

Transitional B Cells: How Well Are the Checkpoints for Specificity Understood?

Anna Vossenkämper · Jo Spencer

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Abstract It is crucial for the immune system to minimise the number of circulating mature self-reactive B cells, in order to reduce the potential for the development of auto-antibody-related autoimmune diseases. Studies of animal models have identified two major checkpoints that ensure that such cells do not contribute to the naïve B cell repertoire. The first is in the bone marrow as B cells develop and the second is in the spleen; B cells that are released from the bone marrow as transitional B cells go through more stringent selection in the spleen before they develop into mature naïve B cells. Transitional B cells and their maturation have mostly been studied in mice. However, recent studies characterised human transitional B cells and found considerable differences to current models. In this review, we will consider these differences alongside known differences in mouse and human splenic function and ask whether human transitional B cells might develop along a different pathway.

Keywords Transitional B cells · Spleen · Checkpoint · Marginal zone

B cell Subsets in Mouse and Human

B cells in mice and humans originate from the bone marrow after birth, with a contribution also from the spleen in mice. The liver is the source of some B lymphocyte progenitors in both species, but only before birth. Newly produced B cells from bone marrow develop into transitional B cells in mice and humans (Carsetti 2000; Gale 1987; Rolink et al. 1993). However, subsequent parallels in B cell subset development are not clear.

In mice, transitional B cells give rise to two B cell subsets that are particularly involved in immune responses to bacterial antigens: B1 B cells that reside in the peritoneal cavity and the splenic marginal zone B cells. Transitional B cells also give rise to the B2 B cells that form the follicular mantle in secondary lymphoid tissues. The latter are the precursors of the effector cells of the B cell arm of the adaptive immune response (Rosado et al. 2009).

In humans, the definitions of B cell subsets and their developmental timeline are not so clear. Bone marrow precursors give rise to transitional B cells, but this stage is followed by considerable uncertainty (Weill et al. 2009). Humans, but not mice, have an IgM-only-B cell subset (i.e. expresses no secondary immunoglobulin isotypes) which is identifiable in the blood on the basis of the expression of B cell memory markers. However, this subset has a separate identity in terms of immunoglobulin configuration compared to class switched memory B cells to which it appears unrelated (Klein et al. 1997; Wu et al. 2010). The IgM-only-B cell subset is thought by some to be a circulating counterpart of marginal zone B cells, since unlike mouse marginal zone B cells, human marginal zone B cells have mutated IgV genes and have been described as memory cells (Dunn-Walters et al. 1995; Weill et al. 2009). However, like mouse marginal zone B cells, those in the human

A. Vossenkämper (✉)
Centre for Immunology and Infectious Disease,
Barts and The London School of Medicine and Dentistry,
Blizard Institute, London E1 2AT, UK
e-mail: a.vossenkaemper@qmul.ac.uk

J. Spencer
Peter Gorer Department of Immunobiology,
King's College London, Guy's Hospital Campus,
St Thomas' St, London SE1 9RT, UK

marginal zone are thought to be involved in immune responses to bacterial antigens (Weill et al. 2009). The human counterpart of another B cell subset in mice, the B1 cells, is similarly unclear, though a recent report has described them as a circulating CD27⁺, CD43⁺ B cell subset in humans (Griffin et al. 2011). Most of these cells express the CD5 antigen which is a feature of most murine B1 B cells, though CD5 is an activation antigen in humans but not mice (Freedman et al. 1989). Below we describe the features of transitional B cells and the spleen in mice and humans and consider the evidence that the spleen plays a significant role in selection of the naïve B cell repertoire from transitional B cell precursors in humans.

A Journey into Maturity: Transitional B Cells

The B cell pool is continuously replenished from the bone marrow throughout life in healthy individuals. As a consequence, B cells with a fresh set of receptors are continuously added to the repertoire. The mechanisms that generate diversity in B cell receptors (BCRs) create a spectrum of specificities that includes reactivity with specific autoantigens and also polyspecificity; a term that reflects the ability of an antibody to bind loosely to multiple different molecular shapes. Newly formed B cells must undergo several selection checkpoints in order to eliminate autoreactive B cells from the population. This process is highly regulated and enables the immune system to maintain a state of self-tolerance and the prevention of tissue damage due to B cells recognising self-antigens. There are two major checkpoints that filter out autoreactive and to some extent polyspecific B cells, ensuring that they make a minimal contribution to the mature recirculating B cell population.

