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Translational Mini-Review Series on B cell subsets in disease. Reconstitution after haematopoietic stem cell transplantation – revelation of B cell developmental pathways and lineage phenotypes

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M. Bemark,*† J. Holmqvist,*† J. Abrahamsson‡ and K. Mellgren‡
*Department of *Microbiology and Immunology and ‡Pediatrics, Institution for Clinical Sciences, University of Gothenburg, and †Department of Clinical Immunology and Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden*

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Correspondence: Dr M. Bemark, Department of Microbiology and Immunology, Gothenburg University, PO Box 435, 40530 Gothenburg, Sweden.
E-mail: mats.bemark@immuno.gu.se

Summary

Haematopoietic stem cell transplantation (HSCT) is an immunological treatment that has been used for more than 40 years to cure a variety of diseases. The procedure is associated with serious side effects, due to the severe impairment of the immune system induced by the treatment. After a conditioning regimen with high-dose chemotherapy, sometimes in combination with total body irradiation, haematopoietic stem cells are transferred from a donor, allowing a donor-derived blood system to form. Here, we discuss the current knowledge of humoral problems and B cell development after HSCT, and relate these to the current understanding of human peripheral B cell development. We describe how these studies have aided the identification of subsets of transitional B cells and also a robust memory B cell phenotype.

Keywords: B cell, haematopoietic stem cell transplantation, lymphocyte development

Haematopoietic stem cell transplantation

Haematopoietic stem cell transplantation (HSCT) following high-dose treatment with cytotoxic drugs and sometimes total body irradiation (TBI) has become a common clinical practice for treatment of malignant and non-malignant diseases over the past 40 years. Today, HSCT is the only curative treatment for certain inherited disorders, including immune deficiencies, haemoglobinopathies and bone marrow failure syndromes, and it is also used to treat haematological and non-haematological malignancies. Patients with malignant disease receiving HSCT are often heavily pretreated with combinations of cytostatic drugs and already have immune dysfunctions prior to transplantation [1]. Patients transplanted for non-malignant diseases have a wide range of immunodeficiency, ranging from a total lack of adaptive immunity in severe combined immunodeficiency (SCID) patients to essentially normal function in patients with haemoglobinopathies. Before infusion of stem cells, patients are conditioned with a regimen consisting of high doses of cytostatic drugs and sometimes also TBI to obtain a total (myeloablative conditioning) or a subtotal myeloablation (reduced intensity conditioning). In malignant disorders, an intense conditioning regimen contributes to the eradication of remaining neoplastic cells, while for non-malignant disorders a regimen that induces tolerance to the graft is sufficient. The graft consists of $CD34^+$ stem cells obtained from related or unrelated donors mixed with a smaller or larger number of mature immune cells. Stem cells obtained from peripheral blood contain larger number of haematopoietic cells and give faster engraftment, but are also associated with an increased incidence of graft-*versus*-host disease (GVHD) [2]. The goal for the procedure is a haematopoietic system that produces fully functional erythrocytes, thrombocytes, myeloid cell lineages and lymphocytes. The first three objectives are usually accomplished within the first months. Lymphocyte numbers and functions are, however, impaired for extended periods. Whereas the development of T cell lineages after HSCT has been defined in considerable detail,

less is known about B cell development and how it relates to the impaired functional immunity.

The establishment of haematopoietic lineages after HSCT

The production of different cell types from grafted haematopoietic stem cells follows a specific pattern [3,4]. The first cells produced are granulocytes and other cells of myeloid lineages [monocytes, macrophages and dendritic cells (DC)], erythrocytes and thrombocytes. These early cells are sometimes functionally impaired. Monocytes may not produce normal amounts of interleukin (IL)-1 and neutrophil functions (e.g. chemotaxis, phagocytosis and bacterial killing) may be attenuated, in particular in patients developing GVHD [5,6]. In addition, subtype deficiencies have been described within certain cell lineages. Myeloid DC and Langerhans cells are, for example, usually found within the first 6 months, whereas $CD123^+$ plasmacytoid DC are rare even 1 year after transplantation [7,8].

Cells of the adaptive immune system form slower than innate cells after HSCT, and functional deficiencies can be detected years after normal numbers of cells are reached [3,4]. As the numbers of $CD8^+$ T cells increase earlier than $CD4^+$ T cells, the CD4/CD8 ratio is initially reversed [9]. This early expansion is dependent on homeostatic peripheral proliferation of memory T cells, both of rare recipient-derived T cells surviving the conditioning regimen and of donor-derived co-transferred T cells, rather than from thymic production [10,11].

