Hierarchical involvement of myeloid derived suppressor cells and monocytes expressing latency-associated peptide in plasma cell dyscrasias

Authors: Tamar Tadmor¹,² Ilana Levy³ and Zahava Vadasz²,⁴

¹Hematology Unit, Bnai-Zion Medical Center, Haifa, Israel. ²The Ruth and Bruce Rappaport Faculty of Medicine, Technion Haifa. ³Internal Medicine B Department, Bnai-Zion Medical Center, Haifa, Israel. ⁴Division of Allergy & Clinical Immunology, Bnai-Zion Medical Center, Haifa, Israel.

Article type: Original article

Keywords: Multiple Myeloma, Monoclonal gammopathy of unknown significance MGUS, myeloid derived suppressor cells, MDSCs, LAP, Latency-associated peptide.

Correspondence: Dr Tamar Tadmor
Hematology Unit, Bnai-Zion Medical Center,
47 Golomb Street, Haifa 31048, Israel.
Phone: +972 48359407
Fax: +972 48359962
Email: tamar.tadmor@b-zion.org.il

Conflict of interest: The author has no conflicts of interest to declare

Disclosures on funding sources: There were no funding sources for the manuscript

Hierarchical involvement of myeloid derived suppressor cells and monocytes expressing latency-associated peptide in plasma cell dyscrasias
**Abstract**

Plasma cell dyscrasias (PCD) are disorders of the plasma cells having in common the production of a monoclonal M-protein. They include a spectrum of conditions which may represent a natural progression of the same disease from monoclonal gammopathy of unknown significance (MGUS), to asymptomatic and symptomatic multiple myeloma and plasma cell leukemia and Waldenström's macroglobulinemia (WM).

In PCD the immune system is actively suppressed through the secretion of suppressive factors and the recruitment of immune suppressive subpopulations. In this study, we examined the expression of two subpopulations of cells with immunosuppressive activity: the monocytic-myeloid derived suppressor cells (MDSCs) and monocytes expressing latency-associated peptide (LAP) in patients with different PCD, and in healthy volunteers. Interestingly, we observed a hierarchical correlation between disease activity and the presence of monocytes with immunosuppressive activity.

These results suggest that MDSCs and monocytes expressing LAP have diverging roles in PCD and may perhaps serve as biomarkers of tumor activity and bulk.
**Introduction:**

Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of immature cells of granulocytic or monocytic origin, which accumulate in a number of disorders including solid tumors and hematological malignancies in particular.\textsuperscript{1,2} MDSCs inhibit T-cell proliferation and cytokine secretion, favoring the recruitment of regulatory T cells (Tregs) and are part of the immune regulatory subpopulations of cells responsible for inhibition of the immune response, thereby facilitating tumor escape.\textsuperscript{1,2}

Latency-associated peptide (LAP) is the N-terminal pro-peptide of the TGF-\(\beta\) precursor which binds non-covalently to TGF-\(\beta\), forming a latent TGF-\(\beta\) complex. When released into the extracellular milieu, LAP forms small latent complexes with transforming growth factor beta 1 (TGF-\(\beta_1\)).\textsuperscript{3–5} TGF-\(\beta\)–LAP complexes are present on the surface of various immune cells and have been shown to play a role in immune regulation, promoting the conversion of naive to activated Tregs which induce “Treg-associated immunosuppression”.\textsuperscript{3–5}

Bolzoni et al. studied the function of CD14/CD16+ monocytes sub-populations sorted from bone marrows of patients with monoclonal gammopathies at different stages of disease. In this report monocytes isolated from patients with multiple myeloma were showed activity, which contributed to enhanced osteoclast activation\textsuperscript{6}.

Multiple myeloma (MM) is the second most common hematological malignancy in the United States and is invariably preceded by “monoclonal gammopathy of unknown significance (MGUS)”. Myeloma cells are critically dependent for their survival, progression and proliferation on the tumor microenvironment, and a number of recent studies have concentrated on targeted therapy of tumor niches pathways\textsuperscript{7–9}.

MM is also associated with immune dysfunction, and several reports have demonstrated increased numbers of MDSCs in the bone marrow microenvironment which contributes to immunosuppression and tumor invasion\textsuperscript{10–16}.

Recently, we studied these two immune subpopulations: monocytic-MDSCs and LAP-expressing monocytes in the peripheral blood of patients with different plasma cell dyscrasias and in healthy volunteers and compared their frequency.
Material and methods:
A total of 27 consecutive patients with plasma cell dyscrasia, classified according to ‘The International Myeloma working Group” (as published in 2009 and updated in 2014-2015)\textsuperscript{14,15}) seen at the Hematology unit of the Bnai Zion Medical Center in Haifa, Israel between 2013-2015, were included in this study. For patients with plasma cell leukemia diagnosis was based upon the percentage (\(\geq 20\%\)) and absolute number (\(\geq 2 \times 10^9/l\)) of plasma cells in the peripheral blood, while Waldenstrom Macroglobulinemia (WM) was defined on the basis of the presence of IgM monoclonal gammopathy and \(\geq 10\%\) bone marrow lympho-plasmacytic infiltration\textsuperscript{17-20}.

