Leukemia-Associated Antigens Are Critical for the Proliferation of Acute Myeloid Leukemia Cells

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Abstract

Acute myeloid leukemia (AML) is the most common acute leukemia in adults. With intensive induction therapy, most patients younger than 60 years achieve complete remission. However, even if these younger patients were treated intensively, more than 50% will relapse. Clinical results of patients older than 60 years are more unfavorable. Therefore, in all patients with AML, the overall survival is still low. In the past decade, several leukemia-associated antigens (LAA) have been identified in patients with acute myeloid leukemia. BAGE, BCL-2, OFA-1LRP, FLT3-ITD, G250, hTERT, PRAME, proteinase 3, RHAMM, survivin, and WT-1 are all LAAs that have been shown to induce CD8+ T-cell recognition and for some antigens also humoral immune responses. Interestingly, most of these LAAs are linked to cell cycle or proliferation. This article discusses the balance between LAA-driven leukemia cell expansion and the elimination of these cells through attacks on LAAs by the immune system. Current knowledge of the function and CD8+ T-cell recognition of LAAs is reviewed and an outlook is given on how to improve T-cell responses to LAAs in acute myeloid leukemia cells.

Background

Immunotherapeutic approaches require the identification of leukemia-associated antigens (LAA) and the accurate characterization of specific T-cell responses against these target structures. During the last decade, several LAAs were identified that induce specific T-cell responses in acute myeloid leukemia (AML) patients like BAGE, BCL-2, OFA-1LRP, FLT3-ITD, G250, human telomerase reverse transcriptase (hTERT), PRAME, RHAMM, proteinase 3, survivin, and WT-1 (1–13). Several epitopes in these LAAs have been shown to induce cell lysis of autologous leukemic blasts by specific cytotoxic T cells (5–11). Moreover, RHAMM and WT-1 induce both serologic and cellular immune reactions (7, 8, 14, 15). The LAAs RHAMM, proteinase 3, and WT-1 were tested in clinical peptide vaccination trials (8, 16–20). In these clinical trials, immunologic and clinical responses could be detected in patients with different hematologic malignancies. The LAAs RHAMM (54%), PRAME (70%), and G250 (60%) showed a high frequency of specific T-cell responses in patients with myeloid leukemias (8).

Several of these LAAs are capable of inducing T-cell responses that are associated with clinical outcome in AML patients. Most LAAs have been shown in vitro to be associated with an increase in the cellular proliferation of leukemic blasts. In contrast to this in vitro function, some of these LAAs were conversely found to be associated with an improved clinical outcome in AML: We found a significant correlation between high G250 mRNA expression levels and a longer overall survival (P = 0.022) based on DNA microarray data of 116 AML patients (8). For the LAA PRAME, we detected a trend for a better clinical outcome of AML patients (8). Interestingly, we found that expression of at least one of the three LAAs, RHAMM, PRAME, or G250, provided the most favorable prognostic score (P = 0.005). Other groups also found an improved overall survival of patients expressing the LAA PRAME. Steinbach et al. (21) found an overexpression of PRAME in 62% (n = 31) of childhood AML patients and that the rates of overall and disease-free survival rates were higher in patients with elevated levels of expression. They also observed that PRAME expression was significantly higher in patients with t(8;21). In accordance with our data in AML patients, the expression of G250/CAM9 has also been shown to be associated with a favorable outcome in renal cell carcinoma (22, 23). However, in contrast to our analysis in AML, the expression of RHAMM was associated with a worse clinical outcome in different solid tumors (20). In chronic myelogenous leukemia patients, expression of proteinase 3 was detected with a high expression level in patients with better clinical outcome (24). Conversely, there are other LAAs that have been reported to be associated with a negative clinical outcome in leukemias. For example, Bergmann et al. showed that high levels of WT1 mRNA in AML were associated with poor long-term outcome (25), whereas others have found no correlation (8, 26, 27). In patients younger than 60 years, the expression of BCL-2 and WT1 was associated with a reduced rate in achieving complete remission and overall survival, in contrast to patients older than 60 years in whom expression of these genes had no effect on survival rates. Many studies have shown that AML patients with FLT3-ITD mutations have poor cure rates due to a lower complete remission rate and a higher relapse rate (28, 29). In normal hematopoiesis, FLT3 is expressed in hematopoietic stem and progenitor cells, and
expression is lost in differentiated cells (30). The expression of survivin was associated with a worse clinical outcome in patients with different solid tumors like renal cell carcinoma and also in patients with AML (31). Guinn et al. found a positive correlation between the expression of the LAA synovial sarcoma X breakpoint 2 interacting protein (SSX2IP) and the poor prognostic indicator FLT-3-ITD \((P = 0.008, t\) test) but not between SSX2IP and other poor prognostic markers such as cytogenetics, abnormalities associated with poor survival, white cell count, age, sex, or survival (32).

