in both children and adults, whereas the CD45RA/CD45RO phenotype predominated in γδ T-ALL. There is a scarcity of data about the CD45 isoforms in γδ and γδ T-ALL, and the only two studies describing the predominance of the CD45RO isoform in T-ALL did not evaluate the γδ, or γδ TCR status of leukemic cells. Since most normal thymocytes express the CD45RA/CD45RO phenotype, the finding that CD45RA/CD45RO was the most common phenotype in γδ, T-ALL was unexpected. Although CD45RA and CD45RO expression discriminates as naive and memory subsets of T-cells, respectively, this differentiation holds best for peripheral blood T cells. In the thymus, where the normal counterparts of T-ALL are found, the expression of these CD45 isoforms is associated with functions related to thymic maturation. Clinically, the CD45 isoform status had no prognostic value when overall survival of patients whose blasts expressed the CD45RA or CD45RO antigens were compared (data not shown).

The expression of membrane CD3 (mCD3), expected to be always detected in TCR ALL, was negative in two cases of γδ T-ALL, whereas cytoplasmic CD3* was present in all cases. Asnafi et al. recently described some T-ALL cases with negativity for mCD3, but with cytoplasmic expression of the TCRβ, antigen, which they called pre-TCR T-ALL. We have no explanation for these TCRβ+/CD3−/mCD3+ cases observed in our series. Although normal γδ T-cells represent a very small proportion of normal thymocytes, this subset is enriched among CD4 and CD8 double-negative T cells. Malignant γδ T-cells, however, expressed CD4, CD8 or both of them in 74% of the cases. Similar results have been previously described, contradicting the intuitive acceptance that the γδ T-ALL blasts should not express CD4 and CD8, as their normal counterparts. Van Dongen et al. described a small subset of normal γδ T-cells in the peripheral blood which was either CD4 or CD8 positive, in addition to the vast majority of double negative cells. Therefore, perhaps this small subset of normal CD4 or CD8 γδ T-cells is particularly prone to malignant transformation. Alternatively, the expression of CD4 or CD8 antigens in γδ T-cells may represent a phenomenon related to the neoplastic transformation. Together, these data give support to the concept that γδ T-ALL represents a distinctive subtype of leukemia, with peculiar clinical, laboratory and immunophenotypic characteristics.

References


Chronic Lymphoproliferative Disorders

Absence of surface CD27 distinguishes hairy cell leukemia from other leukemic B-cell malignancies

Surface expression of CD27 was evaluated in 75 mature leukemic B-cell neoplasms. All cases other than hairy cell leukemia (HCL) expressed CD27. Intensity was significantly higher in chronic lymphocytic leukemia. Lack of CD27 in 17/17 HCL contrasted with expression of this marker in 5/5 splenic lymphomas with villous lymphocytes. Lack of CD27 is a new distinctive feature of HCL among B-cell malignancies.

CD27 is a member of the tumor necrosis factor (TNF) receptor family induced on B lymphocytes after antigen challenge and interacts with CD70 to differentiate mature B cells into plasma cells. CD27 was originally defined as a memory B-cell marker, mainly because of its expression on B-cells with mutated VH-genes. However, CD27 is induced in centroblasts and centrocytes of the germinal center (GC) and retained by post-GC memory B-cells. CD27 is generally conserved after neoplastic transformation on mature B-cell neoplasms, but, differently from the normal situation, is independent of VH-gene status. In chronic lymphocytic leukemia (B-CLL), CD27 is also present as a soluble molecule correlating with tumor load. Immunohistochemistry has confirmed expression of CD27 in mantle cell lymphoma (MCL), Burkitt’s lymphoma, marginal zone lymphoma (MZL) and plasmacytomas/myelomas.
CD27 expression was determined by a 3-color staining technique with the monoclonal antibodies: fluorescein isothiocyanate-conjugated anti-CD27 (PharMingen, CA), peridinin chlorophyll protein-conjugated anti-CD19 and phycoerythrin-conjugated anti-CD11c (HC) or phycoerythrin-conjugated anti-CD5 (for B-CLL and MCL). Haired cells were identified as high side scatter and high forward scatter (hairy cells). (B). FL1 = isotype control. (C) FL1 = CD27 (FL1) expression by HC and non-HC of the same events. (A). R1 FSC<median/SSC<median. R2 FSC<median/SSC>median; R3 FSC>median/SSC<median; R4: non-hairy cells. (E) and (F). CD27 (FL1) expression by HC and non-HC of the same sample.

