Establishment of Functional B Cell Memory Against Parvovirus B19 Capsid Proteins May be Associated With Resolution of Persistent Infection

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Parvovirus B19 (B19) infection can occur during acute lymphoblastic leukemia and persistent viral infection can occur despite intravenous immunoglobulin administration. Here, evidence is presented that resolution of persistent B19 infection in an acute lymphoblastic leukemia patient may be associated with the simultaneous strengthening of antigen-specific B cell memory against the B19 capsid protein VP2 and diminution in the memory response against the B19 non-structural protein 1 (NS1). Determination of antigen-specific B cell memory status may enhance the serological and molecular analyses of persistent B19 infection. J. Med. Virol. 78: 125–128, 2006. © 2005 Wiley-Liss, Inc.

KEY WORDS: ALL; immunocompromised; B cell ELISpot; leukemia; erythro-virus

INTRODUCTION

Parvovirus B19 (B19) is the causative agent of disease in both immunocompetent and immunocompromised individuals [Broliden, 2001; Corcoran and Doyle, 2004]. Specifically with respect to the immunocompromised status, there have been a number of recent reports of B19-associated disease in acute lymphoblastic leukemia of children [Heegaard and Schmiegelow, 2002; Savasan and Ozdeir, 2003; Kerr et al., 2003a; Fattet et al., 2004]. Although it has been suggested that childhood acute lymphoblastic leukemia may be associated with prior B19 exposure [Savasan and Ozdeir, 2003], this causal link remains unproven. In fact, recent work by Isa et al. [2004] has shown that B19 exposure, in utero, is unlikely to be associated with the subsequent onset of acute lymphoblastic leukemia, as judged by the absence of detectable B19 DNA from Guthrie cards of children (n = 54) who subsequently went on to develop acute lymphoblastic leukemia.

Administration of intravenous immunoglobulin is the main treatment for the symptoms associated with persistent B19 infection, such as pancytopenia and chronic anemia, in immunocompromised patients [Broliden, 2001] although the benefit of intravenous immunoglobulin administration in acute lymphoblastic leukemia patients has recently been questioned [Fattet et al., 2004]. Conceivably, the current lack of standardization of B19 IgG levels present in intravenous immunoglobulin may be responsible for this uncertainty with respect to efficacy. To date, most studies on the pattern of B19 serology and viraemia during acute lymphoblastic leukemia have focused solely on the detection of B19 IgG and IgM and viral DNA [Heegaard and Schmiegelow, 2002; El-Mahallawy et al., 2004; Fattet et al., 2004]. Two recent reports [Kerr et al., 2003a; Fattet et al., 2004] have alluded to importance the overall individual patient response, and by implication the cellular immune response, in successful eradication of B19 infection. Evidence is presented in this report that the establishment of B cell memory is coincident with the clearance of circulating B19 and resolution of B19-associated symptoms.

METHODS

A previously well 5-year-old girl presented with pyrexia, cough, bruising, and a purpuric rash. Bone marrow examination confirmed the diagnosis of CD10+ ALL; immunocompromised; B cell ELISpot; leukemia; erythro-virus

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acute lymphoblastic leukemia and she commenced on induction chemotherapy with the MRC ALL 97 (v99) protocol (regimen B). After induction therapy she started consolidation chemotherapy. She responded well, but in week 17 she was anemic and a bone marrow aspirate showed erythroid hypoplasia. Over the following 4 weeks she became transfusion-dependent, requiring nine packed red cell transfusions. Subsequent bone marrow aspirates confirmed erythroid hypoplasia with no relapse of acute lymphoblastic leukemia. Chemotherapy was commenced on T = −3 months (relative to T = 0 for initial B19 serological and nucleic acid testing) and also administered at months 3, 16, and 19. Parental permission was obtained for specimen collection.

Total B19-specific IgG for capsid VP2 (VP2-N) was determined by commercial EIA (Biotrin International, Dublin, Ireland). B19 IgM was assessed according to Brown et al. [1989]. B19 IgG reactivity against linearized VP1 (VP1-D), VP2 (VP2-D), and NS1 was determined using immunoassay formats as described previously [Corcoran et al., 2000]. B19 DNA levels were assessed as described previously [Hicks et al., 1995; Braham et al., 2004] and were quantified using a B19 DNA International Standard preparation [Saldanha et al., 2002]. In addition, cell proliferation studies and cytokine quantitation were carried out as described elsewhere [Corcoran et al., 2000]. B cell memory was analyzed using a B19-specific B cell ELISpot assay as described by Corcoran et al. [2004]. Briefly, nitrocellulose-lined plates (96 well) (Millipore, Bedford, MA) were described by Corcoran et al. [2004]. B cell memory was assessed as described previously [Hicks et al., 1995; Braham et al., 2004] and were quantified using a B19 DNA International Standard preparation [Saldanha et al., 2002]. In addition, cell proliferation studies and cytokine quantitation were carried out as described elsewhere [Corcoran et al., 2000]. B cell memory was analyzed using a B19-specific B cell ELISpot assay as described by Corcoran et al. [2004].

