The Immunological Dilemma: Cellular Innate and Adaptive Immune Response Versus Human Acute Myeloid Leukemia

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Abstract: It is generally accepted that acute myelogenous leukemia (AML) patients are immunocompromized. On the other hand, antileukemic immune reactivity is important for the improved survival of AML patients treated with allogeneic stem cell transplantation and antileukemic immunotherapy. In this article, we review the available studies of disease- and therapy-induced immune dysfunctions in AML patients, including the function of the cellular innate and adaptive immune system of AML patients (i) with newly diagnosed disease before treatment, (ii) immunological functions of AML patients with severe therapy induced cytopenia before hematopoietic reconstitution; and (iii) the immune reconstitution following the initial period of hematopoietic reconstitution after autologous and allogeneic stem cell transplantation. A more detailed knowledge about the immune systems of these patients is essential for an optimal design of future clinical immunotherapy studies in AML.

Keywords: Acute myelogenous leukemia, Immune system, Chemotherapy.

INTRODUCTION

Acute myelogenous leukemia (AML) is characterized by clonal proliferation of immature myeloid precursors and arrest in the maturation of these cells [1]. This results in accumulation of a leukemic blast population in the bone marrow (BM) and eventually in the peripheral blood (PB). The bone marrow infiltration causes a decrease in the production of and thereby a reduction in the peripheral blood levels of mature myeloid cells; resulting in thrombocytopenia, anemia and granulocytopenia.

It is well known that AML patients are immunocompromized and have an increased risk of infections [1]. Fatal infections are an important cause of the treatment-related mortality especially in elderly individuals. AML patients often have neutropenia initially due to the disease and later eventually due to intensive chemotherapy. However, as will be seen from the studies reviewed below these patients can also have additional disease- or therapy-induced dysfunctions of the cellular immune system. One would expect that these dysfunctions differ between patients due to for example differences in AML cell phenotypes, chemotherapy regimen and age-dependent alterations of the immune system.

THE IMMUNE SYSTEM IN PATIENTS WITH UNTREATED AML

Natural Killer Cells: The Importance of AML Cell Burden, NCRdull NK Cell Phenotype and NK-Inhibitory AML Cell Phenotype

Natural killer (NK) cells (Table 1) are a heterogeneous lymphoid population comprising 10–15% of all peripheral lymphocytes, and they seem to be involved in antitumor activity as well as tumor surveillance [2, 3]. Elevated levels of lymphocytes with NK phenotype have been reported at the time of diagnosis in AML [4]. CD56+, CD16+CD2+ and CD16+CD2− NK cells were then found significantly increased in peripheral blood, but only the CD56+ NK cells were additionally increased in bone marrow [4]. When these authors divided the AML cases into two groups according to the absolute number of circulating NK cells, the patients with the highest levels also showed an increased proportion of circulating leukemic blasts [4].

Despite the increased NK cell levels, several other studies report NK cell dysfunction [5-8] or impeded NK cell maturation [9] in patients with cancer, including preleukemic myelodysplastic syndromes (MDS) as well as leukemia. In accordance with this, it has been suggested that NK cells in leukemic patients display a more inhibitory AB haplotype killer cell immunoglobulin-like receptor (KIR) phenotype compared to healthy controls (Table 2, Fig. 1) [10]. Additionally, it is hypothesized that leukemic cells may have evolved an escape mechanism from immune surveillance based on the dominance of inhibitory KIR ligands over activating KIR ligands displayed on the leukemic cells (see Table 3) [11].

Recently, deficient expression of the natural cytotoxicity receptors (NCRdull; NKp30, NKp44 and NKp46) in NK cells from AML patients was reported (Table 3) [5, 12]. This NCRdull phenotype was possibly induced by direct contact with leukemia blasts and NK cells, and not via the cytokine network. Moreover, NCRdull NK cell phenotype was associated with poor survival of AML patients [12]. The influences of the leukemic cells upon the NK lymphocytes still remain unclear. However, based on the above mentioned reports, it is justified to state that both qualitative as well as quantitative disturbances in the NK cell system can be detected in AML patients (Table 2).

Dendritic Cells: Immature Leukemic Dendritic Cells as a Cause of Immunosuppression?

Dendritic cells (DCs) (Table 1) are central in the presentation of tumor antigens to the adaptive arms of the immune
Table 1. Some of the Circulating Cells of the Immune System

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Typically surface markers for distinguishing</th>
<th>Typically associated cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK cells</td>
<td>Natural killer cells</td>
<td>CD56 CD16 CD94</td>
</tr>
<tr>
<td>mDCs</td>
<td>Myeloid dendritic cells</td>
<td>CD11c CD13 CD33</td>
</tr>
<tr>
<td>pDCs</td>
<td>Plasmacytoid dendritic cells</td>
<td>CD123 CD62L CD36</td>
</tr>
<tr>
<td>Adaptive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>T lymphocytes</td>
<td>CD3</td>
</tr>
<tr>
<td>T&lt;sub&gt;H1&lt;/sub&gt;</td>
<td>Helper T cell type 1</td>
<td>CD4 IL12Rβ1β2</td>
</tr>
<tr>
<td>T&lt;sub&gt;H2&lt;/sub&gt;</td>
<td>Helper T cell type 2</td>
<td>CD4 IL12Rβ1</td>
</tr>
<tr>
<td>T&lt;sub&gt;C1&lt;/sub&gt;</td>
<td>Cytotoxic T cell type 1</td>
<td>CD8 CD25 CCR5</td>
</tr>
<tr>
<td>T&lt;sub&gt;C2&lt;/sub&gt;</td>
<td>Cytotoxic T cell type 2</td>
<td>CD8 CD25 CCR4</td>
</tr>
<tr>
<td>T&lt;sub&gt;reg&lt;/sub&gt;</td>
<td>Regulatory T cell</td>
<td>CD4 CD25&lt;sup&gt;S+&lt;/sup&gt;</td>
</tr>
<tr>
<td>NKT</td>
<td>Natural killer T cells</td>
<td>CD3 CD56</td>
</tr>
<tr>
<td>B</td>
<td>B cells</td>
<td>CD19&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

An overview of the immune cells comprising the cellular innate and adaptive immune system, however, there are heterogeneity within each subsets that are not shown in this table.

