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Raphaël Jeger-Madiot, Maud Heredia, Stephanie Graff-Dubois

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Raphaël Jeger-Madiot[1], Maud Heredia[1] and Stéphanie Graff-Dubois[1*]

[1]Sorbonne Université, Inserm, CNRS, Centre d'Immunologie et des Maladies Infectieuses Cimi-Paris, Paris, France.

*Corresponding author

Graff-Dubois

Current address:

UMRS - 959 Inserm - Sorbonne Université

GH Pitié Salpêtrière - Bat. Cervi

83 Bd de l'Hôpital, 75651 Paris CEDEX 13

Telephone number: +33 1 42 17 74 77

Email : stephanie.graff-dubois@sorbonne-universite.fr

Abstract:

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

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

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

Keywords :

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

Introduction:

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

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

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

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

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

The Germinal Center reaction dynamics in response to antigen stimulation

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

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

GC is characterized by two anatomical compartments, the light zone (LZ) and the dark zone (DZ). The LZ is the place of antigen-driven B cell selection and the DZ is the place of intensive B cell proliferation, somatic hypermutation and class switching (13). In LZ, GC B cells bind antigen trapped on FDC and interact with Tfh cells resulting in the selection of high affinity B cells. By displaying different cell-surface densities of peptide-MHCII complexes, B cells compete for interaction with a limited number of Tfh cells which provide signaling for B cell survival. Tfh cells express costimulatory molecules which reinforce their interaction with GC B cells. ICOS is implicated in the recruitment of Tfh cells as well as in their help-delivering functions (17). Recently, dopamine have been identified as a mediator of the Tfh - B cell interaction where dopamine binds to dopamine receptors expressed by GC B cells and induces ICOS-L translocation to the cell surface (18). Tfh cells produce cytokines such as IL-21 and IL-4 which are necessary for GC formation and B cell differentiation into long-lived plasma cells, respectively (19,20). GC Tfh are characterized by the expression of the exhaustion marker PD-1 which limits the Tfh cell recruitment into the GC through the interaction with PD-L1 expressed on bystander B

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

GC reaction homeostasis and potent humoral immunity

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

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

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

HIV infection and GC disruption

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

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

HIV infection supports GC activation

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

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

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

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

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

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

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

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

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

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

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

revealed the presence of CXCR5+ NK cells in virus free lymph nodes where FDC produce high level of IL-15. Anti-IL-15 treatment results in NK cell depletion within lymph nodes and leads to viral DNA increase (59). Potentiality of NK/IL-15 pathway was recently evidenced in human by Garrido *et al*, who showed that blood NK cells of HIV^+ patients simulated with IL-15 were more able to limit viral replication by killing infected cells, as compared to unstimulated NK cells (60). By limiting viral reservoir, NK cells could act on GC reaction by modulating antigenic persistence. If the decrease of NK cell cytotoxicity preserves Tfh cell compartment for high neutralizing breadth, NK cell cytotoxicity appears essential for controlling reservoir establishment in secondary lymphoid organs.

Altogether, these data underline the critical role of these cell actors in GC reaction but future work is required to better understand their role under HIV infection. CXCR5+ CD8+ T and NK cells probably include functionally distinct subsets, and the use of single cell approaches would help to decipher the various functions of in GC.

Conclusion

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

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

Key points:

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

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Conflicts of interest :

None

Figure legends:

Figure 1:

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

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

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

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