Bone Marrow and Peripheral Blood Dendritic Cells From Patients With Multiple Myeloma Are Phenotypically and Functionally Normal Despite the Detection of Kaposi’s Sarcoma Herpesvirus Gene Sequences

Noopur Raje, Jianlin Gong, Dharminder Chauhan, Gerrard Teoh, David Avigan, Zekui Wu, Dongshu Chen, Steven P. Treon, Iain J. Webb, Donald W. Kufe and Kenneth C. Anderson
Bone Marrow and Peripheral Blood Dendritic Cells From Patients With Multiple Myeloma Are Phenotypically and Functionally Normal Despite the Detection of Kaposi’s Sarcoma Herpesvirus Gene Sequences

By Noopur Raje, Jianlin Gong, Dharminder Chauhan, Gerrard Teoh, David Avigan, Zekui Wu, Dongshu Chen, Steven P. Treon, Iain J. Webb, Donald W. Kufe, and Kenneth C. Anderson

Multiple myeloma (MM) cells express idiotypic proteins and other tumor-associated antigens which make them ideal targets for novel immunotherapeutic approaches. However, recent reports show the presence of Kaposi’s sarcoma herpesvirus (KSHV) gene sequences in bone marrow dendritic cells (BMDCs) in MM, raising concerns regarding their antigen-presenting cell (APC) function. In the present study, we sought to identify the ideal source of DCs from MM patients for use in vaccination approaches. We compared the relative frequency, phenotype, and function of BMDCs or peripheral blood dendritic cells (PBDCs) from MM patients versus normal donors. DCs were derived by culture of mononuclear cells in the presence of granulocyte-macrophage colony-stimulating factor and interleukin-4. The yield as well as the comparable APC function and lower detection rate of KSHV gene sequences compared with BMDCs. Whether active KSHV infection is present and important in the pathophysiology of MM remains unclear; however, our study shows that MMDCs remain functional despite the detection of KSHV gene sequences.
KSHV DNA, using in situ hybridization and PCR, within BM core biopsy specimens from patients with MM.12-14 In contrast, several groups failed to detect KSHV DNA in either freshly isolated BM mononuclear cells (BMMCs), BMSCs, or BM biopsy specimens from patients with MM.15-18 and several other studies have not detected antibodies to KSHV in sera from MM patients despite humoral responses to other herpesvirus-PCR amplification product.25,26 Given the potential for innovative immune-based vaccination strategies in MM, most patients are not infected with KSHV, based on lack of KS330233. BMDCs generated from peripheral blood (PB) from patients with MM are not infected with KSHV, based on lack of KS330233 on the viral cyclin D gene sequence, further supports the rationale for novel immune-based therapeutic strategies in MM.

MATERIALS AND METHODS

Patients. BM aspirate samples were freshly obtained from 8 normal donors and 18 MM patients after written informed consent according to institutional guidelines. There were 13 men and 5 women with a median age of 57 (range, 38 to 77) years (Table 1). The majority of patients presented with advanced disease at diagnosis: Durie-Salmon stage IIA (3 patients), IIB (11 patients), and IIIA (3 patients). A single patient presented with plasmacytoma originating in bone. Therapy included combination chemotherapy as well as high-dose alkylating agents with or without total body irradiation followed by autologous or allogeneic stem cell grafting; 2 patients were untreated. The median time from diagnosis to BM sampling was 13 (range, 0 to 50) months. The percentage of mononuclear cells in BM biopsy specimens varied as follows: $<5$% (10 patients); 30% to 40% (3 patients); 50% to 60% (1 patient); 70% (2 patients); and 90% (2 patients). PB samples were obtained from 5 of these 18 MM patients, as well as from 6 normal donors.