Table 2. The Cellular Immune System in Untreated AML Patients

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Abnormality</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural killer cells</td>
<td>Increased CD56&lt;sup&gt;+&lt;/sup&gt;, CD16&lt;sup&gt;+&lt;/sup&gt;CD2&lt;sup&gt;+&lt;/sup&gt;, and CD16&lt;sup&gt;+&lt;/sup&gt;CD2&lt;sup&gt;-&lt;/sup&gt; NK cell levels</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>More inhibitory killer cell immunoglobulin-like receptors (KIRs) expressed</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Deficient expression of natural cytotoxicity receptors (NCR&lt;sup&gt;dim&lt;/sup&gt;)</td>
<td>[5, 12]</td>
</tr>
<tr>
<td></td>
<td>NCR&lt;sup&gt;dim&lt;/sup&gt; expression correlate with worse outcome</td>
<td>[12]</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Quantitative imbalance of mDCs versus pDCs</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Leukemic blasts can be misidentified as DCs</td>
<td>[17]</td>
</tr>
<tr>
<td>T cells</td>
<td>Close to normal CD4/CD8 ratio</td>
<td>[46, 47]</td>
</tr>
<tr>
<td></td>
<td>Normal absolute numbers of CD3&lt;sup&gt;+&lt;/sup&gt;CD8&lt;sup&gt;+&lt;/sup&gt; and CD3&lt;sup&gt;+&lt;/sup&gt;CD8&lt;sup&gt;-&lt;/sup&gt;</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Increased PB CD8&lt;sup&gt;+&lt;/sup&gt;CD57&lt;sup&gt;+&lt;/sup&gt; T cells within the CD8&lt;sup&gt;+&lt;/sup&gt; cells</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>Normal T&lt;sub&gt;H1&lt;/sub&gt; and T&lt;sub&gt;H2&lt;/sub&gt; cytokine profile</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Reduced T cell CD3-ζ expression</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>Functional within a microenvironment dominated by AML cells</td>
<td>[47]</td>
</tr>
<tr>
<td></td>
<td>Decreased V&lt;sub&gt;24&lt;/sub&gt; NKT cells correlated with worse prognosis</td>
<td>[57]</td>
</tr>
<tr>
<td></td>
<td>Increased CD3&lt;sup&gt;+&lt;/sup&gt;CD56&lt;sup&gt;+&lt;/sup&gt; NKT cells in PB</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>Increased proportions of CD4&lt;sup&gt;+&lt;/sup&gt;CD25&lt;sup&gt;Bright&lt;/sup&gt;-T&lt;sub&gt;reg&lt;/sub&gt; cells</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>AML cells IDO expression correlates with increased numbers of T&lt;sub&gt;reg&lt;/sub&gt; cells</td>
<td>[75]</td>
</tr>
</tbody>
</table>

A summary of the hereto characterization of the natural killer (NK) cells, dendritic cells (DCs) and T lymphocytes in untreated AML patients. PB: peripheral blood; IDO: Indoleamine 2,3-dioxygenase; mDC: myeloid DC; pDC: plasmacytoid DC; NKT: natural killer T; T<sub>reg</sub>: regulatory T.
Fig. (1). The AML cell promoting immune dysfunctions.

Some of the immune dysfunctions described in AML are: i) the natural killer (NK) cells seem to display a more inhibitory than activating receptor phenotype, ii) the AML cells display more inhibitory than activating NK cell ligands, iii) the AML cell microenvironment inhibit T cell receptor (TCR) signaling and thus T cell activation, iv) increased expression of indoleamine 2,3-dioxygenase (IDO) correlates with increased numbers of regulatory T (Treg) cells, and v) AML cell resistance to perforin (P) and FasL (CD95L) mediated cell death.

...system, and they are important in maintaining homeostasis and peripheral tolerance [3, 13, 14]. There are several reports of DC defects in cancer patients (reviewed in [15]). Abnormal frequencies as well as abnormal differentiation and/or maturation possibly caused by cancer derived soluble mediators, are among the dysfunctions described [15]. Immature DCs have been described to induce regulatory T cells inducing T cell anergy [16], and because AML cells themselves may be classified as malignancy derived from DC precursors [17], one might hypothesize the leukemic cells to behave as immature DCs (Fig. 2).

A number of studies concerning AML throw light on the generation of leukemic DCs in the context of immunotherapy (reviewed in [18]). There is to our knowledge only one study concerning the remaining non-leukemic DCs in AML; Mohty et al. [19] reported a quantitative imbalance in circulating blood myeloid DCs (mDCs) and plasmacytoid monocytes (pDCs) in 70% of the AML patients examined (Table 2).

In patients with breast cancer the presence of pDCs (CD123+ BDCA2+), revealed by immunohistochemistry of the breast cancer tissue, suggested a disease-promoting activity of pDCs strong production of type I IFN [20]. It is not known whether similar mechanisms are operative in AML, but at least for T cells the release of hematopoietic growth factors may support disease progression (see below).

