

FCGR3A and *FCGR2A* polymorphisms may not correlate with response to alemtuzumab in chronic lymphocytic leukemia

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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 in CLL, suggesting potential divergent mechanisms of action between these 2 diseases. Herein, we examined *FCGR3A* (V/V, n = 4; V/F, n = 10; F/F, n = 19) and *FCGR2A* (A/A, n = 5; H/A, n = 22; H/H,

n = 6) polymorphisms in 36 patients with relapsed CLL who were treated with thrice-weekly alemtuzumab for 12 weeks to assess the potential influence these high-affinity Fc γ R receptor polymorphisms had on response to alemtuzumab. Response to alemtuzumab was similar regardless 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 patients with CLL will be required to definitively determine the predictive value of specific *FCGR* polymorphisms to treatment response. (Blood. 2005;105:289-291)

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Introduction

The CD52 antigen is a 21- to 28-kDa glycopeptide expressed on the surfaces of more than 95% of human lymphocytes, monocytes, and macrophages.¹⁻³ CD52 is also expressed on all chronic lymphocytic leukemia (CLL) cells and indolent B-cell non-Hodgkin lymphoma (NHL) cells.^{4,5} Alemtuzumab (Campath-1H) is a humanized anti-CD52 monoclonal antibody that effectively fixes complement and depletes normal lymphocytes, lymphoma cells, and CLL cells.⁶⁻⁸ Alemtuzumab exhibits clinical activity in previously untreated⁹ and fludarabine-refractory CLL,^{10,11} with a 33% response rate in the pivotal phase 2 study.¹² Antibody binding of CD52 *in vitro* elicits profound complement activation, antibody-dependent cellular cytotoxicity (ADCC), and apoptosis.¹³⁻¹⁵ 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 proapoptotic 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 Fc γ R loci in mice completely abrogated the response to rituximab, whereas knocking out the inhibitory Fc γ RIIb enhanced the response to rituximab in the same xenograft model.¹⁶ 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.¹⁷ 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 Fc γ IIIa and of histidine (H) or arginine (A) at amino acid 131 of Fc γ IIa greatly influences the affinity of IgG for the Fc γ receptor.^{18,19} Expression of the high-affinity V allele at 158 results in tighter binding of Fc γ IIIa to IgG1 and IgG3, whereas the low-affinity F allele is associated with decreased binding of Fc γ IIIa to IgG. Similarly, the high-affinity H allele at 131 results in greater affinity of Fc γ RIIa for IgG2, whereas the low-affinity A allele correlates with decreased binding. Correlation of these high-affinity polymorphisms has been associated with clinical response in 2 studies of NHL.²⁰ In contrast to NHL, we recently demonstrated that these high-affinity polymorphisms do not appear to influence response to single-agent rituximab in CLL.²¹ These findings, along with other studies by our group and others, suggest that apoptosis and CDC may contribute more to rituximab-induced tumor clearance in CLL.²²⁻²⁴

To our knowledge, no studies have examined the correlation of high-affinity polymorphisms with response to alemtuzumab. Herein, we describe a series of patients with CLL treated with alemtuzumab; as in our previous study with rituximab, preliminary

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examination of these polymorphisms suggested little influence on clinical outcome to this antibody therapy.

Patients, materials, and methods

Patient samples and cell processing

Patients with relapsed CLL, as defined by National Cancer Institute (NCI) 96 criteria,²⁵ 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.¹² The alemtuzumab dose was stepped up from 3 mg to 30 mg during the first week and then was given at 30 mg thrice weekly for 12 weeks. Blood counts were monitored weekly. CLL response was assessed by NCI 96 criteria.²⁵

Analysis of *FCGR3A* and *FCGR2A* polymorphisms

Cells were obtained before 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% dimethyl sulfoxide (DMSO), 40% fetal calf serum and 50% RPMI media. DNA was extracted using the QIAamp kit, according to the manufacturer's instructions (Qiagen, Valencia, CA). Assessment of *FCGR3A* and *FCGR2A* polymorphisms was performed as previously described.²⁰ 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 years), and 29 (81%) patients were male. Patients had received a median of 3 previous therapies (range, 1-12), and 29 (81%) patients had fludarabine-refractory disease. Seventy-five percent of the patients had Rai stage IV (n = 24) or III (n = 3) disease. Twelve (33%) patients had deletion of 17p13.1 detected by interphase cytogenetic analysis.

Response

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

Table 1. Patient demographics and response to alemtuzumab therapy

	No. patients (%)
Demographics	
Male sex	29 (81)
Fludarabine refractory	29 (81)
Rai stage III/IV	27 (75)
del(17p13.1)	12 (33)
Response	
Complete	2 (6)
Partial	9 (25)
None	27 (75)

Median age of patients was 61 years (range, 42-74 years). Median number of previous therapies was 3 (range, 1-12). Median duration of response was 9.5 months (range, 3-36 months).

Table 2. Response by *FCGR3A* and *FCGR2A* polymorphisms

Polymorphism	No. responses (ORR%)
V/F 158 <i>FCGR3A</i>	
V/V	4 (25)
V/F	10 (40)
F/F	19 (32)
H/A 131 <i>FCGR2A</i>	
A/A	5 (40)
H/A	22 (32)
H/H	6 (33)

n = 33 responses for each group.

ORR indicates overall response rate.

FCGR3A and *FCGR2A* polymorphisms

FCGR3A and *FCGR2A* polymorphism data were available on 32 patients (Table 2). *FCGR3A* polymorphism information alone was available on 1 patient, and *FCGR2A* information alone was available 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 on 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 patients with CLL based on *FCGR3A* or *FCGR2A* polymorphisms (Table 2). Thus, similar to our previously published study of rituximab in CLL,²¹ our preliminary results suggest that these polymorphisms may not be predictive of 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; rather, the *FCGR* polymorphisms may be of less importance if our data are confirmed by larger, more definitive studies. Indeed, an HTLV leukemia in vivo murine model suggests that ADCC is important.¹⁷ In this study, ATL-bearing FcγR^{-/-} mice failed to respond to a 4-week course of alemtuzumab, with all FcγR knockout mice dying by 22 days irrespective of alemtuzumab therapy. In contrast, alemtuzumab significantly prolonged survival in ATL-bearing wild-type FcγR mice. Although all untreated ATL-bearing wild-type FcγR mice died by 30 days, 8 of 10 wild-type FcγR mice treated with alemtuzumab were alive at 40 days. Other clinical studies performed previously with anti-CD52 antibodies with different immunoglobulin G (IgG) and IgM isoforms also support the contribution of ADCC to the mechanism of action of alemtuzumab.^{26,27} The first, by Dyer et al,²⁷ demonstrated little activity with an IgM anti-CD52 antibody with potent complement-dependent cytotoxicity but absent ADCC mediating ability. The second, by Isaacs et al,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^{17,26,27} 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, which now require larger studies for definitive determination of 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 rituximab and alemtuzumab in our 2 series (herein and in Farag et al²¹), in contrast to previously reported findings in NHL.²⁰ Similarly, we did not

observe an improved response rate to rituximab or alemtuzumab in patients with CLL with the H/H 131 *FCGR2A* polymorphism (herein and in Farag et al²¹), contrary to the findings of a recent report in NHL patients treated with rituximab.²⁸ Preliminary data from our laboratory suggest that alemtuzumab effectively induces apoptosis in CLL through a caspase-dependent mechanism.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 in vivo. Given the promising results of alemtuzumab in refractory CLL and its ability to eliminate highly resistant p53 mutant CLL cells,^{30,31} further investigations of the in vivo mechanism of action of alemtuzumab are warranted.

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