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1 **Germinal centers B-cell reaction and T follicular helper cells in**
2 **response to HIV-1 infection**

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20 **Abstract:**

21 **Purpose of the review:** This review aims to summarize the recent findings on germinal centers
22 (GCs) B-cell reaction and Tfh cells in HIV-1 infection, with particular emphasis on the spatial
23 organization of the GC, follicular cell regulation, and cellular alterations resulting from HIV
24 infection.

25 **Recent findings:** HIV-specific bNAbs are generated by iterative cycles of B cell maturation
26 supported by GC environment. Recent observations underline that GC structural alterations at the
27 earliest stages of HIV infection could impact Tfh and GC B cell homeostasis, thus preventing the
28 rise of efficient humoral immunity. Moreover, despite ART treatment, HIV-derived antigens
29 persist, particularly in follicular CD4+ T cells. Antigenic persistence and variability lead to
30 unregulated chronic stimulation. In this context, regulation of the GC appears of special interest.
31 In addition to follicular T regulatory cells (Tfr), new potent regulators of GC reaction such as
32 follicular CD8 T cells have been recently identified.

33 **Summary:** Altogether these new data provide a better understanding on how HIV infection
34 severely impacts GC reaction. Here we propose several therapeutic approaches to promote the
35 bNAbs development in HIV-infected patients by improving the preservation of GC architecture
36 and its regulation.

37

38 **Keywords :**

39 HIV, germinal center reaction, Tfh cells, follicular CD8+ T cells, Tfr cells

40

41

42 **Introduction:**

43 Within secondary lymphoid organs, Germinal Centers (GC) are highly specialized structures
44 which develop in B follicles in response to antigenic challenge. GC house follicular immune cells
45 which closely cooperate and create the optimal microenvironment for high affinity antibodies
46 production. Among follicular cells, T follicular helper (Tfh) cells migrate to the T-B border and
47 provide the B cell help required for B cell maturation into memory and long-lived plasma cells. In
48 addition, follicular dendritic cells (FDC) are essential to coordinate GC-cell positioning and to
49 participate to the selection of high affinity B cells by presenting antigens (1).

50 HIV infection severely impacts the lymph node structure, its cellular composition and
51 consequently, the GC development (2). In the acute phase, HIV infection leads to GC
52 disorganization and B cell dysfunctions. Despite ART treatment, chronic immune activation and
53 inflammation persist in lymphoid organs, leading to profound structural changes of GC, including
54 collagen deposition in the T cell zone and fibrosis (3). In result, HIV-infected (HIV+) patients
55 develop impaired humoral immunity in response to antigenic challenge (4)**. The local
56 persistence of viral antigens is the result of a high susceptibility of follicular CD4 + T cell subsets
57 to HIV(5). Antigen persistence deeply impacts the functions and the quantity of GC Tfh and GC B
58 cells, resulting in a sustained activation of the GC and inappropriate antibody production. Indeed,
59 autoimmune syndromes and B cell lymphomas are frequently described in HIV infection,
60 suggesting a defective GC regulation under antigen persistence (6).

61 HIV-1 broadly neutralizing antibodies (bNAbs) require high levels of somatic mutations for optimal
62 neutralization potency (7). Thus, their development needs sustained antigenic activation that
63 promotes GC reaction and iterative cycles of B cell maturation (8), suggesting a crucial role of Tfh
64 cells. Indeed, recent studies associated bNAbs development with blood subsets that are clonally
65 and functionally related to Tfh cells. Frequency of circulating Tfh cells defined through their
66 expression of PD-1 and CXCR3 were associated with bNAbs generation in several studies (9–
67 11).

68 In this review, we propose to present recent studies providing a better understanding of GC
69 reaction impairments in the context of HIV infection. Hereafter, we discuss the therapeutic

70 approaches aiming at maintaining optimal balance between antigenic persistence and GC
71 regulation while preserving the GC structure.

72

73 **The Germinal Center reaction dynamics in response to antigen stimulation**

74 Protective humoral immunity relies on the induction of highly antigen-specific antibodies and B
75 cell memory. GC reaction is highly regulated by the spatiotemporal coordination of complex
76 cellular interactions. Recent studies based on high-resolution live-cell imaging combined with
77 single-cell analysis approaches bring breakthroughs in the comprehension of cellular dynamics
78 shaping antibody affinity (12–14).

