Structure and function of lymphoid tissues

Determining germinal centre B cell fate

Dimitra Zotos[1,2] and David M. Tarlinton[1,2]

[1] The Walter and Eliza Hall Institute for Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia
[2] The Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia

The humoral immune system generates immunological memory comprising affinity matured, long-lived memory B cells and plasma cells (PCs), which are generated primarily in germinal centres (GCs). Although many factors are essential in this process, those that specifically govern B cell fate are not fully understood. The provision of T cell help to B cells is key in GC B cell fate determination, and it has become clear recently that this help involves more than direct cell–cell interactions. Recently, the cytokine interleukin (IL)-21 has been identified as a key factor that can modulate the processes within GCs and directly influence B cell fate. In this review, we examine the roles of GC cytokines in the context of cell differentiation.

The fate of an activated B cell is determined by more than just cell–cell interactions

Upon receiving cognate T cell help, B cells have three potential fates. They can differentiate into an extrafollicular focus of antibody-secreting PCs, form a GC within the B cell follicle, or differentiate into early recirculating memory B cells [1–3]. The two predominant pathways are extrafollicular PCs and GCs. The difference between these two pathways is that extrafollicular PCs produce low affinity antibodies that are predominantly short-lived, whereas the end product of GCs is long-lived PCs and memory B cells that have improved affinity for the antigen [1,2,4]. The fate of a responding B cell is governed by B–T cell interactions, which include and are not limited to: antigen-specific (cognate) interaction between the T cell receptor (TCR) and peptide-MHCII on B cells; ligation of CD40 on B cells by CD40 ligand (CD40L; also known as CD154); and provision of B7.1 (CD80) and B7.2 (CD86) by B cells to ligate CD28 on T cells (Figure 1). In addition to cell–cell interactions, CD4^{+} T cells provide help by secreting cytokines, including IL-4 and IL-21. Deficiency in any of these factors profoundly affects the resulting T cell dependent (TD) immune response (Table 1).

This review covers recent developments about the factors influencing the fate of B cells after immunisation. We focus on B cell responses occurring within a T helper (Th)2 environment, meaning that the cytokines IL-4 and IL-21 are major elements of this review. Although the function of IL-4 has been studied over many years, that of IL-21 in regulating B cell differentiation *in vivo* is only recently becoming clear, and of particular interest are reports

showing both independence and interdependence of these two cytokines in influencing aspects of B cell fate.

Activated B cell fate: is it stochastic?

The factors that determine whether a B cell develops into an extrafollicular focus of PCs or enters into a GC reaction are not completely understood. Initially, entry was thought to be stochastic. Immunisation experiments using the hapten 4-hydroxy-3-nitrophenyl (NP) and subsequent sequencing of the Ig_H V genes from GC B cells and adjacent extrafollicular foci of PCs have revealed the same clone may have established both compartments in some cases [5]. One interpretation of this result is that the pathway taken is not influenced by relative affinity for antigen.

Subsequently, evidence supporting selection in B cell fate determination has been reported [6–9]. One group has found that the third complementarity determining region (CDR3) of the V_H genes of early GC B cells and PCs differs in length, with GC B cells having shorter CDR3 compared to PCs [7]. This experiment suggested selection mediated by antigen binding during the bifurcation of B cell differentiation. Another group has demonstrated that transgenic B cells binding antigen with high affinity generate both GCs and extrafollicular PCs, whereas binding a low-affinity antigen generates predominantly GCs [8]. These results suggest that an affinity threshold separates the extrafollicular and intrafollicular pathways, with high affinity favouring extrafollicular PCs and low-affinity intrafollicular GCs [8]. Conversely, others have shown that high-affinity B cells could migrate into GCs and overwhelm established low-affinity clones [9]. These somewhat contradictory findings used two different experimental systems; the first used a constant B cell receptor (BCR) affinity and variable antigen, whereas in the second, the immune response involved competing BCR affinities and a constant antigen. Despite this discordance, both reports have demonstrated a role for selection based on BCR affinity for antigen in determining the fate of B cells.