Although this is a polyclonal proliferation, it is not completely antigen-independent – $CD8^+$ T cell clones recognizing viruses present in the body at the time of transplantation, e.g. cytomegalovirus (CMV) or Epstein–Barr virus (EBV) may expand rapidly [12–14]. During the first months after transplantation a narrow and skewed repertoire of T lymphocytes with memory-like phenotype predominates. *De novo* production of naive cells from the thymus starts later, particularly in adult patients [10]. Therefore, a dominance of oligoclonal T cells with a memory phenotype is observed, sometimes for several years [15]. The generation of naive T cells from donor cells in thymus does not result in full immune reconstitution for at least 1 year after transplantation and is compromised by factors affecting thymic epithelial cells such as irradiation, GVHD and age. Indeed, slow recovery of specific T cells has been shown to have a significant impact on survival of the patients [16].

B cells are rare in peripheral blood during the first months after HSCT and reach close to normal levels within 6–12 months [17]. The CDR3 immunoglobulin (Ig)M spectra, i.e. the distribution of B cells with different lengths of their heavy chain V-D-J regions, are essentially normal with no indication of homeostatic, oligoclonal proliferation within 3–6 months after transplantation [18,19]. Memory B cells expressing CD27 do not expand and subnormal levels are observed during the first 2 years after transplantation [20–22]. Transfer of donor memory B cells that can be reactivated after antigen re-encounter is well documented [23,24]. Low numbers of recipient-derived B cells can also be encountered during the first period after transplantation, especially after reduced intensity conditioning [25]. Because B cell-depleted and non-depleted bone marrow give similar reconstitution in patients who underwent intense myeloablative conditioning, pre-existing mature B cells do not seem to play a major role long-term [17,26,27]. Although only donor-derived B cells circulate in blood, the maintenance of recipient-derived serum antibodies several years after transplantation reflects the resistance and longevity of plasma cells [28–30].

Immunological problems after HSCT

HSCT is associated with major immunological complications. During the first weeks after transplantation, high levels of inflammatory cytokines such as IL-7 and IL-15 induced by the myeloablative conditioning in combination with lymphopenia induce expansion of donor-derived T cells. The presence of specific antigens may favour proliferation of cells directed against mismatched histocompatibility antigens in the host, leading to acute GVHD (aGVHD) [31]. The chronic form of GVHD (cGVHD) can appear later, and donor-derived T cells processed in the thymus are then involved [32]. A certain level of cytotoxic T cell-mediated self-reactivity may still be beneficial in some cases, as the so-called graft-*versus*-leukaemia reaction (GVL), which reduces the risk of relapse, is mediated by cytotoxic T cells and natural killer (NK) cells from the donor and prevents relapse [33].

Another major problem after HSCT is the high incidence of severe infections due to the immunocompromised state of the patients [34]. At the very early pre-engraftment stage, leukopenia in combination with mucosal membrane damage induced by the conditioning regimen put the patients at high risk for infections with bacteria and fungi. Post-engraftment, newly produced cells of the innate immune system confer some protection, but cellular immunity is impaired. This stage is associated with viral infections, in particular herpesvirus such as CMV, but fungal infections are also common. At a later phase, cellular and humoral immunity slowly recovers. In some patients this phase only lasts for a year, while in others it may develop into a chronic problem. The development of GVHD requiring treatment with immunosuppressive agents interferes with immunological reconstitution. Recurrent infections with encapsulated bacteria, such as *Haemophilus influenzae* and *Streptococcus pneumonia*, are common problems even late after HSCT [35–37]. B cell-mediated immunity is important in protection from these bacteria and a deficiency of the humoral immunity is hence likely to be involved [38].

Humoral immunity after HSCT

Although mature B cells are efficiently depleted during conditioning, the levels of circulating IgG antibodies drop slowly [17,39]. This is due to the long half-life of IgG in serum [40] and the survival of many plasma cells after myelodepletion [30]. Eventually, specific plasma cells disappear, resulting in loss of antibodies against antigens encountered before transplantation [41,42]. Why plasma cells are lost relatively quickly in HSCT patients compared to healthy individuals is not clear, but may be due to cytotoxicity of the conditioning regimen [43], damage to supporting cells in the bone marrow such as eosinophils and stroma [44,45] or depletion of plasma cells of recipient origin by donor-derived T cells [30]. In addition, memory B cells may be needed to replenish the pool of long-lived plasma cells [46,47].