79 After antigen recognition, B cells migrate at the T-B border where they establish a cognate
80 antigenic interaction with CXCR5⁺ CD4⁺ T cells. This interaction results in the differentiation of B
81 cells in short-lived extrafollicular plasmablasts and the maturation of T cells that up-regulate BCL-
82 6, ICOS, PD-1 and CXCR5. A small fraction of activated T and B cells migrate into the follicle,
83 through the CXCL13 chemokine gradient provided by the FDC network, giving rise to the early
84 GC (15,16).

85 GC is characterized by two anatomical compartments, the light zone (LZ) and the dark zone (DZ).

86 The LZ is the place of antigen-driven B cell selection and the DZ is the place of intensive B cell
87 proliferation, somatic hypermutation and class switching (13). In LZ, GC B cells bind antigen
88 trapped on FDC and interact with Tfh cells resulting in the selection of high affinity B cells. By
89 displaying different cell-surface densities of peptide-MHCII complexes, B cells compete for
90 interaction with a limited number of Tfh cells which provide signaling for B cell survival. Tfh cells
91 express costimulatory molecules which reinforce their interaction with GC B cells. ICOS is
92 implicated in the recruitment of Tfh cells as well as in their help-delivering functions (17).

93 Recently, dopamine have been identified as a mediator of the Tfh - B cell interaction where
94 dopamine binds to dopamine receptors expressed by GC B cells and induces ICOS-L
95 translocation to the cell surface (18). Tfh cells produce cytokines such as IL-21 and IL-4 which are
96 necessary for GC formation and B cell differentiation into long-lived plasma cells, respectively
97 (19,20). GC Tfh are characterized by the expression of the exhaustion marker PD-1 which limits
98 the Tfh cell recruitment into the GC through the interaction with PD-L1 expressed on bystander B

99 cells (21)**. Meanwhile, with abundant T cell help, bystander (ie non cognate) B cells could
100 participate in GCs and persist long enough to acquire specificity to antigen (22)(23)*,
101 demonstrating that pre-immune BCR engagement is dispensable for GC B cell selection (22).
102 Interestingly, when T cell help is limited, BCR signaling can synergize to potentiate GC B cell
103 selection (23). Hence, the selection of high affinity B cells is ensured by highly regulated cognate
104 antigen or bystander T:B cell interactions. DZ stroma consist of CXCL12-expressing reticular cells
105 (CRCs) that maintain DZ B cells outside the LZ. Within DZ, B cells undergo somatic
106 hypermutation and class swichting to promote antibody diversity while enhancing antigen affinity.
107 Newly generated B cell clones are then selected by GC Tfh cells and FDC in the LZ (24). To
108 achieve their affinity maturation, GC B cells undergo iterative rounds of cyclic re-entry and
109 proliferation of positively selected GC B cells, thus maintaining the GC reaction until the antigen
110 clearance.

111

112 **GC reaction homeostasis and potent humoral immunity**

113 GC homeostasis is critical for the induction of high affinity Ab responses devoided of self-
114 reactivity. Uncontrolled GC reaction leads to the production of autoantibodies, showing the need
115 for a tight regulation of Tfh - B cell interactions (25). A subset of lymphoid CD4+ regulatory T
116 (Treg) cells termed follicular regulatory T (Tfr) cells are characterized by the expression of both
117 Tregs and Tfh cell-associated markers such as Foxp3, CTLA-4, IL-10 and CXCR5. CTLA-4 is a
118 potent regulator of B cell responses by suppressing Tfh cell help via CD28 interaction (26). Once
119 the immune response resolved, concomitant with the reduction of IL-2 concentration, Tfr arise
120 from Treg cell population (27–30). By expressing the IL-1 decoy receptor IL-1R2 and the IL-1
121 antagonist IL-1Ra receptor, Tfr cells limit the IL-1-induced activation of Tfh cells (29). Thus, IL-1
122 axis appears as a new potent regulator of the Tfh / Tfr homeostasis. Tfr cells are found in low
123 numbers in secondary lymphoid organs (SLO) at the steady state, whereas their frequency
124 largely increases with antigenic persistence and ongoing GC reaction (31). Using multiplexed
125 immunohistochemistry, Sayin *et al.* showed that Tfr cells express low levels of PD-1 and are
126 found at the T-B border outside of the GC (32)**. Their findings suggest that Tfr cells prevent

127 inadequate T-B cell interactions, thus controlling the GC entry point. Finally, a recent work on Tfr
128 cells reports an unexpected “helper” effect of Tfr cell-derived IL-10 on GC reaction (33).

129 In sum, tissue-imaging technologies highlight the complex spatial organization of the GC that
130 sustains highly regulated cellular interactions.

