bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

1 # Interleukin-1 receptor antagonist treatment in acute ischaemic stroke
2 # does not alter systemic markers of anti-microbial defence

3

4 **Laura McCulloch[1], Stuart M. Allan[2], Craig J. Smith [3] and Barry W. McColl[1*]**
5
6 [1]UK Dementia Research Institute, University of Edinburgh, Edinburgh, United Kingdom
7 [2]Division of Neuroscience and Experimental Psychology, School of Biological Sciences, Faculty of
8 Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science
9 Centre, Manchester, UK.
10 [3]Division of Cardiovascular Sciences, University of Manchester, Manchester, UK and Greater
11 Manchester Comprehensive Stroke Centre, Manchester Centre for Clinical Neurosciences,
12 Manchester Academic Health Science Centre, Salford Royal NHS Foundation Trust, Salford, UK
13
14 **Corresponding author**
15 Barry W. McColl, UK Dementia Research Institute, University of Edinburgh, Chancellor's Building,
16 49 Little France Crescent, Edinburgh, EH16 4SB
17 Email: barry.mccoll@ed.ac.uk
18

19 ## ABSTRACT

20 **Aim**: Blockade of the cytokine interleukin-1 (IL-1) with IL-1 receptor antagonist (IL-1Ra) is
21 a candidate treatment for stroke entering phase II/III trials, which acts by inhibiting harmful
22 inflammatory responses. Infection is a common complication after stroke that significantly
23 worsens outcome and is related to stroke-induced deficits in systemic immune function
24 thought to be mediated by the sympathetic nervous system. Therefore, immunomodulatory
25 treatments for stroke, such as IL-1Ra, carry a risk of aggravating stroke-associated infection.
26 Our primary objective was to determine if factors associated with antibody-mediated
27 antibacterial defences were further compromised in patients treated with IL-1Ra after stroke.

28 **Methods:** We assessed plasma concentrations of immunoglobulin isotypes and complement
29 components in stroke patients treated with IL-1Ra or placebo and untreated non-stroke
30 controls using multiplex protein assays. Activation of the SNS was determined by measuring
31 noradrenaline, a major SNS mediator.

32 **Results:** There were significantly lower plasma concentrations of IgM, IgA, IgG1 and IgG4
33 in stroke-patients compared to non-stroke controls, however there were no differences
34 between stroke patients treated with placebo or IL-1Ra. Concentrations of complement

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

components associated with the classical pathway were increased and those associated with the alternative pathways decreased in stroke patients, neither being affected by treatment with IL-1Ra. Noradrenaline concentrations were increased after stroke in both placebo and IL-1Ra-treated stroke patients compared to non-stroke controls.

Conclusion: These data show treatment with IL-1Ra after stroke does not alter circulating immunoglobulin and complement concentrations, and is therefore unlikely to further aggravate stroke-associated infection susceptibility through reduced availability of these key anti-microbial mediators.

Keywords: stroke, IL-1Ra, antibodies, complement, infection

INTRODUCTION

Blocking the actions of the inflammatory cytokine interleukin-1 (IL-1) using a highly selective IL-1 receptor antagonist (IL-1Ra) reduced injury and improved outcome in multiple experimental animal models of cerebral ischemia and is in ongoing clinical stroke trials [1-4]. The inflammatory-modifying properties of IL-1Ra may confer protective effects to the brain after stroke, however due to its potential for immunosuppression, it may also compromise systemic immune responses important for defence against infection. Systemic immune dysregulation is particularly important to consider in the context of stroke as patients are highly susceptible to infection which likely involves roles for stroke-induced impairments in some immune functions [5].

We have previously shown deficits in early antibody responses, particularly IgM, associated with innate-like B cells in both experimental animals and stroke patients that may contribute

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

60 to post-stroke infection susceptibility [6]. Il-1β is reported to induce IgM production in innate-
61 like B cells [7], therefore treatment with IL-1Ra may inhibit these important anti-microbial
62 effects. We assessed if markers associated with antibody-mediated antibacterial defences
63 were compromised in patients treated with IL-1Ra after stroke. Plasma IgM, IgG1, IgG4 and
64 IgA immunoglobulin concentrations were reduced after stroke and this was not further altered
65 by treatment with IL-1Ra. Assessment of complement components indicated induction of the
66 classical pathway of complement activation after stroke but inhibition of the alternative
67 pathway without modulation by IL-1Ra. Plasma noradrenaline was increased after stroke and
68 also not influenced by treatment with IL-1Ra. In summary, our data suggest treatment with
69 IL-1Ra is unlikely to aggravate antibody-associated immune function deficits induced by
70 stroke.
71
72 **METHODS**
73 **Participants and study procedures**
74 In brief, patients ≥ 18 years of age with a clinical diagnosis of stroke within 6 h of stroke
75 onset were eligible. Exclusion criteria included National Institutes of Health Stroke Scale
76 (NIHSS) score of ≤ 4, pre-stroke modified Rankin Scale (mRS) score of ≥4 or rapidly
77 improving neurological deficit. Patients were randomly assigned to treatment with
78 recombinant methionylated human IL-1Ra (n=17) or placebo (n=17) stratified by age (<70
79 and ≥ 70 years), baseline stroke severity (NIHSS score 4-9, 10-20, ≥ 21) and time since
80 stroke onset (<4 or ≥ 4 h) but not by sex. IL-1Ra was initially administered as an IV loading
81 dose of 100 mg over 60 seconds followed by 72 h of consecutive infusions at 2 mg/kg/h. Full
82 patient baseline characteristics and stratification of groups are provided in Supplementary
83 Table e-1.

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

84 Non-stroke control patients (n=13) of a similar age range with no previous history of stroke
85 or transient ischemic attack were also recruited. Control patients were living independently
86 at home, free of infection and able to provide written, informed consent. Controls were
87 matched to stroke patients (6 to patients receiving IL-1Ra and 7 to patients receiving placebo)
88 on a basis of age (±5 years), sex and degree of atherosclerosis.
89

90 **Blood sampling**

91 Venous blood samples were collected prior to initiation of treatment (admission), at the next
92 9am time point (if admission was before 7 am or after 11am), and then at 9 am at 24 h, 2 d, 3
93 d, 4 d and at 5-7 d after stroke, into tubes containing a final concentration of 10 μg/ml
94 pyrogen-free heparin and wrapped in cool packs. Control patients were sampled at 9 am and
95 also at matched patient admission time (2 h) if this was not between 7 and 11 am. Samples
96 were centrifuged 1 h after collection at 2000 *xg* for 30 min at 4°C. Plasma was separated and
97 frozen in aliquots at -70 °C until further analysis.