Specific antibodies from long-lived plasma cells are pivotal in sterile immunity after vaccination, through blocking of viruses, bacteria and toxins [48]. The loss of specific antibodies after HSCT necessitates revaccination. Some vaccines, in particular from inactivated viruses and bacteria, give protective responses within a year after grafting [49]. Others, especially carbohydrate-based vaccines, are not efficient until much later [50,51]. Recipient-derived memory B cells sometimes seem to survive the conditioning regimen and give rise to transient monoclonal production of antibodies [30] and functional memory B cells can also be transferred from the donor [23,52]. Thus, regimens where both the donor and the recipient are vaccinated before transplantation have been tested to improve post-transplantation immune responses [53]. As a drawback to this phenomenon, transfer of autoimmune diseases and allergies from the donor has been documented [54].

Antibody subclasses emerge in a distinct order after HSCT, with production of IgM antibodies within a few months, followed by IgG1/IgG3, IgG2/IgG4 and finally IgA [55]. This order recapitulates normal development during the first year of life. Long-term antibody class deficiencies are observed in some patients [56]. B cells from HSCT patients show close to normal *in vitro* responses to polyclonal EBV activation, while they respond to a lesser degree to pokeweed mitogen (PWM) [57,58]. The *in vitro* response to PWM requires T cells to be present, while EBV does not [59,60]. T cells from HSCT patients have a decreased ability to support B cell activation by PWM. Intrinsic deficiencies within the B cell compartment that may hinder PWM responses have also been demonstrated [57,58].

Thus, humoral deficiencies after HSCT are common and can be life-long in some patients, due to intrinsic defects in B cells as well as in supporting cells. Low numbers of naive $CD4^+$ T cells in blood have been documented years after transplantation [15]. Follicular dendritic cells (FDC), a non-haematological cell type supporting germinal centre formation, are also damaged [61,62]. These defects severely impair the production of T cell-dependent antibody responses with germinal centre reactions and B memory cell development. From a clinical perspective, the most important problems late after HSCT, even in the absence of cGVHD, are recurrent infections with encapsulated bacteria and poor responses to polysaccharide vaccines [34]. Antibody responses against polysaccharides are initiated typically through T cell-independent pathways, and intrinsic B cell defects may be critically involved in causing these problems.

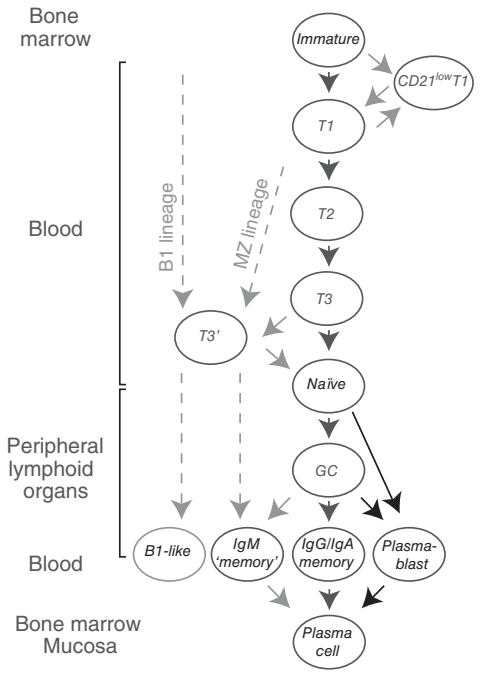
Human B cell differentiation stages and lineages present in peripheral blood

The current model of human peripheral B cell development involves five major consecutive stages: transitional B cells that have just left the bone marrow but are still unable to respond to antigen, naive B cells that are fully mature but have not encountered antigen, germinal centre B cells in lymphoid organs that are actively engaged in immune responses, memory B cells that have encountered antigen and survive for extended periods and plasma cells that produce soluble antibodies (Fig. 1a). In mice, splenic marginal zone B cells (MZB) and peritoneal B1 B cells represent separate lineages [63]. The presence or not of these in humans will also be discussed below. Germinal centre cells and fully mature plasma cells are only rarely present in blood and have been covered in other recent reviews [64]. They will therefore not be discussed further here.