Culture of DCs. BMMCs were isolated by centrifugation on Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ). BMMCs were suspended in RPMI 1640 medium containing 10% heat-inactivated human AB serum, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (10% RPMI); plated on 6 well, flat-bottomed tissue culture plates (Becton Dickinson, Franklin Lakes, NJ); and incubated for 1 to 2 hours at 37°C in a humidified 5% CO₂ atmosphere. Nonadherent cells were harvested, and fresh 10% RPMI medium can be readily obtained either from the BM or PB of MM patients that are phenotypically and functionally equivalent to DCs from normal donors. Most importantly, these DCs remain functional, despite the presence of KSHV gene sequences, providing the rationale for novel immune-based therapeutic strategies in MM.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Stage Dx</th>
<th>BM Status (%plasma cells)</th>
<th>Time From Diagnosis (mo)</th>
<th>Prior Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>3</td>
<td>D/VAD</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>28</td>
<td>VA/D/alogeneic BMT</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>7</td>
<td>D</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>18</td>
<td>DI/CEADP/CD34+ PBSCT</td>
</tr>
<tr>
<td>5</td>
<td>66</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>5</td>
<td>DI/AD</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>IIA</td>
<td>$&gt;5$</td>
<td>15</td>
<td>DI/CD34- PBSCT</td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>11</td>
<td>MP/D</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>13</td>
<td>VA/D/CD34+ PBSCT</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>IIIB</td>
<td>70</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>39</td>
<td>VA/dologeneic BMT</td>
</tr>
<tr>
<td>11</td>
<td>63</td>
<td>IIA</td>
<td>30</td>
<td>10</td>
<td>C</td>
</tr>
<tr>
<td>12</td>
<td>57</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>10</td>
<td>VA/D/CD34+ PBSCT</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
<td>IIIB</td>
<td>$&gt;95$</td>
<td>15</td>
<td>MP/VAD/CD34+ PBSCT</td>
</tr>
<tr>
<td>14</td>
<td>69</td>
<td>*</td>
<td>$&lt;5$</td>
<td>17</td>
<td>XRT/VA/D/CD34+ PBSCT</td>
</tr>
<tr>
<td>15</td>
<td>58</td>
<td>IIIB</td>
<td>50</td>
<td>60</td>
<td>50 MP/VAD/C</td>
</tr>
<tr>
<td>16</td>
<td>62</td>
<td>IIA</td>
<td>70</td>
<td>30</td>
<td>VA/D/CD34+ PBSCT</td>
</tr>
<tr>
<td>17</td>
<td>60</td>
<td>IIA</td>
<td>$&lt;5$</td>
<td>24</td>
<td>VA/D/CD34+ PBSCT</td>
</tr>
<tr>
<td>18</td>
<td>77</td>
<td>IIA</td>
<td>80</td>
<td>90</td>
<td>0 None</td>
</tr>
</tbody>
</table>

DCs were generated from a range of MM patients during different stages of disease with variable treatment histories. Treatment included D (Dexamethasone), VAD (Vincristine, Adriamycin, Dexamethasone), EDAP (Etoposide, Dexamethasone, Ara-C, and Cisplatinum), C (Cyclophosphamide), MP (Melphalan, Prednisolone), BMT (bone marrow transplant), PBSCT (peripheral blood stem cell transplant), and XRT (Radiotherapy).

*Patient 14 had a plasmacytoma of the bone.
containing GM-CSF (1,000 U/mL) and IL-4 (800 U/mL) (Genzyme, Boston, MA) was added to the adherent layer. After 12 to 14 days, loosely adherent cells were harvested and used for further studies. DCs were also cultured either from PB progenitor cells (PBPCs), collected by apheresis after mobilization with cyclophosphamide and G-CSF in 3 MM patients, or from PBMCs in 2 MM patients. Cells were washed three times, resuspended in 10% RPMI medium, and incubated at 37°C for 5 hours to eliminate nonspecific Fc receptor binding. After washing with PBS, cells were incubated with primary murine monoclonal antibodies (MoAbs) reactive with the above Ags for 30 minutes on ice.

For indirect immunofluorescence, cells were washed in phosphate-buffered saline (PBS) and incubated in PBS with 20% human AB serum at room temperature for 20 minutes to eliminate nonspecific Fc receptor binding. After washing with PBS, cells were incubated with primary murine monoclonal antibodies (MoAbs) reactive with the above Ags for 30 minutes on ice. After several washes, the cells were developed with goat anti-murine antibody conjugated with fluorescein isothiocyanate (FITC). Cells were then washed and fixed with 2% paraformaldehyde and evaluated by flow cytometry.

**APC function of DCs in allogeneic MLR.** We next examined the function of DCs from MM and normal BM and PB as APCs in allogeneic MLRs. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR.

