The sensitivity of acute lymphoblastic leukemia cells carrying the t(12;21) translocation to campath-1H-mediated cell lysis

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Background and Objectives. Campath-1H is used in conditioning regimens and more recently as an anti-leukemic therapy in acute lymphoblastic leukemias (ALL). We therefore investigated CD52 expression and campath-1H-mediated lysis of ALL cells in vitro.

Design and Methods. Complement-mediated cytotoxicity assays were performed on freshly isolated neoplastic cells and cell lines using human serum. Antibody-dependent cellular cytotoxicity (ADCC) was performed by calcein-AM release assays.

Results. CD52 was expressed in four out of eight ALL cell lines studied. Among 61 freshly isolated ALL samples CD52 was expressed at varying levels in 87% of cases. Whereas ADCC was equivalent in different CD52+ lines, complement-dependent cytotoxicity (CDC) was variable. The REH cell line bearing the t(12;21) translocation showed 47-60% lysis when treated with 10 µg/mL campath-1H compared to 0-6% for the other cell lines expressing equivalent amounts of CD52. Furthermore all nine ALL samples with t(12;21) showed very high CDC (mean 97%) compared to the other 24 CD52+ cases (mean 24%)(p<0.0001). In t(12;21) samples, efficient CDC was obtained with as little as 1 µg/mL campath-1H. CDC correlated in part with CD52 levels, suggesting that CD52 expression and other yet undefined factors contribute to the particular sensitivity of t(12;21) cells. The resistance of non t(12;21) ALL cases could be overcome to a limited extent by increasing the concentration of campath-1H, blocking the CD55 and CD59 complement inhibitors, and more effectively by combining campath-1H with fludarabine.

Interpretations and Conclusion. We conclude that most ALL samples express CD52 to a variable level and that campath-1H has cytotoxic activity against CD52+ ALL, alone or in combination with cytotoxic drugs.

Key words: acute lymphoblastic leukemia, campath-1H, complement, ADCC, cytotoxicity, CD52.

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CD52 is a surface molecule present on most mature T and B lymphocytes, monocytes and monocyte-derived dendritic cells. It is also expressed on leukemia and lymphoma cells, including B-cell chronic lymphocytic leukemia (B-CLL) derived from these hematopoietic cell populations. Campath-1H® (alemtuzumab), a humanized IgG1k anti-CD52 antibody, has shown therapeutic activity in B-CLL and has been approved for the treatment of fludarabine-resistant cases. Campath-1H is also being used in the context of non-myeloablative reduced intensity conditioning for allogeneic stem cell transplantation, since it can effectively deplete mature potentially alloreactive T lymphocytes as well as dendritic cells. The use of campath-1H in allogeneic stem cell transplantation has resulted in low transplant-related mortality and markedly reduced graft-versus-host disease. The main concerns about the use of campath-1H in this setting is the likely reduction of graft-versus-leukemia activity and the high rate of viral infections, in particular cytomegalovirus, which are associated with this regimen. The mechanism of action of campath-1H is thought to be mostly immune-mediated, through both complement lysis (CDC) and antibody-dependent cellular cytotoxicity (ADCC). Indeed the antibody has been shown to lyse normal T and B lymphocytes, as well as different mature neoplastic B cells such as B-CLL and B-non-Hodgkin's lymphomas very efficiently in vitro in the presence of human complement. The antibody also induces ADCC of T lymphocytes and B-cell lines in vitro and FcγR are required for its in vivo activity in a murine model of leukemia. ADCC using freshly isolated NK cells and B-CLL cells as targets is much less efficient and may require prior activation of the effector cells, for example by stimulation with interleukin-2.
Cytotoxic activity of campath-1H in acute lymphoblastic leukemias

Design and Methods

Cells and reagents

The acute leukemia lines REH, 697, RCH-ACV, Tom-1, KCL, RS4;11, ALL-PO, CEM, SITLI and AIDS-derived Burkitt’s lymphoma ESIII were grown in RPMI1640 medium (Seromed, Berlin, Germany) supplemented with 10% fetal calf serum (FCS, Hyclone, Logan, UT, USA), glutamine (Gibco-BRL, Paisley, Scotland) and penicillin/streptomycin. Campath-1H was a kind gift from Schering SpA, Segrate, Italy. Bone marrow or peripheral blood samples from ALL patients were obtained after informed consent and separated by Ficoll-Hypaque centrifugation and stored frozen in liquid nitrogen until use. All samples were phenotyped for routine clinical diagnosis according to standard protocols. In all cases there were 80-100% leukemic blasts and 1-8% contaminating T cells (mean 3.7%).