The functional role of LAAs in tumor cells is very diverse. Some of the antigens are involved in different critical mechanisms for cell differentiation and proliferation. They play a controversial role: They increase cell growth but they provide targets for effective immune responses against the host tumor. Most of these antigens show differential mRNA and protein expression in tumor cells compared with normal tissue.

**LAAs Are Involved in Different Pathways Relevant for Cell Growth of Leukemic Cells**

**LAAs associated with apoptosis.** Bcl-2, survivin, and proteinase 3 are involved in the regulation of apoptosis. Bcl-2 is one of the antiapoptotic genes that regulate the mitochondrial-mediated pathway of apoptosis (33). Del Poeta et al. showed that an elevated bax/bcl-2 ratio was associated with a longer overall survival and disease-free survival in 225 de novo AML patients by flow cytometry (34). However, earlier studies indicated a poor prognostic association with high bcl-2 expression in AML and with shorter survival, less achievement, and shorter duration of complete remissions (35). Survivin is a member of the inhibitor of apoptosis protein family, which, by immunocytochemistry, was never found in normal samples but was found in almost all AML patient samples examined (36). Wagner et al. found that low expression of survivin-2B correlated with better overall survival and event-free survival of AML patients (37). Proteinase 3 (PR3, myeloblastin) is a synonym for the anti–nuclear cytoplasmatic antigen, which is immunogenic in Wegener’s granulomatosis. PR3 plays a critical role in proliferation of myeloid progenitor cells by regulation of p21 (38) and procaspase-3 (39). Taken together, PR3 regulates critical genes for cell proliferation, differentiation, and apoptosis.

**LAAs involved in the process of mitosis.** The receptor for hyaluronic acid–mediated motility (RHAMM) is a glycosaminoglycan and extracellular matrix molecule that plays a fundamental role in cell growth, differentiation, and motility. Overexpression of RHAMM is essential for Ras-mediated transformation (40). In breast cancer and multiple myeloma, RHAMM expression was found in highly proliferating tumor cells. An association between the potential to metastasize and worse clinical outcomes could be noted (41). We found mRNA expression of RHAMM in AML patients in contrast to normal tissues; strong mRNA expression of RHAMM was only found in the testis, placenta, and thymus (42). Moreover, RHAMM might contribute to the differentiation and maturation of leukemic blasts like CD44, another receptor for hyaluronic acid (43). Therefore, immune therapies targeting RHAMM might target not only an immunogenic antigen but also a gene critically involved in cell cycle, differentiation, and proliferation. The LAA SSX2IP was identified through its interaction with the cancer-testis antigen SSX2 by yeast two-hybrid assays and subsequently shown to be expressed on the surface of AML cells during mitosis (44). Of note, Denniss et al. (44) showed an increased expression of SSX2IP on the surface of myeloid leukemia cells during mitosis, with the blocking of primary AML cells with colcemid leading to an increased frequency of SSX2IP expression. In addition, Guinn et al. found a close correlation between low SSX2IP expression in AML patients harboring a (t(8;21)) and low cdc20 expression (32). CDC20 is one of the anaphase-promoting complex proteins whose low expression is thought to contribute to the aneuploidy seen in these patients.

