CLINICAL RESEARCH

Humoral and Cellular Immunity to Primary H1N1 Infection in Patients with Hematologic Malignancies following Stem Cell Transplantation

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Limited data are available on immunologic responses to primary H1N1 infection in patients with hematologic malignancies. We present a prospective, case-surveillance study of such patients with real-time polymerase chain reaction (RT-PCR) confirmed H1N1-influenza who presented to our institution between September 2009 and January 2010. Ninety-two patients presented with influenza-like symptoms, and 13 had H1N1 infection confirmed by RT-PCR, including 4 allogeneic stem cell transplant recipients (1 with acute myelogenous leukemia, 1 with chronic lymphoblastic leukemia [CLL], 1 with non-Hodgkin lymphoma, and 1 with chronic myelogenous leukemia), 5 patients with multiple myeloma following autologous stem cell transplantation, 1 patient with multiple myeloma perimobilization, 2 patients with NHL post chemotherapy, and 1 patient with CLL. All 13 patients required hospitalization. Six (43%) were admitted to the intensive care unit (ICU), of whom 4 (67%) died. We evaluated B cell and T cell responses to H1N1 infection prospectively in these patients compared with those in 4 otherwise healthy controls. Within 12 weeks of diagnosis, only 6 of 11 patients developed seropositive antibody titers as measured by hemagglutination-inhibition or microneutralization assays, compared with 4 of 4 controls. H1N1-specific T cells were detected in only 2 of 8 evaluable patients compared with 4 of 4 controls. H1N1-specific T cells were functional, capable of producing interferon γ, tumor necrosis factor α, and CD107a mobilization. Furthermore, CD154 was up-regulated on $CD4^+$ T cells in 3 of 4 controls and 2 of 2 patients who had both B cell and T cell responses to H1N1. Post-H1N1 infection, 5 of 8 patients developed seasonal influenza-specific T cells, suggesting cross-reactivity induced by H1N1 infection. These data offer novel insights into humoral and cell-mediated immunologic responses to primary H1N1 infection.

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INTRODUCTION

The emergence in Mexico of swine-origin influenza A (H1N1) virus and its subsequent worldwide spread led to the World Health Organization's declaration of a global pandemic in June 2009. Patients with a hematologic malignancy are at risk from respiratory viral infection [1]. Data are limited on immunologic responses to primary H1N1 infection and its clinical impact in patients with hematologic malignancies [2-5]. We present the results of a prospective, case-surveillance study of hematology-oncology patients and otherwise healthy controls with real-time polymerase chain reaction (RT-PCR)-confirmed H1N1 infection, focusing on the humoral and T cell-mediated immunologic responses to the H1N1 virus. Here, we demonstrate impaired humoral and cellular immune responses to H1N1 infection in patients with a hematologic malignancy.

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METHODS

Case Identification and Clinical Data

Between September 1, 2009, and January 31, 2010, all patients with hematologic malignancies and staff members presenting with flu-like symptoms at our institution were investigated for H1N1 by RT-PCR [6]. Clinical, laboratory, and radiologic data were collected prospectively.

Immunologic Investigations

Serum and peripheral blood mononuclear cells (PBMCs) were collected preinfection and peri-infection and within 12 weeks of diagnosis and cryopreserved. Anti-H1N1 antibodies were measured by hemagglutination-inhibition and microneutralization assays as described previously [7,8]. A titer of >1:32 by hemagglutination-inhibition or >1:160 by microneutralization was defined as a protective antibody response [9,10].