Indirect immunofluorescence flow cytometry using the Coulter Epics XL (Coulter Corp, Miami, FL) was done to assay for expression of DC lineage Ags (CD14, CD40, CD80, CD86, and HLA-DR) [Pharmingen, San Diego, CA] and CD83 (Immunotech, Marseille, France) as well as MM-associated Ags (CD38). Cells were washed in phosphate-buffered saline (PBS) and incubated with 2% paraformaldehyde and evaluated by flow cytometry.

**Characterization of DC phenotype.** Indirect immunofluorescence flow cytometry using the Coulter Epics XL (Coulter Corp, Miami, FL) was done to assay for expression of DC lineage Ags (CD14, CD40, CD80, CD86, and HLA-DR) [Pharmingen, San Diego, CA] and CD83 (Immunotech, Marseille, France) as well as MM-associated Ags (CD38). Cells were washed in phosphate-buffered saline (PBS) and incubated with 2% paraformaldehyde and evaluated by flow cytometry.

**APC function of DCs in allogeneic MLR.** We next examined the function of DCs from MM and normal BM and PB as APCs in allogeneic MLRs. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR.

Indirect immunofluorescence flow cytometry using the Coulter Epics XL (Coulter Corp, Miami, FL) was done to assay for expression of DC lineage Ags (CD14, CD40, CD80, CD86, and HLA-DR) [Pharmingen, San Diego, CA] and CD83 (Immunotech, Marseille, France) as well as MM-associated Ags (CD38). Cells were washed in phosphate-buffered saline (PBS) and incubated with 2% paraformaldehyde and evaluated by flow cytometry.

**Characterization of DC phenotype.** Indirect immunofluorescence flow cytometry using the Coulter Epics XL (Coulter Corp, Miami, FL) was done to assay for expression of DC lineage Ags (CD14, CD40, CD80, CD86, and HLA-DR) [Pharmingen, San Diego, CA] and CD83 (Immunotech, Marseille, France) as well as MM-associated Ags (CD38). Cells were washed in phosphate-buffered saline (PBS) and incubated with 2% paraformaldehyde and evaluated by flow cytometry.

**APC function of DCs in allogeneic MLR.** We next examined the function of DCs from MM and normal BM and PB as APCs in allogeneic MLRs. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR.

Indirect immunofluorescence flow cytometry using the Coulter Epics XL (Coulter Corp, Miami, FL) was done to assay for expression of DC lineage Ags (CD14, CD40, CD80, CD86, and HLA-DR) [Pharmingen, San Diego, CA] and CD83 (Immunotech, Marseille, France) as well as MM-associated Ags (CD38). Cells were washed in phosphate-buffered saline (PBS) and incubated with 2% paraformaldehyde and evaluated by flow cytometry.

**Characterization of DC phenotype.** Indirect immunofluorescence flow cytometry using the Coulter Epics XL (Coulter Corp, Miami, FL) was done to assay for expression of DC lineage Ags (CD14, CD40, CD80, CD86, and HLA-DR) [Pharmingen, San Diego, CA] and CD83 (Immunotech, Marseille, France) as well as MM-associated Ags (CD38). Cells were washed in phosphate-buffered saline (PBS) and incubated with 2% paraformaldehyde and evaluated by flow cytometry.

**APC function of DCs in allogeneic MLR.** We next examined the function of DCs from MM and normal BM and PB as APCs in allogeneic MLRs. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR. Graded numbers of irradiated (3 Gy) DCs were added as APCs in allogeneic MLR.

Indirect immunofluorescence flow cytometry using the Coulter Epics XL (Coulter Corp, Miami, FL) was done to assay for expression of DC lineage Ags (CD14, CD40, CD80, CD86, and HLA-DR) [Pharmingen, San Diego, CA] and CD83 (Immunotech, Marseille, France) as well as MM-associated Ags (CD38). Cells were washed in phosphate-buffered saline (PBS) and incubated with 2% paraformaldehyde and evaluated by flow cytometry.
Specifically, because there were no differences in the phenotype of BMDCs or PBDCs from normal donors versus MM patients, we have compared BMDCs with PBDCs. As can be seen in Table 3, HLA-DR (P = .01), CD86 (P = .003), and CD14 (P = .04) were more highly expressed on PBDCs than on BMDCs.