131

132 **HIV infection and GC disruption**

133 HIV-1 (hereafter HIV) infection dramatically affects GC architecture during the acute phase, thus
134 dampening the rise of potent humoral immunity. In absence of antiretroviral therapy (ART)-
135 treatment, follicular CD4+ T cells are rapidly and continuously exposed to HIV which shows
136 extremely high mutation rate in vivo. HIV disseminates within follicles to constitute latent reservoir
137 after ART treatment introduction. Depending on the delay of ART introduction, severe involution
138 of follicles and ultimate destruction of lymphoid tissues are reported in HIV+ patients (34) where a
139 correlation has been established between the destruction of lymph nodes and the disease
140 progression (35). Unfortunately, introduction of ART reverses only partially structural alterations of
141 the follicles (36).

142 As previously described (37,38), Tfh cells and IgG+ follicular memory B cells were found in higher
143 proportions in lymph nodes from HIV+ volunteers as compared with those from healthy controls
144 (HCs). To better understand the follicular dynamics in ART-treated patients, Moysi et al.
145 investigated by histo-cytometry Tfh and B cell-dynamics before and after trivalent seasonal
146 influenza immunization in HIV+ volunteers on cART (4). HIV+ individuals showed higher Tfh cell
147 frequencies despite a global alteration of the follicular architecture with the loss of GC polarity.
148 Interestingly, the preservation of the FDCs network correlated with higher Tfh frequencies before
149 vaccination and the strength of humoral immunity induced by vaccination (4). However, after
150 influenza vaccination in HIV+ patients, Tfh cell frequency decreased, associated with a loss of
151 FDCs network. Taken together, these observations suggest that, in HIV infection, Tfh and B cell-
152 dynamics are conditioned by the maintenance of the FDC network and the preservation of the GC
153 architecture. Thus, early ART introduction appears critical to limit HIV-induced alterations of the
154 GC.

155

156 **HIV infection supports GC activation**

157 Follicular CD4 T cells constitute a major compartment for HIV infection, replication and production
158 of viral particles in lymph nodes of viremic individuals (38–40). By being highly permissive to HIV-
159 1, Tfh cells are targeted early after infection (5,41). However, tissue-resident Tfh cells accumulate
160 during the chronic phases of infection (38,39,42) suggesting that HIV infection promotes Tfh cell
161 development within a propitious lymphoid environment. HIV persistence in lymphoid tissues
162 promotes immune activation which exacerbates secretion of proinflammatory cytokines as tumor
163 necrosis factor- α , interferon- γ , IL-10 and IL-6 (34). Interestingly, IL-6 has been recently shown to
164 promote extracellular matrix protein 1 (ECM1) which is critical for Tfh cell differentiation (43).
165 Thus, in every stages of HIV infection, immune activation creates a cytokine environment
166 supporting Tfh cell development.

167 HIV infection alters Tfh cell gene signature (4,38,39). In line with previous studies, Moysi et al.
168 reported an increased frequency of Tfh cells expressing cell surface markers such as ICOS and
169 SLAM molecules which are implicated in B cell helper functions. By combining TCR sequencing
170 and mass cytometry analysis, Wendel et al. interrogated the functional phenotype and TCR
171 repertoire composition of Tfh cells isolated from lymph nodes of HIV+ individuals. They evidenced
172 an expansion of gag-reactive TCR in Tfh cell subset (44). HIV-specific Tfh cells presented an
173 activated phenotype and were less polyfunctional as they produced only IL-21, resulting in
174 defective B cell development.

175 Altogether, these results underline the overall activation resulting from the persistence of viral
176 antigens in the GCs of ART-treated patients (4). HIV infection dramatically impacts the
177 homeostasis of lymphoid tissue-resident Tfh cells which are increased in frequency and
178 functionally impaired. Keeping in mind that optimal Tfh cell frequency favors survival of high
179 affinity B cell clones devoid of self-reactivity, Tfh cell regulation appears critical for the induction of
180 high affinity antibodies.

181

182 **Tfh / Tfr cell ratio as a key parameter of regulated GC reaction**

183 After their discovery in 2011 (30,45,46), Tfr cells have been extensively studied in HIV infection.
184 We and others showed that Tfr cells frequency is higher in secondary lymphoid organs of HIV+

185 individuals compared to uninfected individuals (38). Since Tfr cells are important regulators of the
186 GC reaction, Tfh and Tfr cell dynamics are strongly interrelated. After immunization, Tfh/Tfr cell
187 ratio first increases and then progressively decreases due to the expansion of Tfr cell and to the
188 reduction of Tfh cells (Figure 1). Even if the mechanisms implicated in the fine regulation of the
189 Tfh/Tfr cell ratio remain unclear, Tfh/Tfr cell homeostasis appears to depend on the antigen
190 persistence. In a murine model of viral infection, virus with higher replicative capacity induced
191 higher Tfh/Tfr ratio which was, during the peak of the GC response, indicative of the protective,
192 long-lived antibody response (47). In this way, Tfh/Tfr cell ratio is an essential parameter to take
193 into account for the study of GC function and regulation in the context of HIV infection.