Immunofluorescence

Cells were double stained with phycoerythrin-conjugated CD19, CD7, CD8 monoclonal antibodies (BD Biosciences, San Jose, CA, USA), CD55 and CD59 monoclonal antibodies (Serotec, Oxford, UK) and with fluorescein isothiocyanate-labeled CD52 antibody (clone YTH54.5, Serotec) according to standard procedures. All samples were analyzed on a FACscan flow cytometer (BD Biosciences).

Complement-dependent and independent cytotoxicity assays

Complement-dependent cytotoxicity (CDC) assays were performed essentially as described previously. Cell lines were plated at 1×10⁴ cells/well and primary ALL samples at 1×10⁵ cells/well in flat-bottomed 96-well plates in 100 µL StemSpan SFEM serum-free medium (Stem Cell Technologies), in the presence or absence of campath-1H and/or 15-20% human serum as a source of complement. Sets of wells containing medium and serum only were used to establish the background fluorescence for each serum concentration. In some experiments the blocking anti-CD55 and anti-CD59 antibodies BRIC216 and BRIC229 (IGBRL, Birmingham, UK) were also added at a 20 µg/mL final concentration. After 3 hours at 37°C, the total volume was brought to 300 µL in medium containing 1/10 volume of alamar blue solution (Biosource International, Camarillo, CA, USA). Incubation was carried on overnight at 37°C and the plates were read in a fluorimeter (Genios, Tecan) with excitation at 530 nm and emission at 590 nm.

Antibody-dependent cellular cytotoxicity

ADCC assays were performed essentially as described previously, except that target cells were labeled with calcein-AM. Briefly the target cell line was resuspended at 1×10⁶/mL cells in complete growth medium containing 3.5 µM calcein-AM solution (1mM, Sigma-Aldrich, St Louis, MO, USA) and incubated at 37°C for 30 minutes. Cells were then washed twice and resuspended in 1 mL complete growth medium in the presence or absence of 10 µg/mL campath-1H antibody. Incubation was carried out for 20 minutes at room temperature, after which cells were washed again and plated at 10⁴ cells/well in a final volume of 200 µL, in the presence of increasing amounts of freshly isolated peripheral blood mononuclear cells from normal donors or 1% Triton-X100. After 4-5 hours’ incubation at 37°C, 100 µL of supernatant were transferred into a new flat-bottomed 96-well plate and the fluorescence was read at 485 nm excitation and 535 nm emission in a fluorimeter (Genios, Tecan).

Deposition of complement components

In order to measure C3 and C9 deposition on the cell surface, cells were plated at 5×10⁵/mL and incubated in the presence or absence of 10 µg/mL campath-1H and/or 15% human serum. After 1 hour of incubation at 37°C, cells were recovered, washed and stained with anti-C9 monoclonal antibody (clone aE11, a kind gift from Dr. F. Tedesco, Trieste University, Trieste Italy) or anti-C3 antiserum (Quidel Corporation, San Diego, CA, USA) and fluorescein isothiocyanate labeled anti-mouse or anti-goat secondary antibodies (Sigma-Aldrich, Inc.).

Polymerase chain reaction (PCR) analysis of chromosomal translocations

Analysis of the chromosomal translocations t(12;21), t(9;22), t(4;11) and t(1;19) was performed by PCR according to a previously published method. In most cases PCR analysis was confirmed by routine cytogenetic analysis. ALL cases showing none of the above translocations by PCR were classified as negative, although cytogenetic analysis showed that a proportion of these cases had other chromosomal abnormalities.