Transitional cells

Developmentally, mouse transitional B cells are positioned between bone marrow immature B cells and mature peripheral B cells. Induction of apoptosis following BCR receptor signalling makes this stage an important checkpoint for removal of autoreactive specificities [65]. Analogous cells were described more recently in humans based on functional characteristics and expression of several cell surface markers [65–67]. Although rare in adult blood, making up fewer than 5% of the B cells, increased frequencies of transitional B cells are found in cord blood, after bone marrow transplantation, and in patients with systemic lupus erythematosus (SLE), X-linked lymphoproliferative syndrome ((XLP) or common variable immunodeficiency (CVID) [66,67]. When first described in humans, transitional cells were divided into two populations, T1 and T2, based on expression of transitional markers [67,68] (Figs 1b and 2a). It was recently shown that CD21 expression divides the T1 population into two. $CD21^{low}$ T1 cells were suggested to be more immature than $CD21^+$ T1 cells [69]. Because most anergic human B cells that have encountered antigen down-regulate expression of CD21 [70], it is possible that $CD21^{low}$ T1 cells are anergic. An alternative definition of human transitional B cells is that they are $CD27^-IgM^+$ and lack the ABCB1 transporter protein [71] (Fig. 2b). Based on these two ways of defining transitional cells, Palanichamy *et al.* found that cells lacking ABCB1 but

(a)



(b)

	IgM	IgD	CD5	CD10	CD20	CD21	CD23	CD24	CD27	CD38	CD43	ABCB1	CD45RB^{MEM55}
CD21^{low}T1		low	high	+	high	–/low	+/–	high	–	high	–	–	–
T1	high	int	high	+	high	+	+	high	–	high	–	–	–
T2	high	high	low	low	+	+	+	int	–	int	–	–	–
T3	low	high	–	–	+	+	+	low	–	low	–	–	–
T3'	high	high	–	–	+	+	+	low	–	low		–	+
Naïve	low	high	–	–	+	+	+	low	–	low	–	+	–
CD27^{-} IgG/IgA memory	–	–	–					low	–	low		–	+
IgG/IgA memory	–	–	–	–	+	+	low	int	+	–	+	–	+
IgM 'memory'	high	low	–	–	+	+	low	int	+	–	–	–	+
B1-like	high	high	+/–		+	+			+		+		+
Plasma-blast	low/–	low/–			–				high	high	+		+

Fig. 1. Human B cell development. (a) When immature B cells leave the bone marrow, they go through distinct differentiation stages. Transitional (T1, T2 and T3), naive and memory B cells as well as plasmablasts are found in blood. Black arrows indicate differentiation pathways well supported by experimental data, grey arrows pathways less well-defined or only proposed pathways. Recent data suggest that immunoglobulin (Ig)M^{+}CD27^{+} cells may not be memory cells, but rather the human counterpart to marginal zone B or B1 lineages. T3′ cells lack CD27^{-} but express CD45RB^{MEM55} and may be upstream cells in these lineages or cells downstream from T3 cells. (b) The different B cell stages can be distinguished from each other based on expression of cell surface markers. The subtypes express or lack expression, as indicated. Blank spaces indicate that the expression of this marker has not, to our knowledge, been described in the literature.

otherwise conforming to the phenotype of naive cells were enriched after B cell depletion with rituximab [72]. These cells appeared to be at a stage between T2 and naive cells, and were designated T3 cells. We found recently that T3 cells can be divided further into two populations based on CD45RB^{MEM55} expression (Fig. 2b; Bemark *et al.*, submitted; see below). Thus, in humans, three consecutive stages of transitional cells are now recognized – T1, T2 and T3 – and T1 and T3 cells can be divided further based on expression of CD21 and CD45RB^{MEM55}, respectively (Fig. 1a,b).

Naive cells

Naive cells constitute more than half of the blood B cells in healthy adults [73]. They express low levels of IgM, high levels of IgD and lack expression of CD27. In addition, they are the only blood B cells that express the ABCB1 transporter [71]. Activation requires binding of antigen to cell-surface antibodies, CD40 signals from CD4^{+} T cells as well as cytokine stimulation. After activation, some naive B cells will differentiate to early IgM-producing plasma cells while others enter B cell follicles, where they form germinal centres [74]. By regulating the class-switch recombination process, cytokines derived from T cells present during B cell activation determine which antibody class the B cells will produce. After random mutagenesis and selection of high-affinity B cell clones, mutated high-affinity class-switched B cells leave the germinal centre to become long-lived memory cells or long-lived plasma cells.