**Table 1.** Surface expression of CD27 in B-cell malignancies.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of cases</th>
<th>+ cases (%)</th>
<th>CD27 MFI (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCL</td>
<td>17</td>
<td>0 (0)</td>
<td>–</td>
</tr>
<tr>
<td>B-CLL</td>
<td>36</td>
<td>36 (100)</td>
<td>15-253 (78)</td>
</tr>
<tr>
<td>MCL</td>
<td>6</td>
<td>6 (100)</td>
<td>3-11 (6)</td>
</tr>
<tr>
<td>LPL</td>
<td>3</td>
<td>3 (100)</td>
<td>3-61 (10)</td>
</tr>
<tr>
<td>SLVL</td>
<td>5</td>
<td>5 (100)</td>
<td>3-16 (9)</td>
</tr>
<tr>
<td>FL</td>
<td>6</td>
<td>6 (100)</td>
<td>2-19 (9)</td>
</tr>
<tr>
<td>PLL</td>
<td>2</td>
<td>2 (100)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Normal</td>
<td>11</td>
<td>11/11</td>
<td>8-17 (10)*</td>
</tr>
</tbody>
</table>

CD27 expression was mainly based on morphological and phenotypic criteria. However, even the most specific CD103, HC2 and DBA44 antigens, or a scoring system using multiple markers is insufficient to complete diagnosis in some cases, and new diagnostic antibodies are being investigated. In the process of defining the features of HCL, CD27 expression was evaluated on the peripheral blood mononuclear cells (PBMC) from 11 normal individuals and 75 with B-cell neoplasms, including 17 cases of HCL (Table 1). Diagnoses were based on immunohistochemical examination of bone marrow biopsies and morphologic and immunophenotypic findings of the PBMC. Immunophenotypic studies and analysis of CD27 expression were performed using established procedures (Table 1). In normal individuals, CD27 was found on 2.5-50% CD19+ B cells (mean 31%, median 33%). The mean fluorescence index (MFI) of CD27 ranged from 13 to 53 (mean 17, median 14). Within the CD19+/CD5+ population, the MFI of CD27 was lower (range 8-17, mean 10, median 10). All 17 bone marrow biopsy-proven cases of HCL had typical morphology and expressed a stringently defined phenotype of HCL (CD19+, k/B, restriction, CD25+, FMC7+, CD103+, with CD11c+, CD20+, and SmIg at high intensity, and CD23+). No cases of HCL expressed CD27 (Figure 1, Table 1). Conversely, CD27 was expressed in all the remaining 58 B-cell tumors (Table 1). Importantly, in these cases, the histogram plot had an unimodal distribution and the percentage of positive events did not document subpopulations, while it reflected CD27 intensity. In B-CLL, CD27 was expressed on 20-96% leukemic CD19+/CD5+ cells (median 60%, mean 61%). The intensity of CD27 on the leukemic B-CLL cells was significantly higher (MFI range 15-253, mean 32, median 78) than on the CD5+ B cells from normal individuals (χ2 test, p<0.05). The difference in intensity was also significant when compared with that of the entire B-cell population from normal subjects (χ2 test, p<0.05). All other categories always expressed CD27 with an intensity similar to that in normal controls. Importantly, these included 5/5 cases of splenic MZL with villous lymphocytes (SLVL) that expressed CD27 in 37-93% tumor cells (MFI 3-16, mean 9, median 9). Lack of the GC marker CD27 in HCL is an apparent paradox. HCL has several molecular features resembling that of a B-cell activated by antigen. These include mutated VH-genes with intrachromosomal heterogeneity, ongoing switch activity and expression of activation-induced cytokine deaminase. These events generally occur in the GC. However histology and absence of GC markers (e.g. bcl-6, CD10, CD38) point to no interaction of hairy cells with GC elements. In this context, lack of CD27 is concordant with the hypothesis that tumor events of HCL occur outside the GC. Alternatively, the lack of CD27 may be an aberrant event occurring in HCL post-transformation. CD27 transcript levels were found to be negative or lower in HCL than in memory B cells. TNF and other Th1 cytokines produced by hairy cells may be responsible for CD27 abrogation, and hinder plasma cell differentiation through CD70-CD27 ligation.

