

1 **Altered B cells homeostasis in child-onset immunoglobulin A vasculitis**

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24 **Running title** : B cells in IgA vasculitis

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45 **Abstract**

46 **Background:** Immunoglobulin A vasculitis (IgAV), also called Henoch–Schönlein purpura, is
47 a systemic small vessels vasculitis with immunoglobulin A1-dominant immune deposits. B-
48 cells are a heterogeneous population with unique subsets distinguished by their phenotypes and
49 cytokine production. Here, we explored the status of B cell subsets in patients with IgAV.

50 **Methods:** Thirty IgAV patients and fifteen age- and sex-matched healthy individuals were
51 enrolled in this study. Fresh blood samples were collected from both healthy and IgAV patients.
52 Upon the distinct expressions of CD3, CD19, CD20, CD38, CD27 and IgD, peripheral blood
53 mononuclear cells (PBMCs) were initially categorized into plasmablasts and memory B cells.
54 Subsequently, using surface markers including CD138 and IgM, and intracellular markers
55 containing IgM and IgG, plasmablasts and memory B cells were further divided into distinct
56 subgroups. A total of eleven populations were detected using multiple flow cytometry.

57 **Results:** CD3⁻CD19⁺IgD⁺CD27⁻, CD3⁻CD19⁺CD20⁻CD38⁺, CD3⁻CD19⁺CD20⁻CD38⁺IgM⁺,
58 and CD3⁻CD19⁺CD20⁻CD38⁺CD138⁺ B cells were larger in patients with IgAV than in the
59 HCs. Only CD3⁻CD19⁺IgD⁻CD27⁺IgM⁺ B cell counts were reduced in IgAV. The elevated B
60 cell numbers returned to normal after treatment. Plasma and plasmablast B cell numbers
61 correlated with plasma IgA levels. On the contrary, CD3⁻CD19⁺IgD⁻CD27⁺IgM⁺ B cell
62 numbers were negatively proportional to the plasma IgA levels while naïve B cell numbers
63 correlated with plasma and plasmablast B cell counts.

64 **Conclusions:** We hypothesized that immunoglobulin production was abnormally elevated in
65 IgAV and could be explained by altered B-cell subset homeostasis.

66 **Keywords:** B cell subset, IgA vasculitis, Henoch–Schönlein purpura

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88 **Background**

89 Immunoglobulin A vasculitis (IgAV), formerly referred to as Henoch–Schönlein
90 purpura, is the most common form of childhood vasculitis. Skin biopsy of the vasculitis lesions
91 reveal small-vessel leukocytoclastic vasculitis [1, 2]. The scarce epidemiological data for
92 childhood IgAV, mainly pertaining to European populations, indicates annual incidence rates
93 from approximately, 3 to 27 in every 100,000 children [3]. Although IgA1-immune deposits,
94 complement factors and neutrophil infiltration in endothelial cells, are widely accepted as
95 characteristic features of IgAV, the casual pathogenic mechanism is yet to be resolved [4, 5].
96 Additionally, genes also play a crucial role in the pathogenesis of IgAV [6]. It can be triggered
97 by chlamydia, bacteria, viruses, mycoplasma, *Helicobacter pylori*, or infection by parasitic
98 agents. Symptoms include palpable purpura or petechiae, (poly)arthralgia, gastrointestinal
99 disturbances and glomerulonephritis [7]. Usually, IgAV is self-limiting, but glomerulonephritis
100 in some patients may lead to end-stage renal disease. The prognosis of IgAV is predominantly
101 dependent on the extent of kidney damage [4, 8]. Although IgAV is largely considered as a T
102 cell-mediated disease, several studies have already demonstrated that hyperactivation of T cell
103 subsets, as well as a decline in autoreactive natural killer cell numbers, may also be contributing
104 factors as these cells are key players in the humoral immune response. Additionally, increased
105 serum interleukin (IL)-4, -6, and -17 concentrations have also been found in patients with IgAV
106 [9].

107 Although T cells have been be involved in human diseases, data on the pathogenesis of B
108 cell subsets are relatively limited. B lymphocytes play a critical role in adaptive immune
109 response, partly by producing high affinity antibodies to pathogens. However, accumulating
110 evidence suggests the pathogenic role of B cells in autoimmune diseases. B cells have a critical

111 role in the initiation and development of several autoimmune diseases such as systemic lupus
112 erythematosus (SLE). IgAV is also caused by destabilized immunity homeostasis. It is thus
113 interesting to explore if pathogenic mechanisms proposed for those diseases may also be
114 involved in IgAV. Upon activation, class switch and differentiation of B cells are regulated by
115 T cells through cytokines and cognate interactions[10]. Additionally, B cells also regulate T
116 cell activation through antigen presentation, production of cytokines and costimulatory
117 molecules, and recruiting T cell subsets and dendritic cells [11]. B-cells are a heterogeneous
118 population with different subsets distinguished by their phenotypes and cytokine production
119 [12]. Several observations regarding the role of plasma B cell in IgAV have been highlighted
120 [13]. It is important to understand the key role of B cells in IgAV, as this may lead to the
121 development of new therapeutic strategies to prevent disease.

122 However, little information is available on the number of different B cell subsets in
123 patients with IgAV as well as the potential relationship between the subsets. The aim of our
124 study was to describe the altered B cell homeostasis in child-onset IgAV. B cell subsets were
125 determined by flow cytometry using CD19, CD20, CD38, CD138, IgM, and IgG. To the best
126 of our knowledge, we are the first group to show that IgAV patients exhibit an altered peripheral
127 blood B-cell subset distribution.

128 **Methods**

129 *Patients*

130 Written informed consents were obtained from parents or guardians of all study participants.
131 The experimental protocol followed the guidelines of the Declaration of Helsinki and was
132 approved by the Human Ethics Committee of Jilin University (Jilin University, Changchun,

133 China). Thirty patients were prospectively included if they fulfilled the following criteria : (1)
134 children younger than 18 years of age; (2) met the European League Against
135 Rheumatism/Pediatric Rheumatology International Trials Organization/Pediatric
136 Rheumatology European Society criteria for IgAV [14]: palpable purpura (mandatory) and one
137 of following findings: histopathology (typical LCV with predominant IgA deposits or
138 proliferative glomerulonephritis with predominant IgA deposits); abdominal pain (Diffuse
139 abdominal colicky pain, intussusception and gastrointestinal bleeding); arthritis or arthralgia;
140 renal involvement: proteinuria > 0.3 g/24 h or > 30 mmol/mg of urine albumin/creatinine ratio
141 on a spot morning sample, hematuria [> 5 red blood cells (RBCs)/high-powered field or $\geq 2+$
142 on dipstick or RBC casts in urinary sediment.