Combination with cytotoxic drugs

ALL samples were plated at 1×10⁶ cells/well in flat-bottomed 96-well plates in StemSpan SFEM serum-free medium (Stem Cell Technologies), in the presence or absence of 0.2 or 1 µg/mL fludarabine (Schering SpA). After overnight incubation, campath-1H was added at a final concentration of 10 µg/mL with 15% human serum. Incubation was continued for 3 hours at 37°C. The total volume was then brought to 300 µL in medium containing 1/10 volume of alamar blue solution (Biosource International), and incubated overnight at 37°C. The plates were then read in a fluorimeter as in the standard CDC assays.
Statistical analysis

Statistical significance was determined using the Student's T test. Possible correlations between CD52 expression levels and complement-mediated lysis were evaluated by Pearson's coefficient.

Results

CD52 expression in ALL cell lines

In order to generate models in which to study the effect of campath-1H in ALL, a series of eight ALL cell lines were collected and analyzed for CD52 expression by standard immunophenotyping. In addition a previously studied AIDS-derived B lymphoma cell line was used in parallel as a control. The classification and chromosomal rearrangements of the lines are shown in Table 1. The cell lines represent B and T lineage ALL of different subtypes, and carry t(12;21), t(1;19), t(9;22), t(4;11) or c-myc translocations. As shown in Table 1, four out of the eight ALL cell lines expressed CD52 on 67-100% of the cells, two of these weakly with (mean fluorescent intensity [MFI] of 29 for KCL, and of 33 for the RS4;11 cell line) and the other two at higher levels (MFI of 278 and 274 for the REH and SILTI lines, respectively). Expression of CD52 in the latter two lines was equivalent to that observed in the Burkitt’s lymphoma (BL) cell line ESIII (MFI of 313). The other four ALL cell lines expressed CD52 only on a small percentage of cells (TOM-1 and CEM) or were negative (697 and RCH-ACV) (Table 1).

Table 1. Cytogenetic characteristics and immunophenotype of the leukemia cell lines.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Chromosomal Rearrangement</th>
<th>Classification</th>
<th>%CD52 (MFI)</th>
<th>%CD55 (MFI)</th>
<th>%CD59 (MFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REH</td>
<td>t(12;21) TEL/AML1</td>
<td>preB-ALL</td>
<td>99 (278)</td>
<td>44 (23)</td>
<td>100 (303)</td>
</tr>
<tr>
<td>697</td>
<td>t(1;19)(q23;p13) E2A/PBX1</td>
<td>preB-ALL</td>
<td>6 (0)</td>
<td>0 (9)</td>
<td>99 (1617)</td>
</tr>
<tr>
<td>RCH-ACV</td>
<td>t(1;19)(q23;p13) E2A/PBX1</td>
<td>cALL</td>
<td>2 (ND)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SILTI</td>
<td>t(2;8) IgL/C-MYC</td>
<td>B-ALL</td>
<td>100 (274)</td>
<td>63 (45)</td>
<td>62 (197)</td>
</tr>
<tr>
<td>CEM</td>
<td>SIL/TAL deletion</td>
<td>T-ALL</td>
<td>21 (44)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TOM-1</td>
<td>t(9;22) p190</td>
<td>BCR/ABL ALL</td>
<td>24 (200)</td>
<td>86 (180)</td>
<td>85 (161)</td>
</tr>
<tr>
<td>KCL</td>
<td>t(9;22)</td>
<td>BCR/ABL ALL</td>
<td>74 (29)</td>
<td>99 (305)</td>
<td>100 (1200)</td>
</tr>
<tr>
<td>RS4;11</td>
<td>t(4;11) MLL/AF4</td>
<td>ALL-relapse</td>
<td>67 (35)</td>
<td>56 (62)</td>
<td>90 (118)</td>
</tr>
<tr>
<td>ESIII</td>
<td>t(8;14) C-MYC/IgH</td>
<td>BL</td>
<td>100 (313)</td>
<td>51 (29)</td>
<td>100 (556)</td>
</tr>
</tbody>
</table>

ND: not done.