The cohort included: 8 patients with MGUS, 14 with symptomatic MM, 2 with plasma cell leukemia and 3 with WM. Nineteen healthy volunteers served as controls. All samples were taken from treatment naïve patients, before starting any therapy. Written informed consent was obtained from all patients and the study was approved by the hospital Helsinki ethical committee.

Materials:
Mononuclear cells were enriched from whole blood using Ficoll-Hypaque gradient (Lymphoprep, Oslo, Norway). Fluorescence-activated cell sorting (FACS) analysis was performed on these mononuclear cells, using the following antibodies: anti-CD45 PC-5(PE-Cy5), anti-CD14 PE (Phycoerythrin), and anti-HLA-DR FITC (Fluorescein) (BD Biosciences). For staining, 0.5–1 \(\times\) 10\(^6\) mononuclear cells were stained and incubated at room temperature for 30 min in dark with the above-mentioned antibodies according to the manufacturer instructions in 100 \(\mu\)L PBS followed by red blood cell lysis (VersaLyse, Beckman Coulter, Inc. Marseille, France). In addition, MDSCs were characterized using antibodies to CD124 (IL-4Ra) which is the common receptor for interleukin-4 (IL-4). CD14\(^+\)/HLA-DR\(^{neg/low}\) were also gated for expression of LAP using anti-LAP (clone 27232) obtained from R&D Systems (Minneapolis, MN).

Data were acquired on a Beckman Coulter- Cytomics FC500 2 laser and analyzed with CXP Software version 2.2. (Beckman Coulter; Brea, Ca, USA).
Statistical analysis:
All values were expressed as the mean ± SEM. For flow-cytometry data, values between groups of data were tested for statistical significance using the two-tailed Student t-test. Significant p-value was set at less than 0.05.

Results:
The patient cohort included 11 males (41%) and 16 females (59%), median age at diagnosis was 61 (45-86). All patients were diagnosed and followed in the same medical center. Patients’ characteristics are presented in Table 1.

Monocytic MDSC expression:
The mean number of circulating monocytic MDSCs in the peripheral blood was defined by co-expression of positive CD14+ and dim expression of HLA-DR. The average expression for the MGUS cohort was 5.9 % (3.7-8.1), 12.5 % (6.7-27.2) for MM patients, 18.4% in plasma cell leukemia (14.6-22), 17.8% (16.5-19) in WM and 5.5% (2.4-7.9) in healthy controls.
No significant difference was observed between MGUS and healthy volunteers (p=0.39), but comparison with PCD was significant (p=0.002) (Figure 1a). Next, we analyzed the monocyte subpopulation, co-expressing CD124+, another marker of MDSCs. Results obtained using mean numbers for healthy controls and patients with MGUS, MM, plasma cell leukemia and WM were 8.1% (6.1-11), 4.4% (1.6-7.1), 15.7% (2.5-17.5), 18.4% (14.5-22.3) and 19.7% (18.5-20.9) respectively (Figure 1b). Results were statistically significant for all plasma cell dyscrasias when compared to healthy controls (p=0.03).

LAP expression:
The mean number of circulating monocyte/LAP+ cells in the peripheral blood was defined by co-expression of positive CD14+ and LAP. The average expression for the MGUS cohort was 6.5% (3.7-9.1), 15.1 % (12.1-44) for MM patients, 19% in plasma cell leukemia (13.5-23.2), 19.7% (16.9-23) in WM and 7.2% (5.9-9.5) in healthy controls. No significant difference was observed between MGUS and healthy volunteers (p=0.8), but results were significant for other PCD (p=0.018) (Figure 2a, 2b).
Discussion:
Substantial advances in understanding the biology of PCD progression have been performed through the study of the bone marrow (BM) microenvironment. The BM niche appears to play an important role in differentiation, proliferation, migration and survival of plasma cells. It is composed of a heterogenous cellular compartment which includes stromal cells, osteoblasts, osteoclasts, endothelial cells and immune cells. Intercellular interaction appears to induce immune dysfunction, which is also an important feature of MGUS and MM and may promote progression from a pre-malignant state into frank malignancy.

Monocytes, macrophages and mesenchymal stromal cells play a role in MM pathogenesis, where they support survival and proliferation of the neoplastic myeloma cells.

MDSCs are a heterogeneous population of immature myeloid cells at different stages of maturation which play a role in cancer tolerance and function as an immunosuppressive cell subpopulation.

Several studies have analyzed the frequency and function of MDSCs in multiple myeloma, indicating that they promote both myeloma growth as well as osteoclast activity and are involved in cross-talk with T-reg cells resulting in their expansion in the BM microenvironment.

We hypothesize that enhanced activity of a monocyte subpopulation with immunosuppressive activity may play a role in patients with PCD. We were able to demonstrate that in parallel to disease progression from MGUS to MM and PCL the number of monocytic MDSCs appears to increase and that they may express more IL-4R which is critical for suppression of MDSC function through L4Ra–STAT6 pathway and thereby indicative of greater immune-related activity.