143 Given the self-limiting and benign course of IgAV, symptom-oriented and supportive
144 therapies were administered to patients following admission. Glucocorticoids and/or
145 immunosuppressants (such as cyclophosphamide) were administered. Remission following
146 treatment was defined by two criteria: (1) after 2 weeks, all skin purpura improved, with no
147 appearance of new rashes; and (2) All symptoms were alleviated. Only 25 of the total patients
148 entered remission. There were 3 patients with recurrent skin purpura and 2 with obstinate
149 abdominal pain. We randomly selected 15 patients at the remission stage. The prognosis of
150 IgAV is mostly benign; therefore, blood samples were collected from patients who had
151 successfully entered remission.

152 A total of 15 sex- and age-matched healthy controls (HCs) were recruited for the study.
153 All participants underwent a routine blood test: measurement of serum immunoglobulin and
154 complement levels using a specific protein analyzer (BN-II; Siemens, München, Germany),

155 serum C-reactive protein (CRP) level using the QuikRead go CRP kit (Orion Diagnostica,
156 Espoo, Finland), urinary protein level using a P800 biochemical analyzer (Roche, Mannheim,
157 Germany), and urinary RBC and white blood cell (WBC) counts using a UF-1000 automatic
158 urinary sediment analyzer (Sysmex, Kobe, Japan).

159 *Isolation of peripheral blood mononuclear cells*

160 Peripheral blood mononuclear cells (PBMCs) were isolated from HCs and patients with IgAV
161 in the acute and convalescent stages following density-gradient centrifugation using Ficoll-
162 Paque Plus (Amersham Biosciences, Little Chalfont, UK) at $800 \times g$ for 30 min at 25°C.

163 *Flow cytometry*

164 PBMCs at $4 \times 10^6/\text{ml}$ were analyzed by multicolor flow cytometry (FACSAria II; BD
165 Biosciences, Franklin Lakes, NJ, USA). Human PBMCs (10^6 cells/tube) were stained with CD3,
166 CD19, CD20, CD38, CD138, IgD, CD27, IgM and IgG. We detected 11 subpopulations of B
167 cells: CD3-C19⁺, CD3-C19⁺CD20-CD38⁺ (plasmablasts B cell), CD3-C19⁺ CD20-
168 CD38⁺CD138⁺(plasma B cell), CD3-C19⁺CD27-IgD⁺(naïve B cell), CD3-C19⁺CD27-IgD-
169 (double negative B cell), CD3-C19⁺ CD27⁺IgD-(post-switch memory B cell), CD3-C19⁺
170 CD27⁺IgD-IgM⁺, CD3-C19⁺ CD27⁺IgD-IgG⁺,CD3-C19⁺CD27⁺IgD⁺ (pre-switch memory B
171 cell)B cells, at room temperature for 30 min. Subsequently, CD3-C19⁺CD20-CD38⁺ B cells
172 were fixed, permeabilized, and stained with IgG and IgM (a component of the B cell
173 receptor) . Data were processed using FlowJo v.5.7.2 software (Tree Star, Ashland, OR, USA).

174 *Statistical analysis*

175 Data are expressed as the median and range. Kruskal Wallis test was applied to assess the
176 difference among groups. The correlation analysis was evaluated using Spearman's rank

177 correlation test. The difference between the acute and remission stage was analyzed by the
178 Wilcoxon matched pairs test. Statistical analyses were performed using SPSS 22.0 software.
179 Differences in means were considered statistically significant when two-sided P values were <
180 0.05.

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182 **Results**

183 *Clinical characteristics*

184 The demographic and clinical characteristics of the study subjects are shown in Table 1. All
185 patients presented with palpable skin purpura, especially on the lower extremities and buttocks.
186 Upon recruitment, the WBC count ($P < 0.001$), and the platelet ($P = 0.0132$), serum IgA ($P =$
187 0.0243), IgE ($P = 0.0411$), CRP ($P = 0.0213$) and complement C4 ($P = 0.0467$) levels were
188 higher in patients with IgAV than in the HCs (Table 1). No sequelae or other complications
189 were noted.

190 **Table 1.** Demographic and clinical characteristics of the study subjects

	IgAV (n = 30)	Healthy controls (n = 15)
Age, year	6 (3–13)	6 (3–12)
Female/male	14/16	6/9
WBC, $10^9/l$	10.45 (3.27–20.11)*	7.6 (4.23–10.15)
Lymphocytes, $10^9/l$	3.45 (1.8–5.65)	3.52 (1.26–5.12)
Platelet, g/l	379 (165–551)*	258 (134–345)
Serum IgA, g/l	2.25 (0.95–5.97)*	1.56 (0.96–3.89)
Serum IgG, g/l	11.5 (1.2–16.8)	9.1 (0.9–17.1)

Serum IgM, g/l	1.21 (0.78–3.32)	1.16 (0.72–3.13)
Serum IgE, g/l	87.5 (23.9–637.0)*	32.1 (19.3–80.4)
Serum C3, g/l	1.41 (0.91–1.62)	1.45 (1.1–1.78)
Serum C4, g/l	0.38 (0.15–0.48)*	0.28 (0.16–0.55)
Serum CRP (mg/l)	8.5 (1.75–75.00)*	2.5 (0.85–5.2)

191 CRP, C-reactive protein; WBC, white blood cell. * $P < 0.05$ vs. healthy controls.

192 ***Detection of circulating naïve and memory B cells in IgAV patients***

193 To investigate the status of B cells in IgAV, we detected the number of four subsets CD3⁻
194 C19⁺CD27-IgD⁺ (naïve B cell), CD3⁻C19⁺CD27-IgD⁻ (double negative B cell), CD3⁻C19⁺
195 CD27⁺IgD⁻ (post-switch memory B cell) and CD3⁻C19⁺CD27⁺IgD⁺ (pre-switch memory B cell)
196 B cells, which were gated from CD3⁻CD19⁺ B cells in flow cytometry analysis of 30 active
197 IgAV patients, 15 patients in remission and HCs (Fig. 1). Circulating CD3⁻C19⁺ and CD3⁻
198 C19⁺CD27-IgD⁺ B cell-counts were increased in active IgAV patients relative to those in the
199 HCs ($P = 0.0203$, $P = 0.0342$, respectively) (Fig. 2B). However, the number of circulating CD3⁻
200 C19⁺CD27-IgD⁻, CD3⁻C19⁺ CD27⁺IgD⁻ and CD3⁻C19⁺CD27⁺IgD⁺ cells did not differ between
201 the two groups ($P > 0.05$; Fig. 2A). Furthermore, we found that CD3⁻C19⁺ CD27⁺IgD⁻ IgM⁺ B
202 cell counts, but not CD3⁻C19⁺ CD27⁺IgD⁻ IgG⁺ B cells were decreased ($P = 0.0452$; Fig. 2B)
203 in active IgAV compared to that in HCs. All the circulating naïve and memory B cells had no
204 difference between the 15 patients in remission and HCs.

