Renal transplant recipients have elevated frequencies of circulating myeloid-derived suppressor cells

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Abstract

Background. Cancer, particularly cutaneous squamous cell carcinoma (SCC), is a major cause of mortality in renal transplant recipients (RTRs). Myeloid-derived suppressor cells (MDSC) play a central role in suppressing cancer immunosurveillance but their potential mobilisation in RTRs and levels relative to those of other immunoregulatory dendritic cell (DC) populations have not been analysed.

Methods. The circulating frequencies of MDSC and DC were analysed by multicolour flow cytometry in immunocompetent patients without (n = 13) or with (ICI-SCCPos, n = 14) current SCC, normal donors (NDs, n = 34), chronic kidney disease patients (CKD patients, n = 22) and RTRs (n = 31).

Results. Compared to NDs, RTRs had significantly elevated levels of both CD14Neğ and CD14Pos MDSC subsets (P < 0.001), while CKD patients and ICI-SCCPos had significantly elevated levels of only the CD14Neğ,MDSC subset. DC frequencies were significantly decreased in RTRs and CKD patients but were at normal levels in ICI-SCCPos. The MDSC/DC ratio was significantly elevated (P < 0.05) in RTRs (median = 5.7), CKD patients (median = 3.2) and ICI-SCCPos (median = 3.5) relative to NDs (median = 0.7). The use of immunosuppressive drugs in CKD patients and past/current occurrence of SCC in RTRs was associated with significantly increased CD14Neğ,MDSC frequencies. MDSC enriched from RTRs, when cocultured with activated NDs T cells significantly suppressed extracellular IL-10 levels and can, when activated with formyl-methionyl-leucyl-phenylalanine, inhibit T-cell proliferation.

Conclusions. RTRs, CKD patients and ICI-SCCPos have increased MDSC frequencies and MDSC/DC ratios. These changes may impact on cancer immunosurveillance. Therefore, MDSC represent both a potential therapeutic target and prognostic marker in these patients, with respect to the development of SCC and other malignancies.

Keywords: dendritic cell; myeloid-derived suppressor cells; renal transplant; squamous cell carcinoma

Introduction

Renal transplant recipients (RTRs) have a significantly increased risk of cancer. Cutaneous squamous cell carcinoma (SCC) is the most common malignancy in RTRs and is associated with mortality and subsequent cancers [1, 2]. Immunosuppressive drug-mediated impairment of immunosurveillance plays a major role in the increased incidence and aggressiveness of SCC observed in RTRs [1, 3]. This has been attributed to the effect of immunosuppression on T cells but little is known regarding the role of other cell types in suppressing immunosurveillance.

There is evidence that mobilisation of myeloid-derived suppressor cells (MDSC) plays a central role in suppressing cancer immunosurveillance and therefore promoting tumour progression [3–5]. In humans, MDSC are commonly defined as low-density cells that express myeloid markers (CD11b and/or CD33) but lack or only weakly express HLA-DR [4, 6]. Two circulating MDSC subsets have been identified, a monocytoid CD14Pos subset [7–10] and a granulocytic CD14Neğ subset [11–16]. Although increased frequencies of MDSC have been reported in a range of cancers [4, 5], only a single small study has enumerated the levels of both subsets [6], and their numbers and significance in RTRs and SCC is unknown. However, rodent studies suggest that glucocorticoids and kidney allograft tolerance can induce MDSC mobilisation [17, 18].

Dendritic cells (DCs) are another important immunoregulatory population that can either induce or suppress antitumour responses, depending on their ontogeny and differentiation [19, 20]. Two subsets, myeloid and plasma-cytoid, have been identified and, although studies on RTRs have reported reduced DC numbers [21–24], their levels in relation to either SCC occurrence or MDSC frequencies has not been determined.

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The balance of immunoregulatory MDSC and DC populations may modulate the level of cancer immunosurveillance and therefore cancer risk in RTRs. Changes in the immunoregulatory cell populations in RTRs may result from the presence of SCC and/or transplant-related events, such as immunosuppressive therapy and reduced renal function. In order to discriminate between SCC and predominantly transplant-related effects, RTRs were compared with normal donors (NDs), chronic kidney disease patients (CKD patients) and immunocompetent individuals (ICIs) with either SCC or other skin lesions.

**Subjects and methods**

**Participants**

Blood was collected from NDs and patients following informed consent according to Upper South B Ethical Committee (NZ) guidelines. Patient blood was collected at Christchurch Hospital from (i) RTRs who had received transplants between 1972 and 2007 and were attending clinics for either routine follow-up or excision of SCC, (ii) CKD patients (estimated glomerular filtration rate [\(\leq 60\) mL/min/1.73m\(^2\)]) attending nephrology or diabetes clinics and (iii) ICIs attending clinics for excision of skin lesions. All blood was collected prior to excision.

