**FCGR3A and FCGR2A Polymorphisms May Not Correlate with Response to Alemtuzumab (Campath-1H) in Chronic Lymphocytic Leukemia (CLL)**

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Abstract word count: 155

Article word count: 1392

This work is in part supported by the National Cancer Institute (P01 CA95426-01A, CLL Research Consortium P01 CA81534-02), The Sidney Kimmel Cancer Research Foundation, The Leukemia and Lymphoma Society of America, and The D. Warren Brown Foundation. JCB is a Clinical Scholar of the Leukemia and Lymphoma Society of America.
Abstract

The *in vivo* mechanism of action of alemtuzumab (anti-CD52, Campath-1H) remains unclear. With rituximab, *FCGR3A* and *FCGR2A* high affinity polymorphisms have been associated with clinical response in lymphoma but not CLL, suggesting potential divergent mechanisms of action between these two diseases. Herein, we examined *FCGR3A* (V/V, n=4; V/F, n=10; F/F, n=19) and *FCGR2A* polymorphisms (A/A, n=5; H/A, n=22; H/H, n=6) in 36 relapsed CLL patients treated with thrice weekly alemtuzumab for 12 weeks, to assess the potential influence these high affinity FcRγ receptor polymorphisms had on response to alemtuzumab. Response to alemtuzumab was similar irrespective of *FCGR3A* polymorphism (V/V, 25%; V/F, 40%; F/F, 32%) or *FCGR2A* polymorphism (A/A, 40%; H/A, 32%; H/H, 33%). These findings indicate that *FCGR3A* and *FCGR2A* polymorphisms may not predict response to alemtuzumab in CLL. Future studies examining larger cohorts of alemtuzumab treated CLL patients will be required to definitively determine the predictive value of specific *FCGR* polymorphisms to treatment response.
Introduction

The CD52 antigen is a 21-28 kD glycopeptide expressed on the surface of more than 95% of human lymphocytes, monocytes and macrophages. CD52 is also expressed on all chronic lymphocytic leukemia (CLL) cells and indolent B-cell non-Hodgkin’s lymphoma (NHL) cells. Alemtuzumab (Campath-1H) is a humanized anti-CD52 monoclonal antibody that effectively fixes complement and depletes normal lymphocytes, lymphoma cells and CLL cells. Alemtuzumab exhibits clinical activity in previously untreated and fludarabine-refractory CLL, with a 33% response rate in the pivotal phase II study. Antibody binding of CD52 in vitro elicits profound complement activation, antibody-dependent cellular cytotoxicity (ADCC), and apoptosis. To date, detailed studies examining the mechanism of alemtuzumab mediated tumor clearance have not been examined in CLL.

Studies with the anti-CD20 antibody rituximab in NHL suggest that ADCC, complement-dependent cytotoxicity (CDC) and a direct pro-apoptotic effect may contribute to cell death observed with this therapy. Recent studies in NHL have provided strong implication for the role of ADCC in lymphoma tumor clearance. Specifically, in a xenograft model of human lymphoma, knocking out the FcRγ loci in mice completely abrogated the response to rituximab, while knocking out the inhibitory FcRγIlb enhanced the response to rituximab in the same xenograft model. Similar studies with alemtuzumab have been reported with adult T-cell leukemia (ATL) cells in an in vivo murine model demonstrating the importance of ADCC for this tumor type. However, neither this nor any other xenograft model is representative of CLL.
Additional supporting data for the importance of ADCC in the clearance of NHL cells has come from correlating high affinity FCGR polymorphisms with clinical response to rituximab. Indeed, the presence of genomic polymorphisms corresponding to phenotypic expression of valine (V) or phenylalanine (F) at amino acid 158 of FcγIIIa and of histidine (H) or arginine (A) at amino acid 131 of FcγIIa, greatly influences the affinity of IgG for the Fcγ receptor. Expression of the high-affinity V allele at 158 results in tighter binding of FcγIIIa to IgG1 and IgG3, whereas the low-affinity F allele is associated with decreased binding of FcγRIIIa to IgG. Similarly, the high-affinity H allele at 131 results in greater affinity of FcγRIIa for IgG2, while the low-affinity A allele correlates with decreased binding. Correlation of these high affinity polymorphisms has been associated with clinical response in two studies of NHL. In contrast to NHL, we recently demonstrated that these high affinity polymorphisms do not appear to influence response to single agent rituximab in CLL. These findings, along with other studies done by our group and others, suggest that apoptosis and CDC may contribute greater to rituximab-induced tumor clearance in CLL.