T Lymphocytes: Normal Levels and Functional Status

T lymphocytes are a heterogeneous population comprising cell subsets like cytokine-secreting CD4+ T helper (TH1 or TH2) cells, cytotoxic killer CD8+ T (TC1 or...
Table 3. Human NK Cells Surface Receptors, their Ligands and Signaling Type

<table>
<thead>
<tr>
<th>NK cells inhibitory receptors&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Receptor family</th>
<th>Receptor&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Known ligands&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR&lt;sup&gt;+&lt;/sup&gt;</td>
<td>KIR2DL1 (CD158a)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>HLA-C group 2</td>
<td></td>
</tr>
<tr>
<td>KIR&lt;sup&gt;+&lt;/sup&gt;</td>
<td>KIR2DL2/3 (CD158b)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>HLA-C group 1</td>
<td></td>
</tr>
<tr>
<td>KIR</td>
<td>KIR3DL1</td>
<td>HLA-B alleles</td>
<td></td>
</tr>
<tr>
<td>KIR</td>
<td>KIR3DL2</td>
<td>HLA-A alleles</td>
<td></td>
</tr>
<tr>
<td>LIR</td>
<td>LIR-1/IL T2 (CD85j)</td>
<td>HLA class I</td>
<td></td>
</tr>
<tr>
<td>NKG2</td>
<td>NKG2A (CD94/CD159a)</td>
<td>HLA-E</td>
<td></td>
</tr>
<tr>
<td>KLR</td>
<td>KLRG1</td>
<td>E/N/P-cadherin</td>
<td></td>
</tr>
<tr>
<td>NKR</td>
<td>NKR-P1 (CD161)</td>
<td>LLTI</td>
<td></td>
</tr>
<tr>
<td>IGSF</td>
<td>Siglec-7 (CD328)</td>
<td>Sialic acid</td>
<td></td>
</tr>
<tr>
<td>IGSF</td>
<td>Siglec-9 (CD329)</td>
<td>Sialic acid</td>
<td></td>
</tr>
<tr>
<td>IGSF</td>
<td>IRp60 (CD300a)</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NK cells activating receptors&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Receptor family</th>
<th>Receptor&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Known ligands&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR</td>
<td>KIR2DS</td>
<td>HLA class I</td>
<td></td>
</tr>
<tr>
<td>KIR</td>
<td>KIR3DS</td>
<td>HLA class I</td>
<td></td>
</tr>
<tr>
<td>NKG2</td>
<td>NKG2C/E (CD94)</td>
<td>HLA-E</td>
<td></td>
</tr>
<tr>
<td>NKG2*</td>
<td>NKG2D*</td>
<td>MICA/B*, ULBP*</td>
<td></td>
</tr>
<tr>
<td>NCR*</td>
<td>Nkp30*</td>
<td>Unknown*</td>
<td></td>
</tr>
<tr>
<td>NCR*</td>
<td>Nkp44/46*</td>
<td>Influenza HA</td>
<td></td>
</tr>
<tr>
<td>FcγR</td>
<td>CD16</td>
<td>IgG Fc region</td>
<td></td>
</tr>
<tr>
<td>IGSF</td>
<td>2B4 (CD244)</td>
<td>CD48</td>
<td></td>
</tr>
<tr>
<td>TNF-R</td>
<td>CD27</td>
<td>CD70</td>
<td></td>
</tr>
<tr>
<td>CD28</td>
<td>CD28</td>
<td>CD80, CD86</td>
<td></td>
</tr>
<tr>
<td>CD2</td>
<td>CD2</td>
<td>LFA-3 (CD58)</td>
<td></td>
</tr>
</tbody>
</table>

After engagement of the NK cell receptor<sup>1</sup> by the specific ligand<sup>2</sup>, the receptor exerts an inhibitory<sup>3</sup> or activating<sup>4</sup> signal to NK-mediated lysis of target cell [166, 167]. * NK cell receptors upregulated in AML patients [10]. + Downregulated NK cell receptors and ligands in AML patients [12, 105]. KIR: Killer Cell Ig-Like Receptor; LIR: leukocyte Ig-like receptor; KLR: killer cell lectin-like receptor; IGSF: immunoglobulin superfamily; FcγR: Fc gamma receptor; TNF-R: tumor necrosis factor receptor; NCR: natural cytotoxicity receptor.

CD4:CD8 ratio has been reported in certain malignancies [36-40]. This ratio can vary depending on the immunological compartment investigated: lymph node versus peripheral blood versus tumor-infiltrating lymphocytes [38, 40, 41]. Additionally, it is suggested that cancer patients have more memory and less naïve T cells applicable among PBL or tumor infiltrating lymphocytes [42-45].

Only few reports regard the T lymphocyte subsets in patients with de novo AML. Vidriales and colleagues [4] reported normal frequencies of CD4<sup>+</sup>CD45RA<sup>+</sup> and CD4<sup>+</sup>CD29<sup>+</sup> cells in the PB but increased numbers of the cytotoxic CD8<sup>+</sup>CD57<sup>+</sup> subset among CD8<sup>+</sup> cells [4]. A more recent report [46] outlined that the absolute numbers of CD3<sup>+</sup>CD8<sup>+</sup> and CD3<sup>+</sup>CD8<sup>+</sup> (putative CD4<sup>+</sup>) T cells in whole blood of 13 patients with AML were similar to those of healthy controls. However, there was a tendency for higher numbers of CD8<sup>+</sup> T cells in the patients and this was also reflected in lower CD4:CD8 ratios [46]. The close to normal CD4:CD8 ratios were also observed in our own recent studies [47]. Thus, no major quantitative T cell defects have been described for patients with newly diagnosed AML.