**Chaperones.** M-phase phosphoprotein 11 (MPP11) is the human homologue to the murine Id associate 1 (MIDA1) gene that consists of a zuotin homology region and tryptophan-mediated sequences similar to the c-myc oncoprotein (45). This protein interacts with the helix-loop-helix protein of the conserved DNAJ motif of the Id protein (45). MPP11 antisense oligonucleotides inhibited the proliferation of leukemic cells. In primary head and neck squamous cell cancer, MPP11 plays a putative oncogenic role (46). Immunization against MIDA1/ MPP11 resulted in a significant suppression of tumor growth (47). MPP11 is conserved in evolution and belongs to chaperone molecules (48).

**Demethylation.** The cancer/germline (CG) antigen BAGE was shown to be expressed in different solid tumors and more frequently in metastatic melanomas than in primary lesions (1). BAGE is hypomethylated in different cancer cells, and hypomethylation precedes transcriptional activation of BAGE (49). However, the exact role of BAGE expression on cell proliferation needs to be elucidated. Similarly, other CG genes such as HAGE and RAGE-1 have been found to be expressed in <33% AML patient samples (50) and have similarly been shown to be activated by global promoter hypomethylation in other cancers (51). Recently, PASD1 was identified by SEREX in AML (52) and shown to be expressed in 33% of patient samples. Its tissue-restricted pattern is also likely to be due to global promoter hypomethylation in tumor cells and in the testes.

**Tyrrosine kinases and the hypoxia-related gene G250/CA9.** FLT3 is a receptor tyrosine kinase with an important role in the proliferation of hematopoietic progenitor cells. FLT3 is mutated in one third of AML patients, either by internal tandem duplications (ITD) of the juxtamembrane domain or by point mutations usually involving the kinase domain (28–30). These are the two types of mutations that constitutively activate FLT3 (30). FLT3 is a prominent member of the tyrosinase kinase class III family whose other family members include KIT, FMS, and PDGFR. FLT3 has shown to be important for hematopoietic progenitor cell proliferation and survival (28–30). The fusion protein BCR-ABL represents a tyrosine kinase that is involved in the pathogenesis of chronic myeloid leukemia and that constitutes the first molecular structure that was used for tumor-specific targeted therapy (53). More than 95% of chronic myelogenous leukemia patients express the fusion product BCR-ABL that can also induce specific T-cell responses (53). A molecular monitoring of chronic myelogenous leukemia is available through the quantitative reverse transcription-PCR for BCR-ABL expression. The fusion protein BCR-ABL is also a potential target structure for immunotherapeutic approaches (54, 55) and several peptides were
characterized (54, 55). Several group started vaccination trials in chronic myelogenous leukemia patients (56–58). G250/CA9 is a member of the carbonic anhydrase family (59). G250/CA9 is a possible marker for hypoxia in various tumors (60) and it is a gene regulated by the hypoxia-inducible factor α (61). The expression of the LAA G250/CA9 has been shown in various tumor types, whereas it is not expressed in normal tissues, including hematopoietic cells. G250/CA9 is expressed in most clear cell forms of renal cell carcinoma, but not in normal tissue, and may be involved in oncogenesis and the progression of tumors other than renal cell carcinoma (62). In accordance with our data in AML patients, the expression of G250/CA9 has also been shown to be associated with a favorable outcome in renal cell carcinoma (22).

**Differentiation.** PRAME is a dominant repressor of retinoic acid receptor signaling and it binds to retinoic acid receptor in the presence of retinoic acid, thus preventing ligand-induced receptor activation and target gene transcription (63). Retinoic acid induces transcription of a set of target genes through the binding to and activation of its receptor, resulting in differentiation and cell cycle arrest in responsive cells. Loss of retinoic acid responsiveness is therefore beneficial to cancer cells (64). Further supporting our own findings (8), the expression of PRAME has been shown as an indicator of a good prognosis in childhood AML (21), although this effect might have been secondary to its correlation with favorable cytogenetics such as a translocation t(8;21), which has already been previously described (8).

