Fernández, B., Lopes, A. P., Carvalheiro, T., Hartgring, S. A. Y., Tang, M. 522W., … García, S. (2020). Promotion of macrophage activation by Tie2 in the context of the 523inflamed synovia of rheumatoid arthritis and psoriatic arthritis patients. *Rheumatology (Oxford)*, 52459(2), 426-438. DOI: 10.1093/rheumatology/kez315

525Kalinski, P. (2012). Regulation of immune responses by prostaglandin E2. *J. Immunol,* 188, 21-28. 526DOI: 10.4049/jimmunol.1101029

527Kapellos, T. S., Taylor, L., Feuerborn, A., Valaris, S., Hussain, M. T., Rainger, G. E., … Iqbal, A. 528J. (2019). Cannabinoid receptor 2 deficiency exacerbates inflammation and neutrophil recruitment. 529*FASEB J,* 33, 6154-6167. DOI: 10.1096/fj.201802524R

530Kapoor, M., Kojima, F., Qian, M., Yang, L., Crofford, L. J. (2007). Microsomal prostaglandin E 531synthase-1 deficiency is associated with elevated peroxisome proliferator-activated receptor 532gamma: regulation by prostaglandin E2 via the phosphatidylinositol 3-kinase and Akt pathway. *J.* 533*Biol. Chem,* 282, 5356-66. DOI: 10.1074/jbc.M610153200

534Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., Altman, D. G., NC3Rs Reporting 535Guidelines Working Group. (2010). Animal research:in vivo experiments: the arrive guidelines. *Br.* 536*J. Pharmacol,* 160, 1577-1579. DOI: 10.1111/j.1476-5381.2010.00872.x

537Koeberle, A. & Werz, O. (2015). Perspective of microsomal prostaglandin E2 synthase-1 as drug 538target in inflammation-related disorders. *Biochem. Pharmacol,* 98, 1-15. DOI: 53910.1016/j.bcp.2015.06.022

540Kojima, F., Kapoor, M., Yang, L., Fleishaker, E. L., Ward, M. R., Monrad, S. U. … Crofford, L. J. 541(2008). Defective generation of a humoral immune response is associated with a reduced incidence 542and severity of collagen-induced arthritis in microsomal prostaglandin E synthase-1 null mice. *J.* 543*Immunol,* 180, 8361–8368. DOI: 10.4049/jimmunol.180.12.8361

544Korbecki, J., Baranowska-Bosiacka, I., Gutowska, I., Chlubek, D. (2014). Cyclooxygenase 545pathways. *Acta Biochim. Pol,* 61, 639-649. PMID: 25343148

546Korbecki, J., Bobiński, R., Dutka, M. (2019). Self-regulation of the inflammatory response by 547peroxisome proliferator-activated receptors. *Inflammation Research,* 68, 443-458. DOI: 54810.1007/s00011-019-01231-1

549Korotkova, M., Helmers, S. B., Loell, I., Alexanderson, H., Grundtman, C., Dorph, C., … 550Jakobsson, P. J. (2008). Effects of immunosuppressive treatment on microsomal prostaglandin E 551synthase 1 and cyclooxygenases expression in muscle tissue of patients with polymyositis or 552dermatomyositis. *Ann. Rheum. Dis,* 67, 1596-1602. DOI: 10.1136/ard.2007.079525

553Li, X., Afif, H., Cheng, S., Martel-Pelletier, J., Pelletier, J. P., Ranger, P., Fahmi, H. (2005). 554Expression and regulation of microsomal prostaglandin E synthase-1 in human osteoarthritic 555cartilage and chondrocytes. *J. Rheumatol*, 32, 887-895. PMID: 15868626

556Maione, F., Paschalidis, N., Mascolo, N., Dufton, N., Perretti, M., D'Acquisto, F. (2009). 557Interleukin 17 sustains rather than induces inflammation. *Biochem. Pharmacol*, **77**, 878-887. DOI: 55810.1016/j.bcp.2008.11.011

559Maione, F., Cicala, C., Liverani, E., Mascolo, N., Perretti, M., D'Acquisto, F. (2011). IL-17A 560increases ADP-induced platelet aggregation. *Biochem. Biophys. Res. Commun,* 408, 658-662. DOI: 56110.1016/j.bbrc.2011.04.080

562Maione, F. (2016). Commentary: IL-17 in Chronic Inflammation: From Discovery to Targeting. 563*Front. Pharmacol,* 7, 250. DOI: 10.3389/fphar.2016.00250.