Memory B cells

Memory B cells are easier to activate than naive cells, and induce specific IgG production rapidly after antigen re-encounter [75]. Markers to distinguish naive B cells from memory B cells with mutated antibody were lacking for a long time. The discovery that CD27 was expressed on mutated human peripheral blood B cells was therefore important [73]. When the expression of both CD27 and IgM is determined on B cells, two populations of similar sizes can be identified: IgM^{-} class-switched memory and IgM^{high} memory cells. The latter are sometimes separated further based on high or low IgD expression. Whereas it is generally agreed that the IgM^{-} cells are class-switched post-germinal centre memory cells, the origin of IgM^{high}CD27^{+} cells is controversial. It is either argued that IgM^{+} memory cells are bona fide memory B cells generated early during germinal centre formation [76,77] or B cells diversified in the absence

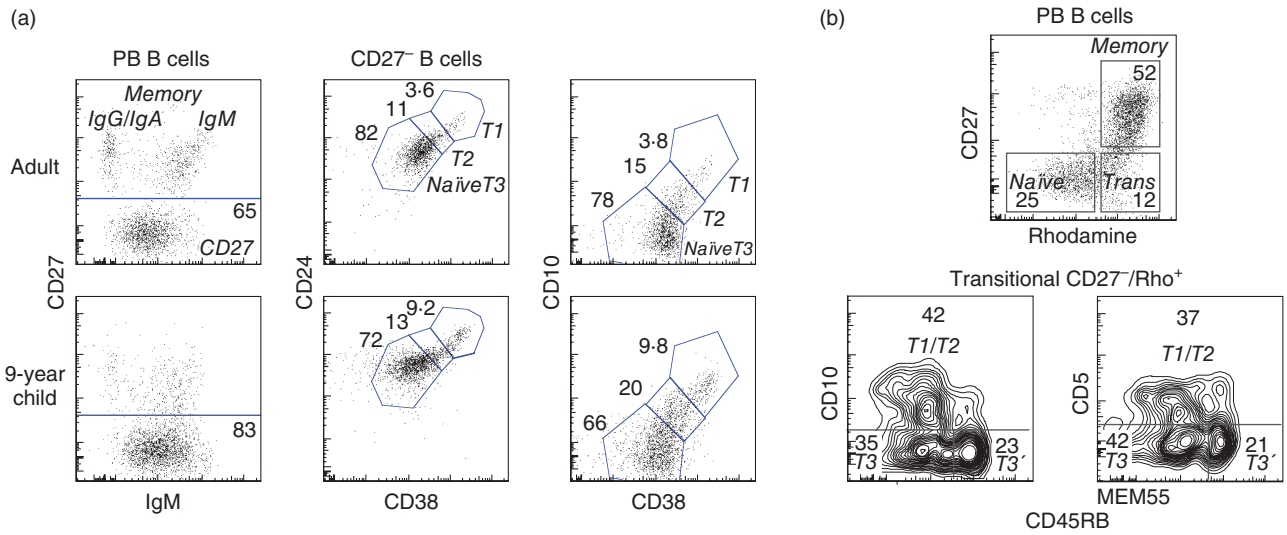


Fig. 2. Identification of peripheral blood B differentiation stages based on expression of surface markers. In (a) peripheral blood cells were stained with antibodies against CD19, CD27, immunoglobulin (Ig)M, CD24, CD38 and CD10. To the left, CD19$^+$ B cells were gated into IgM$^+$CD27$^+$, class-switched memory B cells and CD27$^-$ cells. The CD27$^-$ cells were divided further into T1, T2 and T3/naive cells based on expression of CD24 and CD38 (middle panels) or CD10 and CD38 (right panels). Typical results obtained using peripheral blood from five adults and four children are shown. In (b), peripheral blood cells were preincubated with the dye Rhodamine 123 followed by antibodies against CD19, CD27, CD5, CD10 and CD45RB^{MEM55}. CD19$^+$ B cells were divided into naive, memory and transitional cells based on expression of CD27 and extrusion of the dye (upper panel). The transitional cells could be divided further into T1/T2 cells expressing CD5 and CD10, and two distinct populations (T3 and T3′) that lacked these based on expression of CD45RB^{MEM55} (lower panels). The data represent typical data from three different healthy donors tested.

of an immune response that are similar to cells found in the marginal zone of the spleen [78,79] (see below). In addition to these populations, other minor memory B cell populations have been described. These include CD27$^+$IgM$^-$IgD$^+$ cells and CD27$^-$ class-switched cells [71,73,80]. Recently, our group found that all memory B cell populations, but not naive cells, express CD45RB^{MEM55}, possibly making it a better marker for memory B cells than CD27 [81].

Plasmablasts and plasma cells

Cells immediately preceding plasma cells in development, so-called plasmablasts, home to bone marrow or mucosal surfaces through the blood to develop into plasma cells. They can be identified by their expression of high levels of CD27 and CD38 and lack of CD20. Normally, these cells represent a minor population in peripheral blood, but after infection or vaccination the number of antigen-specific plasmablasts in blood increases rapidly and then disappears quickly [82]. Few fully mature plasma cells are found in blood.