Panoskaltsis et al. [46] measured the intracellular cytokine levels of circulating lymphocytes derived from AML patients. They did not find any significant changes in the IL-4, IL-10, IL-12 or IFNγ levels for the cell subsets derived from patients compared with healthy individuals, suggesting normal T<sub>H1</sub> and T<sub>H2</sub> profiles. However, a nonsignificant trend towards higher absolute numbers and percentages of CD8<sup>+</sup> and CD8<sup>+</sup> lymphocytes with detectable IL-10, IL-12 and IFNγ was observed for the AML patients.

To further investigate whether T cells were qualitatively affected in AML patients, functional analysis of T lymphocytes after in vitro activation through the CD3/TCR complex and CD28 costimulatory molecule, within a microenvironment dominated by autologous AML blast population, was recently reported [47]. When these results were compared with samples from healthy controls on a per cell basis the IFNγ production for the anti-CD3 plus anti-CD28 activated AML samples was not significantly different from the healthy controls, suggesting that at least IFNγ responsiveness of T cells was not modified in the presence of the AML cells [47]. Thus, this last study did not detect any functional T cell defect either, supporting the findings of Panoskaltsis et al. [46].

NKT cells are a heterogeneous group of T lymphocytes expressing NK cell-associated receptors and αβ T cell receptor (TCR) that are lipid or glycolipid antigen-CD1d restricted rather than antigen-MHC restricted [48]. In cancer patients NKT cells may be both reduced and increased in numbers and may also have impaired ability to release cytokines [49-52]. Additionally, NKT cell number and function has been linked to prognosis in some malignancies [53-56]. This may also be true in AML as circulating Vo24<sup>+</sup> NKT cells are decreased in patients with progressive disease [57]. In contrast, increased CD3<sup>+</sup>CD56<sup>+</sup> NKT cells have been found in PB from AML patients, while normal levels were found in BM [4]. Thus disease-induced alterations may differ between NKT cell subsets.

Misoguchi et al. [58] were the first to suggest that immune dysfunction in cancer patients was due to an altered...
The immune system in AML: The Open Hematology Journal, 2007, Volume 1

Composition of the T-cell receptor signaling complex. Today, decreased expression of CD3-ζ (CD3- zeta) in T cells has been demonstrated in several malignancies [59-62]. Reduced CD3-ζ expression in tumor infiltrating lymphocytes even seems to be an independent prognostic factor for patients with oral carcinoma [63]. Buggins et al. [64] reported abnormal expression of CD3-ζ in 64% out of 46 myeloid leukemia patients examined (of which 11 were AML) and successful remission induction was associated with recovery of CD3-ζ expression. Also, the CD3-ζ associated protein tyrosine kinases (p56lck, p59fnl, and ZAP-70) showed variable but often reduced expression in these patients [64]. Additionally, in a murine AML model [65] there were observed reduced responses (proliferation and IL-2 secretion) to mitogenic anti-CD3 but not to PMA/ionomycin (which is TCR independent) as early as one week following the injection of leukemic cells, whereas loss of CD3-ζ protein expression together with intracellular signaling abnormalities (i.e. calcium mobilization and tyrosine kinase activity) were detected only in advanced disease (4 weeks after injection). It was also shown in vitro that leukemia-derived factor(s) stimulated splenic macrophages to secrete a second soluble factor(s) that caused the loss of CD3-ζ [65]. The AML-associated T cell abnormalities are summarized in Table 2.

From the above mentioned studies it is difficult to reach a firm conclusion, partly due to the question of patient heterogeneity and the low number of patients in some of these studies. However, it is justified to conclude that there are no major quantitative defects for the total T cell population and most of the examined T cell subsets in untreated AML, but NKT cells may be an exception. Another exception is the increased frequency of Treg cells (see below). The altered expression of the TCR signaling complex is probably the best example of a functional disturbance of T cells in AML (Fig. 1).

Treg Cells: High Levels may Play a Critical Role in AML-Associated Immunosuppression

Treg cells (Table 1) control autoimmune T cell reactivity in the periphery and may also suppress immune responses against cancer cells (reviewed in [66]). An increased number of Treg cells has been reported for patients with ovarian cancer, lung cancer, breast cancer, gastrointestinal malignancies and lymphoma [67-73]. In concordance with these findings, Wang et al. reported significantly higher proportions of CD4+CD25high Treg cells for PB and BM in AML patients compared with healthy controls [74]. These CD4+CD25high cells were (i) CD45-RA-, CD69-, CD45-RO+, CD95+, intercellular CTLA-4+; (ii) secreted low levels of TNF-α and IL-10 and did not release IL-2, IL-4, IL-5 or IFNγ; and (iii) behaved as Treg cells by inhibiting CD4+CD25+ T cell proliferation and cytokine production during in vitro activation [74]. The AML cell expression of a key enzyme in the tryptophan metabolism; Indoleamine 2,3-dioxygenase (IDO), has been correlated with increased levels of circulating CD4+CD25+FOXP3+ Treg cells in patients with AML at diagnosis [75]. Subsequent in vitro experiments indicated that AML cells induce T-cell anergy by directly converting CD4+CD25+ Treg cells into CD4+CD25+ Treg cells through an IDO-dependent mechanism [75]. Thus, CD4+CD25+ Treg cells are increased and possibly play a critical role in immunosuppression in AML (Fig. 1).

LEUKEMIA ASSOCIATED ANTIGENS: A POSSIBLE ROLE IN DIAGNOSTIC CLASSIFICATION, PROGNOSTIC EVALUATION OR IMMUNOTHERAPEUTIC TARGETING?