205 ***Circulating plasmablasts and plasma B cells in IgAV patients***

206 Next, we detected the cell number of four subsets, i.e. CD3⁻C19⁺ CD20⁻CD38⁺ (plasmablasts
207 B cell), CD3⁻C19⁺ CD20⁻CD38⁺CD138⁺ (plasma B cell), CD3⁻C19⁺ CD20⁻CD38⁺IgM⁺, CD3⁻

208 C19⁺ CD20⁻CD38⁺ IgG⁺ B cells from 30 active patients with IgAV and HCs (Fig. 1B).
209 Circulating CD3⁻C19⁺ CD20⁻CD38⁺ (plasmablasts B cell), CD3⁻C19⁺ CD20⁻CD38⁺CD138⁺
210 (plasma B cell), CD3⁻C19⁺ CD20⁻CD38⁺IgM⁺ and CD3⁻C19⁺ CD20⁻CD38⁺IgG⁺ B cells in
211 active IgAV were greater than that in HCs (P = 0.0105, P = 0.0023, P = 0.0036 and P = 0.0145,
212 respectively) (Fig. 2A). All the plasmablasts and plasma B cells had no difference between the
213 15 patients in remission and HCs.

214 *Alterations in B cell subsets following treatment*

215 Following symptom-oriented and supportive therapies, the majority of patients successfully
216 went into remission. We examined the above B cell subsets in 15 patients in remission (Fig.
217 3). Naïve, plasmablasts, plasma B cells were reduced relative to the absolute value in the active
218 stage (P = 0.0383, 0.0026, and 0.0020, respectively). There were no changes in CD3⁻
219 C19⁺CD27⁺IgD⁻ (P > 0.05; Fig. 3C) and CD3⁻C19⁺CD27⁺IgD⁻IgM⁺ (P > 0.05; Fig. 3E) levels
220 during remission.

221 *Association between B cell subsets and clinical parameters*

222 We investigated whether alterations in B cell subsets were associated with disease etiology and
223 progression, and found that the number of CD19⁺CD20⁻CD38⁺ (r = 0.4320, P = 0.0171),
224 CD19⁺CD20⁻CD38⁺CD138⁺ (r = 0.5316, P = 0.0025), CD19⁺CD20⁻CD38⁺IgM⁺ (r = 0.5847,
225 P = 0.0007), but not of CD20⁻IgD⁺CD27⁻ (r = 0.1393, P = 0.4628) B cells was positively
226 correlated with serum IgA levels (Fig. 4A). By contrast, circulating CD19⁺IgD⁻CD27⁺IgM⁺ B
227 cell counts were inversely related to serum IgA levels (r = -0.3755, P = 0.0409; Fig. 4A).
228 Additionally, CD19⁺CD20⁻CD38⁺ (r = 0.0545, P = 0.7749), CD19⁺CD20⁻CD38⁺CD138⁺ (r =
229 -0.1008, P = 0.5962), CD19⁺CD20⁻CD38⁺IgM⁺ (r = 0.1174, P = 0.5369), CD19⁺IgD⁺CD27⁻

230 (r = 0.0520, P = 0.7850) and CD19⁺IgD⁺CD27-IgM⁺ (r = -0.0092, P = 0.6019) cell subsets
231 showed no association with serum C4 in the IgAV, respectively. We also explored the
232 relationship between B cell subsets and serum IgA level/C4 in the HCs, there is no statistical
233 significance (data not shown).

234 *The relationship among the different B cell subsets*

235 Meanwhile, we analyzed the potential relationship between the numbers of the circulating B
236 cell subsets in the IgAV patients. Circulating CD19⁺IgD⁺CD27⁻ naïve B cell counts correlated
237 with the number of CD19⁺CD20⁻CD38⁺ (r = 0.6620, P = 0.0002), CD19⁺CD20⁻CD38⁺CD138⁺
238 (r = 0.6571, P < 0.0001), CD19⁺CD20⁻CD38⁺IgM⁺ (r = 0.4821, P = 0.0070) B cells. But there
239 was no relationship observed between CD19⁺CD20⁻CD38⁺ (r = 0.1927, P = 0.3077),
240 CD19⁺CD20⁻CD38⁺CD138⁺ (r = -0.3103, P = 0.0784), CD19⁺IgD⁺CD27⁻(r = 0.1592, P =
241 0.4007) and CD19⁺IgD⁻CD27⁺IgM⁺ B cells.

242 **Discussion**

243 IgAV is a common vasculitis with an early age of onset, triggered by environmental and
244 genetic factors, and is associated with a history of often URTI. Although the exact
245 pathogenesis has not yet been elucidated, interaction between the T and B cell lineages is
246 considered a key underlying cause. It has long been presumed that aberrant deposition of
247 glycosylated IgA₁ and complement activation contribute to IgAV. In this study, increased
248 levels of IgA were found in the patient's peripheral blood, IgA is mostly known as the major
249 antibody subset present in mucosal areas, where it plays a key role in mucosal defense.
250 Approximately 90% of the IgA present in the circulation is IgA1 while <10% is IgA2. It is thus
251 plausible that the level of IgA in peripheral blood is equivalent to IgA1. When immune

252 complexes are deposited, they activate the complement pathway. Complement is present in
253 inactive form in the circulation, and three pathways can lead to the activation of complement.
254 However, it has been demonstrated that IgA can induce the mannan-binding lectin and
255 alternative complement pathways. GalNAc on the surface of pathogens may facilitate the
256 production of cross-reactive IgA and IgG, which recognize Gd-IgA1 (Galactose-deficient IgA1)
257 [15]. We believe that Gd-IgA1 complexes are deposited in the vasculature due to the aberrant
258 elevation of B cell numbers, thereby inducing neutrophil migration and activation with
259 concomitant tissue damage. Vascular damage is induced by IgA via inflammatory processes
260 including antibody-dependent cell-mediated cytotoxicity (ADCC), reactive oxygen species
261 (ROS) production, and neutrophil extracellular traps (NETs) formation. Additionally, IgA
262 stimulation of neutrophils leads to the release of LTB₄, inducing subsequent neutrophil
263 migration in a positive feedback loop [15].