APC function of DCs in allogeneic MLR. To determine whether these phenotypic differences correlated with variations in APC functional repertoire, we next assessed the relative function of BMDCs and PBDCs in allogeneic MLR. T cells were cultured with graded numbers of irradiated DCs for 5 days, and T-cell proliferation was measured by [3H]TdR uptake. Results from 3 normal BMDCs and 3 MM BMDCs are shown in Fig 3A. [3H]TdR uptake was not statistically different for normal BMDCs versus MM BMDCs at DC:T-cell ratios of 1:20 (P = .85), 1:60 (P = .86), 1:120 (P = .69), and 1:180 (P = .25).

PBDCs from normal donors and PBDCs from MM patients were similarly tested for their APC function in MLR (Fig 3B). As noted for BMDCs, there were no statistical differences in [3H]TdR uptake at DC:T-cell ratios of 1:20 (P = .8), 1:60 (P = .72), 1:120 (P = .81), and 1:180 (P = .15) for normal donor PBDCs versus MM patient PBDCs. We next compared APC function of BMDCs with PBDCs, including both MM and normal DCs. APC function of BMDCs and PBDCs were equivalent at DC:T-cell ratios of 1:20 (P = .38), 1:60 (P = .33), 1:120 (P = .49), and 1:180 (P = .70).

PCR analysis for KSHV gene sequences. Given recent reports suggesting detection of KSHV gene sequences in MM BMDCs, we next isolated DNA from BMDCs or PBDCs to assay for the presence of KSHV gene sequence by PCR. Initial PCR was performed using primers specific for the 233-bp KSHV gene sequence (KS330233), followed by a nested PCR with a second set of primers to yield a 186-bp amplification product internal to the 233-bp fragment, as in our prior studies. Genomic DNA from the KSHV-infected BCBL-1 cell line served as a positive control. Representative results from 10 MM BMDCs and 3 normal BMDCs are shown in Fig 4A. All samples are negative for the 233-bp product, except BCBL-1 (lane 1), which represents the positive control. Nested PCR yields a 186-bp amplification product detectable in KSHV infected BCBL-1 cell line (lane 1) and in DCs generated from 8 of 10 MM BMs (lanes 3 through 6, 7, 9, 10, and 11), which is absent in normal donor BMDCs (lanes 12 through 14). Amplifi-

### Table 2. Phenotypic Profile of DCs From MM Patients Versus Normal Donors

<table>
<thead>
<tr>
<th>Cell-Surface Antigen</th>
<th>Normal Donor DC (% of Ag expression)</th>
<th>MMDC (% of Ag expression)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>88.6 ± 5.1</td>
<td>87.4 ± 5.4</td>
<td>.67</td>
</tr>
<tr>
<td>CD40</td>
<td>64 ± 19.8</td>
<td>79.9 ± 11.8</td>
<td>.44</td>
</tr>
<tr>
<td>CD54</td>
<td>77.5 ± 12.2</td>
<td>72.7 ± 10.5</td>
<td>.59</td>
</tr>
<tr>
<td>CD80</td>
<td>69.8 ± 10.8</td>
<td>75.0 ± 10.1</td>
<td>.43</td>
</tr>
<tr>
<td>CD86</td>
<td>54.8 ± 30.9</td>
<td>65.8 ± 16.3</td>
<td>.48</td>
</tr>
<tr>
<td>CD14</td>
<td>8.8 ± 11.5</td>
<td>8.3 ± 6.5</td>
<td>.99</td>
</tr>
<tr>
<td>CD38</td>
<td>3.3 ± 1.5</td>
<td>2.6 ± 0.9</td>
<td>.46</td>
</tr>
<tr>
<td>PB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>94.7 ± 2.1</td>
<td>91.5 ± 5.1</td>
<td>.24</td>
</tr>
<tr>
<td>CD40</td>
<td>76.9 ± 26.4</td>
<td>80.7 ± 15.3</td>
<td>.87</td>
</tr>
<tr>
<td>CD54</td>
<td>84.9 ± 15.1</td>
<td>58.2 ± 26.0</td>
<td>.14</td>
</tr>
<tr>
<td>CD80</td>
<td>60.2 ± 32.1</td>
<td>47.3 ± 23.0</td>
<td>.56</td>
</tr>
<tr>
<td>CD86</td>
<td>91.9 ± 2.9</td>
<td>80.1 ± 7.4</td>
<td>.41</td>
</tr>
<tr>
<td>CD14</td>
<td>47.1 ± 13.7</td>
<td>35.3 ± 19.9</td>
<td>.44</td>
</tr>
<tr>
<td>CD38</td>
<td>3.5 ± 0.7</td>
<td>2.3 ± 0.6</td>
<td>.18</td>
</tr>
</tbody>
</table>