The REH cell line is highly sensitive to campath-1H and complement-mediated lysis

Since CDC is thought to be an important mechanism of action of campath-1H,23 we next investigated whether CD52-positive ALL cell lines could be lysed by campath-1H in the presence or absence of human serum as a source of complement. For this purpose we used the two cell lines showing high CD52 expression, REH and SILTI, in parallel with the BL line ESIII. One of the lines expressing CD52 more weakly, KCL, was also tested. For CDC assays 13% or 20% human serum was used with campath-1H at both the therapeutic concentration of 10 µg/mL and at the high dose of 100 µg/mL. The results show that campath-1H and complement lysed the REH cell line much more efficiently than SILTI or ESIII (Figure 1A) even though all three cell lines express equivalent levels of CD52 (Table 1). Indeed lysis of REH with 13% serum was 47% and 91% at 10 and 100 µg/mL campath-1H, respectively, compared to 0-6% and 0-27% for the other three cell lines. As expected from its lower expression levels of CD52, the KCL cell line was not lysed with either 10 or 100 µg/mL campath-1H. Increasing the serum concentration to 20% did not significantly alter the cytotoxicity against REH and only marginally increased lysis of the other cell lines at 100 µg/mL (Figure 1A). The standard concentration of 13% was therefore chosen in most subsequent experiments.

In order to verify that lysis was specific, the REH cell line, which does not express the CD20 molecule, was
The pattern of expression of these cells.

MFI (range)  
Average +a +

Figure 2. Campath-1H-mediated ADCC of REH and ESIII cell lines. ADCC was analyzed using freshly isolated peripheral blood mononuclear cells from normal volunteers and calcein-AM labeled REH or ESIII cells at 1:1 to 60:1 effector:target ratios and in the presence (closed symbols) or absence (open symbols) of 10 µg/mL campath-1H (C). The results are the means and standard deviations of triplicate wells and are representative of two independent experiments.

The particularly high sensitivity of the REH cell line compared to the others to complement lysis could have been due to different levels of expression of the complement inhibitors CD55 and CD59, which are known to inhibit monoclonal antibody-mediated CDC on tumor cells. The pattern of expression of these antigens in shown in Table 1. Although the levels of CD55 and CD59 varied significantly between cell lines, expression of the complement inhibitors did not appear to be responsible for the different susceptibility of the cells to lysis. Indeed the REH and ESIII cell lines express CD55, CD56 and CD59 at approximately the same levels yet showed widely different sensitivity to campath-1H-mediated CDC (Table 1, Figure 1A). Furthermore, we investigated the effect of CDC of functionally blocking the CD55 and CD59 molecules with specific antibodies. As shown in Figure 1A, blocking either CD55 or CD59 singly had little effect on CDC in most cell lines examined. Blocking both antigens simultaneously increased lysis from 5 to 40% in the SILTI line and from 0 to 20% in the KCL cell line, but had no significant effect in ESIII. CDC of REH cells in the presence of anti-CD55 and CD59 was 70% compared to 47% in their absence. Adding CD55 and CD59 blocking antibodies without campath-1H had no effect on lysis, as expected since these antibodies do not activate complement by themselves (data not shown). Thus blocking both CD55 and CD59 increased campath-1H-mediated lysis to some extent in most cell lines, but did not reduce the difference observed between REH and other lines. We conclude that REH shows a particularly high susceptibility to campath-1H-mediated CDC which does not appear to be due to different levels of expression of CD52, or to increased activity of CD55 and CD59, but rather to some intrinsic property of the cells.

Another mechanism of action of Campath-1H is likely to be ADCC mediated by NK cells. We therefore investigated whether REH cells also show a particularly high sensitivity to ADCC, using peripheral blood mononuclear cells as the source of NK effector cells. As shown in Figure 2, ADCC of REH cells was equivalent to that observed with ESIII cells with about 50% lysis at a 60:1 effector to target ratio. The KCL cell line, which expresses lower levels of CD52, was also tested but ADCC could not be evaluated in this case because of the high background lysis (90%) observed in the absence of campath-1H. We conclude that the extent of ADCC-mediated lysis of the REH cell line is similar to that of other CD52-positive cell lines.