More recently, it has been reported that a BCR with low affinity for antigen promotes apoptosis over extrafollicular PC expansion [10], questioning once again a role for BCR affinity in B cell fate selection. Interestingly, a separate study has found this to be true for GCs, with high affinity for antigen enhancing survival [11]. These studies imply that selection based on BCR affinity may reflect persistence of B cell clones rather than triggering a particular differentiation event.

Aside from the antigen and BCR affinity, T cell help is also key in B cell differentiation determination (Table 1)
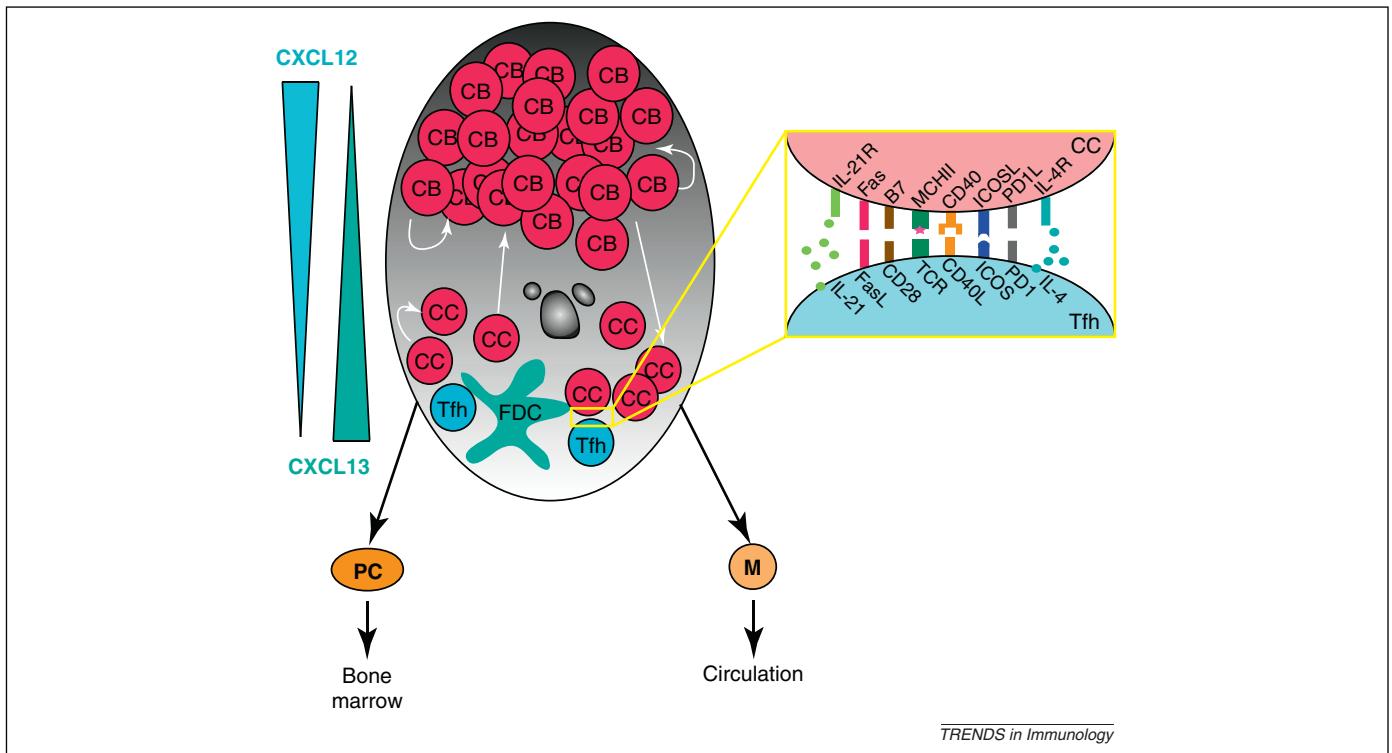
Figure 1. The GC reaction. From left to right: chemokine gradient within the GC, GC reaction and potential fates, and Tfh and B cell interactions. Proliferating B cells within the GC are called centroblasts (CBs). CBs are located in the dark zone (black shading) where they proliferate and undergo SMH of their B cell receptor. CBs that migrate to the light zone (grey shading) are referred to as centrocytes (CCs). CCs downregulate CXCR4 and migrate to the light zone in response to CXCL13, which is produced by FDCs. CCs have several potential fates (arrows). These include: **(i)** apoptosis, if cells are unable to bind antigen and/or gain Tfh cell help; **(ii)** return to the dark zone for further proliferation and SMH; and **(iii)** selection to differentiate into long-lived PCs or memory B cells (M). Long-lived PCs reside in the bone marrow, whereas memory B cells can recirculate. The factors that govern CC fate or selection are not fully understood; some key interactions between CCs and Tfh cells are shown on the right.