DCs from MM patients and normal donors, from either BM or PB, were tested by immunofluorescence for cell-surface expression of HLA-DR, CD40, CD54, CD80, CD86, CD14, and CD38. Statistical differences were tested using the Student’s t-test.
cation with β actin primers confirmed adequacy and integrity of DNA in each lane. Overall, KSHV-specific amplicons were detected by nested PCR in BMDCs from 16 of 18 (88.8%) MM patients and 3 of 8 (37.5%) normal donors.

DNA isolated from 5 MM PBDCs and 6 normal donor PBDCs was similarly tested for the presence of the KSHV gene sequences. Results from 4 normal donor PBDCs and 5 MM patient PBDCs are shown in Fig 4B. Lanes 1 represents the BCBL-1 cell line, which served as a positive control. Lanes 2 through 6 represent DNA from MM patient PBDCs and lanes 7 through 10 represent DNA from normal donor PBDCs. All samples are negative for KS330233 except the BCBL-1 cell line (lane 1). Lanes 1 (BCBL-1) and 2 (MM PBDC) are positive for the 186-bp product of nested PCR, but the remainder of PBDCs from both MM patients and normal donors are PCR negative. Amplification with β actin primers confirmed integrity of DNA. Overall KSHV gene sequences were detected in PBDCs from 1 of 5 (20%) MM patients and 2 of 6 (33%) normal donors.

To probe for other KSHV gene sequences in BMDCs, we next performed nested PCR on genomic DNA from 5 MM BMDCs and 2 normal donor BMDCs using primers that amplify gene sequences specific for viral cyclin D (Fig 5). The BCBL-1 cell line (lane 1) and 2 MM patient samples (lanes 3 and 6) were positive for viral cyclin D. Amplification with β actin primers confirmed integrity of DNA.

Sequencing of the 186-bp product confirmed 96% to 98% homology to the published KSHV gene sequence with patient-specific point mutations ruling out artifact and possible contamination. Sequencing of the viral cyclin D product confirmed 91% and 92% homology to the published sequence with distinct patient specific point mutations.

**DISCUSSION**

MM cells express specific idiotypic proteins and other associated Ags, which may serve as potential targets for immunotherapy. However, patients with MM are known to be immunocompromised, with defects in both humoral and cellular immunity. Moreover, early attempts at immunization with pneumococcal and other vaccines in patients with MM were unsuccessful. However, it has recently been shown that MM cells themselves can serve as effective APCs and that vaccination with idiotypic protein or using DCs pulsed with
idiotype can induce specific responses. The recent report of KSHV infection in cells of DC lineage within long-term BMSCs and BM biopsy specimens from patients with MM, although controversial, suggested potential sequelae of viral infection on MM APC function. Previous studies have shown the feasibility of generating large numbers of functional DCs from apheresis cells from MM patients and have compared PBDCs from MM patients with those from normal donors. However, in these studies DCs were either KSHV PCR or not examined. Given the great potential of novel immune therapeutic approaches in MM, we in the present study compared phenotype, functional repertoire, and KSHV gene expression in DCs from either BM or PB of MM patients with those derived from normal donors. We have shown that DCs from either BM or PB of patients with MM are equivalent in phenotypic profile and function with those from normal donors, despite the presence of KSHV gene sequences. These studies provide the framework for incorporation of DCs in novel immune treatment strategies for MM.

In our previous studies, we identified KSHV DNA sequences in long-term BMSC cultures from 92% (24 of 26) MM patients,
in cells expressing CD68, CD83, and fascin. Furthermore, these PCR results were confirmed with southern blotting and DNA sequence analysis. Given these and Rettig et al’s prior report, we in this study examined DCs from BM and PB cultured with GM-CSF and IL-4, in terms of phenotype, function, and KSHV gene expression. Patients at various stages of therapy, including those who were heavily treated and had persistent infiltration of BM with MM cells, were examined. Our results demonstrate that DCs can be readily generated from either BM or PB of MM patients in the presence of IL-4 and GM-CSF. These DCs are monocyte derived with an immature DC phenotype, evidenced by lack of cell-surface expression of CD83. Interestingly, we found that generation of BM-derived DCs, identified by both antigenic profile and functional repertoire, required 12 to 14 days of culture compared with only 7 to 10 days for PBDCs. Importantly, DCs from either source were equivalent in terms of phenotypic profile and APC function; moreover, they were equivalent to DCs derived from either BM or PB from normal donors. Therefore, our studies show that the presence of KSHV gene sequences does not adversely impact on function of DCs grown from either BM or PB using GM-CSF and IL-4.