264 In our previous work, we demonstrated that Tfh cell subpopulations contributed
265 differentially to IgAV pathogenesis and remission [9]. T follicular helper (TFH) cells are
266 specialist providers of B cell help, notably by the secretion of IL-21. Upon activation, B cells interact
267 with TFH cells and can promote the maturation of B cell response within GCs, thereby leading
268 to autoantibody secretion. Although not completely understood, the situation is somewhat
269 similar in the B cell compartment. In fact, the expansion of CD19⁺ B cells has been implicated
270 in IgAV by a previous study [13]. In support of this data, we detected a greater level of B cell
271 subsets in IgAV and believe that an altered balance of circulating B cell subsets may be
272 associated with IgAV.

273 B cells, as part of the adaptive immunity, are responsible for the humoral responses against

274 pathogens and produce a significant amount of antibodies [16]. Dysregulated frequency of T
275 and B cells can be found in various human autoimmune diseases such as rheumatoid arthritis,
276 systemic lupus erythematosus, and multiple sclerosis, with different cytokines, transcription
277 factors. From studies conducted previously in our lab, we learned that IgAV is mainly mediated
278 by T helper type 2 cell immunity. In this study, we show a slew of evidence demonstrating the
279 involvement of B cells and their antibody production in the IgAV course.

280 B cells contribute to disease pathogenesis in autoimmunity by presenting antigens as
281 well as stimulation via cytokines to T cells [17-21]. B cells also play an immunomodulatory
282 role in regulating the immune response by secreting cytokines that inhibit disease onset and/or
283 progression [10, 22]. Recent progresses in B-cell activation and differentiation suggested a
284 complex scenario with multiple steps in the generation of long-lived plasma cells (PCs) and
285 memory B cells in the follicles of germinal centers (GCs) as well as in extra-follicular
286 plasmablasts [16, 23-25]. B cell activation is triggered by antigen recognition through B-cell
287 receptor (BCR) either directly or with the help of antigen presenting cells (APCs) in peripheral
288 lymphoid organs, and is achieved by the activation of intracellular signaling pathways and
289 subsequent target gene expression [23]. The activated B cells migrate to the B-T area of
290 lymphoid organs where they undergo a limited expansion upon cognate interaction with
291 antigen-primed T cells. A fraction of memory B cells differentiate into short-lived plasmablasts
292 providing prompt responses to antigens, while others initiate the formation of GC in secondary
293 follicles. Notably, we also observed that IgAV patients have significantly higher levels of
294 peripheral blood CD19⁺CD20⁻CD38⁺ and CD19⁺CD20⁻CD38⁺CD138⁺ B cells when compared
295 to healthy individuals. However, after treatment these B cell subpopulations return to normal

296 levels suggesting their participation in the pathogenic process of IgAV and that they may be
297 used for monitoring disease progression. At the same time, the abnormal increase in naïve B
298 cells can also promote the production of plasma B cells. With strong correlation between
299 peripheral CD19⁺CD38⁺, CD19⁺CD38⁺CD138⁺ B cells, and total IgA levels in IgAV patients.
300 We concluded that plasma B cells are a distinct B cell subset contributing to abnormal IgA
301 production in IgAV. Our results highlight the association between systemic immunity and
302 IgAV pathogenesis. Furthermore, treatments resulted in significant decrease in the proportion
303 of peripheral plasmablasts. The CD19⁺CD38⁺, CD19⁺CD38⁺CD138⁺ B cell subsets may
304 facilitate the production of IgA. T-B cell interactions can increase the production of IgA which
305 aggravates vascular damage and that in turn further activates the immune system. Thus, all the
306 cells and cytokines get involved in a vicious circle of immune activation.

307 Although the exact function of all the B cell subset is not entirely understood, it is known that
308 it belongs to the memory B cell compartment because of the high levels of somatic
309 hypermutation. Moreover, it has been suggested that these cells might contribute to
310 inflammation by induction of T cell responses and the production of proinflammatory
311 cytokines. It is possible that these B cells are linked with the production of higher affinity
312 antibodies relevant in inflammation. Indeed, subsets of memory B cell have been reported to
313 dysregulated in other autoimmune diseases such as systemic lupus erythematosus and multiple
314 sclerosis[18]. Based on the reported pathogenic role of B cell in other autoimmune diseases[11],
315 we anticipated that also plays an important role in etiology of IgAV. In the present study, the
316 frequency of IgD⁺CD27⁻ naïve B cells were higher in IgAV patients than controls but have no
317 correlation with the serum level of IgA. Alternatively, naïve B cells may not play a major role

318 in the pathogenesis of IgAV. Nevertheless, naïve B cell numbers as well as the level of plasma
319 IgA correlated with CD19⁺CD20⁻CD38⁺, CD19⁺CD20⁻CD38⁺CD138⁺ and CD19⁺CD20⁻
320 CD38⁺IgM⁺ B cells. Therefore, we speculate that naïve B cell subset is indirectly involved in
321 the pathogenesis of IgAV by promoting the generation of plasmablasts B cells. We
322 characterized the numbers of circulating CD19⁺IgD⁻CD27⁺, CD19⁺IgD⁻CD27⁻,
323 CD19⁺IgD⁺CD27⁺ B cells and found no significant difference between IgAV patients and HCs.
324 It is possible that antigen may activate memory B cells, which differentiate into plasma cells,
325 leading to antigen-specific IgA production and the pathogenesis of IgAV. We observed a
326 significant reduction in the number of CD19⁺IgD⁻CD27⁺IgM⁺ memory B cells. Furthermore,
327 CD19⁺IgD⁻CD27⁺IgM⁺ memory B cells is negatively correlated with the level of plasma IgA.
328 Classically, the IgD⁻CD27⁺ memory B cells have switched their IgM to IgG, IgA or IgE, IgM⁺
329 memory B cells maybe the frontline responders by directly giving rise to IgM-secreting cells.
330 It is possible that antigen may activate memory B cells, which differentiate into plasma cells,
331 leading to antigen-specific immunoglobulin production and the pathogenesis of IgAV.
332 Therefore, CD19⁺IgD⁻CD27⁺IgM⁺ memory B cells are exhausted and may be a sensitive
333 marker for evaluating plasma cells. Collectively, our data suggest that the decreased numbers
334 of this distinct group of memory B cells may be associated with the development of IgAV.