The first checkpoint for specificity occurs in the bone marrow, where selection of B cells is mainly achieved by eliminating autoreactive B cells. B cells that react with self-antigens present in the bone marrow will be removed by mechanisms that include anergy (Brink et al. 1992; Hartley et al. 1991), clonal deletion (Nemazee and Buerki 1989; Nemazee and Burki 1989) and receptor-editing (Gay et al. 1993), particularly editing of the Ig light chain. Receptor editing replaces one gene rearrangement with another in immature B cells. The configuration of the light chain loci allows the potential for sequential rearrangements on the same allele, through rearrangements involving 5'V segments and 3'J segments to those involved in the original VJ join. There is evidence of light chain editing in mouse models and also in human cells where the most V proximal IGKJ segment is more common in the unproductive than the productive rearrangements. Positive selection requires the cytokine BLyS (B lymphocyte

stimulator, also known as B cell activating factor). The essential role of BLyS in promoting B cell survival has been reviewed thoroughly elsewhere (Harless et al. 2001; Mackay et al. 2010). Moreover, current data suggest that tonic signals via BCRs are critical for immature B cells to suppress Rag expression and to maintain their stage of development (Tze et al. 2005). Only a small percentage of transitional B cells manage to get through the first tolerance check and will then enter the periphery.

The second checkpoint for selection is the spleen where transitional B cells need to receive survival signals before they differentiate into mature naïve B cells and marginal zone B cells (Loder et al. 1999). For murine transitional B cells it has been reported that maturation also takes place in the bone marrow (Cariappa et al. 2007). The maturation of human B cell subsets is not so clearly understood and this will be discussed below following comparative analysis of splenic morphology and function and a description of B cell maturation in mouse spleen.

Maturation of Transitional B Cells in Mouse Spleen

The classification of murine transitional B cells into subclasses T0, T1, T2, and T3 is based on the expression of CD23, IgM and the developmental marker AA4 (Allman et al. 2001). T1 cells (sIgM^{high} sIgD^{low} CD23^{low} CD21/35^{low}) are found in blood, bone marrow and spleen, whereas T2 (sIgM^{high} sIgD^{high} CD23⁺ CD21/35^{int/high}) cells are only found in the spleen and are thought to be derived from the T1 cells (Allman et al. 2001; Sims et al. 2005). T3 (CD23⁺ IgM^{low}) cells seem to be an anergic splenic population that does not progress into the mature stage (Merrell et al. 2006; Srivastava et al. 2005; Teague et al. 2007). All transitional B cells populations were found to be non-proliferative *in vivo* (Allman et al. 2001). To enter the white pulp of the spleen, murine transitional B cells require signalling via the GTPases Rac1 and Rac2. Mice lacking the Rac GTPases show the accumulation of T0-like B cells in the blood. In addition, the development of T0 cells into T1 IgD⁺ B cells was blocked in both the spleen and bone marrow (Henderson et al. 2010). This developmental block rendered the T0-like cells unable to interact with the integrins LFA-1 and VLA-4, a process necessary to enter the spleen (Henderson et al. 2010; Lo et al. 2003). Differentiation from the T1 to the T2 stage requires SWAP-70, a guanine-nucleotide-exchange factor, and the T1 cells lacking SWAP-70 are unable to come to the marginal zone (Chopin et al. 2010). SWAP-70 negatively regulates $\alpha_1\beta_2$ and $\alpha_4\beta_1$ integrin function and lack of SWAP-70 resulted in T1 cells getting held in the red pulp and the follicles. The latter finding led to speculation if transitional B cells need to pass through the follicles before they enter the marginal zone.

The two integrins $\alpha_L\beta_2$ and $\alpha_4\beta_1$ seem to be important for retaining IgM^{high} cells in the marginal zone, as inhibition of both molecules resulted in release of the cells into the red pulp (Chopin et al. 2010).

Transitional B cells eventually enter different splenic compartments and develop into follicular or marginal zone B cells. Though the precise lineage scheme is not understood, it is considered that in mice, marginal zone B cells form at a branch in the B cell maturation pathway and they are a subset or separate developmental lineage of naïve B cells to those that occupy the follicular mantle. The situation can be confused by the observed presence of memory cells in the marginal zone. These cells may reside in the marginal zone or they may be in transit (Liu et al. 1988).