98 **Standard Protocol Approvals, Registrations, and Patient Consents**

99 This study involved tertiary analysis of plasma samples taken from a randomised, placebo-
100 controlled phase II trial originally designed to determine the safety and biological activity of
101 intravenous (IV) IL-1Ra [4]. The online clinical trials registries ClinicalTrials.gov and ISRCTN
102 went live online during the year 2000, at which time online trial registration was a relatively
103 new recommendation. The original IV IL-1Ra trial was set-up in 2000, and commenced Feb
104 2001 and therefore this trial was not officially registered. Ethical approval for reanalysis of
105 the samples was obtained through the Health Research Authority National Research and
106 Ethics Service Committee (16/NW/0853).
107

108 **Luminex analysis of immunoglobulins and complement components**

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

109 Immunoglobulins and complement components were measured in plasma samples using
110 MILLIPLEX® multiplex assays. Patient details were blinded from samples and coded
111 samples were randomised across plates for analysis. The MILLIPLEX®$_{MAP}$ Human
112 Isotyping Magnetic Bead Panel- Isotyping Multiplex Assay (HGAMMAG-301K-06, Merck
113 Millipore Corporation, Billerica, MA, USA) was used to measure IgG1, IgG2, IgG3, IgG4,
114 IgA and IgM. MILLIPLEX®$_{MAP}$ Human Complement Panel 1 was used to measure C2, C4b,
115 C5, C9, Mannose-binding lectin (MBL), Factor D (Adipsin) and Factor I (HCMP1MAG-
116 19K, Merck Millipore Corporation). Many samples had concentrations of Factor D and
117 Factor I below the detection range of the standard curve and so results for these analytes are
118 not reported. MILLIPLEX®$_{MAP}$ Human Complement Panel 2 was used to measure C1q, C3,
119 C3b/ iC3b, C4, Factor B, Properdin and Factor H (HCMP2MAG-19K, Merck Millipore
120 Corporation). Samples were assayed as singlets and all samples, standards and quality
121 controls were prepared in accordance with the manufacturer's instructions. Samples were
122 incubated with beads on plate for 1 h (Isotyping assay) or overnight (Complement assays) at
123 4°C and washes carried out using a magnetic plate washer. Plates were analysed using a
124 Magpix™ Luminex® machine and Luminex xPonent® software.

125

126 **Measurement of Noradrenaline**

127 Noradrenaline was measured in plasma samples using a Noradrenaline ELISA kit (BA E-
128 5200; LDN®, Nordhorn, Germany). Patient details were blinded from samples and coded
129 samples were randomised across plates for analysis. Samples were assayed as singlets and all
130 samples, standards and quality controls were prepared in accordance with the manufacturer's
131 instructions where noradrenaline is extracted from plasma using a cis-diol-specific affinity
132 gel, acylated, enzymatically converted and then measured by ELISA. Optical density at 450
133 nm was measured using an MRX microplate Reader (Dynatech Labs, Chantilly, VA).

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

134
135
136
137 **Statistical analyses**

138 All immunoglobulin and complement components were measured in μg/ml and the
139 D'Agostino and Pearson omnibus test was used to determine Gaussian distribution of sample
140 data. As data were non-normally distributed, sample values were $\log_{10}$-transformed. As the
141 precise kinetics of individual patient responses may vary, the maximal and minimal
142 concentrations of each mediator in the first 7 d after stroke were compared to non-stroke
143 controls. Maximal and minimal concentrations from IL-1Ra-treated and placebo-treated
144 stroke patients and non-stroke controls were compared by one-way ANOVA with Bonferonni
145 correction. Noradrenaline concentrations were measured in ng/ml and the D'Agostino and
146 Pearson omnibus test was used to confirm Gaussian distribution of sample data. Maximal
147 and minimal noradrenaline concentration from IL-1Ra-treated and placebo-treated stroke
148 patients and non-stroke controls were compared by one-way ANOVA with Bonferonni
149 correction. Data analysis was performed using GraphPad Prism 6.0 statistical analysis
150 software and for all experiments, values of $P \leq 0.05$ were accepted as statistically significant.
151

152 **Data availability**

153 Anonymised data will be shared on reasonable request from any qualified investigator
154

155 **RESULTS**

156 **Plasma IgM concentration is reduced after stroke and is not affected by treatment with**
157 **IL-1Ra**

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

158 Immunoglobulin M (IgM) is the predominant immunoglobulin isotype associated with early
159 B cell antibody responses to infection by innate-like B cells which we have previously shown
160 to be depleted after experimental stroke in mice [6, 8, 9]. Lower minimum concentrations of IgM
161 were measured after stroke in comparison to non-stroke controls, and no difference was
162 found between placebo and IL-1Ra treated patients. (**Figure 1A**). Maximum IgM
163 concentrations in the first 7 days after stroke were also assessed and did not significantly
164 differ in IL-1Ra or placebo treated patients in comparison to non-stroke controls (**Figure e-**
165 **1A**). This indicates that the reduced minimum IgM concentration measured over the first 7d
166 reflects an actual reduction in circulating IgM in stroke patients and is not an artefact of
167 increased variance in IgM concentration after stroke.
168

169 **Plasma IgA, IgG1 and IgG4 concentrations are reduced after stroke and are not**
170 **affected by treatment with IL-1Ra**

171 Minimum IgG1 concentration was significantly reduced in both placebo-treated and IL-1Ra-
172 treated stroke patients in comparison to non-stroke controls (**Figure 1B**). Minimum IgG4
173 (**Figure 1C**) and IgA (**Figure 1D**) concentrations were significantly reduced in placebo-
174 treated stroke patients only. However, there was no significant difference in these
175 immunoglobulins between placebo-treated and IL-1Ra-treated patients. Minimum
176 concentrations of IgG2 (**Figure 1E**) and IgG3 (**Figure 1F**) were not significantly altered in
177 IL-1Ra or placebo treated patients in comparison to non-stroke controls. Maximal circulating
178 concentrations of all immunoglobulin isotypes measured in the first 7 days after stroke were
179 also compared to non-stroke controls and no significant differences were measured in any
180 immunoglobulin isotypes (**Figure e-1**).
181

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

182 **Concentrations of complement components are differentially affected by stroke and not**
183 **affected by treatment with IL-1Ra**

184 As complement components are directly associated with the antibacterial functions of
185 immunoglobulins, we investigated stroke-induced changes in circulating complement
186 components and if any changes observed were further influenced by treatment with IL-1Ra.
187 Stroke induced a significant reduction in the minimum concentrations of C3b/ iC3b (**Figure**
188 **2A**), C3 (**Figure2B**), C4 (**Figure 2C**), Factor H (**Figure 2D**) and Properdin (**Figure 2E**)
189 measured in the first 7 days after stroke in both placebo and IL-1Ra treated patients in
190 comparison to non-stroke controls. Maximum circulating concentrations of these
191 complement components measured in the first 7 days after stroke were also compared to non-
192 stroke controls and no significant differences were seen (**Fig e-2A-E**).