564Maione, F., Iqbal, A. J., Raucci, F., Letek, M., Bauer, M., D'Acquisto, F. (2018a) Repetitive 565Exposure of IL-17 Into the Murine Air Pouch Favors the Recruitment of Inflammatory Monocytes 566and the Release of IL-16 and TREM-1 in the Inflammatory Fluids. *Immunol,* 9, 2752. DOI: 56710.3389/fimmu.2018.02752

568Maione, F., Piccolo, M., De Vita, S., Chini, M. G., Cristiano, C., De Caro, C., … Bifulco, G. 569(2018b). Down regulation of pro-inflammatory pathways by tanshinone IIA and cryptotanshinone 570in a non-genetic mouse model of Alzheimer's disease. *Pharmacol Res*, 129, 482-490. DOI: 10.1016/ 571j.phrs.2017.11.018

572Maione, F., Casillo, G. M., Raucci, F., Iqbal, A. J., Mascolo, N. (2020). The functional link between 573microsomal prostaglandin E synthase-1 (mPGES-1) and peroxisome proliferator-activated receptor 574γ (PPARγ) in the onset of inflammation. *Pharmacol Res*, 157, 104807. DOI: 57510.1016/j.phrs.2020.104807

576McGrath, J. C. & Lilley, E. (2015). Implementing guidelines on reporting research using animals 577(arrive etc.): new requirements for publication in BJP. *Br. J. Pharmacol,* 172, 3189-3193. DOI: 57810.1111/bph.12955

579Mosca M., Polentarutti, N., Mangano, G., Apicella, C., Doni, A., Mancini, F., … Mantovani, A. 580(2007). Regulation of the Microsomal Prostaglandin E synthase-1 in Polarized Mononuclear 581Phagocytes and Its Constitutive Expression in Neutrophils. *J. Leukoc. Biol,* 82, 320-326. DOI: 58210.1189/jlb.0906576

583Murakami, M. (2011). Lipid mediators in life science. *Exp. Anim,* 60, 7-20 DOI: 58410.1538/expanim.60.7

585Napimoga, M. H., Vieira, S. M., Dal-Secco, D., Freitas, A., Souto, F. O., Mestriner, F. L., … 586Cunha, F. Q. (2008). Peroxisome proliferator-activated receptor-gamma ligand, 15-deoxy-587Delta12,14-prostaglandin J2, reduces neutrophil migration via a nitric oxide pathway. *J. Immunol,* 588180, 609-617. DOI: 10.4049/jimmunol.180.1.609

589Navarini, L., Costa, L., Tasso, M., Chimenti, M. S., Currado, D., Fonti, G. L., … Caso, F. (2020). 590Retention rates and identification of factors associated with anti-TNFα, anti-IL17, and anti-591IL12/23R agents discontinuation in psoriatic arthritis patients: results from a real-world clinical 592setting. *Clin. Rheumatol*. DOI: 10.1007/s10067-020-05027-1

593Onishi, R. M. & Gaffen, S. L. (2010). Interleukin-17 and its target genes: mechanisms of 594interleukin-17 function in disease. *Immunology,* 129, 311-321. DOI: 10.1111/j.1365-5952567.2009.03240.x

596Park, J. Y., Pillinger, M. H., Abramson, S. B. (2006). Prostaglandin E2 synthesis and secretion: the 597role of PGE2 synthases. *Clin. Immunol*, 119, 229-240. DOI: 10.1016/j.clim.2006.01.016

598Perretti, M., Cooper, D., Dalli, J., Norling L. V. (2017). Immune resolution mechanisms in 599inflammatory arthritis. *Nat. Rev. Rheumatol*, 13, 87-99. DOI: 10.1038/nrrheum.2016.193

600Podaru, M. N., Fields, L., Kainuma, S., Ichihara, Y., Hussain, M., Ito, T., … Suzuki, K. (2019). 601Reparative Macrophage Transplantation for Myocardial Repair: A Refinement of Bone Marrow 602Mononuclear Cell-Based Therapy. *Basic Res. Cardiol*, 114. DOI: 10.1007/s00395-019-0742-1

603Posadas, I., Terencio, M. C., Guillén, I., Ferrándiz, M. L., Coloma, J., Payá, M., Alcaraz, M. J. 604(2000). Co-regulation Between cyclo-oxygenase-2 and Inducible Nitric Oxide Synthase Expression 605in the Time-Course of Murine Inflammation. *Naunyn Schmiedebergs Arch. Pharmacol.* **361**, 98-606106. DOI: 10.1007/s002109900150

607Raucci, F., Iqbal, A. J., Saviano, A., Minosi, P., Piccolo, M., Irace, C., … Maione, F. (2019a). IL-60817A neutralizing antibody regulates monosodium urate crystal-induced gouty inflammation. 609*Pharmacol. Res,* 147.