Human Transitional B Cells

Considerable progress has been made in understanding and characterising the different transitional B cell subsets in humans. About 40% of transitional B cells in human blood are self-reactive and will not enter the pool of mature B cells (Meffre et al. 2004; Wardemann et al. 2003). Sims et al. (2005) characterised cells in human peripheral blood that showed a very similar phenotype to murine T1 transitional B cells. Cells in this population were CD38⁺, IgD⁺, CD10⁺, CD27⁻, CD44^{low}, CD24^{hi}, CD21^{low}, CD23^{low}, IgM^{hi}, and CD62L^{low}. These cells also expressed CD5 and CD43, markers that are not found on murine T1 cells (Sims et al. 2005), but are associated with a proposed human B1 lineage (Liu et al. 1988). The authors ruled out that these cells are pre-germinal centre B cells (which are also CD38⁺, IgD⁺) by demonstrating that T1 B cells do not express CD27 and CD77, two markers that are typical for germinal centre B cells. Although it was not possible to separate human transitional B cells from subsets that correspond to the murine T1 and T2 populations, CD21 was found to be a marker that can be used to identify two distinct transitional B cell populations in human blood (Suryani et al. 2010). Based on the initial gating on CD20⁺CD27⁻CD10⁺ transitional B cells, the detected populations were either CD21^{low} or CD21^{hi}. These two populations could be isolated from peripheral blood, cord blood, and the spleen. However, the frequencies in which these populations were present differed considerably. CD21^{low} cells were most abundant in the human spleen and the least present in peripheral blood, in which CD21^{hi} cells appeared in the highest frequency. Genetic analysis revealed that CD21^{hi} transitional B cells were more mature than the CD21^{low} cells. In addition, in patients who underwent hematopoietic stem cell transplantation, the majority of the transitional B cells that appeared in the blood were CD21^{low}, suggesting that CD21^{low} cells

differentiate into CD21^{hi} cells (Suryani et al. 2010). A third population has been found besides human T1 and T2 cells. These so-called T3 cells were able to differentiate into mature B cells in vitro and characterised as being CD24^{int}CD38^{int}IgD⁺CD27⁻. This population was transient and especially high during reconstitution in patients after B cell depletion therapy and over time replaced by mature naïve B cells (Palanichamy et al. 2009), suggesting that T3 cells are the late stage of transitional B cells before they differentiate into mature naïve cells.

The Toll-like receptor 9 (TLR9) is found on B cells and plasmacytoid dendritic cells and binds the CpG motif in unmethylated bacterial DNA (Hemmi et al. 2000; Hornung et al. 2002). Human transitional B cells express TLR9 to a higher level than IgM memory and mature B cells (Capolunghi et al. 2008). Transitional B cells isolated from cord blood as well as peripheral blood from adult donors were shown to respond to TLR9 stimulation with a low frequency of somatic hypermutation in vitro (Aranburu et al. 2010; Capolunghi et al. 2008). Intriguingly, a subset of transitional B cells differentiated into IgM-secreting plasma cells when cultured with CpG for 7 days, whereas other subsets showed the phenotype of memory B cells (CD24^{bright}CD38⁻CD27⁺) or mature naïve B cells (CD24⁻CD38⁻CD27⁺) (Capolunghi et al. 2008). These data suggest that transitional B cells play an important role in building up a fast anti-bacterial response by differentiating into mature B cells and plasma cells upon TLR9 activation. This process might be particularly beneficial in newborns.

Differences between Mouse and Human Spleen

Can we assume that B cell maturation in the spleen from transitional to mature B cell phenotypes as observed in murine studies also occurs in humans? When studying B cell development in both species, one has to consider the differences that exist in the architecture of the lymphoid tissue in mouse and human spleen. Histological images of the spleen of both species are shown in Fig. 1.

Structurally, the white pulp in the murine spleen is highly abundant, whereas in human spleen proportionally more red pulp is found, which acts as a blood filter for erythrocytes (Cesta 2006). The white pulp is composed of the periarteriolar lymphoid sheath (PALS; a T cell compartment around the central arterioles), the B cell follicles, and the marginal zone. Secondly, in the human spleen there is no marginal sinus which in rodents separates the marginal zone from the follicles and the PALS. In mice, the marginal zone surrounds the entire white pulp (follicles and T cell area) whereas in the human spleen, the marginal zone is divided in an inner and outer zone by fibroblasts

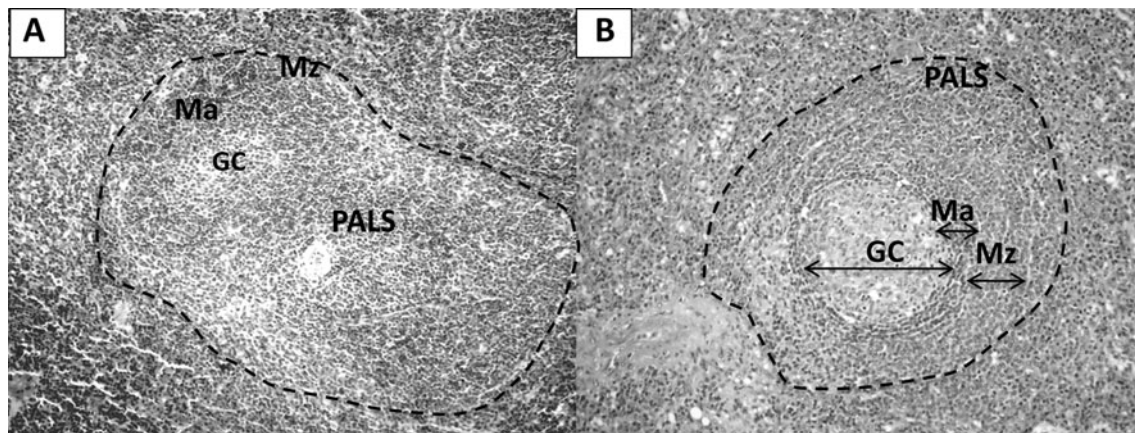


Fig. 1 Paraffin sections of mouse spleen (**a**) and human spleen (**b**) at the same magnification ($\times 100$) to illustrate some basic microanatomical differences between them. The boundary between the white pulp and the red pulp is indicated by a dotted line. White pulp constitutes a greater proportion of mouse than human splenic tissue. The human red pulp is highly vascularised comprising sinusoids and