193

194 In contrast, stroke induced a significant increase in maximal circulating concentrations of
195 C1q (**Figure 3A**), C5 (**Figure 3D**) and C9 (**Figure 3E**) in both IL-1Ra and placebo treated
196 patients measured in the first 7 days after stroke in comparison to non-stroke controls.
197 Maximum concentrations of C2 (**Figure 3B**) and C4b (**Figure 3C**) were increased in
198 placebo-treated patients only however no significant difference was apparent between
199 placebo treated and IL-1Ra treated patients for these factors suggesting IL-1Ra treatment
200 exerts no effects additional to stroke. Minimum concentrations of these complement
201 components measured in the first week after stroke were also compared to non-stroke
202 controls and no significant differences were seen (**Figure e-3A-E**).

203

204 Minimal and maximal levels of factor B, mannose-binding lectin (MBL) and C5a measured
205 in the first week after stroke were also compared to non-stroke controls. Concentrations of

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

206 Factor B (**Figure 4A, B**) and MBL (**Figure 4C, D**) were not significantly altered by stroke or
207 by treatment with IL-1-Ra.
208
209
210
211 **Plasma noradrenaline concentration is increased after stroke and is not affected by**
212 **treatment with IL-1Ra**
213 Splenic noradrenaline levels are increased after experimental stroke and may be toxic to IgM
214 producing B cells [6]. Maximum noradrenaline concentration measured in the first 7 days after
215 stroke was increased in both placebo and IL-1Ra treated patients in comparison to non-stroke
216 controls (**Figure 5A**). Treatment with IL-1Ra had no additional effect on noradrenaline
217 concentration when compared to placebo. Minimum noradrenaline concentration measured
218 in the first 7 days after stroke was also measured and was not significantly different to non-
219 stroke controls (**Figure e-4**), or affected by IL-1Ra treatment.
220
221 **DISCUSSION**
222 The IL-1 family of cytokines play a critical role in host defence to pathogens by signalling to
223 a variety of host cells to induce downstream effects including, but not limited to, pro-
224 inflammatory cytokine and chemokine production, immune cell recruitment and upregulation
225 of vascular adhesion molecules [10, 11]. However, in conditions of sterile inflammation and
226 tissue injury, such as stroke, these effects can aggravate primary tissue damage and impair
227 injury repair mechanisms. Blocking IL-1 signalling has shown improved outcome in both
228 experimental animal and patient stroke studies [1, 4, 12]. However, the immunosuppressive
229 effects of blocking IL-1 signalling after stroke may additionally inhibit systemic responses to
230 infection, further increasing the risk of infection in patients who are already immune

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

231 compromised [13, 14]. Indeed, meta-analysis studies have shown an increased risk of serious
232 infection in rheumatoid arthritis patients treated for prolonged periods with the IL-1 blocking
233 drug anakinra [13]. However as of yet this has not been observed in stroke patients potentially
234 reflecting differences in the duration of treatment. No statistically significant differences in
235 infection incidence were seen between IL-1Ra and placebo treated patients in this study with
236 5/17 IL-1Ra treated patients experiencing infection between admission and d7 and 4/17
237 infections in placebo treated patients. Consistent with this pattern we have shown here that
238 relatively short duration of treatment with IL-1Ra after acute stroke did not further affect
239 stroke-induced changes to circulating immunoglobulin, complement or noradrenaline
240 concentrations and is therefore unlikely to further compromise immune defence against
241 infection through reducing the availability of these antibacterial mediators.
242
243 IL-1 cytokine family members are reported to have variable effects on B cell antibody
244 production. IL-1β was reported to be important for the rapid production of anti-bacterial IgM
245 by innate-like B cells important for early containment of infection prior to the induction of
246 adaptive immune responses [7, 15]. This would suggest treatment with IL-1Ra after stroke could
247 further compromise the early production of IgM in innate-like B cells which are already
248 known to be reduced in number after stroke [6]. However, this effect of IL-1Ra on IgM
249 concentrations was not seen. We know that experimental stroke results in a significant loss
250 of many populations of B cells and associated IgM [6], therefore it is possible that the effects of
251 the stroke itself on B cells overwhelm any additional effects of cytokines that could
252 moderately enhance or inhibit immunoglobulin production. Furthermore, we do not know if
253 remaining B cells are functionally impaired and therefore able to respond to IL-1β signalling
254 as they would under normal homeostatic conditions. We have previously reported that stroke
255 is associated with reduced circulating IgM concentrations in comparison to non-stroke

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

256 controls [6], an effect reproduced here. Further studies will be required to determine if IgM, or
257 any of the mediators assessed in this study, would be useful as biomarkers to determine
258 which patients are likely to develop infection after stroke.
259
260 We have shown, for the first time that circulating IgG1, IgG4 and IgA concentrations were
261 reduced in the first 7 d after stroke in comparison to non-stroke controls. This is in
262 agreement with previous data showing that pan-IgG concentrations were reduced in patients
263 after stroke although subclasses of IgG were not assessed in that study and no reduction in
264 IgA was found at the 7 d time point assessed [16]. IgA is the most predominant
265 immunoglobulin isotype at mucosal surfaces including the respiratory tract and is crucial for
266 antibacterial protection at these sites [17]. Given the early reduction of IgA in placebo-treated
267 stroke patients, determining the effect of stroke on IgA-producing B cells at infection
268 susceptible sites such as the lung mucosa could further elucidate if this has an important role
269 in post-stroke infection susceptibility.
270
271 In contrast to the short half-life of IgA and IgM [17-19], the half-lives of IgG1 and IgG4 are
272 reported to be 21 d and therefore an early reduction in IgG concentration is not compatible
273 with a lack of *de novo* production after stroke due to loss of B cells [20]. Previous studies have
274 suggested that reduced total-IgG after stroke may be associated with increased loss or
275 catabolism of IgG which could account for reductions in concentration occurring more
276 rapidly than its natural half-life [21]. An alternative explanation could be that reduced IgG
277 concentration is indicative of vascular risk factors and inflammatory changes preceding
278 stroke that are associated with stroke risk. However, control patients in this study were
279 matched for risk factors including their degree of atherosclerosis and would be expected to
280 show similar changes to stroke patients if these were associated with risk factors.

