Allogeneic hematopoietic stem cell transplantation as immunotherapy

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CHAPTER 2

AML relapse after rituximab treatment for GvHD: crucial role for B cells in GvL responses

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Allogeneic hematopoietic stem cell transplantation (HSCT) is curative when a potent graft versus leukemia/lymphoma (GvL) response is induced, but is often complicated by graft versus host disease (GvHD). GvL responses and GvHD are lymphocyte driven because lymphocyte depletion with anti-thymocyte globulin (ATG) or MabCampath (anti-CD52) averted GvHD at the expense of much higher relapse rates. Initial efforts to understand the mechanisms of GvL and GvHD focused on T cells,[1,2] and later donor NK cells were recognized as important anti-tumor effector cells.[3] Observations that GvHD and GvL responses were associated with the appearance of host- and tumor- specific antibodies[4-8] and that B cell depletion with the CD20-binding monoclonal antibody rituximab was effective in some patients with steroid-refractory GvHD suggested that B cells are involved in GvHD pathobiology.[9-13] By inference it can be hypothesized that B cells are also involved in GvL allogeneic-immune responses however this remains to be determined.

Here we studied the B cell repertoire of a patient that had developed a potent GvL response that was however abrogated after B cell depletion with rituximab for extensive GvHD. The patient, a 39-year-old male with a history of alkylating chemotherapeutic treatment for testicular choriocarcinoma, presented at our out-patient clinic with a therapy related myelodysplastic syndrome / refractory anemia with excess blasts (MDS/RAEB-1) with 5-10% blasts (CD34+/CD117+), a complex karyotype (monosomy 5, 11 and 17), and structural chromosomal aberrations in 7p, 12p, 15p and 22p. After one cycle of remission-induction chemotherapy with idarubicine and cytarabine he received a non-myeloablative allogeneic HSCT (conditioning: fludarabine and 2Gy total body irradiation) from a 10 out of 10 matched unrelated donor. At the time of allogeneic HSCT his bone marrow persistently showed 5-10% CD34+/CD117+ blasts (Figure 1A, panel 1). In the weeks following allogeneic HSCT he obtained full donor-chimerism with significantly improved peripheral blood counts. However, 8 weeks after allogeneic HSCT his AML relapsed with 20% bone marrow infiltration of CD34+/CD117+ leukemic blasts (Figure 1A, panel 2). To induce a GvL response, mycophenolic acid and cyclosporine were rapidly tapered, upon which he obtained complete hematologic and cytogenetic remission (Figure 1A, panel 3). Re- obtaining complete remission through cessation of immunosuppressive therapy, without chemotherapy, demonstrates the potency of the graft vs tumor response in this patient. This GvL effect came, however, at the cost of extensive GvHD of the skin, oropharynx, liver and intestine that initially responded well to corticosteroids and topical calcineurin inhibition. During steroid tapering however, GvHD of the oropharynx and skin worsened. Rituximab (375 mg/m^2, four weekly infusions) and mycophenolic acid were added after which GvHD of the skin slowly resolved. Three months after receiving the 4th infusion of rituximab the patient reported severe pain in the pelvis. An MRI scan showed multiple osteolytic lesions throughout

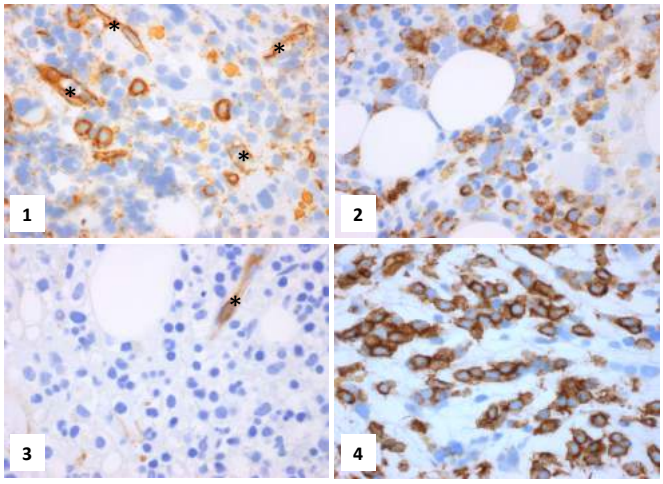
the pelvis and both femora extending beyond the bone structures. Biopsy of these lesions showed CD34+/CD117+ leukemic blasts (Figure 1A, panel 4). The patient refrained from further intensive treatment and passed away five months later. A schematic representation of this patient's clinical history is depicted in Figure 1B.