CD52 expression in freshly isolated ALL cells

In order to investigate the possible therapeutic activity of campath-1H in ALL, we first determined whether freshly isolated ALL cells of different subtypes express the CD52 antigen. The results obtained on 61 samples obtained from ALL patients at diagnosis are summarized in Table 2. Twenty-six of the cases analyzed were pediatric ALL whereas the other 35 were adult cases. All cases of ALL were randomly chosen for the analysis, except for the 11 cases bearing the t(12;21) translocation which were specifically selected for the study, in view of the results obtained with the t(12;21) REH cell line. These were all pediatric samples, given the higher frequency of this translocation in chil-

Table 2. CD52 expression according to ALL subtype.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Age (range)</th>
<th>Cases</th>
<th>% CD52+</th>
<th>MFI (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGIL (B lineage)</td>
<td>Pro B ALL</td>
<td>10-61</td>
<td>5/9</td>
<td>56%</td>
</tr>
<tr>
<td></td>
<td>cALL</td>
<td>2-67</td>
<td>22/23</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td>Pre B ALL</td>
<td>2-84</td>
<td>16/17</td>
<td>94%</td>
</tr>
<tr>
<td></td>
<td>B ALL</td>
<td>43-47</td>
<td>2/2</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>2-84</td>
<td>45/51</td>
<td>88%</td>
</tr>
<tr>
<td>Chromosome rearrangement (B lineage)</td>
<td>t(4;11)</td>
<td>22-61</td>
<td>0/4</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>t(12;21)</td>
<td>2-8</td>
<td>12/12</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>t(9;22)</td>
<td>8-51</td>
<td>8/8</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>t(1;19)</td>
<td>2-31</td>
<td>3/3</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>None detected</td>
<td>2-84</td>
<td>22/24</td>
<td>92%</td>
</tr>
<tr>
<td>T lineage</td>
<td>pro T ALL</td>
<td>18-64</td>
<td>3/5</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>Pre T ALL</td>
<td>25-71</td>
<td>4/4</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Cortical T</td>
<td>5</td>
<td>1/1</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>5-71</td>
<td>8/10</td>
<td>80%</td>
</tr>
</tbody>
</table>

*Positivity was defined as samples with at least 40% CD52+ cells. Only CD52+ positive samples (over 40% positivity) were considered for MFI values. NA: not applicable.
Of the ALL cases analyzed, 9 were pro-B, 23 common ALL, 17 pre-B and 2 mature B ALL, 5 pro T, 4 pre T and 1 cortical T ALL, according to the EGIL classification. Table 2 reports the percentage CD52 expression and average intensity of fluorescence in the different subgroups of B and T lineage ALL, divided according to immunophenotype (EGIL) or to chromosomal rearrangement. The results show that 88% of B lineage ALL and 80% of T lineage ALL express CD52. The percentage positivity varied from 41% to 99% (mean 90%), with lower percentage expression in a few cases reflecting a weak but homogeneous expression of CD52 on all leukemic cells. Patients scored as negative showed 2-4% positivity on the leukemic cells. When analyzing the different subgroups, CD52 expression appeared to correlate with differentiation stage since 56% of pro B ALL were CD52+, compared to 94-100% of the more mature subgroups. Similarly, in T lineage ALL, 60% of pro T ALL were CD52+ compared to 100% of the more mature T leukemias. Although numbers are too small to draw firm conclusions, there also appeared to be a relationship between CD52 expression and chromosomal rearrangements, since all four cases of t(4;11) pro B ALL were CD52 negative whereas the five non t(4;11) pro-B ALL were positive. Mean fluorescence intensities were variable between and within subgroups, with t(12;21) cases showing the highest average intensity of fluorescence (809 compared to 183-490 for other subtypes; Table 2). Examples of high (panel A), medium (panel B) and low CD52 expression (panel C) on B lineage ALL (one t(12;21) and two negative cases, respectively), are shown in Figure 3. In conclusion, CD52 is expressed on the vast majority of freshly isolated ALL samples at diagnosis, although at variable intensities.