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

281 Understanding the kinetics of individual immunoglobulin subset changes both preceding, and
282 as a result of stroke, and their associations with post-stroke infections, could be invaluable in
283 providing new therapeutic targets to reduce incidence of infection and improve outcome in
284 patients.

285

286 The complement system has a crucial role in enhancing humoral immune defence and
287 protecting from bacterial infection via interactions with both the innate and adaptive immune
288 systems [22]. As activation of complement is closely associated with efficient
289 immunoglobulin-mediated clearance of pathogens, we determined if these pathways were
290 compromised by stroke. We have assessed for the first time, individual concentrations of
291 multiple complement components covering all pathways of complement activation after
292 stroke. These exploratory data suggest there are no overall deficits in complement activation
293 after stroke. Complement activation pathways converge at multiple points, however their
294 initial activation mechanisms are distinct. The classical complement pathway is activated
295 when IgM or IgG immune complexes bind to C1 (composed of C1q, C1r and C1s) [22, 23].
296 Maximum circulating concentration of complement components associated with the classical
297 and lectin pathways of activation, C1q, C2 and C4b and end stage mediators common to all
298 pathways, C5 and C9 were increased in the first 7 d after stroke in comparison to non-stroke
299 controls. As concentrations of MBL itself was not significantly altered by stroke, this
300 suggests the classical complement pathway is specifically activated after stroke.

301

302 In contrast, the alternative pathway of complement activation is initiated by microbial cell
303 surfaces and polysachharide antigen and results in a cascade that generates C3 [22, 23].
304 Complement components that were significantly downregulated after stroke, C3b/ iC3b, C3,
305 Factor H (fH) and Properdin, are more associated with the alternative pathway of

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

306 complement activation, suggesting that the alternative pathway is suppressed. These data are
307 in agreement with previous studies investigating systemic CRP, C3c and C4 complement
308 concentrations in the serum of patients 24 h after ischemic stroke which concluded the
309 classical pathway of complement activation was activated in the first 24 h after ischemic
310 stroke whereas C3c, associated with the alternative pathway, was reduced [24, 25]. The roles of
311 individual pathways of complement activation in infection susceptibility after stroke remains
312 to be determined but these data suggest overall deficits in complement concentration are
313 unlikely to contribute to reduced antibody-mediated clearance of pathogens that may occur
314 after stroke further supporting reduced circulating immunoglobulins as an important
315 influence on infection susceptibility.
316

317 In this study, circulating noradrenaline concentrations measured in the first week after stroke
318 were increased in comparison to non-stroke controls but were not influenced by treatment
319 with IL-1Ra. This is in agreement with previous studies showing activation of the
320 sympathetic nervous system in both stroke and subarachnoid haemorrhage patients that
321 resulted in increased plasma noradrenaline concentrations that persisted up to 10 days [26-28].
322 Our previous studies have shown that after experimental stroke, activation of the sympathetic
323 nervous system and release of noradrenaline within the spleen is toxic to resident B cells and
324 preventing noradrenaline signalling using the β-blocker propranolol prevented B cell and
325 IgM loss and resulted in reduced infectious burden [6]. The cytokine IL-1β is also increased in
326 the spleen after stroke and is reported to activate peripheral nerves, including the splenic
327 nerve, and increase production of splenic noradrenaline [29, 30]. However blockade of IL-1β
328 signalling did not alter circulating concentrations of noradrenaline after stroke.
329

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

330 In summary, we have shown that treatment with IL-1Ra after stroke does not affect
331 circulating concentrations of immunoglobulins, complement components or noradrenaline
332 and is therefore unlikely to further increase patient susceptibility to infection via pathways in
333 which these mediators are key participants. This is in agreement with data from IL-1Ra
334 Phase 2 trials in which treatment of stroke patients with IL-1Ra did not aggravate incidence
335 of infection [4, 31]. These data suggest that blocking IL-1 in a stroke context may not be
336 concerning from the perspective of increasing infection risk in patients. Additionally, the
337 reductions in circulating immunoglobulin concentrations detected after stroke in this study
338 further support that antibody mediated immune defence may be an important therapeutic
339 target to reduce the burden of infection after stroke.

340

341 **Acknowledgements**

342

343 We thank Dr Hedley Emsley for recruitment of patients and data collection during the
344 original stroke patient study, and for all the participating patients and controls for their
345 participation and consent. We also thank Sharon Hulme for assistance with ethical
346 applications and sample transfer. We thank Merck Millipore Corporation, Billerica, MA,
347 USA for kind provision of the Milliplex®$_{MAP}$ immunoglobulin isotyping and complement
348 panel kits used in this study.