The preliminary results we report here are in keeping with those of a recent study that also demonstrated increased activity of CD14/CD16+ monocytes in different monoclonal gammopathies in an hierarchical pattern. Indeed, these CD14/C16+ monocytes isolated from multiple myeloma patients appear to contribute to bone disease and osteoclastogenesis via IL21 overexpression.

Recently, a novel regulatory cell subset population has also been described: T-regs and immature dendritic cells which express the human latency-associated peptide (LAP+) have not yet been studied extensively but based on our lab’s preliminary results, showing
high expression of LAP on the surface of CD14+ mononuclear cells isolated from patients with ankylosing spondylitis, we decided to examine this phenomenon in patients with PCD. Here we indeed show that monocytes isolated from these patients have higher expression of LAP+ and that the frequency of its expression was correlated with disease progression. Our results may have additional significance as a biomarker of disease activity and we are currently initiating a study analyzing these two sub-populations after therapy in symptomatic patients with PCD. In addition, it has been reported that when effective therapy for PCD is given, as with the immune-regulatory lenalidomide and more recently treatment with daratumumab, immunosuppressive MDSCs, Tregs, and Bregs are reduced while the expression of CD4+ T-helper cells and CD8+ cytotoxic T cells is increased supporting a numeric correlation between their frequency and disease activity. Our study obviously has several limitations including the limited size of the cohort; the fact that these immune-suppressive populations were isolated from peripheral blood and not bone marrow, as well as the lack of functional assays. In conclusion, we observed a hierarchical correlation between the subtypes of PCD categories and the recruitment of two subpopulations of monocytes -the monocytic-myeloid derived suppressor cells and monocytes expressing latency-associated peptide with immunosuppressive activity. These results suggest that MDSCs and LAP play diverging roles in PCD and may have a potential role as markers of tumor activity. Our results require further validation and we are now performing a following study to validate them and analyze the effect of therapy on these two subpopulations.
References:


**Figure Legend:**

Table 1: patients’ demographic, clinical and laboratory characteristics. MM - multiple myeloma, WM- waldenstroom macroglobulinemia, PCL- plasma cell leukemia, MGUS- monoclonal gammopathy of unknown significance.

**Figure 1:** Flow-cytometry analysis of peripheral blood from patients with different plasma cell dyscrasias in comparison to healthy controls.

1a: Co-expression of CD14+/ HLA dr- dim

1b: Co-expression of CD14+/ CD124+, both represent the average of MDSC percentage identified in peripheral blood of each cohort.

1c: An example of FACS analysis presenting peripheral blood infiltrated by MDSC in MGUS, MM and plasma cell leukemia patients.

**Figure 2:** Flow-cytometry analysis of peripheral blood from patients with different plasma cell dyscrasias in comparison to healthy controls for the expression of LAP on monocytes.

2a: Co-expression of CD14+/ LAP+. Results represent the average percentage identify in blood of each cohort.

2b: An example of FACS analysis presenting peripheral blood infiltrated by monocytes/LAP+ cell in a healthy control and a multiple myeloma patient.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MM</th>
<th>WM</th>
<th>PCL</th>
<th>MGUS</th>
<th>Healthy Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Age</td>
<td>67.3±13.4</td>
<td>74.3±6.7</td>
<td>75.5±4.9</td>
<td>67.9±15.5</td>
<td>48.1±18.8</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.8±1.7</td>
<td>11.4±3.6</td>
<td>11.4±3.6</td>
<td>12.1±2.7</td>
<td>13.2±1.4</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.4±1.3</td>
<td>0.9±0.3</td>
<td>0.9±0.1</td>
<td>1.4±1.3</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.5±1.9</td>
<td>10.4±1.3</td>
<td>9.8±0.4</td>
<td>8.9±1.5</td>
<td>9.5±0.2</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7±0.7</td>
<td>3.9±0.9</td>
<td>4.1±0.5</td>
<td>3.8±0.8</td>
<td>4.3±0.4</td>
</tr>
<tr>
<td>Beta-2-microglobulin (mg/L)</td>
<td>8.1±7.3</td>
<td>2.9±1.1</td>
<td>2.3±0</td>
<td>3.2±1.6</td>
<td>Unknown</td>
</tr>
<tr>
<td>M spike</td>
<td>IgG kappa g/dL</td>
<td>5 (36%)</td>
<td>-</td>
<td>-</td>
<td>8 (100%)</td>
</tr>
<tr>
<td></td>
<td>IgG lambda g/dL</td>
<td>2.9±3.3</td>
<td>-</td>
<td>-</td>
<td>1.1±0.98</td>
</tr>
<tr>
<td></td>
<td>IgA kappa g/dL</td>
<td>2 (14%)</td>
<td>1 (50%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>g/dL</td>
<td>2.7±3.1</td>
<td>0.1±0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 1: patients’ demographic, clinical and laboratory characteristics. MM - multiple myeloma, WM- waldenstrom macroglobulinemia, PCL- plasma cell leukemia, MGUS- monoclonal gammopathy of unknown significance.