610Raucci F., Iqbal, A. J., Saviano, A., Casillo, G. M., Russo, M., Lezama, D., … Maione, F. (2019b). 611In-depth immunophenotyping data relating to IL-17Ab modulation of circulating Treg/Th17 cells 612and of in situ infiltrated inflammatory monocytes in the onset of gouty inflammation. *Data Brief,* 61325, 104381. DOI: 10.1016/j.dib.2019.104381. eCollection 2019 Aug

614Raucci F., Mansour A. A., Casillo, G. M., Saviano, A., Caso, F., Scarpa, R., … Maione, F. (2020). 615Interleukin-17A (IL-17A), a key molecule of innate and adaptive immunity, and its potential 616involvement in COVID-19-related thrombotic and vascular mechanisms. *Autoimmun Rev,* 19(7), 617102572. DOI: 10.1016/j.autrev.2020.102572.

618Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., Glass, C. K. (1998). The peroxisome proliferator-619activated receptor-gamma is a negative regulator of macrophage activation. *Nature,* 391, 79-82. 620DOI: 10.1038/34178

621Samuelsson, B., Morgenstern, R., Jakobsson, P. J. (2007). Membrane prostaglandin E synthase-1: a 622novel therapeutic target. *Pharmacol. Rev,* 59, 207-224. DOI: 10.1124/pr.59.3.1

623Serhan, C. N. (2014). Pro-resolving lipid mediators are leads for resolution physiology. *Nature,* 624510, 92-101. DOI: 10.1038/nature13479

625Sreeramkumar, V., Fresno, M., Cuesta, N. (2012). Prostaglandin E2 and T cells: friends or foes? 626*Immunol. Cell. Biol,* 90, 579-586. DOI: 10.1038/icb.2011.75

627Sreeramkumar, V., Hons, M., Punzón, C., Stein, J. V., Sancho, D., Fresno, M., Cuesta, N. (2016). 628Efficient T-cell priming and activation requires signaling through prostaglandin E2 (EP) receptors. 629*Immuno.l Cell. Biol,* 94**,** 39-51. DOI: 10.1038/icb.2015.62

630Stichtenoth, D. O., Thorén, S., Bian, H., Peters-Golden, M., Jakobsson, P. J., Crofford, L. J. (2001). 631Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and

632glucocorticoids in primary rheumatoid synovial cells. *J. Immunol,* 167**,** 469-474. DOI: 63310.4049/jimmunol.167.1.469

634Thommesen, L., Sjursen, W., Gåsvik, K., Hanssen, W., Brekke, O. L., Skattebøl, L., … Laegreid, 635A. (1998). Selective inhibitors of cytosolic or secretory phospholipase A2 block TNF-induced 636activation of transcription factor nuclear factor-kappa B and expression of ICAM-1. *J Immunol,* 637161, 3421-3430. PMID: 9759860

638Tontonoz, P. & Spiegelman, B. M. (2008). Fat and beyond: the diverse biology of PPARgamma. 639*Annu. Rev. Biochem,* **77**, 289-312. DOI: 10.1146/annurev.biochem.77.061307.091829

640Trebino, C. E., Stock, J. L., Gibbons, C. P., Naiman, B. M., Wachtmann, T. S., Umland, J. P., … 641Audoly, L. P. (2003). Impaired inflammatory and pain responses in mice lacking an inducible 642prostaglandin E synthase. *Proc. Natl. Acad. Sci. U. S. A*, 100, 9044-9049. DOI: 64310.1073/pnas.1332766100

644Tsai, J. S., Chen, C. Y., Chen, Y. L., Chuang, L. M. (2010). Rosiglitazone Inhibits 645Monocyte/Macrophage Adhesion Through De Novo Adiponectin Production in Human Monocytes. 646*J. Cell. Biochem*, 110, 1410-1419. DOI: 10.1002/jcb.22657

647Uematsu, S., Matsumoto, M., Takeda, K., Akira, S. (2002). Lipopolysaccharide-dependent 648prostaglandin E(2) production is regulated by the glutathione-dependent prostaglandin E(2) 649synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. *J. Immunol,* 168, 5811-6505816. DOI: 10.4049/jimmunol.168.11.5811

651Westman, M., Korotkova, M., Klint, E. A., Stark, A., Audoly, L. P., Klareskog, L., … Jakobsson, P. 652J. (2004). Expression of microsomal prostaglandin E synthase 1 in rheumatoid arthritis synovium. 653*Arthritis Rheum,* 50, 1774-1780. DOI: 10.1002/art.20286