349
350
351 **Abbreviations**

352

353 **CRP** C-Reactive protein

354 **IL-1Ra** IL-1 receptor antagonist

355 **MBL** mannose-binding lectin

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

356 **NIHSS** National Institute of Health Stroke Scale

357 **SNS** sympathetic nervous system

358 **WBC** White blood cell

359

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

360 **References**

361 1. Sobowale OA, Parry-Jones AR, Smith CJ, Tyrrell PJ, Rothwell NJ, Allan SM. Interleukin-1 in
362 Stroke. From Bench to Bedside 2016;47:2160-2167.
363 2. Touzani O, Boutin H, Chuquet J, Rothwell NJ. Potential mechanisms of interleukin-1
364 involvement in cerebral ischaemia. Journal of Neuroimmunology 1999;100:203-215.
365 3. Pradillo JM, Denes A, Greenhalgh AD, et al. Delayed Administration of Interleukin-1 Receptor
366 Antagonist Reduces Ischemic Brain Damage and Inflammation in Comorbid Rats. Journal of Cerebral
367 Blood Flow & Metabolism 2012;32:1810-1819.
368 4. Emsley HCA, Smith CJ, Georgiou RF, et al. A randomised phase II study of interleukin-1
369 receptor antagonist in acute stroke patients. Journal of Neurology, Neurosurgery & Psychiatry
370 2005;76:1366-1372.
371 5. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med
372 2011;17:796-808.
373 6. McCulloch L, Smith CJ, McColl BW. Adrenergic-mediated loss of splenic marginal zone B cells
374 contributes to infection susceptibility after stroke. Nature Communications 2017;8:15051.
375 7. del Barrio L, Sahoo M, Lantier L, Reynolds JM, Ceballos-Olvera I, Re F. Production of Anti-LPS
376 IgM by B1a B Cells Depends on IL-1β and Is Protective against Lung Infection with Francisella
377 tularensis LVS. PLOS Pathogens 2015;11:e1004706.
378 8. Martin F, Oliver AM, Kearney JF. Marginal Zone and B1 B Cells Unite in the Early Response
379 against T-Independent Blood-Borne Particulate Antigens. Immunity 2001;14:617-629.
380 9. Baumgarth N, Herman OC, Jager GC, Brown L, Herzenberg LA, Herzenberg LA. Innate and
381 acquired humoral immunities to influenza virus are mediated by distinct arms of the immune
382 system. Proceedings of the National Academy of Sciences 1999;96:2250-2255.
383 10. Palomo J, Dietrich D, Martin P, Palmer G, Gabay C. The interleukin (IL)-1 cytokine family –
384 Balance between agonists and antagonists in inflammatory diseases. Cytokine 2015;76:25-37.
385 11. Dinarello CA, Simon A, van der Meer JWM. Treating inflammation by blocking interleukin-1
386 in a broad spectrum of diseases. Nature Reviews Drug Discovery 2012;11:633.
387 12. Rothwell NJ. Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic
388 potential. Brain, Behavior, and Immunity 2003;17:152-157.
389 13. Salliot C, Dougados M, Gossec L. Risk of serious infections during rituximab, abatacept and
390 anakinra treatments for rheumatoid arthritis: meta-analyses of randomised placebo-controlled
391 trials. Annals of the Rheumatic Diseases 2009;68:25-32.
392 14. Westendorp W, Nederkoorn P, Vermeij J-D, Dijkgraaf M, van de Beek D. Post-stroke
393 infection: A systematic review and meta-analysis. BMC Neurology 2011;11:110.
394 15. Zouali M, Richard Y. Marginal zone B-cells, a gatekeeper of innate immunity. Frontiers in
395 Immunology 2011;2.
396 16. Liesz A, Roth S, Zorn M, Sun L, Hofmann K, Veltkamp R. Acquired Immunoglobulin G
397 deficiency in stroke patients and experimental brain ischemia. Experimental Neurology 2015;271:46-
398 52.
399 17. Mkaddem SB, Christou I, Rossato E, Berthelot L, Lehuen A, Monteiro RC. IgA, IgA Receptors,
400 and Their Anti-inflammatory Properties. In: Daeron M, Nimmerjahn F, eds. Fc Receptors. Cham:
401 Springer International Publishing, 2014: 221-235.
402 18. Fahey JL, Sell S. THE IMMUNOGLOBULINS OF MICE: V. THE METABOLIC (CATABOLIC)
403 PROPERTIES OF FIVE IMMUNOGLOBULIN CLASSES. The Journal of Experimental Medicine
404 1965;122:41-58.
405 19. Sigounas G, Harindranath N, Donadel G, Notkins AL. Half-life of polyreactive antibodies.
406 Journal of Clinical Immunology 1994;14:134-140.
407 20. Morell A, Terry WD, Waldmann TA. IgG subclasses: Physical properties, genetics and
408 biological functions. J Clin Invest 1970;1970:673-680.
409 21. Liesz A, Dalpke A, Mracsko E, et al. DAMP Signaling is a Key Pathway Inducing Immune
410 Modulation after Brain Injury. The Journal of Neuroscience 2015;35:583-598.

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

411 22. Kemper C, Atkinson JP. T-cell regulation: with complements from innate immunity. Nature
412 Reviews Immunology 2006;7:9.
413 23. Holers MV. Complement and Its Receptors: New Insights into Human Disease. Annual
414 Review of Immunology 2014;32:433-459.
415 24. Pedersen ED, Waje-Andreassen U, Vedeler CA, Aamodt G, Mollnes TE. Systemic complement
416 activation following human acute ischaemic stroke. Clinical & Experimental Immunology
417 2004;137:117-122.
418 25. Di Napoli M. Systemic Complement Activation in Ischemic Stroke. Stroke 2001;32:1443-
419 1448.
420 26. Naredi S, Lambert G, Edén E, et al. Increased sympathetic nervous activity in patients with
421 non-traumatic subarachnoid hemorrhage. Stroke 2018;31:901-906.
422 27. Urra X, Cervera Á, Obach V, Climent N, Planas AM, Chamorro Á. Monocytes Are Major
423 Players in the Prognosis and Risk of Infection After Acute Stroke. Stroke 2009;40:1262-1268.
424 28. Chamorro A, Amaro S, Vargas M, et al. Catecholamines, infection, and death in acute
425 ischemic stroke. J Neurol Sci 2007;252.
426 29. Schwarting S, Litwak S, Hao W, Bähr M, Weise J, Neumann H. Hematopoietic Stem Cells
427 Reduce Postischemic Inflammation and Ameliorate Ischemic Brain Injury. Stroke 2008;39:2867-2875.
428 30. Niijima A, Hori T, Aou S, Oomura Y. The effects of interleukin-1β on the activity of adrenal,
429 splenic and renal sympathetic nerves in the rat. Journal of the Autonomic Nervous System
430 1991;36:183-192.
431 31. Smith CJ, Hulme S, Vail A, et al. SCIL-STROKE (Subcutaneous Interleukin-1 Receptor
432 Antagonist in Ischemic Stroke). A Randomized Controlled Phase 2 Trial 2018;49:1210-1216.

433
434

bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

435 **Figure 1 Reduced plasma IgM , IgA, IgG1 and IgG4 after stroke is not affected by IL-1Ra**

436 **(A)** Minimum IgM concentration measured in the first 7 d after stroke was lower in both placebo and
437 IL-1Ra treated patients in comparison to healthy controls. Data show mean ±SD, * $P<0.05$, **
438 $P<0.01$, one-way ANOVA with Bonferonni correction. **(B)** Minimum concentration of IgG1 and
439 measured in the first 7 d after stroke was reduced in both placebo and IL-1Ra treated patients in
440 comparison to healthy controls. Minimum IgG4 **(C)** and IgA **(D)** concentrations were reduced in
441 placebo-treated stroke patients in comparison to healthy controls. There was no significant difference
442 between placebo-treated and IL-1Ra-treated stroke patients. No significant difference in IgG2 **(E)** and
443 IgG3 **(F)** concentration was detected between placebo-treated and IL-1Ra-treated stroke patients in
444 comparison to healthy controls. Data show mean ±SD, * $P<0.05$; ** $P<0.01$; one-way ANOVA with
445 Bonferonni correction.