Marginal zone and B1 B cells

The human subsets defined above, except for IgM^{high}CD27$^+$ B cells, are similar to developmental stages described for mouse follicular B2 cells. In mice, two separate B cell lineages associated with innate immunity exist; peritoneal B1 B cells and marginal zone B cells (MZB), that have distinct developmental pathways [63]. These are sometimes referred to as innate-like B cells, as they have a separate repertoire of unmutated antibody specificities and respond to antigens in the absence of T cell help. Mouse MZB share their early differentiation with follicular B2 cells, but are selected during the transitional stage to enter the marginal zone of the spleen. B1 B cells are generated early during ontogeny and home to the peritoneum where they form a self-renewing compartment [83]. If these subsets are also present in humans has been debated, specially whether human MZB and B1 cells are present among the IgM^{high}CD27$^+$ cells in blood.

Weill and colleagues suggested in 2004 that non-class-switched CD27$^+$ human blood B cells are the circulating counterpart of cells present in the splenic marginal zone rather than memory cells generated in germinal centres [84]. Subsequently, the same group showed that these subsets may diversify their antibody genes through somatic hypermutation in the absence of typical immune responses [85]. In favour of a non-memory origin, mass sequencing of antibody genes did not reveal related sequences between IgM$^+$ and IgM$^-$ CD27$^+$ B cells in human blood, and found little evidence for clonal expansion among IgM$^-$CD27$^+$ B cells [79]. Reports have also suggested that the function of circulating IgM^{high}CD27$^+$ B cells and marginal zone human B cells may be similar to the one described for MZB in mice, i.e. to respond to polysaccharides in T independent response [78]. Mouse MZB are non-mutated, mainly sessile B cells, clearly differentiating them from human circulating IgM^{high}CD27$^+$ B

cells, and many groups still prefer to interpret them as memory cells [76,77].

Unlike the situation in mice, only a small percentage of cells isolated from the human peritoneum express B cell markers, making up a population of less than 10^5 cells in healthy adults [86]. Of these, some express CD5 [87], a marker for B1 cells in mice, but it is questionable if these are B1 cells, as the largest CD5-expressing human B cell population is transitional cells. B cells in the human peritoneum do not seem to contribute to mucosal IgA-producing plasma cells, a feature associated with mouse B1 cells [88]. This does not exclude the possibility of B1-like cells in other organs. Griffins *et al.* described recently a small population of unmutated IgM^+ B cells present in both cord blood and healthy adults that expressed CD27 together with CD43 and shared functional characteristics with mouse B1 cells [89]. Although this is to be confirmed by other groups, it may explain the importance of $IgM^{high}CD27^+$ B cells in the responses to encapsulated bacteria [38].

Thus, although it is not proven indisputably that humans have B cell lineages distinct from follicular B cells, several recent studies suggest that this may be the case. In particular, these studies have suggested that such cells can be identified among circulating $IgM^{high}CD27^+$ human B cells. The $IgM^{high}CD27^+$ population may hence not be homogeneous but rather made up of several subtypes of cells with distinct characteristics, possibly explaining some of the conflicting data with regard to the origin and function of this subtype.

$CD45RB^{MEM55}$ is expressed differentially during B cell differentiation

We demonstrated recently that $CD45RB^{MEM55}$ is expressed differentially during B cell differentiation [81]. Although CD45 is expressed on all haematopoietic cells, it has three exons, RA, RB and RC, which are expressed differentially in many of them during differentiation as a consequence of regulated splicing [90]. The splicing pattern of CD45 does not change during peripheral human B cell development, with the longest CD45RABC form being dominant at all stages. However, the expression of the $CD45RB^{MEM55}$ epitope changes through developmentally regulated glycosylation in human B cells, with high expression on essentially all $CD27^+$ B cells but not on naive or transitional B cells [81]. The $CD45RB^{MEM55}$ epitope is also present on a minor population of $CD27^-$ B cells that express high levels of IgM (Fig. 2b). These cells are present in increased numbers in young children and in cord blood, and constitute 25–50% of all B cells even 1 year after HSCT, indicating that these are immature B cells (Bemark *et al.*, submitted). Interestingly, these $IgM^{high}CD27^-CD45RB^{MEM55+}$ cells lack transitional cell

Table 1. Cell surface markers showing abnormal expression on B cells after haematopoietic stem cell transplantation (HSCT).