RESULTS

Initial serological testing (T = 0), using a B19 VP2 capsid-based immunoassay, for B19 IgG/M proved negative, however B19 DNA positivity was confirmed by both real-time PCR and by dot blot hybridization [Hicks et al., 1995; Braham et al., 2004] (Fig. 1). Treatment with intravenous immunoglobulin was commenced, and although logarithmic reductions in B19 viral load were observed post-treatment, viral clearance was not achieved, as judged by a continual elevation in plasma B19 DNA levels, until month 31 (Fig. 1). Serological analysis of a specimen obtained from the individual at T = 1 (Fig. 1) demonstrated the presence of high levels of intravenous immunoglobulin-derived B19 VP2 IgG (index value 5.76; reactivity > or = 1.1), in addition to detecting the presence of B19 NS1 IgG (Index value 3.22). No IgG was detected which was reactive against linear epitopes of either VP1 or VP2 proteins (VP1-D and VP2-D; data not shown). At T = 2, the B19 VP2 IgG index value was 2.31, while that for B19 NS1 had decreased to 0.77 (seronegative status).

Freshly isolated peripheral blood mononuclear cells (PBMCs), from the T1 specimen, were stimulated ex vivo, with both B19 VP1 and VP2 proteins. Although phytohaemagglutinin (PHA) stimulation resulted in significant stimulation index (SI (mean ± standard deviation): 1062.92 ± 508.81, n = 3), no detectable T cell stimulation was evident following exposure of PBMCs to either VP1 (SI: 0.3 ± 0.2) or VP2 (SI: 1.7 ± 0.7). Cytokine analysis of culture supernatants likewise indicated a deficit in the presence of cytokines associated with either a Th1 (interferon-γ) or Th2 (IL-4 and IL-5) immune response following B19 antigen stimulation (data not shown). Interferon-γ was detectable in culture supernatants following PBMC stimulation with PHA (data not shown). Subsequent analysis of a second specimen (T2) obtained 5 months after the initial sample, demonstrated the continued absence of a detectable cellular immune response against both B19 VP1 and VP2 proteins. Interestingly, significant PBMC proliferation was evident following stimulation with B19 NS1 protein (SI: 8.7 ± 1.7) (Fig. 2A). Again, however, there was no evidence of B19-specific cytokine production (IL-2, interferon-γ, IL-4, and IL-5) against B19 VP1, VP2, or NS1 proteins.
stimulatory indices (SI) and are the mean phytohaemaggultinin (PHA), at T = VP2 (VP2-N), and NS1 in addition to the positive control mitogen, proliferative response to human parvovirus B19 proteins VP1 (VP1-D), denatured VP1 (VP1-D), and NS1. Human IgG detection of NS1 IgG has also been shown to be present during infection only [von Poblotzki et al., 1995]. The detection of NS1 IgG reactivity, in addition to evidence of T cell immunity and B cell memory directed against B19 NS1. Furthermore, evidence is presented that the establishment and enhancement of B cell memory, directed against the capsid proteins of B19, was associated with diminution of B19 viremia and resolution of related disease symptoms in the acute lymphoblastic leukemia patient. It is evident that multiple administration of intravenous immunoglobulin throughout the treatment period was insufficient to eliminate B19 viraemia, indeed the viral load remained between $10^2$ and $10^{10}$ IU B19 DNA/ml until the final intravenous immunoglobulin treatment at month 23. The precise origin (i.e., patient- or intravenous immunoglobulin-derived) of B19 VP2 IgG detectable in specimen T1 is unclear, although the presence of B19 NS1 IgG does indicate that patient antibody production capability was intact. Nonetheless, the use of intravenous immunoglobulin therapy for the elimination B19 viraemia remains unproven, indeed a recent study [Fattet et al., 2004], which reported variable success with intravenous immunoglobulin treatment in acute lymphoblastic leukemia, recommended the treatment only in cases of persistent pancytopenia or clinical manifestations of B19 infection. However, intravenous immunoglobulin therapy has been shown to be successful for the treatment of B19-associated chronic fatigue syndrome [Kerr et al., 2003b]. Ultimately, the efficacy of intravenous immunoglobulin therapy for the elimination of B19 viremia will only become apparent when the level of B19 IgG present in intravenous immunoglobulin preparations is reported in terms of B19 IgG IU/ml as previous studies have indicated variable B19 IgG levels (64.7 ± 17.5 IU/ml; n = 30) in pooled solvent-detergent treated plasma [Daly et al., 2002].

