IL-21 boosts germinal center response through independent actions on T and B cells in a concentration-dependent manner

Mariana C.G Miranda Waldetario[1]

[1]Icahn School of Medicine at Mount Sinai Department of Pediatrics

January 31, 2023

Abstract

Germinal centers (GC) are the sites of B cell clonal expansion, somatic hypermutation and clonal selection, a process that leads to the production of antibodies of higher affinity [1](#ref-0001). Efforts have been made to understand the kinetic of events controlling the GC and the production of specific antibodies in protective as well in pathogenic responses, such as autoimmunity and allergy. The ability of newly mutated GC clones to capture and present antigen to T follicular helper cells (Tfh) in the light zone of the GC is crucial for clonal survival and selection. Tfh cells produce IL-21, a key cytokine for the GC reaction and antibody responses [2](#ref-0002). However, it was not understood how IL-21 acts independently on T and B cells to mediate the GC reaction. In this study Quast and colleagues [3](#ref-0003) contribute to elucidate the specific role of IL-21 on the GC reaction and how IL-21 bioavailability affects the outcome of the GC response. They demonstrate that IL-21 influences Tfh cell differentiation and expansion early, before the GC establishment, as well later during GC development, through both autocrine and paracrine mechanisms, regardless of cognate T-B cell interactions.

IL-21 boosts germinal center response through independent actions on T and B cells in a concentration-dependent manner

Mariana C.G Miranda Waldetario[1,2]

[1]Icahn School of Medicine at Mount Sinai, Jaffe Food Allergy Institute, New York, New York, USA

[2]Icahn School of Medicine at Mount Sinai, Precision Immunology Institute, New York, New York, USA

Acknowledgments

I would like to thank Dr. Maria A. Curotto de Lafaille for critically review this manuscript and Dr. Anna Globinska for the figures design.

Germinal centers (GC) are the sites of B cell clonal expansion, somatic hypermutation and clonal selection, a process that leads to the production of antibodies of higher affinity [1]. Efforts have been made to understand the kinetic of events controlling the GC and the production of specific antibodies in protective as well in pathogenic responses, such as autoimmunity and allergy. The ability of newly mutated GC clones to capture and present antigen to T follicular helper cells (Tfh) in the light zone of the GC is crucial for clonal survival and selection. Tfh cells produce IL-21, a key cytokine for the GC reaction and antibody responses [2]. However, it was not understood how IL-21 acts independently on T and B cells to mediate the GC reaction. In this study Quast and colleagues [3]contribute to elucidate the specific role of IL-21 on the GC reaction and how IL-21 bioavailability affects the outcome of the GC response. They demonstrate that IL-21 influences Tfh cell differentiation and expansion early, before the GC establishment, as well later during GC development, through both autocrine and paracrine mechanisms, regardless of cognate T-B cell interactions.

The lack of IL-21 signaling profoundly affects the GC persistence and output, resulting in reduced Tfh cell differentiation, B cell expansion, affinity maturation and plasma cell differentiation[4,5]

The intricate nature of the GC makes it challenging to precisely determine the direct and indirect effects of IL-21 on each component of the GC reaction.

To specifically modulate the IL-21 production (T cells) and sensing (T and B cells) in the GC, Quast and colleagues[3] used a mouse model in which mice deficient in both IL-21R and IL-21 received adoptively transferred IL-21R sufficient ($Il21r^{+/+}$) or deficient ($Il21r^{-/-}$) hen egg lysozyme (HEL) specific B cells, together with ovalbumin (OVA)-specific IL-21 sufficient ($Il21^{Gfp/+}$) or IL-21 deficient ($Il21^{Gfp/Gfp}$) T cells. Following by immunization with cross linked HEL^2 x OVA_{pep}, resulting in a robust splenic GC response (Fig. 1). Alone, $Il21^{Gfp/Gfp}$ T cells had poor proliferation and Tfh differentiation, but when transferred together with $Il21^{Gfp/+}$ T cells, both IL-21 competent and IL-21 deficient T cells expanded similarly in the first days following immunization. These results suggested that IL-21 in autocrine or paracrine manner controlled the differentiation and expansion of Tfh cells. This was independent of B cells responding or not to IL-21. Moreover, higher proportion of T secreting IL-21, which increased IL-21 availability in the microenvironment, directly impacted Tfh density and consequently, the magnitude of the GC responses (Fig. 1).