446

447 **Figure 2 Treatment with IL-1Ra has no effect on complement components downregulated after**
448 **stroke**

449 Minimum concentrations of **(A)** C3b/ iC3b, **(B)** C3, **(C)** C4, **(D)** Factor H and **(E)** Properdin were
450 measured in the first 7 d after stroke were reduced in both placebo and IL-1Ra treated patients in
451 comparison to healthy controls. Data show mean ±SD, * $P<0.05$; ** $P<0.01$; one-way ANOVA with
452 Bonferonni correction.

453

454 **Figure 3 Treatment with IL-1Ra has no effect on complement components upregulated after**
455 **stroke**

456 Maximum concentrations of **(A)** C1q, **(B)** C2, **(C)** C4b, **(D)** C5 and **(E)** C9 were measured in the first
457 7 d after stroke were increased in both placebo and IL-1Ra treated patients in comparison to healthy
458 controls. Data show mean ±SD, * $P<0.05$; ** $P<0.01$; one-way ANOVA with Bonferonni correction.

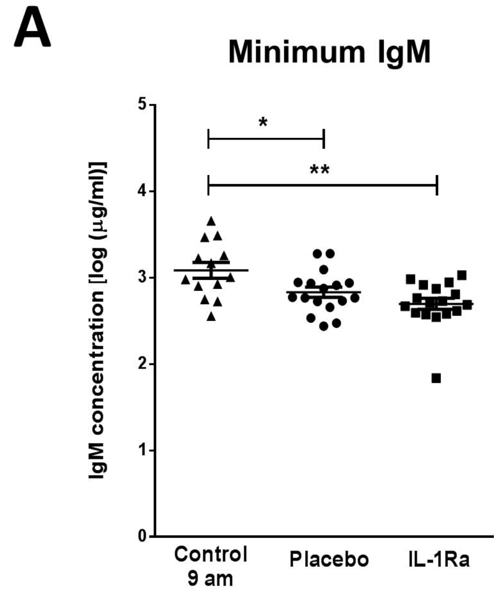
459

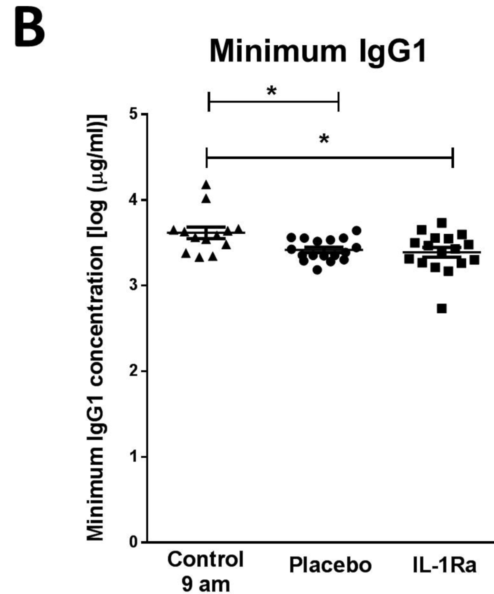
460 **Figure 4 Treatment with IL-1Ra has no additional effect on complement components unaffected**
461 **by stroke**

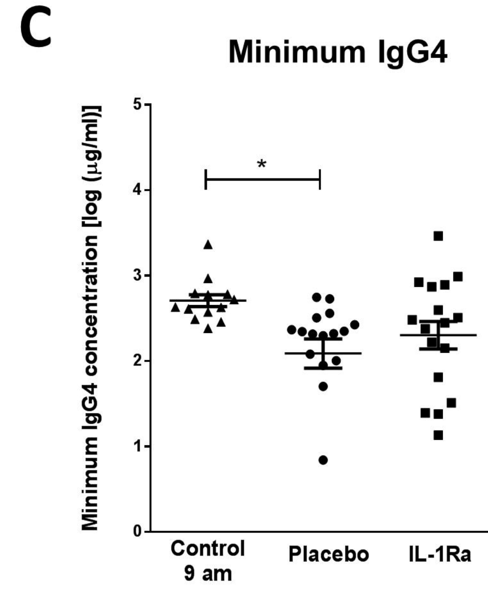
bioRxiv preprint doi: https://doi.org/10.1101/587881; this version posted March 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

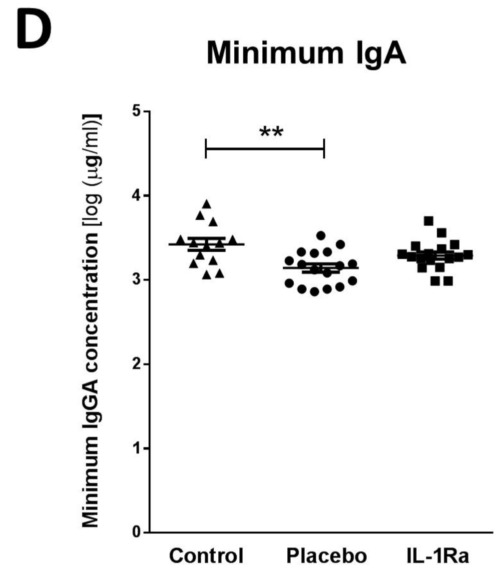
462 Minimal and maximum concentrations of **(A, B)** Factor B and **(C, D)** MBL were measured in the first
463 7 d after stroke were unchanged in both placebo and IL-1Ra treated patients in comparison to healthy
464 controls. Data show mean ±SD, one-way ANOVA with Bonferonni correction.
465
466 **Figure 5 Plasma noradrenaline concentration is increased after stroke and is not affected by**
467 **treatment with IL-1Ra**
468 **(A)** Maximal noradrenaline concentration measured in the first 7 d after stroke was significantly
469 higher in both placebo and IL-1Ra treated patients in comparison to controls. Data show mean ± SD
470 $*P<0.05$; $**P<0.01$; one-way ANOVA.
471
472
473