Complement-mediated lysis of ALL cells

Given the expression of CD52 on freshly isolated ALL cells, we next investigated whether campath-1H was effective at inducing complement-mediated lysis in these cells. CDC experiments were performed on samples from 33 patients, from whom enough cells were available, using 10 µg/mL campath-1H and 13% human serum, i.e. the conditions previously shown to be effective on the cell lines. The results are shown in Figure 4. Each bar represents lysis of a single sample and patients are grouped according to translocations. Thirty patients had B lineage ALL and three had T ALL. Amongst B lineage ALL, nine carried a t(12;21), seven a t(9;22), three a t(1;19) and eleven none of these translocations (neg). It is evident from Figure 4 that all t(12;21) samples were lysed very effectively by campath-1H, with lysis ranging from 85 to 100% (mean 96.6%). In contrast lysis of other ALL samples was low to intermediate, ranging from 0 to 80% (mean 24% for all non-t(12;21) samples). The mean lysis for each subgroup is indicated above the bars in Figure 4 and ranges from 3% for the T-ALL to 44.6% for the t(9;22) sam-

Figure 3. CD52 expression of representative patients. Patients’ samples were stained with FITC-labeled anti-CD52 or control antibody and analyzed on the FACS. The histograms of CD52 (black profiles) and controls (grey profiles) are shown for three representative patients with high (A), intermediate (B) and low CD52 expression (C). The mean fluorescence intensities (MFI) for CD52 are indicated in each panel.

Figure 4. Complement-mediated lysis of ALL patients’ samples. CDC was evaluated using 10 µg/mL campath-1H and 13% human serum as a source of complement in 33 ALL samples. The results are the means and standard deviations of triplicate wells for each patient. The patients are grouped according to their chromosomal translocations with codes indicating the EGIL classification. Mean lysis for each subgroup of patients’ is indicated above the bars. Neg indicates that no translocation was detected by polymerase chain reaction.
The difference in lysis between t(12;21) cases and all other cases was statistically highly significant \((p<0.0001)\). Eleven cases for which enough cells were available were also analyzed in the absence of serum or in presence of heat-inactivated serum. The results demonstrated that lysis is complement dependent (data not shown).

In order to further analyze the sensitivity of ALL cells to CDC, we constructed dose-response curves on selected cases, using campath-1H concentrations ranging from 0.1 to 100 µg/mL. Analysis of three t(12;21) cases showed that near maximal or maximal lysis was observed already at 1 µg/mL but lysis was low or undetectable at 0.1 µg/mL (Figure 5A). In contrast the three non-t(12;21) ALL cases tested at 1-100 µg/mL campath-1H showed little lysis at the low dose of antibody (1 µg/mL), but lysis increased at the highest concentration, with one case (patient n. 27) showing 85% lysis at an antibody dose of 100 µg/mL (Figure 5B). All other cases were tested with only 10 and 100 µg/mL campath-1H and the results of the mean lysis for each subgroup of patients are shown in Figure 5C. Lysis increased on average about 2-fold with 100 µg/mL compared to 10 µg/mL campath-1H in the three groups of B lineage ALL cells analyzed, with the three (9;22) cases showing the highest mean lysis (reaching 72% at 100 µg/mL campath-1H). In contrast the two T ALL cases tested were not lysed significantly at either concentration of monoclonal antibody (Figure 5C).

The experiments performed on cell lines had shown that the t(12;21) cell line REH was highly sensitive to campath-1H, independently of its expression of the target antigen. In contrast the MFI of t(12;21) ALL cases was on average higher than that of other ALL (Table 2)\((p=0.0001)\). We therefore analyzed the possible correlation between CD52 expression levels (MFI) and lysis in freshly isolated ALL cases. As shown in Figure 6, there was some correlation, albeit not very strong, between lysis and CD52 intensity, with a Pearson’s correlation coefficient of 0.62. Although the numbers are relatively small (33 cases analyzed), these data suggest that campath-1H-mediated CDC in ALL is at least partially dependent upon C52 expression levels.