Several mutated or overexpressed proteins seem to be processed and presented to the immune system as tumor antigens leading to cellular and/or humoral responses [76]. One of the most extensively studied cancer-associated antigens is p53 [77], but more recent studies have demonstrated that
autoantibodies against a wide range of autoantigens can be detected in patients with malignant disorders [76, 78]. Zhang et al. investigated a group of 174 cancer patients for autoantibodies against 7 known antigens, each autoantibody could then be detected in 10-20% of the patients and approximately half of the patients had at least one autoantibody [76]. Cancer-associated humoral autoimmunity thus seems to be a common phenomenon, but autoimmune disease is uncommon in these patients [79].

The number of autoantibodies known to occur in AML patients is few compared to other malignancies. However, humoral responses against the following antigens have been detected at an increased frequency in AML patients compared with healthy controls (Table 4): Wilms tumor gene product (WT1) [80, 81], single-stranded DNA (ssDNA) [82], anticardiolipin antibodies (ACA) [83], the M-phase phosphoprotein 11 (MPP11) [84], receptor for hyaluronan-mediated motility (RHAMM) [85], myc associated zinc finger (MAZ) [86], per ARNT SIM (PAS) domain containing 1 (PASD1) [87], cyclin B1 [88] and Rhamm-like protein [89]. Some of these autoantibodies have originally been detected in tumor microenvironments [95, 96]. Such tumor-derived factors can for instance induce apoptosis (e.g. CCL5/CXCL5) and thereby contribute to local immunosuppression. Similar mechanisms seem to be operative in AML (Table 5). In addition to the above mentioned IDO-dependent Treg cell induction [75], the AML microenvironment seems to be immunosuppressive by inhibiting T cell cytotoxic activity [101, 102]. AML culture supernatants have in addition shown a TGF-β, IL-10 and VEGF independent inhibition of T cell activation, T cell proliferation and Th1 cytokine production [102].

A brief summary of some of the antibody/B cells (humoral) and spontaneous cytotoxic T (Tc) cell recognized leukemia associated antigens (LAAs) described in AML patients. WT-1: Wilms tumor gene product-1; MPP-11: the M-phase phosphoprotein 11; PASD1: per ARNT SIM (PAS) domain containing 1; RHAMM: receptor for hyaluronan acid-mediated motility; MAZ: myc associated zinc finger; Bcl-2: B-cell CLL lymphoma 2.

### Table 4. Leukemia Associated Antigens in Untreated AML Patients

| Humoral recognized antigens | Leukemia associated antigens | Ref.
<table>
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<tbody>
<tr>
<td>WT-1, MPP-11, PASD1, RHAMM, MAZ, cyclin-B1, Rhamm-like protein</td>
<td>WT-1, proteinase3, RHAMM, Bcl-2</td>
<td>[81, 84, 85, 87-89]</td>
</tr>
</tbody>
</table>

A brief summary of some of the antibody/B cells (humoral) and spontaneous cytotoxic T (Tc) cell recognized leukemia associated antigens (LAAs) described in AML patients. WT-1: Wilms tumor gene product-1; MPP-11: the M-phase phosphoprotein 11; PASD1: per ARNT SIM (PAS) domain containing 1; RHAMM: receptor for hyaluronan acid-mediated motility; MAZ: myc associated zinc finger; Bcl-2: B-cell CLL lymphoma 2.

### THE CYTOKINE NETWORK: IMMUNOREGULATORY FACTORS ARE CONSTITUTIVELY RELEASED BY PRIMARY HUMAN AML CELLS

Numerous immunosuppressive factors like TGF-β, IL-10, vascular endothelial growth factor (VEGF), Fas-L and Fas have been detected in tumor microenvironments [95, 96]. Such tumor-derived factors can for instance induce apoptosis in T cells [96-99] or inhibit a Th1 response [100] and thereby contribute to local immunosuppression. Similar mechanisms seem to be operative in AML (Table 5). In addition to the above mentioned IDO-dependent Treg cell induction [75], the AML microenvironment seems to be immunosuppressive by inhibiting T cell cytotoxic activity [101, 102]. AML culture supernatants have in addition shown a TGF-β, IL-10 and VEGF independent inhibition of T cell activation, T cell proliferation and Th1 cytokine production [102].

AML cell supernatants have been shown in experimental models to affect major signaling pathways involved in T cell activation and proliferation (summarized in Table 5): i) reduced nuclear translocation of the transcription factors NFATc and NF-κB; ii) delayed activation of the mitogen-activated protein (MAP) kinase c-Jun N-terminal kinase 1/2 (JNK-1/2); iii) no phosphorylation of the cell cycle related proteins retinoblastoma protein (pRb), cyclin-dependent kinase-6/4 (CDK-6/4) cyclin D and Rb family member p130; and iv) no induction of the transcription factor c-Myc, the cell cycle protein cyclin D3 and the Rb family member p107. Calcium mobilization, the MAP kinase extracellular signal-regulated kinase-1/2 (ERK-1/2) and p38, as well as the signal transducer and activator of transcription protein-5 (STAT-5) remained unaffected [102]. Taken together these observations clearly demonstrate that AML-derived soluble mediators have extensive effects on the intracellular T cell signalling (Fig. 1).