654Yousefnia, S., Momenzadeh, S., Seyed Forootan, F., Ghaedi, K., Nasr Esfahani, M. H. (2018). The 655influence of peroxisome proliferator-activated receptor γ (PPARγ) ligands on cancer cell 656tumorigenicity. *Gene,* 649, 14-22. DOI: 10.1016/j.gene.2018.01.018

657Zhou, L., Braat, H., Faber, K. N., Dijkstra, G., Peppelenbosch, M. P. (2009). Monocytes and their 658pathophysiological role in Crohn's disease. *Cellular and Molecular Life Sciences,* 66, 192-202. 659DOI: 10.1007/s00018-008-8308-7

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663**FIGURE LEGENDS**

664**Fig. 1 PF and TRO, in a time- and dose-dependent manner, revert leukocytes accumulation** 665**and activation at site of inflammation:** Mice were treated with IL-17 vehicle (CTRL), IL-17 (1 666μg/pouch) alone (IL-17) or co-administrated with PF 9184 (PF, 1-9 μg/pouch) and Troglitazone 667(TRO, 1-9 μg/pouch) and thereafter total cell number from pouches inflammatory exudates was 668evaluated at 4 (**A**), 24 (**B**) and 48 (**C**) h. At the same time-point (**D-F**), supernatants from cell pellet 669lysate were tested for myeloperoxidase activity. Subsequently, inflammatory fluids from all 670experimental conditions were assayed by Elisa for PGE_2 (**G-H**), PGD_2 (**I-J**), and PGJ_2 (**K-L**) levels 671at both 4 and 24 h. Data were expressed as millions of cells for pouch (**A-C**), units/mg of protein

672(**D-F**) or pg/pouch (**G-L**) and presented as means ± S.D. of n=7 mice per group. Statistical analysis 673was conducted by one-way ANOVA followed by Bonferroni's for multiple comparisons. $^{\#}$P≤0.05, 674$^{\#\#}$P≤0.01, $^{\#\#\#}$P≤0.005 *vs* CTRL group; *P≤0.05, **P≤0.01 *vs* IL-17 group.

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676**Fig. 2 mPGES-1 and PPAR-γ enzymatic pathway modulation on the onset of ongoing** 677**inflammation**: Whole cellular pellets homogenates from air pouch experiments in all experimental 678conditions were assayed by western blot for IL-17 Receptor (IL-17R) (**A**), COX-2 (**B**), NF-Kb (**C**), 679PPAR-γ (**D**), IκB-α (**F**), mPGDS-1(**G**), mPGES-1 (**H**) expression at 4 and 24 h. Western blot 680imagines are representative of three separate experiments with similar results. Cumulative 681densitometric values (at the bottom of the Figure) are expressed as OD Ratio with actin (**E**) for both 6824 and 24 h. Values are presented as means ± S.D. of three experiments with n=7 mice per group. 683Statistical analysis was conducted by one-way ANOVA followed by Bonferroni's for multiple 684comparisons. $^{\#}$P≤0.05, $^{\#\#}$P≤0.01, $^{\#\#\#}$P≤0.005 *vs* CTRL group; *P≤0.05, **P≤0.01, ***P≤0.005 *vs* IL-68517 group.

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687**Fig. 3 Flow cytometry strategy applied to identify the modulation of neutrophils after IL-17** 688**stimulation:** Mice were treated with IL-17 vehicle (CTRL), IL-17 (1 μg/pouch) alone (IL-17) or in 689co-administrated with PF 9184 (PF, 9 μg/pouch) and Troglitazone (TRO, 9 μg/pouch). Cells 690collected from the pouch cavities were washed, gated in their totality (**A**, *gate* R1) and singlet (**B,** 691*gate* R2) before the identification of CD45 positive (**C**, CD45^{+ve}) population (*gate* R3). CD45^{+ve} 692cells (**C**) were then plotted for Ly6C and Ly6G expression at 4 (**D-G**) and 24 h (**I-L**) to identify 693CD45^{+ve}/Ly6C^{+ve}/Ly6G^{+ve} as neutrophils. Histograms values (expressed as million for pouch) 694indicate the total positive cells, in the different experimental conditions, of 695CD45^{+ve}/Ly6C^{+ve}/Ly6G^{+ve} (**H,M**) at 4 and 24 h. FACS pictures are representative of independent 696experiments with similar results. Values are presented as means ± S.D. of three separate 697experiments with n=7 mice per group. Statistical analysis was conducted by one-way ANOVA 698followed by Bonferroni's for multiple comparisons. $^{\#\#}$P≤0.01, $^{\#\#\#}$P≤0.005 *vs* CTRL group; *P≤0.05, 699**P≤0.01 *vs* IL-17 group.