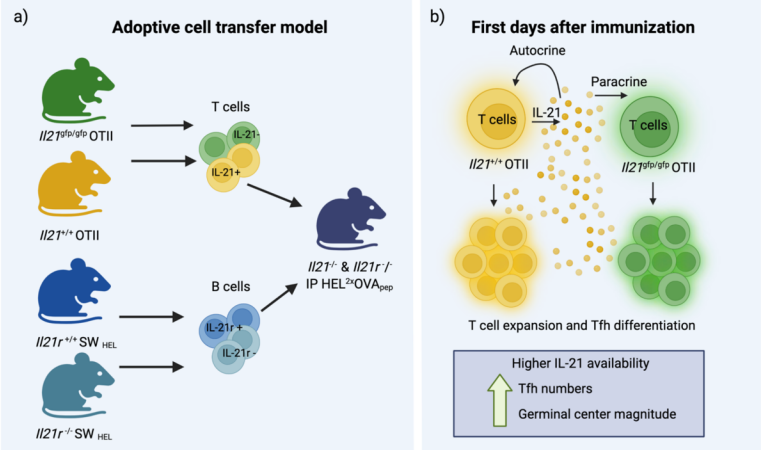

Figure 1: a) Mouse model of adoptive cell transfer used to genetically manipulate the IL-21 production and sensing transferring ovalbumin (OVA)-specific IL-21 sufficient ($Il21^{Gfp/+}$) or IL-21 deficient ($Il21^{Gfp/Gfp}$) T cells together with IL-21R sufficient ($Il21r^{+/+}$) or deficient ($Il21r^{-/-}$) hen egg lysozyme (HEL) specific B cells followed by immunization with the cross linked peptide HEL^2 x OVA_{pep}. **b)** Early on in the immune response, the availability of IL-21 in the microenvironment plays a critical role in regulating T cell expansion and Tfh differentiation, impacting the density of Tfh cells and ultimately determining the magnitude of the GC response.

After the establishment of the GC, increasing IL-21 availability promoted the centroblast phenotype in GC B cells, confirming a previous report [6].

To determine if the IL-21 activity in GC B cells was restricted to cognate T cell secreting IL-21 during the T-B synapse, mice deficient in both IL-21R and IL-21 adoptively received OVA-specific T cells able to produce and sense IL-21 ($Il21r^{+/+}Il21^{Gfp/+}$), together with$Il21r^{+/+}$ B1-8 B cells, which are specific for the

hapten NP. The recipient mice were then dually immunized with OVA and NP-KLH (Fig. 2). In this system NP specific B cells could not receive IL-21 from endogenous cognate T cells that recognized KLH, but IL-21 secreted by the non-cognate OVA specific T cells, in a dose dependent manner, supported the NP specific B cell proliferation, centroblast identity and plasma cell differentiation after immunization. This demonstrated for the first time that IL-21 acts locally in a non-cognate fashion to sustain GC B cell proliferation and differentiation.

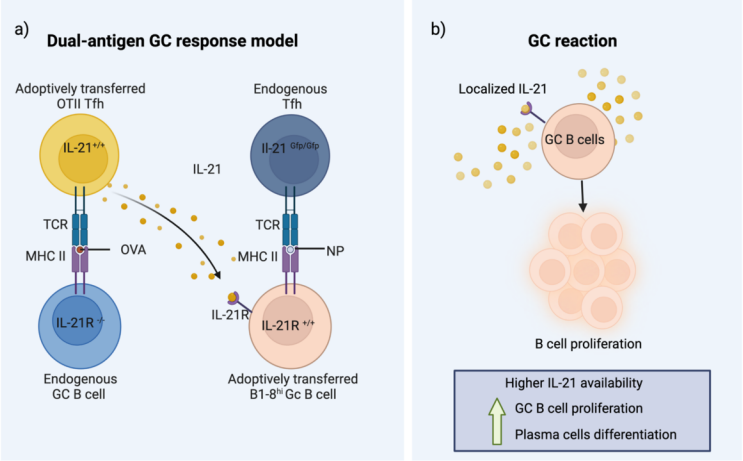

Figure 2: a) Experimental model used to investigate the impact of IL-21 produced by non-cognate T cells on GC B cells. The IL-21 produced by adoptively transferred OVA-specific OTII T cells was sensed by non cognate B1-8hi NP-specific B cells. **b)** The availability of IL-21 in the local microenvironment influences the fate of GC B cells, with higher levels leading to increased proliferation, and plasma cell formation.

Quast and colleagues showed that IL-21 acts locally on T and B cells, independently of direct cell-to-cell interaction. The concentration of IL-21 in the microenvironment, both before the establishment of the GC and during the GC reaction, determines the trajectory and dynamics of the immune response. Greater IL-21 availability enhances the early expansion of Tfh cells in the GC, which directly impacts the size of the GC and amplifies the B cell response, leading to the formation of plasma cells.

1. Victora GD, Nussenzweig MC. Germinal Centers. *Annu Rev Immunol* . Apr 26 2022;40:413-442. doi:10.1146/annurev-immunol-120419-022408

2. Spolski R, Leonard WJ. Interleukin-21: a double-edged sword with therapeutic potential. *Nat Rev Drug Discov* . May 2014;13(5):379-95. doi:10.1038/nrd4296

3. Quast I, Dvorscek AR, Pattaroni C, et al. Interleukin-21, acting beyond the immunological synapse, independently controls T follicular helper and germinal center B cells. *Immunity* . Aug 9 2022;55(8):1414-1430 e5. doi:10.1016/j.immuni.2022.06.020

4. Linterman MA, Beaton L, Yu D, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J Exp Med* . Feb 15 2010;207(2):353-63. doi:10.1084/jem.20091738

5. Zotos D, Coquet JM, Zhang Y, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J Exp Med* . Feb 15 2010;207(2):365-78. doi:10.1084/jem.20091777

6. Zotos D, Quast I, Li-Wai-Suen CSN, et al. The concerted change in the distribution of cell cycle phases and zone composition in germinal centers is regulated by IL-21. *Nat Commun* . Dec 9 2021;12(1):7160. doi:10.1038/s41467-021-27477-0