Antigen	Number of cells*	Comments	References
mIgM	A	High levels of membrane bound IgM on B cells post-HSCT	[98–100]
mIgD	A	Slightly increased levels of membrane-bound IgD post-HSCT	[98]
		Few $mIgD^-$ class-switched cells 1 year after transplantation	[21]
CD1c	+	Increased numbers first year post-HSCT	[97]
CD5	+	Increased numbers first year post-HSCT	[96–98,101]
		Increased expression of IgM, CD20 and HLA-DR on $CD5^+$ B cells	[101]
		Normal expression of CD11a, CD44, CD54 and CD62L on $CD5^+$ B cells	[102]
	N	Normal numbers first year post-HSCT	[103]
CD10	+	Increased numbers post-HSCT	[95,104,105]
	N	Normal numbers post-HSCT	[97,103,106]
CD11a	–	Decreased numbers of $CD5^-$ B cells that express CD11a 4 months after transplantation	[102]
CD21	–	Decreased numbers first half-year post-GSCT	[27,93,98,99,105]
	N	Normal numbers of cells first 10 months post-HSCT	[97,103]
CD23	–	Low numbers first half-year post-HSCT, then normal or slightly increased numbers of cells	[26]
	+	Increased numbers of cells first 10 months, then normal numbers	[97,103,104]
	N	Normal numbers early and late post-HSCT	[98]
CD27	–	Low numbers post-HSCT. Both IgM^+ and class-switched cells affected	[20,91–94]
CD38	+	Increased numbers first half-year post-HSCT	[97–99,106]
CD44	–	Low numbers of $CD5^-$ B cells that express CD44 4 months after transplantation	[102]
CD54	–	Low numbers of $CD5^-$ B cells that express CD54 4 months after transplantation	[102]
CD62L	–	Low numbers first 10 months	[98,103]
		Low numbers of $CD5^-$ B cells express CD62L 4 months after transplantation	[102]
	N	Normal numbers post-HSCT	[97]
HLA-DR	N	Normal numbers post-HSCT	[97,106]

*The number of B cells in blood expressing the respective marker: A: all B cells; N: normal expression compared to healthy controls; –: decreased percentage of B cells in blood compared to healthy control; +: increased percentage of B cells in blood compared to healthy control; HLA-DR: human leucocyte antigen D-relayed; Ig: immunoglobulin.

markers such as CD5 and CD10 but, in similar with transitional cells, do not express ABCB1.

Development of B cell subtypes after HSCT

Some recent studies have investigated the development of B cell subsets after HSCT following the classifications outlined above. These have found low numbers of class-switched and IgM$^+$ CD27-expressing memory B cells in peripheral blood for extended periods after transplantation [20,91–94]. In contrast, the number of transitional cells was high [66,94,95]. Studies performed before the current schedule of B cell stages, however, also determined the expression of many cell surface markers on B cells (Table 1). These studies can now be reconciled with the current understanding of B cell development. Many of these early studies found increased expression of CD5, CD38 and IgM and lowered levels of CD62L expressing cells, all features of transitional B cells. In addition, few class-switched cells were found, in line with a delay in memory cell formation.

It should, however, be pointed out that phenotypic features of transitional human B cells change after HSCT (Fig. 3c; Bemark *et al.*; submitted). Although IgM^{high} cells in blood constitute the majority of B cells 1 year after transplantation, fewer than half of these express other transitional markers (Fig. 3a–c). Many IgM^{high}CD27$^-$ B cells instead express CD45RB^{MEM55}, identifying them as the T3′ population described above. The slow development of CD27$^+$ B cells after HSCT is of interest (Fig. 3b). The establishment of class-switched memory cells mimics the production of class-switched antibodies in serum, and most probably the ability to form germinal centres in peripheral lymphoid organs. With the proposed role of IgM$^+$CD27$^+$ B cells in response to encapsulated bacteria, their slow occurrence may be linked to infections after HSCT. Furthermore, the suggestion that IgM^{high}CD27$^+$ B cells are from a separate lineage to follicular B cells indicate that these lineages may establish slowly or not at all after HSCT.

Interestingly, many phenotypic changes encountered on B cells after HSCT are more pronounced in patients developing GVHD, and the B cell reconstitution is, in this case, also delayed [91–94,96–99]. Thus, monitoring of phenotypic changes on B cells following HSCT may not only give clues about the maturity of the immune system, but may also give early indications if GVHD is developing.