Although initially thought to be a marker of persistent infection only [von Poblotzki et al., 1995], the detection of NS1 IgG has also been shown to be present during acute B19 infection in 61%–69% of individuals [Hemauer et al., 2000; Ennis et al., 2001]. Moreover, significant T cell responses to NS1 have also been reported in recently infected individuals and patients who developed chronic arthropathy following B19 infection [Mitchell et al., 2001]. It is clear from the present study, however, that immunity to NS1 does not appear to be consistent with clearance of B19 infection, at least in this individual suffering from acute

**DISCUSSION**

Persistent infection with parvovirus B19 (B19) is described in an acute lymphoblastic leukemia patient despite the administration of intravenous immunoglobulin therapy. The persistent B19 infection was associated with the detection of NS1 IgG reactivity, in addition to evidence of T cell immunity and B cell memory directed against B19 NS1. Furthermore, evidence is presented that the establishment and enhancement of B cell memory, directed against the capsid proteins of B19, was associated with diminution of B19 viremia and resolution of related disease symptoms in the acute lymphoblastic leukemia patient. It can be seen from Figure 2B that no B cell memory is evident against either linear epitopes of B19 VP1 or VP2 proteins, however significant B cell memory is detectable against the B19 NS1 protein (123.3 ± 20.2 SFC/10⁶ cells; mean ± SD) in specimen T1. In addition, a small though detectable level of B cell memory was detectable against B19 VP2 capsid protein (5.3 ± 5.7 SFC/10⁶ cells; mean ± SD). Interestingly, upon analysis of specimen T2, it was observed that the memory B cell level against the NS1 protein had diminished (tenfold). However, the level of detectable memory against capsid VP2, and to a lesser extent the VP1 unique region, had increased with respect to the initial analysis, (VP2-N: 13 ± 2, VP1-D: 3 ± 2, VP2-D: 1.7 ± 0.6, NS1-D: 9 ± 3.6 SFC/10⁶ cells). This apparent development of a functional host response against parvovirus B19 capsid protein VP2 was accompanied by a resolution of viral disease symptoms and diminution of plasma B19 DNA levels to less than $10^2$ IU B19 DNA/ml.

**Fig. 2.** Evaluation of PBMC proliferative response and antigen-specific B cell memory in acute lymphoblastic leukemia. A: Specific proliferative response to human parvovirus B19 proteins VP1 (VP1-D), VP2 (VP2-N), and NS1 in addition to the positive control mitogen, phytohaemaggultinin (PHA), at T = 2. Results are expressed as stimulatory indices (SI) and are the mean ± SD of triplicate wells. B: Parvovirus B19-antigen-specific B cell memory at T = 1 and 2. Results of B cell memory are expressed as B19-specific spot-forming cells (SFCs)/number of starting cells and are the mean ± SD of triplicate wells. Individual ELISpot microplates were coated with the following B19 proteins: native (capsid) VP2 (VP2-N), denatured VP2 (VP2-D), denatured VP1 (VP1-D), and NS1. Human IgG detection using rabbit anti-human IgG was used to confirm that total B cell activation had occurred (data not shown). Note: B19 NS1 protein response = 123.3 ± 20.2 SFC/10⁶ cells in specimen T1.
lymphoblastic leukemia. In addition, the presence of high titer B19 VP2 IgG, in the absence of detectable cellular immunity, also appears to be insufficient to completely ameliorate B19 viraemia. However, the establishment of B cell memory against capsid VP2, and to a lesser extent VP1, may play a role in the clearance of B19 viremia. We have previously reported evidence of B cell memory against B19 capsid proteins in immunocompetent individuals [Corcoran et al., 2004], and the data presented above represent the first determination of a similar pattern of B cell memory in acute lymphoblastic leukemia, coincident with the resolution of B19 viremia and symptoms. Assessment of antigen-specific B cell memory also facilitates discrimination between intravenous immunoglobulin- and individual-derived B19 IgG, respectively, which is significant in determining the seroconversion status of the individual. Interestingly, no evidence of B cell memory was evident against B19 NS1 in five immunocompetent individuals [Corcoran et al., 2004] thereby suggesting that the presence of B cell memory against this protein may be indicative of memory dysfunction in acute lymphoblastic leukemia. Resolution of B19 viraemia to less than $10^2$ IU/ml B19 DNA in the patient was coincident with a threefold increase in detectable B cell memory against capsid VP2 (and a tenfold reduction with respect to NS1).

In conclusion, we suggest that determination of antigen-specific B cell memory should accompany the serological and molecular investigation of persistent B19 viraemia, and associated disease manifestations, in immunocompromised individuals.

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