To our knowledge, no studies have examined the correlation of high affinity polymorphisms with response to alemtuzumab. Herein, we describe a series of CLL patients treated with alemtuzumab; as in our previous study with rituximab, preliminary examination of these polymorphisms suggested little influence upon clinical outcome to this antibody therapy.

**Patients, materials and methods**

**Patient samples and cell processing.** Patients with relapsed CLL, as defined by NCI 96 criteria, were enrolled and provided written consent to participate in this
previously reported institutional review board (Johns Hopkins University and The Ohio State University)-approved protocol. Alemtuzumab was administered as previously reported for the CAM211 study. The alemtuzumab dose was stepped up from 3 mg to 30 mg during the first week, and then given 30 mg thrice weekly for 12 weeks. Blood counts were monitored weekly. CLL response was assessed by NCI 96 criteria.

Analysis of FCGR3A and FCGR2A polymorphisms. Cells were obtained prior to alemtuzumab treatment, and mononuclear cells were isolated from blood using density-gradient centrifugation (Ficoll-Paque Plus, Pharmacia Biotech, Piscataway, NJ). Cells were then viably cryopreserved in 10% DMSO, 40% fetal calf serum and 50% RPMI media. DNA was extracted using the QIAamp kit, according to the manufacturer’s instructions (Qiagen Inc., Valencia CA). Assessment of FCGR3A and FCGR2A polymorphisms was performed as previously described. All samples were analyzed in duplicate with identical results.

Results

Patient population. Thirty-six patients with relapsed CLL who received alemtuzumab were examined (Table 1). Median age was 61 years (range 42-74), and 29 patients (81%) were male. Patients had received a median of 3 prior therapies (range 1-12), and 29 patients (81%) were fludarabine-refractory. Seventy-five percent of patients were Rai stage IV (n=24) or III (n=3). Twelve patients (33%) had deletion of 17p13.1 detected by interphase cytogenetic analysis.

Response. Eleven responses (31%) were observed, including 2 complete (CR) and 9 partial responses (PR). One patient who achieved CR was taken to autologous
stem cell transplant; median duration of response in the other 10 patients was 9.5 months (range 3-36). Results are summarized in Table 1.

**FCGR3A and FCGR2A polymorphisms.** FCGR3A and FCGR2A polymorphism data were both available on 32 patients (Table 2). FCGR3A polymorphism information alone was available on 1 patient, and FCGR2A information alone on 1 patient. Two patients had no polymorphism data. Analysis of V/F 158 FCGR3A showed V/V (n=4), V/F (n=10) and F/F (n=19), and analysis of H/A 131 FCGR2A showed A/A (n=5), H/A (n=22) and H/H (n=6). There was no concordance between FCGR3A and FCGR2A polymorphisms. No significant difference in response to alemtuzumab based upon V/F 158 FCGR3A polymorphism was observed; response rates were 25% (V/V), 40% (V/F) and 32% (F/F). Similarly, H/A 131 FCGR2A polymorphism did not predict response to alemtuzumab, with response rates of 40% (A/A), 32% (H/A) and 33% (H/H).

**Discussion**

Our report is the first preliminary investigation of the impact of FCGR polymorphisms on clinical response to alemtuzumab. No difference in response to alemtuzumab was observed in our 36 CLL patients based on FCGR3A or FCGR2A polymorphisms (Table 2). Thus, similar to our previously published study of rituximab in CLL, our preliminary results suggest that these polymorphisms may not be predictive for improved response to alemtuzumab in CLL.