Deposition of C3 and C9 fragments on ALL cells

The complement cascade can be divided in two major phases, the first, initiating with C1q binding and
activation and leading to deposition of C3 fragments on the cell surface and the second, subject to the activity of several cell surface inhibitors, leading to insertion of polymerized C9 in the cell membrane. In order to determine whether t(12;21) and non-t(12;21) cells differ mostly in the activation or downstream phase of the complement cascade, deposition of C3 and C9 was measured on a t(12;21) showing 100% lysis (patient n. 38) versus 28% positive for C3 and 81% versus 45% of cells positive for C9, respectively (Table 3). Similar results were obtained with the REH and ESIII cell lines. These results confirm that in sensitive ALL cells, a higher level complement activation takes place resulting in greater deposition of both C3 and C9 and, consequently, more efficient lysis.

**Overcoming resistance to Campath-1H-mediated CDC in ALL**

We have shown above that increasing campath-1H concentration could augment lysis about 2-fold in B lineage ALL cases. We wanted to find other means to enhance cytotoxicity in the more resistant ALL cases. One strategy could be to block the CD55 and CD59 complement inhibitors. CD59 was found to be expressed on all ALL samples analyzed whereas CD55 expression was more variable, ranging from 13 to 96% (data not shown). Lysis of 12 ALL samples resistant to CDC was therefore performed with 10 µg/mL campath-1H in the presence or absence of antibodies that block the CD55 and CD59 molecules, either singly or together. The patients' samples used showed 65-99% positivity for CD52, and MFI ranging from 96 to 929. As shown in Figure 7A, blocking CD55 and/or CD59 increased lysis 1.7 to 2.5 fold compared to control lysis, reaching a mean of 27% lysis with both blocking antibodies (p=0.01 for the presence versus absence of CD55 and CD59). These data show that resistance of non-t(12;21) ALL is unlikely to be due to the activity of the CD55 and CD59 molecules, since still relatively limited lysis was obtained after blocking both molecules, even though some increased cytotoxicity was observed (Figure 7A). Given the different mechanism of action of campath-1H compared to standard chemotherapeutic drugs, we also analyzed the effect of combining campath-1H with fludarabine, using four ALL cases showing suboptimal lysis (14-69%) with campath-1H alone. These were one t(1;19) and three t(9;22) ALL. The data in Figure 7B show that addition of fludarabine to campath-1H and complement leads to a significant increase in cytotoxicity (p=0.004 with 1 µg/mL fludarabine compared to campath-1H alone), the effects of campath-1H and fludarabine being approximately additive. Thus a mean of about 80% lysis could be obtained by combining campath-1H and 1 µg/mL fludarabine (Figure 7B). We conclude that a significant response can be obtained also in the less sensitive ALL cases expressing CD52 by combining campath-1H with standard cytotoxic drugs.

**Discussion**

In this study of 61 ALL samples, including 26 pediatric and 35 adult cases, we show that CD52 is expressed on 88% of freshly isolated B lineage ALL and 80% of T ALL. Levels of CD52 expression varied in different samples, and correlated in part with disease subtype. Thus all t(4;11) pro-B ALL were CD52 negative whereas common and pre B ALL cells bearing the t(12;21) translocation (TEL/AML1) showed, on average, higher mean expression levels of CD52 than did other subtypes. In order to determine whether campath-1H has cytotoxic activity against acute leukemia cells, we measured the capacity of the antibody to lyse CD52+ ALL cells through complement activation. We found that the antibody did indeed mediate CDC, with variable efficacy in different leukemia subgroups. ALL cells bearing the t(12;21) translocation were found to be particularly sensitive to campath-1H and complement-mediated lysis with a mean 97% lysis at 10 µg/mL antibody, a concentration reached in vivo and near maximal lysis observed already with 1 µg/mL antibody. Other ALL subtypes were clearly more resistant than the t(12;21), with lysis varying between samples from 0 to 80%. It is worth...
Complement-mediated lysis is likely to be an important mechanism of action of campath-1H. CDC is known to depend upon target antigen levels. The correlation between campath-1H-mediated lysis and CD52 expression levels in freshly isolated ALL cells did indeed suggest that more intense expression of CD52 on t(12;21) cells was at least in part responsible for their high complement response. In confirmation of this, measurement of C3 and C9 deposition suggested that the t(12;21) cells activate the first steps of the complement cascade more efficiently than do other ALL subtypes. However, the fact that several non-t(12;21) ALL cells as well as two cell lines that expressed high levels of CD52 were nonetheless resistant to CDC indicates that other yet undetermined factors contribute to the resistance or susceptibility of t(12;21) cells to CDC. These factors do not appear to be related to differential expression of the complement inhibitors CD55 and CD59, since blocking these molecules increased CDC only to a limited extent, and because their expression was not different between ALL subgroups. The t(12;21) translocation involves the TEL-AML1 genes encoding transcription regulators.