To the best of our knowledge only one case report has been published describing disease relapse after rituximab treatment for GvHD.[14] This 22-year old man with pre-B-ALL (acute lymphoid leukemia) received 4 cycles of rituximab for therapy-refractory chronic GvHD of the skin. One month after the last rituximab infusion the pre-B-ALL relapsed. This is in line with observations in a murine allogeneic HSCT model, where survival of mice with ALL was associated with the development of potent antibody responses directed against ALL.[15]

These observations, including ours, suggest that B cells can be critically involved in GvL responses. To investigate this we generated B cell clones from the patients' PBMC that were collected 3 months after allogeneic HSCT, when the patient had obtained complete remission of relapsed AML, 2 months before the start of rituximab therapy (indicated by asterix in Figure 1B). PBMC that had been viably frozen at that time point in the context of the allogeneic HSCT biobank that the patient participated in were thawed and CD27+ IgG B cells were sort-purified. At this early time point after allogeneic HSCT lymphocyte numbers are low and frozen PBMC from ten ml EDTA whole blood yielded only 500 B cells. CD27+ IgG B cells were immortalized through retroviral transduction with Bcl-xL and Bcl-6 and expanded as described previously.[16] Supernatants of these B cell clones contain antibodies produced by the B cell clones and these supernatants were then screened for binding to AML cell lines with goat-anti-human IgG-H+L-AF647 as a secondary antibody. This retrieved an AML-specific IgG1-κ B cell clone (AT15-001) that did bind to the AML cell lines THP-1, Molm13 and MonoMac6 and to AML blasts isolated from newly diagnosed AML patients (Figure 2A), but did not bind to healthy-donor-derived PBMC or BM, the T cell line Jurkat, tissue cell lines such as Caco-2 (colon), HepG2 (liver) and BJ (foreskin) or skin fibroblasts (Figure 2B). This B cell clone was of donor origin, as determined by micro-chimerism analysis of genomic polymorphisms of short tandem repeat DNA loci. Compared to the germline sequence the antibody showed somatic hypermutations, 12 in the variable regions of the heavy chain and 4 replacement mutations in the light chain, indicating that that the B cell clone was antigen experienced.

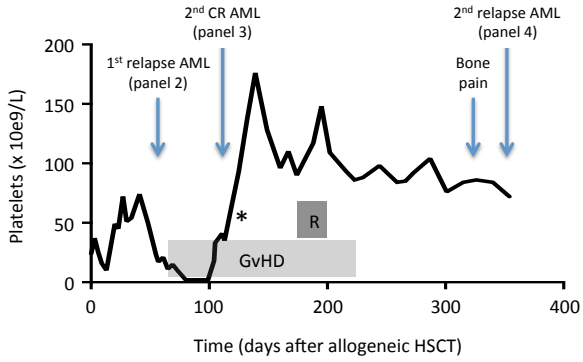
Figure 1. Clinical history.

A



Histology of bone marrow biopsies demonstrating CD34+ myeloblasts (brown staining) and endothelium (asterisks) at the time of allogeneic HSCT (panel 1), first relapse (panel 2), second remission (no myeloblasts; panel 3) and second relapse (panel 4).

B



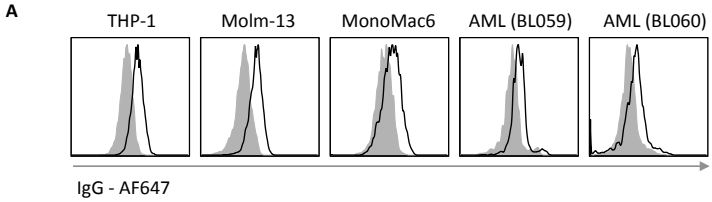
Schematic overview of the medical history of the patient. Following allogeneic HSCT (at time point 0) peripheral blood platelet numbers (solid black line) improved, as did erythrocyte and leukocyte numbers (not shown). Two months later platelet counts collapsed and AML relapse was diagnosed (arrow). Upon rapid tapering of cyclosporine and mycophenolic acid and without additional chemotherapy peripheral blood numbers improved and bone marrow biopsy confirmed complete remission (arrow). At the same time extensive GvHD developed of the skin (and to a lesser extent of liver and intestine) that was steroid- refractory, but did respond to rituximab. About 100 days after rituximab treatment the patient reported bone pain (arrow). MRI revealed osteolytic lesions; biopsy demonstrated AML relapse in bone and bone marrow (arrow). Asterix indicates timepoint of which frozen and stored PBMC were available for B cell analyses. R: rituximab (4 cycles). Panels refer to panels in Figure 1A.

The appearance of leukemia-specific antibodies in allogeneic HSCT recipients with relapsed AML, CML or CLL who obtained durable remission after donor-lymphocyte infusion has been described.[7,8,17] Their appearance clearly correlated with clinical responses, suggesting that they functionally contributed to GvL allo-immunity. However, these antibodies were directed against intracellular targets and therefore most likely reflect a bystander result of effective cytotoxicity of other immune cells against tumor cells. The AML-specific antibody that was identified in our patient at a time of maximum GvL response is clearly different, in that it binds to a target that is expressed on the surface of AML cells.