Wilms’ tumor gene 1 (WT1) is expressed in most AML patients (25, 42) and also in the early CD34+ cells of normal hematopoiesis; its expression is correlated with a poor clinical prognosis of AML patients (25). WT1 inhibits cell differentiation of normal hematopoietic progenitor cells and leukemic blasts (65, 66). There is emerging evidence for WT1 gene mutations in AML (67, 68) that does not affect the known CD8+ T-cell epitopes used in clinical vaccination trials. AML1-ETO induces acute myeloblastic leukemia in cooperation with WT1 (69). However, WT1 also targets regulatory genes related to the process of apoptosis (70). In our own cDNA microarray analysis, we could not detect an association of WT1 and survival in 116 primary AML samples (8).

**Telomerase activity.** The telomerase catalytic subunit (hTERT) is a tumor-associated antigen expressed in different human cancers, and its expression correlates with telomerase

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**Fig. 1.** Immunogenic LAA marked in orange might exercise a positive effect on the proliferation of leukemia cells (top part) or hamper the apoptosis of leukemia cells, thus increasing the tumor burden in leukemia patients. RAR, retinoic acid receptor.
activity (71). hTERT plays an important role in leukemogenesis (72), and hTERT expression and telomere length correlate with the occurrence of chromosomal abnormalities (73). hTERT peptides binding to HLA-A*0201, inducing specific CTL responses, were characterized (9).

Clinical-Translational Advances

LAAs have a dual role: increase of cell proliferation and induction of specific T-cell responses. Nearly all LAAs that have been shown to induce specific CTL responses in AML patients are critically involved in mechanisms responsible for tumor growth in vitro such as proliferation, inhibition of apoptosis, differentiation, and demethylation (Fig. 1). Surprisingly, the expression of most of these antigens that induce tumor cell growth in vitro is not associated with a worse clinical outcome. In contrast, expression of some of these genes or simultaneous expression of several LAAs is even associated with a better clinical outcome of patients with AML. Different groups showed that specific CD8+ T cells of AML patients can lyse autologous leukemic blasts expressing LAAs. The immune system targets these antigens to control tumor growth. These immunologic mechanisms might overcome some of the effects of LAAs on cell cycle and tumor growth. However, less is known about the mechanisms that are physiologically involved in the effective rejection of single tumor cells before developing a clinically detectable tumor load. Potentially, these mechanisms are quite different and only tumor cells that are able to proliferate can develop central mechanisms of tumor escape.

However, tumor-restricted or tumor-specifically expressed LAAs represent excellent target structures for cytotoxic T cells to eliminate malignant cells. Up-regulation of gene expression constitutes an important mechanism in cell cycle regulation. The recognition of most LAAs also depends on the expression level of the antigen (8, 16, 17): LAAs that induce specific T-cell responses in AML patients are tumor specific (FLT3-ITD, BCR-ABL), tumor specifically expressed (G250/CA9, hTERT, BAGE, RHAMM, WT-1), or highly tumor-restricted expressed and/or overexpressed in tumor cells and leukemic blasts (MPP11, proteinase 3, BCL-2, survivin). Tumor cells are in an area of tension between genes that induce or increase malignant cell proliferation and CTLs that attack the malignant cell expressing these LAAs.

Obstacles to immune responses to LAAs. Several mechanisms have a negative effect on the immune responses against LAAs. Cytostatic drugs induce cytopenia in the peripheral blood as well as in the bone marrow but the functional impairment of T cells after reconstitution remains to be elucidated. Several studies have shown that the circulating T cells detected early after chemotherapy are functional (74, 75). T cells of AML patients after intensive chemotherapy show an increased proliferation when stimulated (76).