\( t(12;21) \) ALL are also usually more sensitive to chemotherapeutic drugs perhaps due to down-regulation of the multidrug resistance protein 1.\(^{26} \) The mechanism by which this translocation may influence the sensitivity to campath-1H-mediated CDC remains to be determined. Although \( t(12;21) \) ALL (25-30% of all pediatric cases) are associated with a favorable outcome following conventional chemotherapy programs, still 25% of them will eventually relapse. Since only about half are eligible for allogeneic stem cell transplantation, campath-1H may represent a useful additional therapeutic strategy for these relapsed patients.

Even though other subgroups of ALL showed less effective complement susceptibility compared to \( t(12;21) \) cells, lysis of these cells was measurable (20-80%) in more than half of cases and could be increased with higher antibody concentrations. Perhaps more clinically relevant is our observation that efficient cytotoxicity could be obtained by combining campath-1H with complement with standard chemotherapeutic drugs such as fludarabine. The additive effect observed is presumably due to the different mechanisms of action of these drugs, as previously demonstrated using other monoclonal antibodies.\(^{26} \) Indeed fludarabine is a nucleoside analog inducing apoptosis whereas complement activation leads to the formation of membrane pores. Thus, in combination with fludarabine, campath-1H could have significant therapeutic activity in CD52+ ALL. Interestingly, preliminary data suggest that campath-1H may indeed have activity as an anti-leukemic agent in ALL.\(^{18-20} \)

Campath-1H and fludarabine are included in different conditioning regimens before allogeneic stem cell transplantation. Clinical studies have shown that this combination leads to efficient engraftment of stem cells, prolonged immune suppression, reduced graft-versus-host disease and good control of lymphoid malignancies.\(^{18-20} \) This last point is remarkable taking into account the detrimental effect of the antibody on donor T lymphocytes and dendritic cells, resulting in a likely reduced graft-versus-leukemia effect, which should have increased the likelihood of disease relapse.\(^{18} \) Thus, our finding that campath-1H has cytotoxic activity in ALL alone or combined with cytotoxic drugs is of particular interest in this context. It is important to note, however, that the level of CD52 expression on normal T cells is higher than that observed in most ALL studied here.\(^{28} \) Thus, in the standard schedule of campath-1H used in allogeneic stem cell transplantation, the amounts of antibody available for the anti-leukemia effect may not always be optimal due to absorption by normal T cells. In contrast, the dose-response curve for lysis of \( t(12;21) \) cells was similar to that observed using normal peripheral blood T cells as targets (MM, unpublished observations), suggesting that campath-1H may indeed have favorable therapeutic activity, at least in this subtype of ALL. In addition to CDC, we showed that campath-1H can also mediate ADCC against ALL cells to an extent similar to that observed against more mature B cell leukemias and lymphomas,\(^{18} \) suggesting that this additional mechanism may also contribute to the overall anti-leukemic activity of this antibody.

To conclude, we propose that expression of CD52 on most ALL cases as well as the cytotoxic activity of campath1H in this disease warrant further clinical studies, both in the context of conventional chemotherapy and within conditioning regimens prior to hematopoietic stem cell transplantation.
References