Concluding remarks

HSCT patients provide a rare opportunity to study early peripheral cell development through consecutive blood

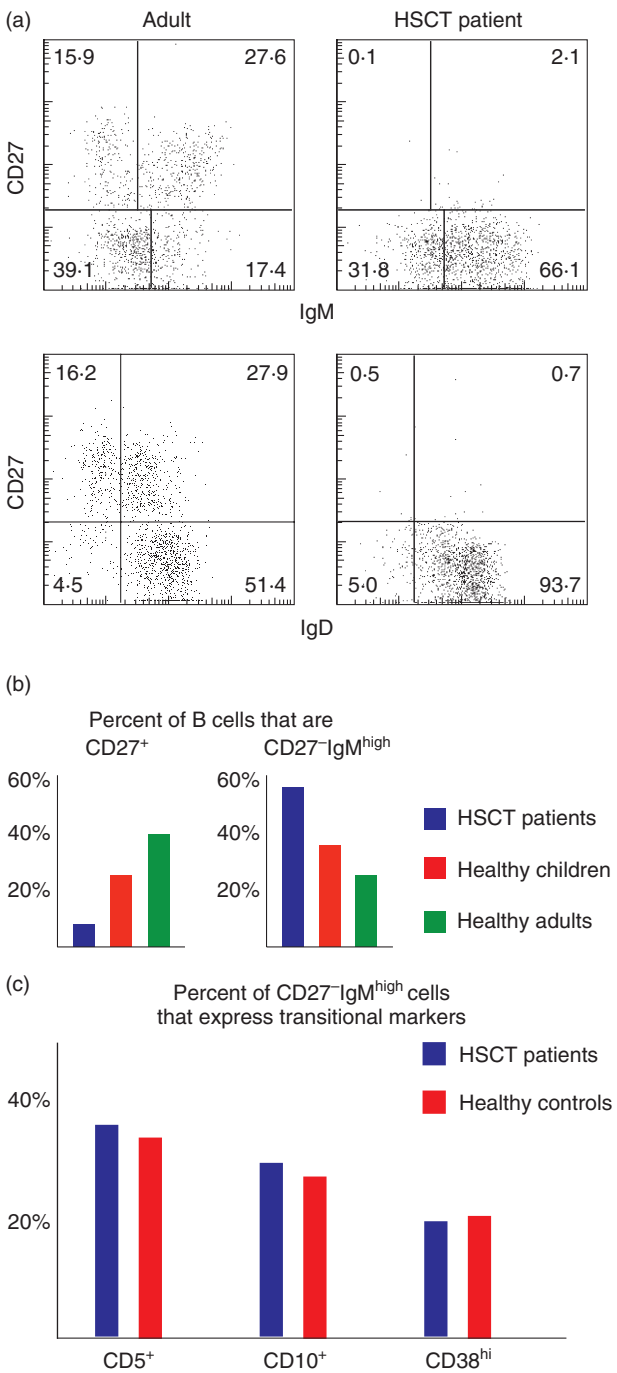


Fig. 3. Subtyping of B cells in children who have undergone haematopoietic stem cell transplantation (HSCT). (a) In healthy adults, the CD19$^+$ B cells can be divided into immunoglobulin (Ig)M-IgD^{low}CD27$^+$ and IgM$^-$IgD$^-$CD27$^+$ memory cells and IgM^{low}IgD^{high}CD27$^-$ naive cells, with few cells being IgM^{high}CD27$^-$. In paediatric patients who have undergone HSCT, IgM^{high}IgD^{high}CD27$^-$ B cells is a major population even 1 year after transplantation when normal numbers of B cells has been reached in blood. In (b) are shown the mean frequency of CD27$^+$ B cells and IgM^{high}CD27$^+$ B cells in healthy children ($n = 9$), healthy adults ($n = 3$) or children who have undergone HSCT 1 year prior to the analysis ($n = 10$). (c) Few of the IgM^{high}CD27$^+$ B cells express markers typical for transitional cells in healthy controls ($n = 4$) or paediatric HSCT patients ($n = 4$).

samples from a single donor during reconstitution, allowing refinement of our knowledge of B cell subset development and complexity. Deficiencies associated with poor B cell responses can result in long-term problems. Therefore, monitoring the development of B cell subsets may prove clinically relevant, giving insights into how deficiencies develop and to tailor the care of the patients. Such measurements could, for example, monitor infection sensitivity, development of GVHD, determine when vaccinations are best performed and track responses to vaccines or infections. Absolute and relative frequencies of immature, naive, class-switched memory B cells or plasma blasts in blood are candidates for such predictions. In addition, as the true identity of $IgM^{high}CD27^{+}$ B cells is now being unravelled, measurements of these may give insights into why some HSCT patients experience repeated infections with encapsulated bacteria. Few recent clinical studies have been performed where the presence or absence of B cell subtypes in HSCT patients are linked to clinical problem, and future studies that address these issues are therefore called for.

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