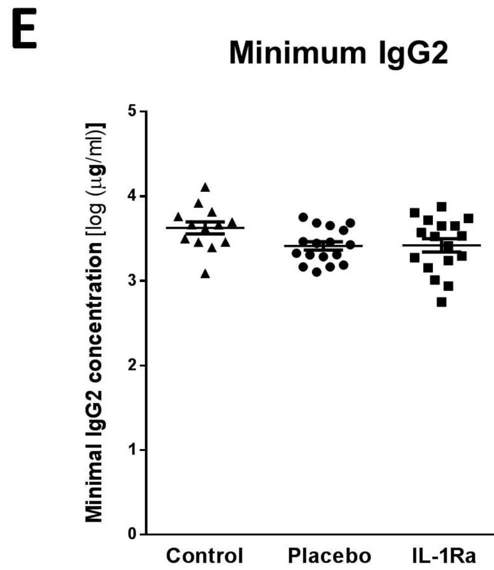
Figure 1











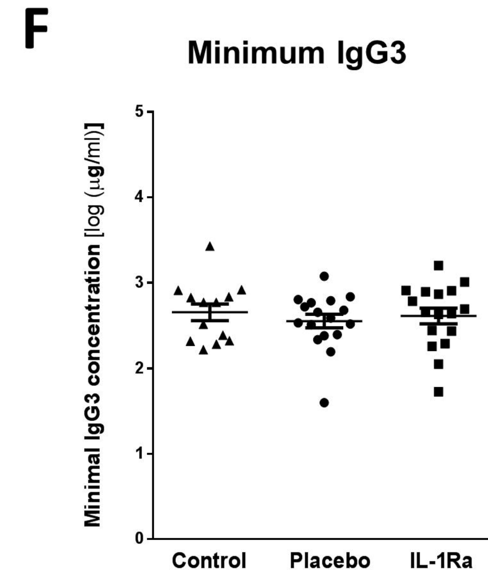
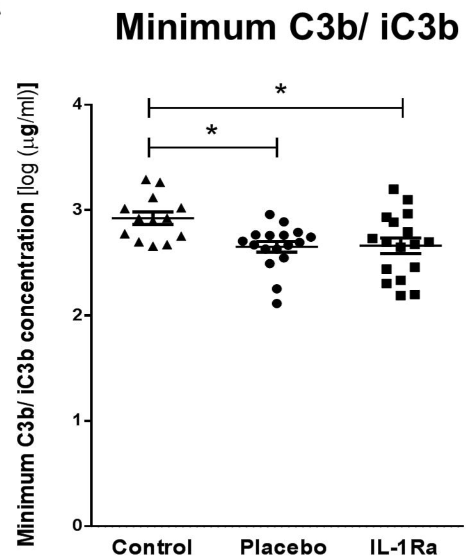
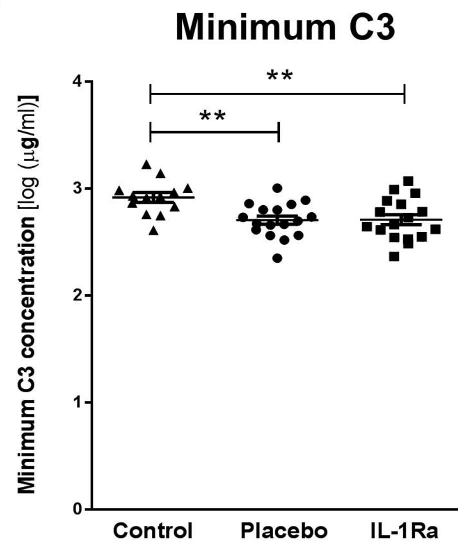
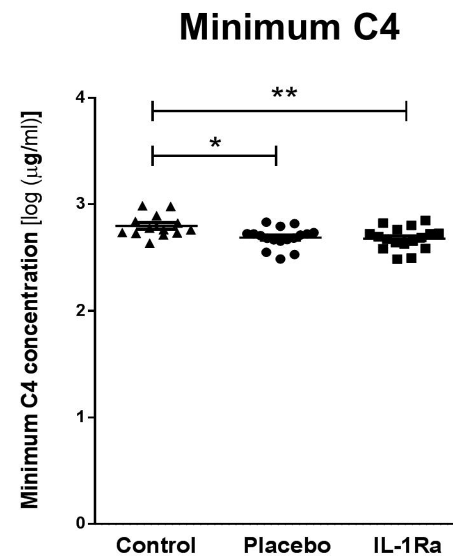
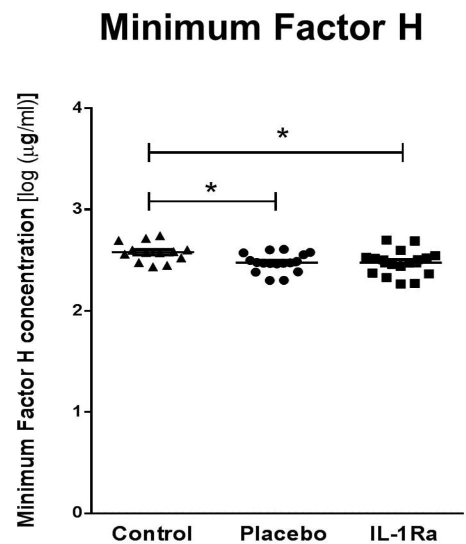