### AML CELLS: IMMUNOLOGICAL ESCAPE PHENOTYPE AND RESISTANCE TO IMMUNOLOGICAL MEDIATED CELL DEATH

In addition to proposed immunosuppressive factors constitutively released by AML cells, an immune escape phenotype of AML cells has been suggested (see Table 5). We have already mentioned the NCR<sup>+</sup> NK lymphocyte phenotype that is most possibly induced by direct contact with the leukemic blasts [12] and the high expression of inhibitory over activating KIR ligands on the AML cells [11] (Fig. 1). Moreover, ligands for NK cell-activating receptors are expressed only at very low levels in leukemic cells in 80 percent of patients [105]. In vitro administration of differentiation-promoting drugs upregulate NK cell-activating receptor expression and enhances the susceptibility of AML cells to
NK cell-mediated lysis [105, 106]. Thus, the NK cell-escape phenotype also can be overcome, but differentiation-promoting drugs may also prevent NK cytotoxicity by down-regulation of NK cell activating receptors [107].

The two major mechanisms by which immune effector cells exert their cellular cytotoxicity is through granule exocytosis and Fas (CD95) ligation [108]. Granzymes and perforin are the major components of the cytotoxic granules in both T and NK cells [108]. A perforin- and Fas-mediated cell death resistance has been suggested for AML cells [96, 109, 110] (Fig. 1), but due to the small numbers of patients included in these studies no firm conclusion can be made regarding the clinical significance.

The expression of the major histocompatibility complex (MHC) genome area class I and II is crucial for LAA presentation. HLA class I molecules are reduced in AML blasts compared to normal monocytes [111]. Myeloid progenitor cells seem to temporarily express HLA class II molecules during differentiation to granulocytes and macrophages [112]. Primary human AML cells also express HLA class II molecules [113, 114]. A recent study outlines the importance of class-II associated invariant chain peptide (CLIP) expression and the HLA-DO:HLA-DM ratio for prediction of clinical outcome of AML patients [114]. Based on these findings the authors suggested that HLA-DR+/CLIP+ AML cells associates with adverse prognosis [114]. High expression of costimulatory molecules associates with adverse prognosis [115]. The negative effect of high CD86 (B7-2) expression has also been reported previously [116], whereas the results for CD80 (B7-1) expression are conflicting [111, 113, 115-117]. The association of adverse prognosis with high expression of certain costimulatory molecules, is contradictory to the hypothesis that high levels of costimulatory molecules are favorable for immunological recognition and destruction of AML cells and therefore would be expected to be associated with a good prognosis.

The costimulatory cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) is a negative regulator of T cell activation. Recently, CTLA-4 was reported constitutively expressed by 80% of AML samples tested [118, 119]. Blocking either PD-1, CD80 or CTLA-4 in vivo prolonged the survival of naive mice engrafted with AML cells [120].

To conclude, additional studies are clearly needed to further elucidate the role of the T cell costimulatory or inhibitory molecules in AML to clarify whether these molecules have effects in immunoregulation, or whether high expression of these molecules is only a part of a functional AML cell phenotype associated with chemoresistance or a more aggressive disease.

### INTENSIVE AML CHEMOTHERAPY: EARLY IMMUNOLOGICAL RECONSTITUTION IS A GOOD PROGNOSTIC FACTOR

**Immune Function in AML Patients with Severe Chemotherapy-Induced Cytopenia**

Cytotoxic antineoplastic therapy disrupts T cell homeostasis and is the primary contributor to the severe clinical immunodeficiency observed in AML patients [121]. The

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Observations</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Soluble mediators</td>
<td>Reduce T cell CD3-ζ expression.</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>Inhibit T cell activation, proliferation, T_{H1} cytokine production, and cytotoxic activity</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td>Inhibit major signaling pathways involved in T cell activation and proliferation;</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td>• Transcription factors (NFATc, NFκ-B, c-Myc)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Map kinase (JNK-1/2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Rb family members (pRb, p130, p107)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Cell cycle related proteins (CDK-6/4, cyclin D3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemokine release clusters have been identified</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>• CCL2-4/CXCL1/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• CCL5/CXCL9-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• CCL13/17/22/24/CXCL5</td>
<td></td>
</tr>
<tr>
<td>Direct cell-cell contact</td>
<td>Deficiently expression of activating ligands for NK cell-activating receptors</td>
<td>[11, 105]</td>
</tr>
<tr>
<td></td>
<td>High IDO expression correlate with high numbers of T_{reg} cells</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>Perforin- and Fas-mediated cell death resistance</td>
<td>[109]</td>
</tr>
<tr>
<td></td>
<td>Reduced expression of HLA class I molecules</td>
<td>[111]</td>
</tr>
<tr>
<td></td>
<td>MHC class II HLA-DR+/CLIP+ AML cells associates with adverse prognosis</td>
<td>[114]</td>
</tr>
<tr>
<td></td>
<td>High expression of costimulatory molecules associates with adverse prognosis</td>
<td>[115]</td>
</tr>
</tbody>
</table>

IDO: Indoleamine 2,3-dioxygenase, CLIP: class-II associated invariant chain peptide.
impact of the immune system for outcome after chemotherapy was recently elucidated by Behl et al. [122], finding that rapid absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in AML.

In general, patients receiving chemotherapy for various malignant disorders seem to develop a CD4⁺ T lymphopenia together with high serum levels of the pleiotropic cytokine IL-7 [123, 124]. In contrast, AML patients with severe chemotherapy-induced leukopenia and CD4⁺ T lymphopenia, showed decreased IL-7 serum levels, and the detection of circulating IL-7-responsive T cells in these patients indicated that variations in systemic IL-7 levels are functionally important and may contribute to the T cell defect in lymphopenic AML patients [125]. Other studies have demonstrated that administration of recombinant IL-7 to humans restored CD4⁺ T lymphocytes and decrease the percentage of CD4⁺ Treg cells [126]. The possible clinical use of IL-7 therapy in AML has not been investigated.

