Brief report

Neutrophils express CD52 and exhibit complement-mediated lysis in the presence of alemtuzumab

Lyn R. Ambrose,1 Anne-Sophie Morel,1 and Anthony N. Warrens1,2

Departments of 1Immunology and 2Renal Medicine, Division of Medicine, Imperial College London, London, United Kingdom

Neutrophils is a recognized adverse event in patients treated with the humanized anti-CD52 monoclonal antibody alemtuzumab. However, as it is widely believed that neutrophils do not express CD52, the etiology of alemtuzumab-associated neutropenia is unclear. We have found that neutrophils express both mRNA coding for CD52 and the protein itself on the cell surface. We confirmed cell-surface expression using 3 different anti-CD52 antibodies, and note that neutrophils express lower levels of CD52 than lymphocytes and eosinophils. Further, incubation of alemtuzumab with neutrophils results in dose-dependent, complement-mediated lysis in the presence of both heterologous and autologous complement. These data offer an explanation for the etiology of alemtuzumab-associated neutropenia. In a climate of increased use of alemtuzumab in leukemia and other disease states, as well as in transplantation, these data highlight the need for increased vigilance of emerging neutropenia in patients treated with alemtuzumab. (Blood. 2009;114:3052-3055)

Introduction

Neutropenia is a recognized adverse event in patients treated with alemtuzumab, a humanized monoclonal CD52-specific antibody.1 A highly lytic antibody, alemtuzumab mediates cytotoxicity by antibody-dependent cell-mediated cytolysis and potent activation of human complement.2 It is approved for use in chronic lymphocytic leukemia (CLL)3 but is also used in non-Hodgkin lymphoma,4 T-cell malignancies,4 rheumatoid arthritis,5 vasculitis,6 scleroderma,7 eosinophilia,8 and prevention of graft-versus-host-disease and graft rejection in bone marrow,9 stem cell,10 and solid organ transplantation.11-13

Alemtuzumab administration is sometimes associated with a cytokine-release syndrome, which can include pyrexia, headaches, nausea, urticaria, and rashes.2 Myelotoxicity may result in anemia, thrombocytopenia, and neutropenia.14 In particular, postalemtuzumab neutropenia occurs in both fludarabine-refractory1 and treatment-naive14 CLL. The etiology of postalemtuzumab neutropenia, and its associated morbidity and mortality, is poorly understood.15

The obvious mechanism for postalemtuzumab neutropenia would be through neutrophil CD52 expression. However, it is widely believed that neutrophils do not express CD52,11 unlike T and B lymphocytes, natural killer (NK) cells, monocytes, dendritic cells, and male reproductive tract cells.2 Within the granulocyte population, it has been reported that eosinophils, but not neutrophils, express CD52.16 We revisited this question and have shown that neutrophils contain CD52 mRNA, express surface CD52, and are susceptible to complement-mediated lysis in the presence of alemtuzumab. The level of CD52 on neutrophils is lower than on eosinophils and T and B lymphocytes, which could be why it has been difficult to detect.

Methods

Cell isolation

Blood was separated into peripheral blood mononuclear cells (PBMCs) and granulocytes using density-gradient centrifugation over Polymorphprep (Axis-Shield). RPMI-1640 with 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine (Invitrogen), and either 2% or 10% human male AB serum (Biowest) was used for all washes and incubations. Eosinophils were negatively selected from the granulocytes using CD16-conjugated magnetic beads (Miltenyi Biotec). Permission to use human blood samples was granted by the institutional ethics review board of Imperial College London and donor informed consent was obtained in accordance with the Declaration of Helsinki.

Flow cytometry

Cells were stained for 30 minutes at 4°C with saturating concentrations of a specific monoclonal antibody or an isotype-matched control (supplemental Table 1, available on the Blood website; see the Supplemental Materials link at the top of the online article), acquired on a FACSCalibur flow cytometer, and analyzed using CellQuest software (BD Biosciences).

Reverse-transcription–polymerase chain reaction

Total RNA was isolated from cells using an RNeasy mini kit (QIAGEN). A total volume of 15 μL containing 5 μg RNA, 0.5 μg Oligo-dT primer (Invitrogen), and water was incubated at 65°C for 10 minutes and cooled. To this, the following was added: 1 μL dNTP mix (each 10 mM; Promega), 200 U Moloney murine leukemia virus reverse transcriptase, 7 μL 5× buffer, 5 μM DTT (Invitrogen), and 1 μL RNase ribonuclease inhibitor (Promega), and the volume made up to 35 μL with water. To perform first-strand cDNA synthesis, this 35-μL mixture was incubated at 37°C for 90 minutes. cDNA was amplified in a 25-μL reaction mixture containing 0.625 U Taq DNA polymerase, reaction buffer (Eppendorf), 0.2 mM each
dNTP, 0.2 μM each primer, and water. The polymerase chain reaction was incubated in a Primus 96 Plus thermocycler (MWG Biotech) with initial denaturation (95°C, 4 minutes), 35 cycles of denaturation, annealing and extension (95°C, 30 seconds; 55°C, 30 seconds; 68°C, 1 minute), and final extension (72°C, 10 minutes).

Complement-dependent cytotoxicity

Alemtuzumab-induced complement-dependent cytotoxicity was measured using a standard technique: 2 x 10⁶ cells were incubated in Terasaki trays with 1 to 300 μg/mL alemtuzumab for 30 minutes. Standard rabbit complement (5 μL; Cedarlane Laboratories) or autologous serum (5 μL) was added and incubated for 60 minutes. Cells were stained with 5 μL vital dye mix (propidium iodide, acridine orange, ink) and read under fluorescence microscopy. The anti–HLA class I mAb (W6/32; Sigma-Aldrich) was the positive control; the negative control was male AB serum (Biowest).