Figure 2









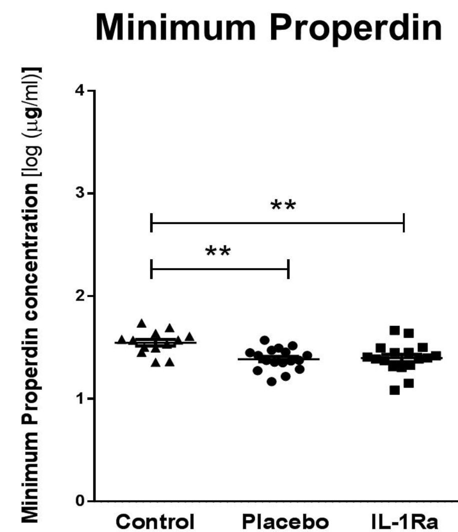
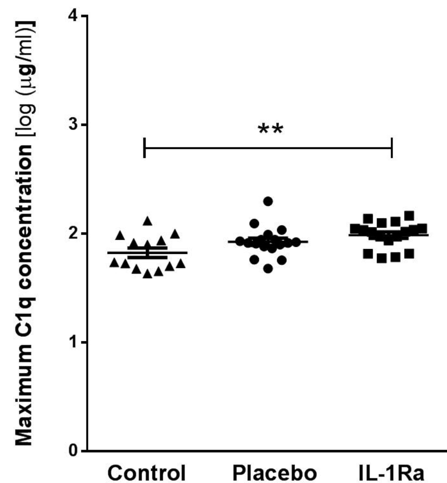
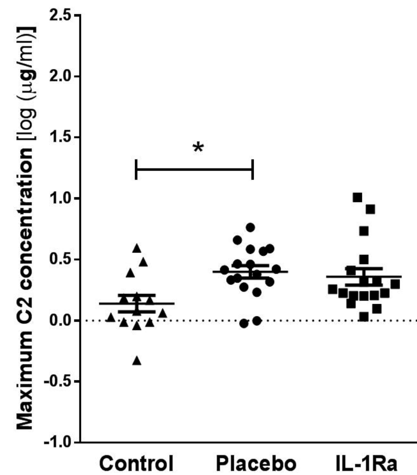
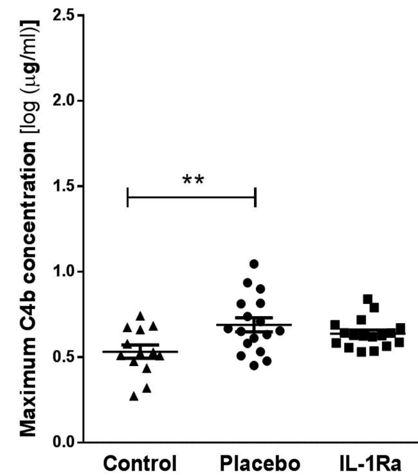
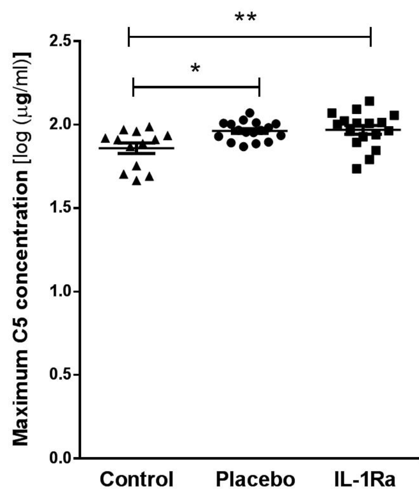


Figure 3









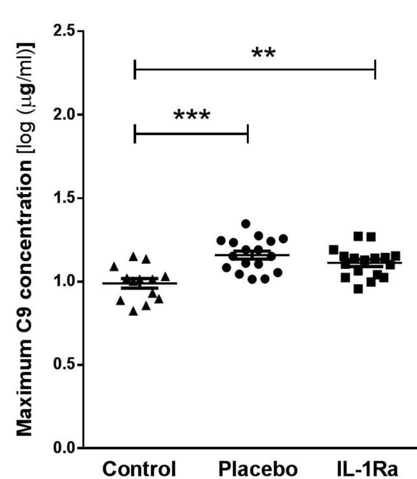
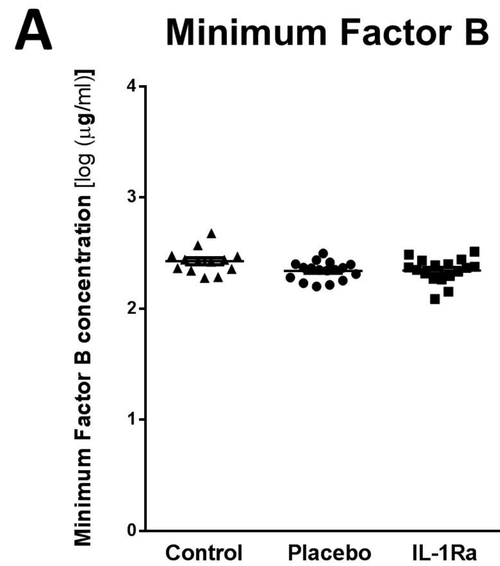
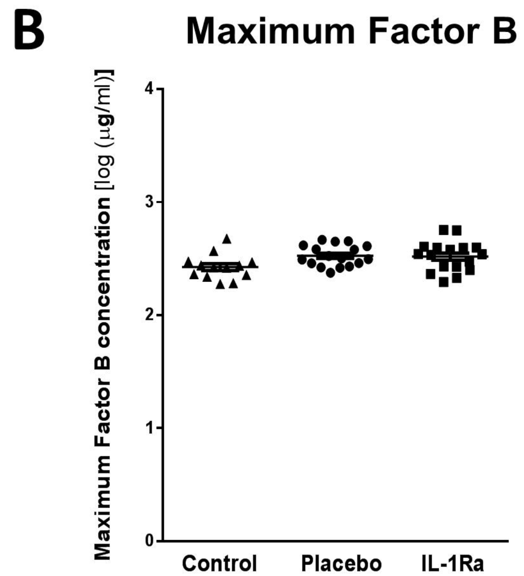
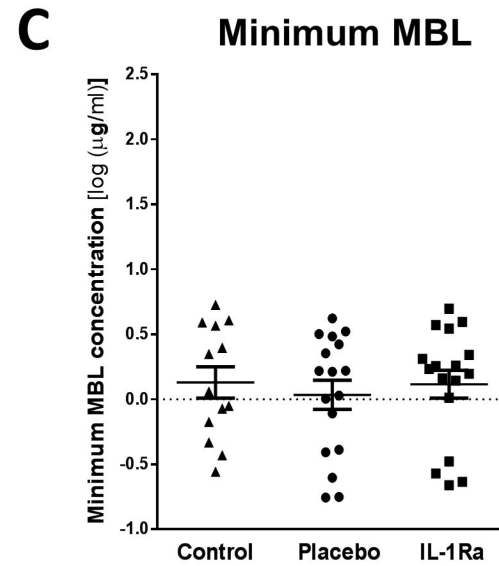


Figure 4







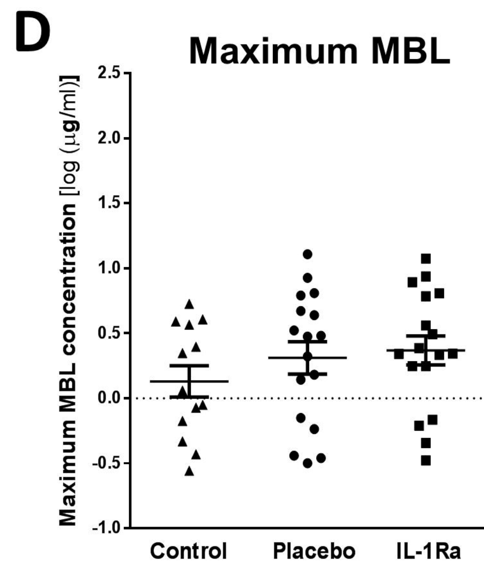


Figure 5