Whether KSHV gene sequences are present in MM remains controversial. We previously found KSHV gene sequences to be present in the majority (92%) of long-term MM BMSCs. In this study DNA sequencing confirmed specificity of the PCR results. In addition, amplification and sequencing of other KSHV gene sequences including T1.1 and viral cyclin D further confirmed the presence of KSHV DNA. In the present study, we have amplified two distinct sequences in the KSHV genome, ORF 26 and viral cyclin D, using a nested PCR approach. Stringent PCR conditions were used to prevent contamination. Sequencing of the PCR products in 5 MM patients and 1 normal donor for orf 26 and 2 MM patients for viral cyclin D confirmed specificity of the PCR. In addition, patient-specific mutations were noted, ruling out PCR contamination. Therefore, these results confirm the detection of KSHV gene sequences by PCR and are consistent with prior reports. The contrasting results reported by several groups may be attributable to differences in PCR methodology and the sensitivity of the techniques used. For example, the recent report that functional clinical-grade DCs from patients with MM are not infected with KSHV used PCR for K330 or, which also failed to detect KSHV gene sequences in our study. Only with nested PCR did we detect KSHV sequences in MMDCs. It is possible that the virus is present in a very low copy number and at the threshold of PCR detection.

In contrast to the report by Rettig et al, we have in the present study identified the KSHV gene sequence in 3 of 8 normal BMDCs and 2 of 6 normal PBDCs, suggesting that use of nested PCR with enhanced sensitivity detects KSHV sequences in a significant fraction of normal individuals. KSHV gene sequences have also been previously identified in PBMCs of children and healthy donors. Moreover, although controversial, this virus has also been identified in a number of tissue specimens, including lymph nodes, brain, prostate, and semen, in clinical settings other than human immunodeficiency virus infection (HIV) and immunosuppression, suggesting that it may be ubiquitous as is EBV. These findings, coupled with the lack of serologic response to KSHV in patients with MM in our previous report and multiple other studies, further highlight the need to look for evidence other than PCR detection to answer the important question of the role of KSHV in the pathophysiology of MM. The finding that viral IL-6, like human IL-6, induces DNA synthesis of human MM cell lines in vitro, suggests a potential role for KSHV in tumor cell proliferation and survival, which is the subject of ongoing studies. A recent report showing KSHV antibodies in MM patients with the use of improved latent nuclear antigen immunoblot assay and ORF 55 immunoblot assay further supports an association between MM and KSHV infection. However, this improved assay needs to be used to confirm these results in a larger series of MM patients. Until evidence is provided demonstrating either the presence of viral transcripts and biologically active viral gene products in MM or other functional sequelae of KSHV infection, PCR detection of KSHV gene sequences must be interpreted with caution.

The lack of clinically evident anti-MM immunity in MM patients, coupled with our finding that DCs in MM are effective APCs in vitro, suggests that defects in Ag presentation may be uniquely present in vivo in these patients. For example, it is possible that DCs in MM patients may not take up, process, and present Ag due to an inhibitory effect of cytokines, ie, IL-10 or vascular endothelial growth factor secreted by tumor cells. The ability to generate large numbers DCs using different cytokine cocktails ex vivo, as described in this study, will facilitate their use in various immunization protocols. To exploit their APC function, DCs are being pulsed with whole-tumor Ag, naked DNA, or whole-tumor RNA fused with tumor cells, or genetically modified before vaccination. Already, these modified DCs have induced Ag-specific, major histocompatibility complex–restricted cytotoxic T lymphocyte responses in animals, with associated antitumor activity in both prophylaxis and treatment models. Therefore, the stage is set to test analogous approaches in clinical trials. Indeed, vaccinations using DCs pulsed with idiotype have already triggered tumor-specific response in patients with non-Hodgkin’s lymphoma and chronic myeloid leukemia, and similar studies are presently ongoing in MM patients.

REFERENCES