194 In SIV infection, monkeys show an increase of Tfh/Tfr cell ratio, from acute to chronic phase of
195 the disease (48)*. In humans, such longitudinal studies are more difficult to conduct in secondary
196 lymphoid organs. However using tonsils from HIV+ patients, Roeder et al. showed that children,
197 who are known to be particularly potent to develop bNAbs, present a higher frequency of follicular
198 Tfh and Tfr cells but a smaller Tfh/Tfr cell ratio than adults, suggesting a central role of the GC
199 regulation for bNAbs emergence (49). GC activation is first characterized by an increase of the
200 Tfh cells which have to be regulated by a proportionate increase of Tfr cells. Indeed, Fan *et al*
201 recently showed the development of auto-immune antibodies in SIV+ monkeys with persistent
202 high Tfh/Tfr cell ratio (50). The persistence of a high Tfh/Tfr cell ratio could result from a
203 continuous GC priming with neoantigens deriving from high rate of mutation associated with HIV
204 replication. Interestingly, autoreactivity associated with HIV-infection correlate with bNAbs
205 development (51,52) (Figure 1). This emphasizes the ambivalent role of antigenic persistence
206 and ongoing GC reaction in the development of efficient humoral immunity against HIV.
207 Altogether, these studies support the need for a Tfh cell regulation, easily measurable by
208 evaluation of the Tfh/Tfr cell ratio. In addition to Tfr cells, new follicular cell subsets have been
209 identified to play a role in GC regulation.

210

211 **CXCR5+ CD8+ T and NK cells in the control of GC reaction**

212 CXCR5+ CD8+ T cells have been recently identified as potent regulators of the GC reaction
213 under HIV infection. A recent study confirmed the presence of CXCR5+ CD8+ T cells in

214 secondary lymphoid organs from SIV+ monkeys (53). Follicular CD8+ T cells have also been
215 evidenced in lymph nodes from HIV+ individuals where local immune activation drives their
216 accumulation (54). However, the role of CXCR5+ CD8+ T cells in GC reaction under HIV infection
217 is not fully understood. Using *in vitro* tonsils HIV infection as well as *in vivo* SIV infection, Miles et
218 al evidenced regulatory functions associated with this cell subset. In human tonsils, CXCR5+
219 CD8+ T cells limit Tfh B-helper functions through a tim-3 dependent axis. Additionally, CXCR5+
220 CD8+ T cells induce Tfh cell apoptosis through a HLA-E dependent pathway (55). Interestingly,
221 the overall frequency of CXCR5+ CD8+ T cells is similar in both children and adults but the
222 frequency of HIV-specific CXCR5+ CD8+ T cells is higher in children (49). Children with high
223 neutralization breadth display more polyfunctional circulating CXCR5+ CD8+ T cells than those
224 with low neutralization breadth (49). Altogether, these data suggest a strong relationship between
225 follicular CD8+ T cells, Tfh cell regulation and the neutralization breadth. CXCR5+ CD8+ T cells
226 also play a crucial role in the elimination of HIV-infected cells. Petrovas et al. showed that
227 CXCR5+ CD8+ T cells presented higher cytotoxic abilities compared to conventional CXCR5-
228 CD8+ T cells (54). By eliminating HIV-infected cells, HIV-specific CXCR5+ CD8+ T cells are
229 supposed to limit viral antigen persistence (54). Cytotoxic CXCR5+ CD8+ T cells appear as
230 strong regulators of GC regulation through Tfh/Tfr cell ratio modulation by killing HIV-infected Tfh
231 and Tfr cells and thus limiting antigen persistence. Finally, showing that CXCR5+ CD8+ T cells is
232 a heterogenous population, Shen et al recently identified a CXCR5+CD8+ T cell subset that does
233 not express cytotoxic molecules but instead supports B cells maturation through IL-21 secretion
234 and CD40L expression (56). CXCR5+ CD8+ T cells appear as new actors of GC reaction
235 dynamics but their functions remain complex.