Results and discussion

In all 13 individuals studied, we have found that neutrophils contain CD52 mRNA (supplemental Figure 3) and express surface CD52 (Figure 1A), albeit at lower levels than lymphocytes (Figure 1B) or eosinophils (Figure 1C). On the basis of relative mean fluorescent intensities, we estimate that neutrophils have 22% the CD52 of lymphocytes. Anti-CD52 titration demonstrated that, at lower mAb concentrations, at which lymphocytes still appear positive (Figure 1Bii), neutrophils appear negative (Figure 1Biii). We speculate that neutrophil CD52 may have hitherto remained undetected due to the use of antibody concentrations that were nonsaturating at such low-level expression.

We confirmed specificity of the cell-surface protein using 3 different anti-CD52 antibodies, recognizing at least 2 different epitopes (supplemental Figure 2). Alemtuzumab binds neutrophil CD52 (Figure 2A-B) and induces dose-dependent complement-mediated lysis in the presence of either heterologous or autologous complement (Figure 2Ci-ii, respectively), and the concentration of alemtuzumab observed therapeutically certainly exceeds that needed to cause both complement-dependent and antibody-dependent cell-mediated cytotoxicity in vitro.

Alemtuzumab’s ability to activate autologous complement, despite the presence of cell-surface complement regulators, represents a potential mechanism by which alemtuzumab may be therapeutically effective. Incidentally, we did not observe the down-regulation of CD16 that is characteristic of neutrophil activation (data not shown), making it unlikely that the adverse effects of alemtuzumab could be attributed to it activating neutrophils. In a multicenter trial of alemtuzumab in CLL (n = 149),
77% of patients developed neutropenia and 9.5% required granulocyte colony-stimulating factor (G-CSF). Our data provide a potential mechanism for neutropenia in alemtuzumab-treated patients.

In the only trial to use alemtuzumab with concurrent, prophylactic G-CSF, 64% (9/14) of patients developed grade 3 to 4 neutropenia. Four developed late-onset neutropenia (week 10), which was unresponsive to increased doses of G-CSF but reversed within 3 to 5 weeks of withdrawing alemtuzumab. It is unclear whether the unresponsiveness to G-CSF was due to alemtuzumab-mediated consumption of mature neutrophils, or alemtuzumab-mediated interference with bone marrow neutrophil development and release. Gilleece and Dexter reported that alemtuzumab treatment does not affect myeloid progenitor cells, but others reported that Campath-1 (alemtuzumab's rat precursor; Genzyme) reduces granulocyte-macrophage colony-forming cells. In addition, it is possible that alemtuzumab-mediated depletion of CD52+ neutrophils favors selection of CD52+ neutrophil clones, a process known to occur in T cells, and for other neutrophil surface proteins.

Neutropenia after alemtuzumab therapy in solid organ transplant recipients is not widely reported, perhaps due to the frequent concomitant use of (neutrophilia-inducing) steroids. Where neutropenia is reported, use of other bone marrow–suppressing agents often renders etiologic conclusions impossible. Considering the current trend toward steroid-free immunosuppression in solid organ transplantation, it is possible that alemtuzumab-associated neutropenia may be unmasked. In the largest series of live donor renal transplant recipients undergoing alemtuzumab and steroid induction followed by tacrolimus monotherapy (n = 205), 15% experienced 40 episodes of neutropenia requiring G-CSF.

Of course, patients being treated with alemtuzumab are at risk of neutropenia from causes other than the alemtuzumab: the underlying bone marrow disease itself, as a complication of immunosuppression, such as Epstein-Barr virus infection, or as an adverse effect of other drugs used.

Our data are of relevance to eosinophil studies that have used CD52 as a marker to positively select eosinophils from a granulocyte population during purification. Failure to double-stain the
resulting population for CD16 may result in not detecting contaminating (CD52+ neutrophils. In addition, if activated during purification, these neutrophils may express only low levels of CD16, and be mistaken for (CD16- neutrophils. These data offer an explanation for the etiology of alemtuzumab-associated neutropenia. Our data do not suggest avoiding or discontinuing alemtuzumab, nor do published guidelines. Alemtuzumab is an important and effective treatment and its adverse events are generally predictable, transient, and manageable. These data simply highlight the need for vigilance for neutropenia after alemtuzumab, especially in patients undergoing medium- to long-term treatment and in solid organ transplantation regimens avoiding steroids.

Acknowledgments

We thank Dr Matt Butler for useful scientific and technical discussions and Drs Mark Little, Efrem Eren, and Paul Brookes for review of the paper.

References


We are grateful to the National Institutes for Health Research (NIHR) Biomedical Research Center funding scheme for supporting this work.

This work was funded by a British Transplantation Society PhD Research Fellowship and a grant from the Charitable Funds of Hammersmith and Queen Charlotte’s Hospital to L.R.A.

Authorship

Contribution: L.R.A. designed, performed, and analyzed the research and wrote the paper; A.-S.M. contributed to the design and analysis of the molecular work and donated reagents; and A.N.W. contributed to the design and analysis of the research to the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Lyn Ambrose, Immunology Unit, Rm 236, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT, United Kingdom; e-mail: lynambrose@googlemail.com.