335 Abnormal B-cell activation and differentiation in antibody-driven autoimmune diseases
336 is one of the hallmarks with the continuous production of autoantibodies [23]. B cells can
337 participate in the pathogenesis of autoimmune diseases via several mechanisms: as cytokine
338 producers, antigen-presenting cells, or autoantibody secretors. Elevated IgA is one of the key
339 features of IgAV but it is unclear how B cell activated in IgAV. We suspected that these B cell

340 numbers increased via the activation of signaling mediated through BCR and/or its associated
341 molecules. We and others have reported that a combination of BCR-triggering, costimulatory
342 signals including either CD40L, soluble BAFF or IL-21 and Toll-like receptors (TLRs)
343 stimulation induces the most robust B cell activation and differentiation. B cell activating factor
344 (BAFF, also known as TNF ligand superfamily member 13B) is a key cytokine that promotes
345 the maturation, proliferation and survival of B cells [26]. BAFF has been suspected to play a
346 role in progressive systemic sclerosis (pSS) on the basis of elevated BAFF levels in the serum
347 of patients with pSS and the correlation of BAFF levels with levels of antiSSA/Ro and anti-
348 SSB/La antibodies and RF [27]. BAFF could provide a link between activation of the innate
349 immune system and the adaptive immune system (mainly via B cell stimulation. We speculate
350 that BAFF may be associated with IgAV.

351 Although IgAV is benign and self-limiting, and majority of patients enter remission, the
352 degree of renal damage determines the long-term prognosis of the IgAV patients, particularly
353 males > 10 years of age with severe gastrointestinal symptoms (abdominal pain,
354 gastrointestinal bleeding, and severe bowel angina), arthritis/arthralgia, persistent purpura or
355 relapse, WBC > $15 \times 10^9/L$, platelets > $500 \times 10^9/L$, elevated ASO, and low C3 [28]. Henoch-
356 Schönlein purpura nephritis (HSPN) is a major cause of mortality and morbidity in children
357 with HSP, which occurs in 30%-80% of patients during the first three weeks of the initial
358 presentation [29]. For the refractory and severe IgAVN patients, the unraveling of the exact
359 pathogenesis of IgAV will provide directions for prevention of disease, identification of
360 biomarkers and future therapeutic remedies. B cell-targeted approaches for treating immune
361 diseases of the kidney and other organs have gained significant momentum [10, 30]. With our

362 study demonstrating that IgAV patients have abnormal circulation of B cell subsets, B cells
363 gain prominence as the causative agents in IgAV pathogenesis and the over-activation of B
364 cells is the result of a multistep process. Environmental triggers occurring in the presence of
365 genetic and epigenetic dysregulation of IgAV patients lead to the stimulation of specific B cell
366 subsets, particularly plasmablasts and plasma cells. For this reason, it has been considered as a
367 candidate therapeutic target. This is reinforced by the therapeutic efficacy of rituximab, an anti-
368 CD20 monoclonal antibody that specifically depletes B cells. Understanding its function and
369 signaling mechanisms would provide more tools to look for additional therapeutic targets.
370 However, it is well known that IgAV is clinically heterogeneous which leads to failure in
371 developing efficacious targeted therapies. Some studies suggest that rituximab is an effective
372 and safe therapeutic option for adult-onset IgAV [31]. Similarly, B cell-depleting therapy may
373 also be an alternative treatment for patients with IgAN or IgAVN and nephritic–nephrotic
374 syndrome[32]. Identification of the multiple factors that support B cell activation has led to the
375 development of promising targeted therapies, especially for the intractable patients.

376 Our study had some limitations such as a relatively small sample size and the lack of
377 functional study of memory B cells and plasmablasts in the pathogenic process of IgAV. We
378 are also interested in further investigating the values of these subsets of cells in the kidney
379 lesion to understand their roles in the pathogenesis of IgAV. Biomarkers, associated with the
380 risk of nephritis and renal sequelae, must also be investigated in future.

381 **Conclusions**

382 In summary, we show that abnormal distribution of B cell subsets in IgAV patients may play
383 a causal role in development of the disease and should be targeted by future therapeutic efforts.

384 **Declarations**

385 **Abbreviations**

386 **IgAV:** Immunoglobulin A vasculitis

387 **GC:** germinal center

388 **HC:** healthy control

389 **PBMC:** Peripheral blood mononuclear cell

390 **BCR:** B-cell receptor

391 **Ethics approval and consent to participate**

392 Written informed consents were obtained from parents or guardians of all study participants.

393 The experimental protocol followed the guidelines of the Declaration of Helsinki and was

394 approved by the Human Ethics Committee of Jilin University (Jilin University, Changchun,

395 China).

396 **Consent for publication**

397 All the authors agreed to publish.

398 **Availability of data and material**

399 The data used and analyzed in the present study are available from the corresponding author

400 on reasonable request.

401 **Competing interests**

402 The authors declare that they have no competing interest.

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406 **Authors' contributions**

407 DL and YJ carried out the experiments, and analyzed, and interpreted the data. JW, JL, MX
408 and CL were responsible for the collection of blood samples and the interpretation of the data
409 from the clinical perspective. SY contributed to the conception and design of the study, the
410 analysis and interpretation of the data, and drafting and revising the manuscript. All authors
411 read and approved the final manuscript.

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415

416 **References**

417

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485

486 **Figure legends**

487 **Figure 1.** Detection of circulating B cell subsets by flow cytometry. Peripheral blood
488 mononuclear cells (PBMCs) were isolated from patients with immunoglobulin A vasculitis
489 (IgAV) (n = 30) and age and gender-matched healthy controls (HCs; n = 15), labeled with
490 fluorophore-conjugated antibodies, and analyzed by flow cytometry. (A) A gating strategy
491 was used to identify IgD⁻CD27⁻, IgD⁺CD27⁻, IgD⁻CD27⁺, IgD⁻CD27⁺IgM⁺, IgD⁻CD27⁺IgG⁺
492 and IgD⁺CD27⁺ B cell subsets in CD3⁻CD19⁺ B cell. (B): Gating strategy used to identify
493 CD20⁻CD38⁺, CD20⁻CD38⁺IgM⁺, CD20⁻CD38⁺IgG⁺, CD20⁻CD38⁺CD138⁺ B cell subsets in
494 CD3⁻CD19⁺ B cell.