The findings of our study should not be interpreted to minimize the importance of ADCC in mediating alemtuzumab tumor clearance, but rather the FCGR polymorphisms may be of less importance provided our data are confirmed by larger, more definitive
studies. Indeed, an HTLV leukemia in vivo murine model suggests that ADCC is important.\textsuperscript{17} In this study ATL-bearing FcRγ/- mice failed to respond to a 4-week course of alemtuzumab, with all FcRγ knockout mice dying by 22 days irrespective of alemtuzumab therapy. In contrast, alemtuzumab significantly prolonged survival in ATL-bearing wildtype FcRγ mice. While all untreated ATL-bearing wildtype FcRγ mice died by 30 days, 8 of 10 wildtype FcRγ mice treated with alemtuzumab were alive at 40 days. Other clinical studies performed previously with anti-CD52 antibodies with different IgG and IgM isoforms also support the contribution of ADCC to the mechanism of action of alemtuzumab.\textsuperscript{26,27} The first by Dyer and colleagues demonstrated little activity with an IgM anti-CD52 antibody with potent complement dependent cytotoxicity, but absent ADCC mediating ability.\textsuperscript{27} The second study by Isaacs and colleagues \textsuperscript{26} administered an IgG4 anti-CD52 antibody followed 8 days later by an IgG1 anti-CD52 antibody in patients with refractory rheumatoid arthritis. This study demonstrated modest CD4 cell depletion with the IgG4 antibody that should not mediate complement or ADCC but marked depletion with later treatment using the IgG1 antibody.

Thus, the data presented herein and previously reported by others\textsuperscript{26,27,17} suggest that alemtuzumab may exert its effects through several pathways not inclusive or exclusive of ADCC. With respect to the importance of FCGR polymorphisms, this study represents an initial assessment of these that now require larger studies to definitively determine their importance in predicting response to alemtuzumab. Interestingly, however, the FCGR3A polymorphism (V/V 158) associated with high-affinity binding to IgG correlated with the lowest response rate to both rituximab and alemtuzumab in our
two series (herein and in\textsuperscript{21}), in contrast to previously reported findings in NHL.\textsuperscript{20} Similarly, we did not observe an improved response rate to rituximab or alemtuzumab in CLL patients with the H/H 131 \textit{FCGR2A} polymorphism (herein and in\textsuperscript{21}), contrary to the findings of a recent report in NHL patients treated with rituximab.\textsuperscript{28} Preliminary data from our laboratory suggest that alemtuzumab effectively induces apoptosis in CLL through a caspase-dependent mechanism.\textsuperscript{29} The CD52 antigen is also expressed at high density on CLL cells, and it is feasible that CDC may also partially contribute to alemtuzumab-induced tumor clearance \textit{in vivo}. Given the promising results of alemtuzumab in refractory CLL and its ability to eliminate highly resistant p53 mutant CLL cells,\textsuperscript{30,31} further investigations of the \textit{in vivo} mechanism of action of alemtuzumab are warranted.
Table 1. Patient Demographics and Response to Alemtuzumab Therapy

**Demographics (n=36)**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Count</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
<td>61</td>
<td>(42-74)</td>
</tr>
<tr>
<td>Male, number (%)</td>
<td>29</td>
<td>(81%)</td>
</tr>
<tr>
<td>Prior therapies, number (range)</td>
<td>3</td>
<td>(1-12)</td>
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<tr>
<td>Fludarabine-refractory, number (%)</td>
<td>29</td>
<td>(81%)</td>
</tr>
<tr>
<td>Rai stage III/IV, number (%)</td>
<td>27</td>
<td>(75%)</td>
</tr>
<tr>
<td>Del(17p13.1), number (%)</td>
<td>12</td>
<td>(33%)</td>
</tr>
</tbody>
</table>

**Response (n=36)**

<table>
<thead>
<tr>
<th>Response Type</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response (CR)</td>
<td>2</td>
<td>(6%)</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>9</td>
<td>(25%)</td>
</tr>
<tr>
<td>No response (NR)</td>
<td>27</td>
<td>(75%)</td>
</tr>
<tr>
<td>Median duration of response (n=10), Months (range)</td>
<td>9.5</td>
<td>(3-36)</td>
</tr>
</tbody>
</table>
Table 2. Response by FCGR3A and FCGR2A Polymorphism

### Response by V/F 158 FCGR3A polymorphism (n=33)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>ORR</th>
</tr>
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<tbody>
<tr>
<td>V/V</td>
<td>n=4</td>
<td>25%</td>
</tr>
<tr>
<td>V/F</td>
<td>n=10</td>
<td>40%</td>
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<tr>
<td>F/F</td>
<td>n=19</td>
<td>32%</td>
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</table>

### Response by H/A 131 FCGR2A polymorphism (n=33)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>ORR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>n=5</td>
<td>40%</td>
</tr>
<tr>
<td>H/A</td>
<td>n=22</td>
<td>32%</td>
</tr>
<tr>
<td>H/H</td>
<td>n=6</td>
<td>33%</td>
</tr>
</tbody>
</table>
References


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