cords that have a filtration function, and also capillaries. In the white pulp in humans the marginal zone surrounds the naïve cells of the mantle zone. In mice, the marginal zone relates to the outer boundary of the white pulp. *GC* germinal centre, *Ma* mantle zone, *Mz* marginal zone, *PALS* periarteriolar lymphoid sheath

and only surrounds the follicle (Mebius and Kraal 2005). In humans, the marginal zone is separated from the red pulp by the perfollicular zone which mainly contains capillaries (Crivellato et al. 2004). Further, primary follicles are more abundant in the human spleen where they dominate the white pulp. The PALS in humans is discontinuous and most prominent around only the larger arteries. The follicles are mainly populated by B cells, but also hold follicular dendritic cells and macrophages. Upon antigen encounter, germinal centres develop where activated B cells clonally expand and undergo somatic hypermutation and isotype switching. The marginal zone serves as an entrance area for cells entering the white pulp from the blood stream. Details about the cellular structure, cellular organisation, and chemokine environment in the murine marginal zone were summarised by Mebius and Kraal (2005), whereas an overview of the human marginal zone is found in Steiniger et al. (2001) and Wilkins and Wright (2000).

In addition to structural differences between mouse and human spleen described above, B cell development from precursors, and also maturation of the B1a B cell subsets that is associated with mucosal IgA production occurs in the spleen of mice and not in humans (Rosado et al. 2009), implying a marked difference in functional capability within the structure of mouse and human spleen.

Relationship between Human Transitional B Cells and the Spleen

Differences in the repertoire and functional properties of transitional and mature naïve B cells exist and the

repertoire and binding capability of naïve and transitional B cells are different (Yurasov et al. 2005). However, does this evidence of repertoire development necessarily implicate involvement of the spleen?

In studies of the maturation of murine transitional B cells, T1 and T2 cells are features of the splenic tissue. However, T1, T2 and T3 cells have been observed in both human cord blood and bone marrow prompting the suggestion that maturation can be extrasplenic (Marie-Cardine et al. 2008; Palanichamy et al. 2009). Whilst some observe transitional B cells in human spleen, others do not (Marie-Cardine et al. 2008; Suryani et al. 2010; Weill et al. 2009).

It has been suggested that the splenic marginal zone is directly derived from transitional B cells in humans as in mice (Weill et al. 2009). However, human splenic marginal zone B cells accumulate over the first 2 years of life (Timens et al. 1989) and have somatic mutations in their immunoglobulin heavy chain variable region (IGHV) genes (Dunn-Walters et al. 1995). No hypermutation of IGHV genes in transitional B cells has been described, other than the very low frequencies associated with cellular activation (Rosado et al. 2009). It is difficult to see how human splenic marginal zone B cells could be derived directly by maturation alone from transitional B cell precursors, thus potentially removing the possibility of a direct differentiation step from transitional B cell to marginal zone B cell in man.

Relative entry from transitional into follicular or marginal zone compartments in mice is dependent on the Bruton's tyrosine kinase (Btk) that signals down stream of BCR ligation (Cariappa et al. 2001; Martin and Kearney 2000). In humans, Btk has a much more critical role in B cell development; mutations in Btk result in X-linked

agammaglobulinemia, a disease characterised by very low peripheral blood B cell numbers and virtually absent plasma cells (de Weers et al. 1993; Vetrie et al. 1993). It is therefore beyond doubt that human and mouse B cells have profound differences in differentiation, lineage and function.

Conclusion

In summary, we should not assume that B cell development from transitional B cell to mature B cells proceeds along the same pathway in humans as in mice, or that it necessarily involves the spleen. There is a lack of direct equivalence in mature B cell subsets in humans and mice, and where apparent equality does exist, such as the splenic marginal zone B cells, there are marked differences on closer inspection that relate directly to pathways of differentiation. Coupled to this, there are fundamental differences in mouse and human splenic anatomy. Clearly, experimentation in human subjects and using human cells poses significant challenges, but we should not assume that mouse models always provide adequate answers, especially when there may be indicators to the contrary.

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Conflict of interest The authors declare no conflict of interest.

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