495

496 **Figure 2.** Comparison of different B cell subsets between active and remission in
497 immunoglobulin A vasculitis (IgAV) and HC. NS, not significant.

498

499 **Figure 3.** Treatment-induced changes in B cell subsets. Following treatment, 15 patients
500 exhibited remission from disease. B cell counts were compared between active and remission
501 stages.

502

503 **Figure 4.** Correlation between and among B cell subsets and serum IgA or plasma C4 levels.

504 Correlations between indicated B cell subsets and (A) serum IgA level, (B) plasma C4, (C)

505 among B cell subsets were analyzed by Spearman's rank correlation test.

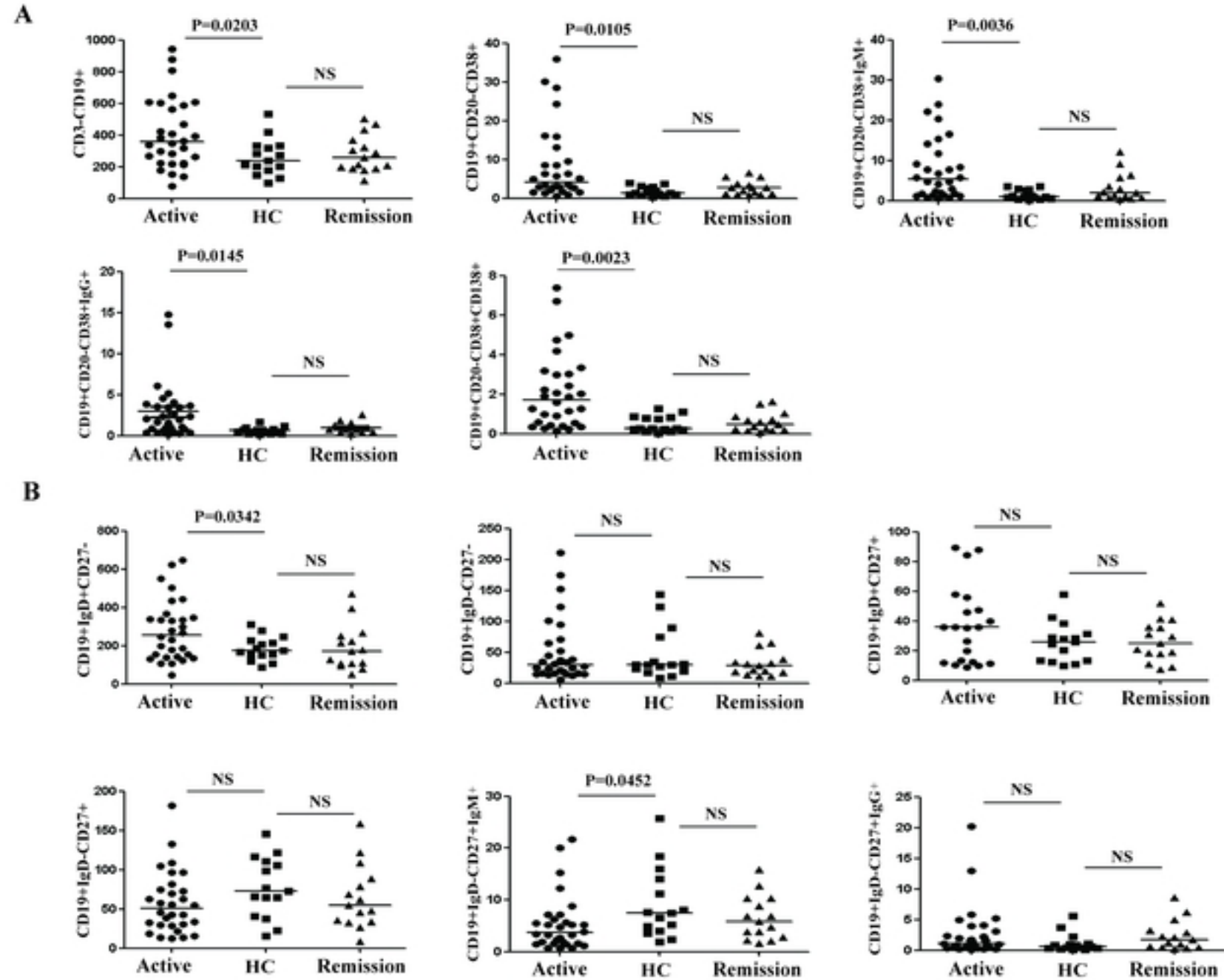


Fig2

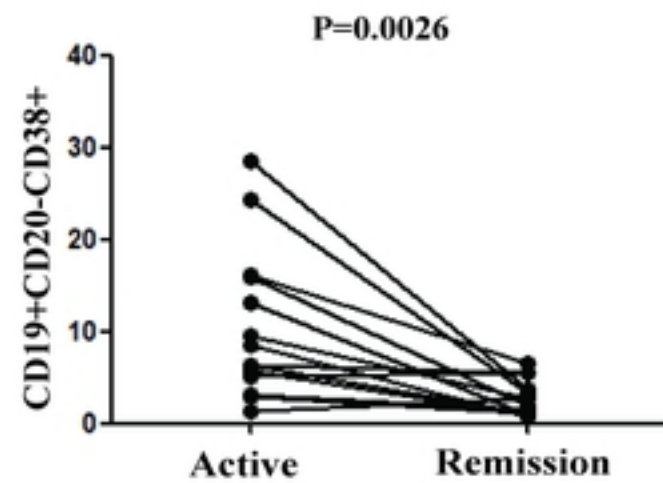
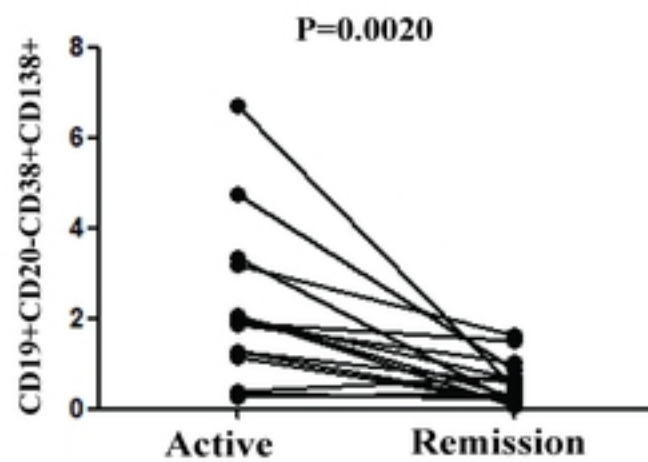
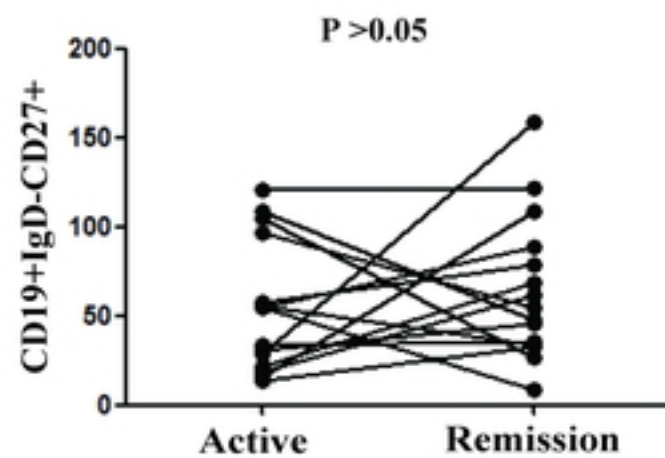
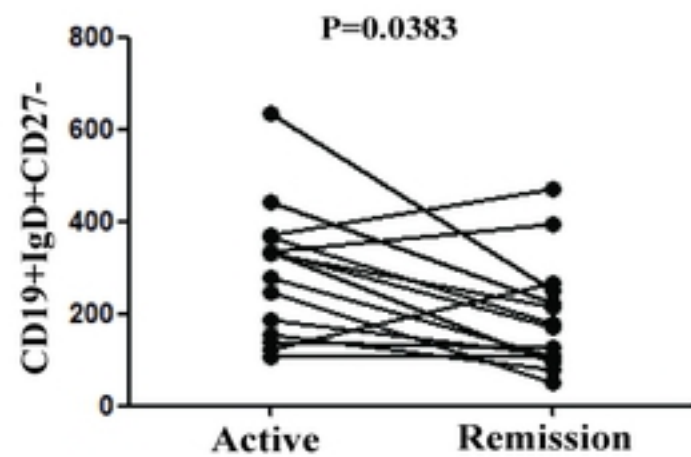
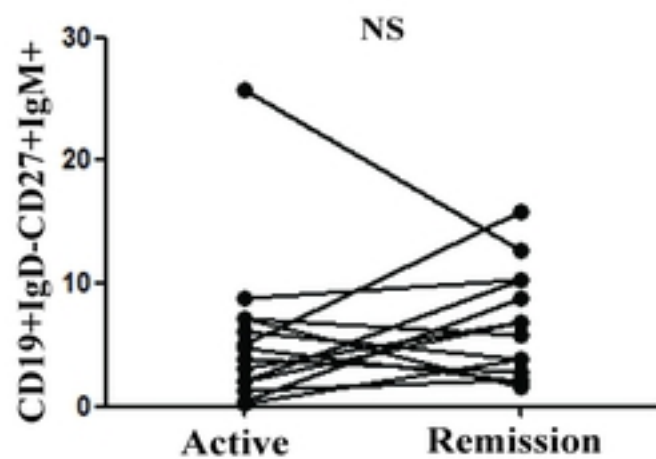
A**B****C****D****E**