236 Already known for their antiviral activity, NK cells are taking a growing importance for their role
237 during HIV infection. In HIV+ individuals, a loss of cytotoxic abilities in a blood NK cell subset is
238 associated with neutralizing antibody responses. This study suggests that, in blood, the decrease
239 of activated Tfh cell killing mediated by NK cells leads to a higher Tfh cell availability and bNAbs
240 emergence (57). Consistently with this work, Rydynski *et al* showed in mice the NK cell role in the
241 regulation of GC reaction where NK cells could limit Tfh cell frequency through a perforin
242 dependent mechanism (58). In nonpathogenic SIV infection of African green monkeys, Huot *et al*

243 revealed the presence of CXCR5+ NK cells in virus free lymph nodes where FDC produce high
244 level of IL-15. Anti-IL-15 treatment results in NK cell depletion within lymph nodes and leads to
245 viral DNA increase (59). Potentiality of NK/IL-15 pathway was recently evidenced in human by
246 Garrido *et al*, who showed that blood NK cells of HIV⁺ patients simulated with IL-15 were more
247 able to limit viral replication by killing infected cells, as compared to unstimulated NK cells (60).
248 By limiting viral reservoir, NK cells could act on GC reaction by modulating antigenic persistence.
249 If the decrease of NK cell cytotoxicity preserves Tfh cell compartment for high neutralizing
250 breadth, NK cell cytotoxicity appears essential for controlling reservoir establishment in secondary
251 lymphoid organs.
252 Altogether, these data underline the critical role of these cell actors in GC reaction but future work
253 is required to better understand their role under HIV infection. CXCR5+ CD8+ T and NK cells
254 probably include functionally distinct subsets, and the use of single cell approaches would help to
255 decipher the various functions of in GC.

256

257 **Conclusion**

258 The development of HIV-specific bNAbs requires sustained antigenic activation that promotes
259 iterative cycles of B cell maturation and GC reaction. In absence of ART treatment, HIV rapidly
260 invades follicles. Consequently, GC are dramatically altered in their structure and cell distribution,
261 leading to inappropriate activated GC reaction. Production of cytokines and chimiokines promote
262 the development of functionally altered Tfh cells. Tfh cell increase may dampen the selection of
263 high affinity HIV-specific B cells and in some extent, promotes the induction of autoreactive B
264 cells. Lessons from recent studies indicate that early ART introduction would preserve the Tfh cell
265 functions. As a consequence, the frequencies of HIV Env gp140–reactive intestinal B cells is
266 higher in patients who were treated early as compared with patients who were treated during the
267 chronic phase of HIV infection (61)**. Another, non-exclusive idea is that the presence of viral
268 antigens within the follicles is required to maintain ongoing GC reaction and favor bNAbs
269 development. Therefore, the goal for ART treatment introduction is to reach the right balance
270 between on the one hand, low antigen persistence incompatible with the maintenance of the GC
271 reaction and, on the other hand, the establishment of HIV reservoir resulting in unregulated

272 ongoing GC reaction. In the latter case, one approach is to reinforce the regulation of GC in order
273 to control altered Tfh cells. Thus, the use of replicative-based virus vectors is a promising vaccine
274 strategy to promote antigen persistence and the induction of Tfh/Tfr cell ratio compatible with the
275 induction of bNAbs. Therefore strategies promoting expansion of regulatory cell subsets
276 combined with systems allowing constant antigen delivery appear to be particularly attractive
277 (Figure 1).

278

279 Key points:

- 280 • HIV infection rapidly alters the GC structure
- 281 • FDC network alterations impair Tfh cell homeostasis
- 282 • GC B-cell reaction is overactivated by viral persistence
- 283 • Tfh/Tfr cell ratio seems to be an indicative marker of bNAbs development
- 284 • Promising strategies aim at promoting antigen persistence in structurally preserved GC

285

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291 **Conflicts of interest :**

292 None

293

294 **Figure legends:**

295 Figure 1:

296 Heading: Key role of regulation in GC reaction for bNAbs emergence under antigenic persistence.

297 Legend: In physiological situation, GC reaction resolution appears after antigen clearance and
298 regulation. Tfh/Tfr ratio decreases in time with Tfh cells reduction and Tfr cells increase. HIV
299 infection leads in most patients to a pathological GC reaction where antibody responses are
300 inadequate and sometimes associated with auto-immunity. This pathological GC reaction seems

301 to appear because of antigen persistence combined with a lack of regulation, reflected by
302 continuously increased Tfh/Tfr cell ratio during disease progression. bNAbs development is
303 associated with efficient GC regulation reflected by a decreased Tfh/Tfr cell ratio over time. Thus,
304 therapeutics approaches allowing an optimal balance between antigen persistence and GC
305 regulation appear attractive.

306

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