Assessment of the functionality of antigen-specific $CD8^+$ and $CD4^+$ T cells was performed by intracellular cytokine staining for interferon (INF)-γ and tumor necrosis factor (TNF)-α and evaluation of CD154 expression and CD107a cytotoxicity as described previously [11,12]. In brief, PBMCs were thawed and stimulated for 24 hours with or without the H1N1 vaccine [A/California/07/2009(H1N1)v-like strain; Baxter, Berkshire, UK] and seasonal influenza vaccine [A/Brisbane/59/2007(H1N1)-, A/Brisbane/10/2007 (H3N2)- and B/Florida/30/2008-like strain; CSL Biotherapies, Hattersheim, Germany]. Brefeldin-A (10 μg/mL; Sigma-Aldrich, St Louis, MO) was added for another 5 hours. PBMCs were washed and stained with anti-CD3 and anti-CD8 antibodies, fixed/permeablized (BD Biosciences, Oxford, UK), and stained with anti–IFN-γ and anti–TNF-α antibodies (all from BD/Pharmingen, San Diego, CA). For CD154 and CD107a staining, PBMCs were stimulated for 18 hours with or without the H1N1 vaccine solution or seasonal influenza vaccine. Cells were incubated for 6 hours with 0.7 μL/mL of monensin, anti-CD154, and anti-CD107a antibodies (all BD/Pharmingen). PBMCs were washed and stained with anti-CD3 and anti-CD4 antibodies. A response was considered positive if the percentage of antigen-specific IFN-γ–, TNF-α–, or CD107a-expressing T cells was ≥2-fold higher than background (unstimulated PBMCs) and if there was a minimum of 0.05% antigen-specific T cells (after subtracting the background).

All work was carried out under approval from the local Ethics Committee, with informed consent obtained from all participants.

Statistical Analysis

This study was a prospective, observational study. One study endpoint was to prospectively analyze the associations and outcomes for patients who were not admitted to the intensive care unit (ICU) and survived with those for patients who either died or were admitted to the ICU. A second endpoint was to analyze and compare the antibody and T cell responses to H1N1 infection in patients and controls. Clinical and laboratory endpoint data were analyzed using the two-sided Fisher's exact test (for noncontinuous variables) or the Mann-Whitney *U* test (for continuous variables) to compare the characteristics of evaluable patients who required ICU admission versus those who did not. Antibody and T cell responses in patients and controls were compared using Fisher's exact test, with responses defined as positive or negative according to the cutoff values defined earlier. All reported *P* values are two-sided, with values ≤.05 considered to indicate statistical significance. All calculations were performed using SPSS version 17.0 (SPSS Inc, Chicago, IL).

RESULTS

H1N1 Clinical Data and Outcomes

Between September 1, 2009, and January 31, 2010, a total of 173 samples from 92 patients with a hematologic malignancy were evaluated by RT-PCR for clinically suspected H1N1 influenza. H1N1 infection was confirmed in 13 patients. Seven staff members working in the Hematology-Oncology Department (controls) presenting with flu-like symptoms over the same time period were screened for H1N1. H1N1 infection was confirmed in 4 cases. Table 1 present data on the patients and controls with confirmed H1N1 infection. The median age was 53 years (range 34-75 years) for patients and 29 years (range 27-55 years) for controls. Four patients had undergone a previous allogeneic stem cell transplantation (allo-SCT), including 1 patient with chronic lymphocytic leukemia (CLL) on day +53 after sibling reduced-intensity conditioning (RIC) allo-SCT (patient 1), 1 patient with non-Hodgkin lymphoma (NHL) on day +54 after volunteer unrelated donor (VUD) RIC (patient 2), 1 patient with acute myelogenous leukemia (AML) 18 months post-VUD RIC (patient 3), and 1 patient with chronic myelogenous leukemia (CML) 3 years after myeloablative conditioning (MAC) allo-SCT (patient 4). Patients 1 and 2 were receiving cyclosporine at the time of H1N1 diagnosis. Five patients had undergone a previous autologous SCT (auto-SCT) for multiple myeloma (MM) (patients 5-9), with widely varying intervals from transplantation to contraction of H1N1 (day +8, day +9, 4 months, 36 months, and 6 years post-transplantation). Three of the 4 patients who had not undergone transplantation (patients 10, 11, and 13), had received chemotherapy or rituximab within 1 month of onset of influenza symptoms, including 1 patient with MM peri-mobilization.