Fig3

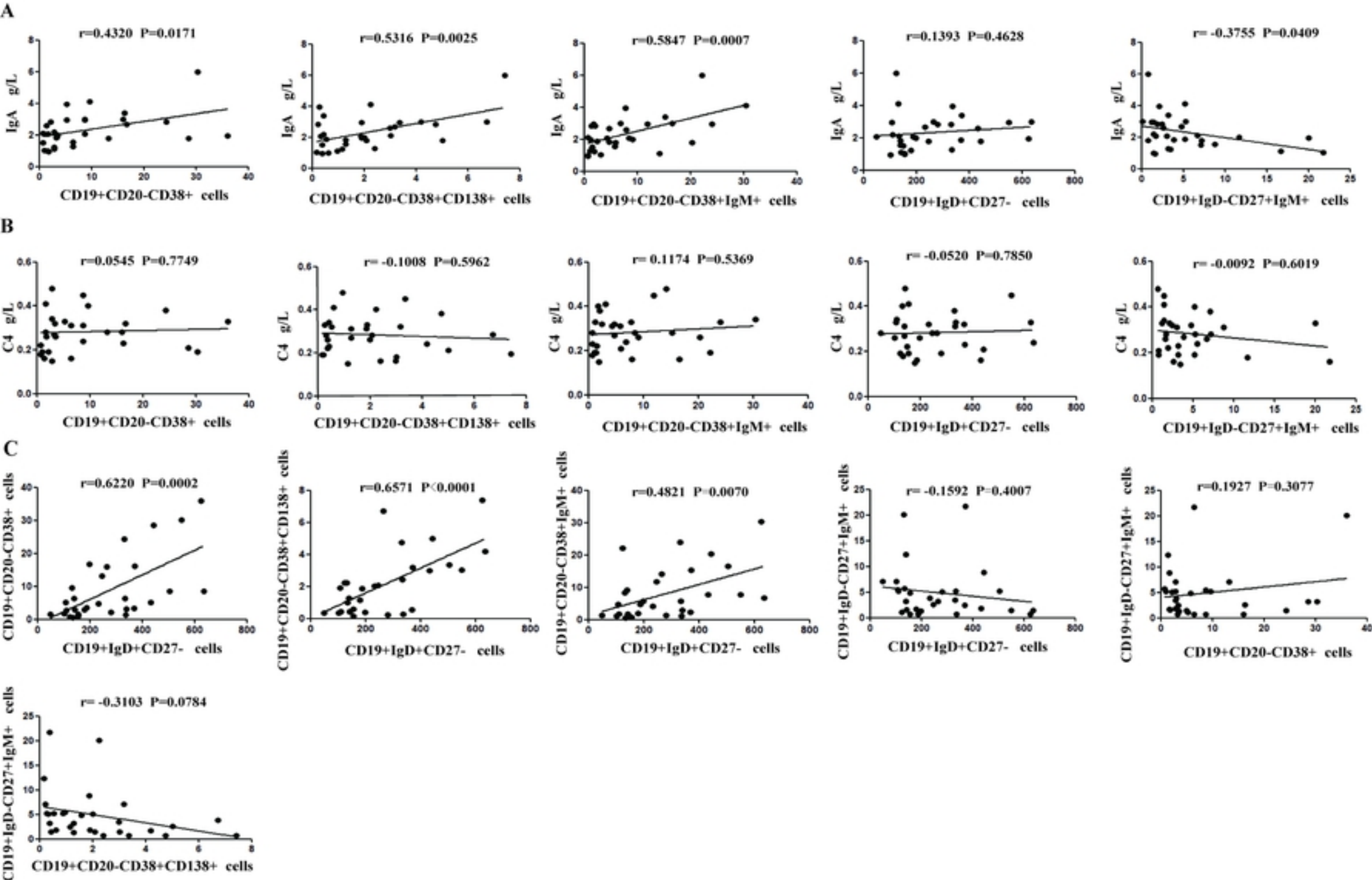


Fig4

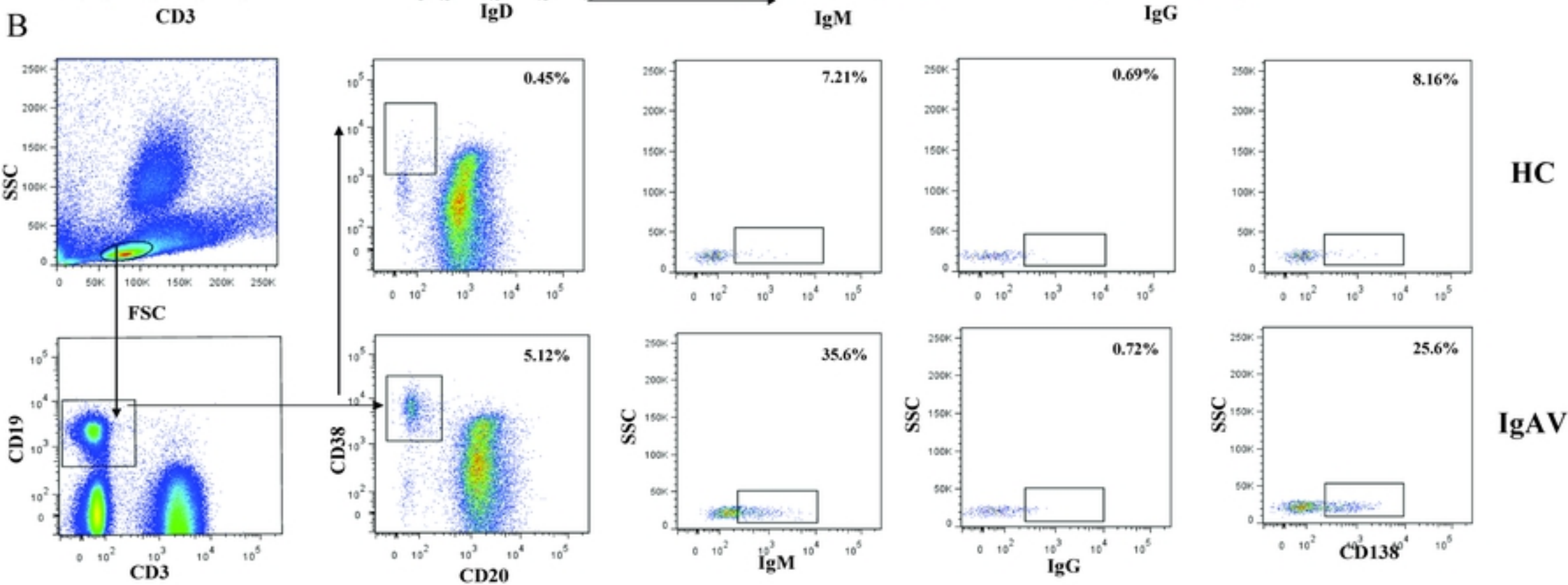
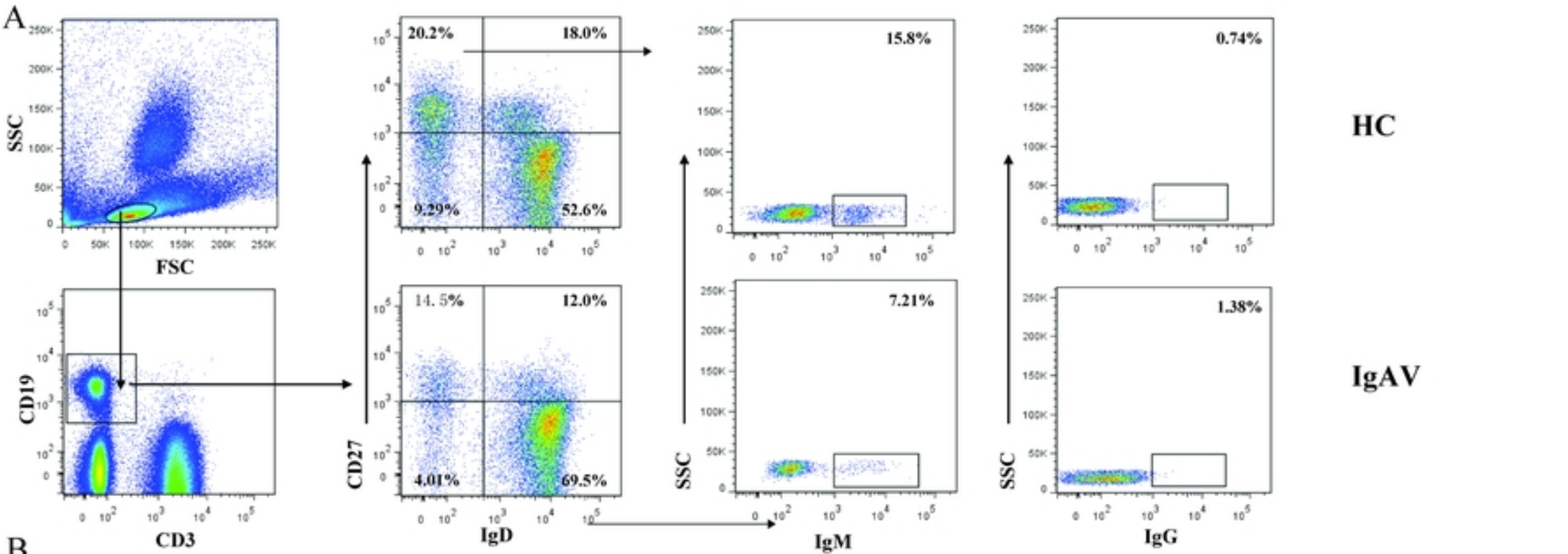


Fig1