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701**Fig. 4 PF and TRO selectively modulate the recruitment of inflammatory monocytes and** 702**reparative macrophages:** Mice were treated with IL-17 vehicle (CTRL), IL-17 (1 μg/pouch) alone 703(IL-17) or in co-administrated with PF 9184 (PF, 9 μg/pouch) and Troglitazone (TRO, 9 μg/pouch). 704Cells collected from the pouch cavities were washed, gated in their totality (**A,N**, *gate* R1) and 705singlet (**B,O**, *gate* R2) before the identification of CD11b positive (**C,P** CD11b^{+ve}) population (*gate* 706R3). CD11b^{+ve} (**C**) cells were then plotted for Ly6C and CD115 expression at 4 (**D-G**) and 24 h (**I-** 707**L**) to distinguish CD11b^{+ve}/CD115^{+ve}/Ly6C^{low} patrolling monocytes (*gate* R4) from 708CD11b^{+ve}/CD115^{+ve}/Ly6C^{hi} inflammatory monocytes (*gate* R5). Therefore, CD11b^{+ve} cells were then 709plotted for CD206 and F480 expression at both 4 (**Q-T**) and 24 h (**V-Y**) to identify 710CD206^{+ve}/F480^{+ve} population. Histograms values (expressed as million for pouch) indicate the total 711positive cells, in the different experimental conditions, of CD11b^{+ve}/CD115^{+ve}/Ly6C^{hi} (**H,M**) and 712CD11b^{+ve}/CD206^{+ve}/F480^{+ve} (**U,Z**) at 4 and 24 h. FACS pictures are representative of independent 713experiments with similar results. Values are presented as means ± S.D. of three separate 714experiments with n=7 mice per group. Statistical analysis was conducted by one-way ANOVA

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715followed by Bonferroni's for multiple comparisons. $^{\#}P \leq 0.05$, $^{\#\#\#}P \leq 0.005$ *vs* CTRL group; *$P \leq 0.05$, 716**$P \leq 0.01$ *vs* IL-17 group.

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718**Fig. 5 Co-administration of PF and TRO with IL-17 into the air pouch decreases the release of** 719**cyto-chemokines in the inflammatory fluid:** Inflammatory supernatants obtained from the pouch 720cavities were assayed using a Proteome Profiler cytokine array at both 4 and 24 h for CTRL 721(respectively **A, F**), IL-17 (1 μg/pouch, respectively **B, G**), IL-17 + PF (9 μg/pouch, respectively **C,** 722**H**) and IL-17 + TRO (9 μg, respectively **D, I**). Densitometric analysis is presented as heatmap at 4 723(**E**) and 24 h (**J**). Data (expressed as INT/mm$_2$) are presented as means ± S.D. of positive spots of 724three independent experiments with n=7 mice per group.

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726**Fig. 6 Protective effect of PF and TRO on murine isolated, and IL-17-stimulated,** 727**macrophage**: Whole cellular pellets homogenates from murine macrophages stimulated with 728increasing concentration of IL-17 (0.5-500 ng/ml) (**A**) or with IL-17 at 50 ng/ml in presence or 729absence of PF 9184 (PF, 50 μM) and Troglitazone (TRO, 50 μM) at 4 and 24 h (**B**) were analysed, 730by western blot, for IL-17R, COX-2, PPAR-γ, mPGES-1 and actin expression. Western blot 731imagines are representative of three separate experiments with similar results. Cumulative 732densitometric values (at the bottom of the Figure **A,B**) are expressed as OD Ratio with actin for 733both 4 and 24 h. Values are presented as means ± S.D. of three experiments. Statistical analysis was 734conducted by one-way ANOVA followed by Bonferroni's for multiple comparisons. $^{\#}P \leq 0.05$, 735$^{\#\#}P \leq 0.01$, $^{\#\#\#}P \leq 0.005$ *vs* CTRL group; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.005$ *vs* IL-17 group.

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737**Fig. 7 Schematic representation of involvement if IL-17/mPGES-1/PPAR-γ axis on the onset** 738**and resolution of inflammation:** IL-17 injection into air pouch recruits neutrophils and, more 739specifically, inflammatory monocytes, producing a massive release of pro-inflammatory cyto-740chemokines at both 4 and 24 h. However, the co-administration of IL-17 with an mPGES-1 741antagonist (PF) or PPAR-γ agonist (TRO) shifts the *equilibrium* between COX-2/mPGES-1 and 742PPAR-γ/mPGDS-1 pathways, downregulating PGE$_2$ and upregulating PGD$_2$/PGJ$_2$ levels via NF-κB.

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