From the diagnostic point of view, all tumor categories other than HCL expressed the CD27 molecule. We con-

**Figure 1.** CD27 expression in HCL. CD27 is not expressed on the hairy cells (HC). HC were identified as SSC<median/FSC<median and CD19+/CD11c+ B cells, and distinguished from the remaining normal PBMC. The anti-CD27 antibody binds to normal PBMC but not to tumor HC. (A). R1 FSC<median/SSC<median PBMC; R2 FSC>median/SSC>median PBMC (hairy cells). (B). FL1 = isotype control. (C). FL1 = CD27 expression. CD27 is expressed only by cells with low FSC. (D). FL2 = CD11c expression. FL3 = CD19 expression. R3: CD19+/CD11c hairy cells; R4: non-hairy cells. (E) and (F). CD27 (FL1) expression by HC and non-HC of the same sample.
Letters to the Editor

Delayed response to fludarabine in lymphoplasmacytic lymphoma/Waldenström’s macroglobulinemia

We retrospectively reviewed time to response and incidence of delayed responses in 13 patients with lymphoplasmacytic lymphoma/Waldenström’s macroglobulinemia (LPL/WM) treated with fludarabine with or without cyclophosphamide. During follow-up post-treatment, seven delayed responses (54%) were observed, improving the initial overall response rate of 61% to a final response rate of 77%.

Several reports document the efficacy of fludarabine in lymphoplasmacytic lymphoma (LPL) and Waldenström’s macroglobulinemia (WM) in both untreated patients and those with relapsed/refractory disease, but few have focused on the timing of response. We retrospectively reviewed time to response and incidence of delayed responses in 13 patients with LPL (including 7 with WM) treated with fludarabine with or without cyclophosphamide. The patients’ characteristics are summarized in Table 1. Three patients were previously untreated; 10 had relapsed after or were refractory to alkylating agents. Eight patients received single agent fludarabine (25 mg/m² intravenously (iv) for 5 days or 40 mg/m² orally (p.o.) for 5 days) every 4 weeks for a median of 6 courses (range 4-6). Five patients received fludarabine (25 mg/m² i.v. for 3 days or 24 mg/m² os for 5 days) with cyclophosphamide (FC) (250 mg/m² iv for 3 days or 150 mg/m² p.o. for 5 days) every 4 weeks for a median of 8 courses (range 5-9). The number of courses was determined by clinical and laboratory evidence of what was thought to be the maximal achievable response. Conventional criteria for complete response (CR) and partial response (PR) were used. We also defined serologic CR (sCR) as the absence of detectable serum and urine paraprotein by immunofixation, resolution of organomegaly, but with residual bone marrow (BM) disease (<50%). Patients not fulfilling these criteria were non-responders (NR). Any subsequent improvement in response during treatment-free follow-up constituted a delayed response.

The overall response rate assessed 1 month after completing therapy was 61% (23% sCR; 38% PR). Seven delayed responses (54%) were observed: 2/7 NR achieved PR two and seven months from the end of therapy and 2/5 with a PR reached sCR two and five months after completion of fludarabine (Table 1). In addition, 3 patients with a PR experienced further reduction in their paraprotein level, although insufficient to attain sCR: one patient (#12) achieved an 84% reduction 25 months after stopping FC and two others (#13, #7) 89% and 90% reduction 9 and 5 months after stopping therapy, respectively (Figure 1). The final response rate improved to 77% (10/13), including all 5 patients treated with FC and 5 of the 8 treated with fludarabine. All 3 previously untreated patients and 7 of 10 previously treated patients responded. The sCR rate was 38%; BM histology showed nodular PR (#4, #9, #10) or PR (#11) in 4 and CR (#8) in one. Thus, the final CR rate was 8%. Nodal disease resolved during treatment in all responders regardless of the tim-

References