For AML patients with chemotherapy-induced cytophenia most circulating leukocytes are T lymphocytes, whereas B lymphocytes and monocytes show a wide variation among patients but usually represent less than 10% of the leukocytes (Table 6) [127]. Most of the circulating T cells in cancer patients with chemotherapy-induced cytophenia seem to express the activation markers HLA-DR as well as CD25 and CD69, and this is also true in human AML [128, 129]. Following chemotherapy there seems to be an absence of CD4⁺CD45RA⁺ T cells, all the remaining CD4⁺ T cells expressing the CD45RO⁺ isofrm [121, 128].

There have also been some reports regarding qualitative/functional evaluation of T cells, these include the examination of cytokine release and cytokine responsiveness of in vitro expanded clonogenic T cells derived from AML patients with therapy-induced cytophenia [129-133]. These clonogenic cells constitute a minor population of circulating T cells including CD4⁺ and CD8⁺ TCRαβ cells [130], and the cells secrete a wide range of immunoregulatory cytokines [131]. The frequency of these clonogenic T cells is often reduced compared to healthy individuals [130].

Very few studies have included functional analysis of circulating T cells other than the clonogenic minority. However, there are studies of the proliferative responsiveness of T cell derived from AML patients with severe leucopenia evaluated in a whole blood assay [127, 134]. In this assay activated T cells were found to have proliferative responsiveness equal to healthy controls in the presence of optimal stimulation with anti-CD3 plus anti-CD28, but responses were significantly reduced in the patient samples when using anti-CD3 stimulation only. Furthermore, the responses were significantly lower for ALL than for the AML patients [127].

More recently, we reported that the in vitro activated T cells in this whole blood assay show a broad cytokine release profile including high and significantly correlated release of IFNγ and GM-CSF [135]. The anti-CD3 plus anti-CD28-stimulated IFNγ release, but not GM-CSF release, was significantly lower for AML patients than for healthy individuals. This difference is probably caused by combined qualitative and quantitative defects within the remaining T cell population in the AML patients [127, 134]. Additionally, higher IFNγ and GM-CSF levels were significantly associated with older age, an observation clearly demonstrating that age therapeutic-dependent alterations have to be considered when clinical trials of immunotherapy are designed [135]. Significantly higher T cell proliferation and cytokine release were also observed when the novel drug PEP005 (a protein kinase C agonist) was added, thus indicating that pharmacological immunotargeting could be possible in these severely compromised patients [135].

**Immune Reconstitution after Conventional Intensive Chemotherapy: the Impact of Thymic Atrophy**

Effective immunological functions after chemotherapy and myeloablative therapy depend upon reconstitution of antigen specific T cells. For the recovery of T cells renewed thymopoiesis is crucial [136]. Thymus involution rapidly reduces its function to <5% of its maximal capacity after puberty [137, 138]. Therefore, when T cell levels are severely decreased after chemotherapy, the ability to regenerate the T cell repertoire is severely compromised and worsening occurs with increasing age [139]. Thus, the T cell re-

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**Table 6. Immune System in AML Patients with Chemotherapy-Induced Cytopenia**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Abnormality</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>NK cells</td>
<td>No studies</td>
<td></td>
</tr>
<tr>
<td>DCs</td>
<td>No studies</td>
<td></td>
</tr>
<tr>
<td>T cells</td>
<td>Circulating IL-7 responsive T cells</td>
<td>[125]</td>
</tr>
<tr>
<td></td>
<td>Most T cells express CD45RO, HLA-DR, CD25, CD69</td>
<td>[127]</td>
</tr>
<tr>
<td></td>
<td>Reduced frequencies of clonogenic T cells</td>
<td>[130]</td>
</tr>
<tr>
<td></td>
<td>In vitro aCD3+aCD28 T cell proliferative response equal to healthy control</td>
<td>[127]</td>
</tr>
<tr>
<td></td>
<td>In vitro aCD3+aCD28 T cell IFNγ secreting response lower than healthy controls</td>
<td>[135]</td>
</tr>
<tr>
<td></td>
<td>T cell directed pharmacological targeting may be possible</td>
<td>[135]</td>
</tr>
<tr>
<td>B cells</td>
<td>Generally less than 10% of the leukocytes</td>
<td>[127]</td>
</tr>
</tbody>
</table>

NK: natural killer cells; DCs: dendritic cells; aCD3: antibody against CD3; aCD28: antibody against CD28.
generation can be divided into two different pathways mainly dependent upon age. Firstly, in young patients HSCs generate T cell committed HPCs (T-HPCs: CD34+CD7+CD3- ) in the BM that transmigrate to the thymus where the T-HPCs down-regulate the CD34 antigen, up-regulate the expression of T cell receptors (TCRs) and CD3 antigen and finally differentiate into naive CD4+ and CD8+ T cells [140, 141]. Alternatively, adult patients with thymic atrophy regenerate the T cell compartment principally by antigen induced conversion of naive to memory T cells and subsequent expansion [141-143]. This implies that adults show a delay in peripheral T cell regeneration as well as increased incidence of opportunistic infections compared to younger individuals [144]. Among suggested approaches to treat such immunodeficiency states in adults are the use of sex-steroid ablation or androgen blockade to activate thymic regeneration which may lead to the reversal of age-related thymic atrophy [145-147]. However, fatal infections are uncommon after hematopoietic reconstitution, and only low- or nontoxic strategies to enhance immune reconstitution are therefore justified.

Effective immunological function after chemotherapy also depends upon reconstitution of antigen specific antibody production by B lymphocytes as well as NK cell reconstitution. In contrast to T cells, B cells and NK cells differentiate essentially in the bone marrow and thus depend upon an intact BM microenvironment. Additionally tissue-specific NK cell development (e.g. thymus) is hypothesized to exist [148], and thus thymic atrophy and age might also affect NK cell restoration.