(reviewed in [12]). An inability of B cells to interact with cognate CD4+ T cells and receive necessary co-stimulation and cytokines has a dramatic impact on the fate of B cells. For example, T cell deficiency in the signalling lymphocytic activation molecule (SLAM)-associated protein (SAP) interferes with the formation of stable B cell/T cell interactions [13,14], which profoundly reduces GC B cell frequency but only mildly effects extrafollicular PC formation [15].

Although the cues that determine activated B cell fate are not clear, the changes required for a B cell to become an extrafollicular PC or enter a GC and to localise correctly are better understood (Table 1). Expression of chemokine receptors must be altered to allow the migration of blasting B cells into a primary follicle to form a GC, or alternatively to migrate to an extrafollicular site and differentiate into a PC (reviewed in [16,17]). Primarily, the upregulation of an orphan G-protein-coupled receptor called Epstein–Barr-virus-induced molecule 2 (Ebi2) and loss of chemokine CXC receptor (CXCR)5 and chemokine CC receptor (CCR)7 on blasting B cells are key for their extrafollicular

Table 1. Selection of mouse genetic modifications that affect T cell dependent immune responses

Gene knockout	Component affected	Proposed mechanism	Outcome	Reference
Bcl6	GC Formation	Loss of Blimp1 repression	No GCs, little CSR, germline memory	[30–32,109]
B7.1 and *B7.2*	B–T Collaboration	Defective T cell help	No GCs	[126–128]
Blimp1	PC Formation	Loss of Bcl6 repression	Enlarged GCs, no PCs	[129]
Cd19	B cell signalling	Defective B cell signalling	GCs form but are functionally impaired	[130]
Cd28	B–T collaboration	Defective T cell help	No GCs, reduced PCs	[128]
Cd40, Cd40l	B–T collaboration	Defective T cell help	No GCs	[131,132]
Cxcr4	Organ architecture	Loss of DZ and LZ polarisation	No segregation of B and T cells	[115]
Cxcr5, Cxcl13	Organ architecture	Loss of DZ and LZ polarisation	No segregation of B and T cells	[115,133,134]
Ebi2	Organ architecture	Defective extrafollicular cell migration	Mislocalised extrafollicular PCs	[18,19]
Icos, Icosl	B–T collaboration	Defective T cell help	No GCs, reduced PCs	[126,135–137]
Il-4	B–T collaboration	Defective T cell help	Reduced PCs and IgG1 CSR	[84]
Il-21, Il-21R	B–T collaboration	Defective T cell help	Reduced PCs, GCs form but are poorly maintained	[87,98,99]
Irf4	B–T collaboration	No AID expression and proliferation	No GCs, reduced PCs	[138]
Irf8	Organ architecture	Decreased Bcl6 and AID expression	Poor organisation of GCs	[22]
Mhcll	B–T collaboration	Defective antigen presentation	No GCs	[139]
Sap	B–T collaboration	Unstable T:B cell interaction	Smaller and fewer GCs	[13]