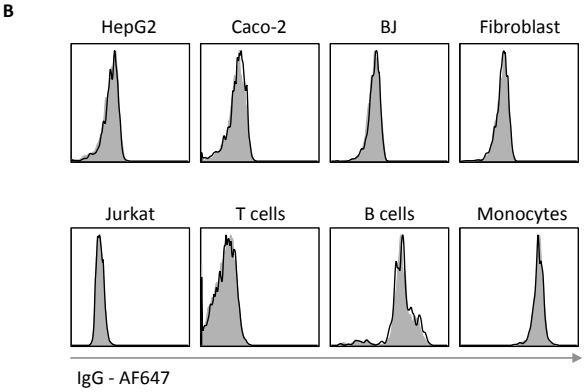
Nevertheless, the mere presence of an antibody even when directed against surface antigens does not prove functional involvement of B cells in anti-tumor responses. B lymphocytes express a variety of functions in human immunity. They can serve as T helper cells or as antigen presenting cells, modulating the activity and maintenance of T cells.[18] In addition, B cells produce antigen-specific monoclonal antibodies that serve to opsonize targets that can then be killed by antibody dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). We tested the killing capacity of the AML-specific antibody by incubating Calcein-AM-labeled AML cell lines and primary blasts with AML or control antibodies and rabbit serum complement for 1 hour at 37°C. FACS calibration beads were added in a 50/50 volume ratio to acquire a standard amount of beads with FACS. We then calculated the amount of dead cells as: 100- ((Dapi negative, Calcein-AM positive cells (AML antibodies)/Dapi negative, Calcein-AM positive cells (buffer control)) × 100). We found that the AML-specific antibody retrieved from our patient induced CDC of target cells including the AML cell line Molm13 and freshly isolated leukemic blasts from patients with MDS and AML (Figure 2C). While we cannot prove causality, we hypothesize that the AML-specific antibody that we isolated from this patient directly contributed to the B cell-mediated anti leukemia effect by inducing CDC of AML blasts. This antibody is probably derived from a short-lived antibody producing plasma cell clone that became deprived of memory CD20+ B cell supply after rituximab treatment, similar to what has been described in patients with auto-immune disease.[19] Our observations do not preclude a role for leukemia-specific T cells that may have provided B cell help, or may have generated a cytotoxic response.

In conclusion, we here describe an allogeneic HSCT recipient who mounted an AML-specific antibody response directed against a cell surface expressed antigen with an antibody that is capable of inducing CDC of AML blasts in vitro. This GvL response was lost after rituximab treatment. We conclude therefore that B lymphocytes can mount a functional anti-tumor response following allogeneic HSCT.

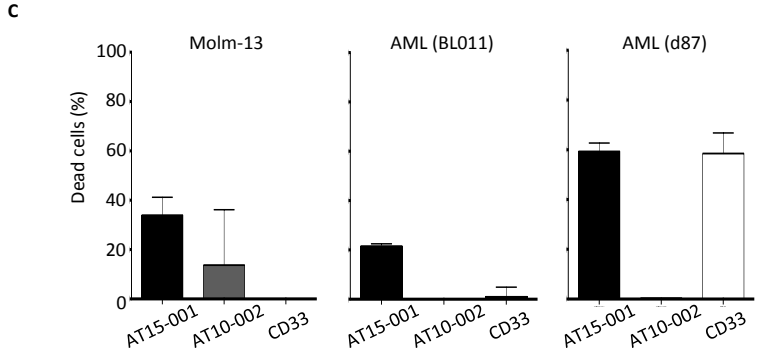
Figure 2. Graft vs leukemia response by AML-specific antibodies that induce CDC.



Histograms demonstrating specific binding of the AML-specific antibody AT15-001 isolated from the patient to AML cell lines THP-1, Molm13, Mono-Mac6 and AML blasts freshly isolated from newly diagnosed patients with AML (BL059 and BL060).



This AML-specific antibody did not bind to liver (HepG2 cell line), colon (Caco2), skin (BJ and fibroblasts), the T cell line Jurkat or bone marrow and PBMC obtained from healthy individuals.



The AML-specific antibody AT15-001 induced CDC of calcein-AM labeled target cells when incubated with complement and effector cells. As target cells the AML cell line Molm13 and blasts from 2 patients with AML (BL011 and d87) were used.

As negative controls, we used in-house generated antibodies against influenza (AT10-002; shaded histograms in (A) and (B)) and AML (CD33).

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