**Complete Haematological Remission and Recovery of the Immunesystem**

Very few studies have examined the T cell system in AML patients after hematopoietic reconstitution. Long-lasting T cell defects can occur after intensive chemotherapy for other malignancies, especially in adult patients [121]. One would expect similar defects to occur after AML therapy. However, as mentioned above, the abnormal CD3-ζ expression on T cells from AML patients was recovered when achieving remission [64]. Furthermore, low expression of the activating natural cytotoxicity receptors (NCR) on NK cells (see above) in AML patients was completely or partially restored in patients achieving remission [12]. These studies suggest that the continued presence of leukemia cells are necessary for abnormal CD3-ζ expression on T cells and NCR down-regulation on NK cell.

**IMMUNE RECONSTITUTION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: INFLUENCED BY STEM CELL MOBILIZATION REGIMES?**

Similar to the significance of the immune system reconstitution after induction therapy [122], early lymphocyte recovery is a predictive factor for prolonged survival after autologous stem cell transplantation (auto-SCT) for AML patients [149].

The recirculation pattern of injected leukocyte subpopulations during the first 24 hours after auto-SCT of AML differs between leukocyte subsets [150]; i) the number of CD3+ T lymphocytes increases during this period, ii) CD56+ NK cells decrease rapidly after auto-SCT and remain low throughout the observation period of 24 hours, and iii) B lymphocyte levels are also low during the first 24 hours [150].

The early reconstitution of the T lymphocyte system after both auto- and allo-SCT is mainly due to the peripheral expansion of mature T cells transferred with the graft [142, 151]. The recovery of polyclonal T lymphocytes occurs gradually, and complete reconstitution of humoral and cellular immunity may take more than one year [152]. Similar to the observations in patients only receiving intensive chemotherapy, there seems to be an absence of CD4+CD45RA+ T cells early after auto-transplantation with a remaining population of CD4+CD45RO+ circulating T lymphocytes [121, 128, 153]. One study has suggested that BM-resident memory T cells are resistant to both pretransplant chemotherapy and ex vivo pharmacological purging and thus may contribute to the immune reconstitution after auto-SCT [154].

G-CSF is the growth factor most commonly used for stem cell mobilization. T cells in PB after HSC mobilization with G-CSF have been analyzed: some studies indicate that G-CSF doubles the total number of circulating T cell without major changes in the fractions of various T cell subsets [141, 155]. However, other functional studies [156-158] indicate that G-CSF might affect the cytotoxic T cells by stimulating these lymphocytes to preferentially release type 2 cytokines (IL-4 and IL-10) rather than type I (IFNγ, TNF-α, IL-2) upon activation. G-CSF may in addition increase the number of circulating Treg cells [159].

**THE EARLY IMMUNE RECOVERY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS IMPORTANT FOR SUCCESSFUL TRANSPLANT OUTCOME**

Allogeneic stem cell transplantation (allo-SCT) can be a curative treatment for younger AML patients. Bone marrow (BM), cord blood (CB), and G-CSF-mobilized peripheral blood progenitor cell (PBPCs) can be used. A comparison of the cellular composition of leukapheresis products (LPs), CB allografts and BM allografts demonstrated that the composition of the graft lymphocytes differed considerably. T, B and NK cells were highest in LPs and lowest in CB allografts [141]. Also, early immune recovery is possibly enhanced following blood stem cell allografting compared with BM allografting; this is probably due to the large number of mature lymphocytes in blood stem cell allografts [160].

Recently, lymphocyte recovery at 3, 6 and 12 months after allo-SCT, was characterized for patients with various hematological malignancies, including AML [161]. In summary, this study revealed: i) a rapid CD56+ NK cell recovery at 3 months, ii) the CD19+ B cell recovery was slow as only 45% of patients recovered CD19+ cell counts above 5x10^9/L at 3 months, iii) an inverted CD4:CD8 ratio was observed during the first year, iv) the CD4+ helper T cell reconstitution at 3 months was associated with improved overall survival when using the cutoff value of 2000x10^6/L CD4+ helper T cells, v) rapid CD4+ helper T cell recovery was also associated with a higher CD4+ helper T cell transplant dose and HLA-matched sibling donors, vi) an early CD4+ helper T cell recovery at 3 months correlated with a subsequent faster helper T cell recovery until 12 months but
not with faster B cell recovery, and vii) rapid recovery of CD4+ helper T cells at 3 months was a favorable prognostic factor in terms of overall survival and non-relapse mortality [161]. In addition to the non-relapse mortality reduction the donor T cells are important for the graft-versus-leukemia (GvL) effect together with NK cells [162]. The general principles of GvL and GvHD are outlined in detail elsewhere [163].

Ruggeri et al. [162] have recently underlined the importance of KIR ligand mismatching, i.e. donor NK cell recognition of “missing self” on recipient targets, essential for triggering NK cell alloreactions beneficially for haplotype-mismatched transplantation outcome [162]. However, this is not supported by other studies [164, 165], and thus the effect of KIR ligand mismatching on the outcome of unrelated donor transplantation for AML patients, still remains obscure.

CONCLUDING REMARKS

Both the cellular innate and adaptive immune system in human AML patients differ from healthy individuals. AML patients are immunocompromized initially due to the disease and later due to intensive chemotherapy. We have included several examples of the importance of the immunological compartment and clinical significance in AML patients before, during and after treatment. However, future studies will be necessary to clarify the impact of the many immunological conditions for clinical relevance.

ACKNOWLEDGEMENTS

This work was supported by the Norwegian Cancer Society.

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