Impairment of the transporter associated with antigen processing and lack of MHIClass I molecules were frequently observed in different human cancers (76). In AML, CD40 and CD80 molecules were deficient, but HLA molecules and CD86 were preserved on AML blasts and constitute a prerequisite for AML blast recognition by the immune system (77).

The cytokine milieu is of crucial importance for leukemia cell/T-cell interactions: In chronic lymphocytic leukemia patients, leukemic cells express high levels of immunomodulatory factors, including transforming growth factor-β and interleukin-10 (IL-10) that suppress a T-cell response to antigens (78). Moreover, IL-15 plays a key role in the maturation of antigen-presenting cells like dendritic cells (79). AML blasts release chemotactic chemokines such as CXCL10 or CCL5 that inhibit the migration of T cells in the bone marrow to control the proliferation of AML blasts (80). FasL-positive AML cells can induce apoptosis of T lymphocytes in vitro (81, 82). The local recruitment of antileukemic T cells to the AML microcompartment is also essential for the control of leukemia. T-cell anergy is induced by insufficient B7 costimulation, inhibition by ligands such as programmed death ligand-1 (PD-1), or metabolic dysregulation by enzymes such as indoleamine-2,3-dioxygenase (83). IDO+ AML cells can be active immunosuppressors (84). Recently, the focus has moved to the role of regulatory T cells, which have an immunosuppressive effect on LAA-specific CD8+ T cells. Regulatory T cells, characterized by coexpression of CD4 and IL-2R CD25, as well as by the forkhead family member FoxP3 (85, 86), may produce transforming growth factor-β and IL-10, thus inhibiting an antileukemic response. The importance of FoxP3 in the context of AML is underlined by the recent finding that it interacts with AML1/RUNX (87).

All these mechanisms may impair the efficacy of tumor cell control by T lymphocytes and might therefore be relevant to the immune response induced against LAAs and, eventually, the clinical outcome of AML patients (8).

Strategies to overcome immune deficiency against leukemic cells. Today, different immunotherapeutic approaches have been developed to improve the efficiency of spontaneous immunologic antitumor responses like peptide vaccination, application of dendritic cells, cytokines, adoptive immunotherapy (donor lymphocyte infusion), gene transfer, and whole-cell vaccines (20). Moreover, to overcome T-cell defects induced by crucial factors of the microenvironment, possible strategies might consist of the inhibition of VEGF or CXCL10 or targeting FasL or a choice of optimal adjuvants. Peptides derived from LAAs are already under clinical investigation for AML patients in current peptide vaccination trials, including the proteinase 3-derived peptide PR1, WT1-derived peptides, and the RHAMM-derived peptide R3 (16–19). Immunologic and clinical responses could be detected in these clinical trials. However, the search for the most immunogenic peptides, optimal conditions, and appropriate adjuvants (CpG motifs, cytokines like IL-15, granulocyte macrophage colony-stimulating factor, and/or Freund’s incomplete adjuvant) have to be performed to improve the responses in clinical trials.

For G250/CA9, PRAME, and RHAMM, the expression of at least one of these antigens was associated with a favorable outcome in AML patients, in contrast to patients without expression of these LAAs (8). Simultaneous expression of LAAs might increase the efficiency of T-cell responses and might overcome the tumor escape mechanisms developed by tumor cells. Therefore, the use of a polyvalent vaccine could reduce the risk of immune escape during immunotherapeutic approaches. As several LAAs are crucial for different cell cycle pathways, targeting them simultaneously might control tumor cell proliferation in a more efficient way. In several immunotherapeutic approaches, not only immunologic but also clinical responses could be detected (16, 17, 18, 19). After allogeneic
stem cell transplantation, strong graft-versus-leukemia effects could induce a stable complete remission. Further immunotherapeutic maneuvers as indicated above might be effective to control minimal residual disease of patients with different hematologic malignancies.

Taken together, LAs induce specific T-cell responses and are involved in crucial mechanisms for cell growth of leukemic cells. Because of this dual role, LAs constitute exquisite target structures for targeted therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

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