**Isolation of peripheral blood populations**

Centrifugation (20 min, 900 g) over Ficoll/Paque (Amersham Pharmacia Biotech, Uppsala, Sweden) was used to prepare the low-density fraction (LDF) of peripheral blood cells. Granulocytes were obtained from high-density fraction following NH\(_4\)Cl lysis of red blood cells.

**Table 1. Clinical characteristics and cell population frequencies of study participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal donors</th>
<th>RTRs</th>
<th>SCC(_{Neg})</th>
<th>SCC(_{Pos})</th>
<th>CKD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>34</td>
<td>31</td>
<td>13</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>65 (41–82)</td>
<td>60 (40–80)</td>
<td>64 (40–86)</td>
<td>81 (54–91)</td>
<td>56 (25–77)</td>
</tr>
<tr>
<td><strong>Male sex (%)</strong></td>
<td>44</td>
<td>55</td>
<td>62</td>
<td>57</td>
<td>54</td>
</tr>
<tr>
<td><strong>Current SCC (%)</strong></td>
<td>n.d.</td>
<td>22</td>
<td>0</td>
<td>100</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Prior/current SCC (%)</strong></td>
<td>n.d.</td>
<td>68</td>
<td>0</td>
<td>100</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Years post-transplant</strong></td>
<td>10 (0.3–38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MDSC</strong></td>
<td>17.0 (7.1–31.8)</td>
<td>30.4 (17.1–75.9)</td>
<td>19.6 (13.6–31.0)</td>
<td>27.8 (14.3–77.4)</td>
<td>23.9 (10.3–79.4)</td>
</tr>
<tr>
<td><strong>CD33(_{Pos})</strong></td>
<td>1.6 (0.6–3.8)</td>
<td>7.3 (1.8–50.0)</td>
<td>2.2 (0.6–5.2)</td>
<td>4.5 (0.9–43.2)</td>
<td>3.9 (1.2–38.1)</td>
</tr>
<tr>
<td><strong>CD14(_{Neg})</strong></td>
<td>0.3 (0.1–1.9)</td>
<td>2.9 (0.1–14.8)</td>
<td>0.3 (0.2–2.3)</td>
<td>0.9 (0.1–10.5)</td>
<td>1.0 (0.8–4.9)</td>
</tr>
<tr>
<td><strong>Ratio(_{Pos}/Neg})</strong></td>
<td>1.2 (0.4–3.5)</td>
<td>2.7 (0.9–35.2)</td>
<td>1.7 (0.6–3.3)</td>
<td>3.7 (0.6–32.7)</td>
<td>2.5 (0.5–37.3)</td>
</tr>
<tr>
<td><strong>DC</strong></td>
<td>0.3 (0.1–1.9)</td>
<td>0.7 (0.1–8.23)</td>
<td>0.2 (0.1–1.3)</td>
<td>0.3 (0.0–0.8)</td>
<td>0.4 (0.2–2.9)</td>
</tr>
<tr>
<td><strong>CD11c(_{Pos})</strong></td>
<td>1.8 (1.1–3.5)</td>
<td>1.2 (0.4–4.0)</td>
<td>2.2 (0.7–3.1)</td>
<td>1.9 (0.4–4.1)</td>
<td>1.2 (0.3–3.5)</td>
</tr>
<tr>
<td><strong>CD11c(_{Neg})</strong></td>
<td>0.4 (0.3–1.0)</td>
<td>0.3 (0.1–0.6)</td>
<td>0.5 (0.2–1.2)</td>
<td>0.5 (0.2–0.9)</td>
<td>0.3 (0.0–0.7)</td>
</tr>
<tr>
<td><strong>CD11c(_{Pos}/Neg})</strong></td>
<td>1.4 (0.6–2.6)</td>
<td>0.9 (0.2–3.9)</td>
<td>1.4 (0.5–2.4)</td>
<td>1.5 (0.3–3.7)</td>
<td>0.9 (0.3–3.4)</td>
</tr>
<tr>
<td><strong>Ratio(_{Pos}/Neg})</strong></td>
<td>0.3 (0.1–1.0)</td>
<td>0.4 (0–1.3)</td>
<td>0.3 (0.2–0.9)</td>
<td>0.4 (0.1–0.9)</td>
<td>0.3 (0–4.4)</td>
</tr>
<tr>
<td><strong>MDSC/DC</strong></td>
<td>0.7 (0.2–2.7)</td>
<td>5.9 (0.7–46.9)</td>
<td>1.0 (0.4–7.1)</td>
<td>4.5 (0.9–43.2)</td>
<td>3.9 (1.2–38.1)</td>
</tr>
</tbody>
</table>

\(\text{n.d., not done; bold indicates values significantly different from NDs using Kurskal–Wallis test.}\)

\(\text{Percentage of studied group with indicated characteristic.}\)

\(\text{AZA, azathioprine; Pred, prednisone; CsA, cyclosporine; Tac, tacrolimus; MMF, mycophenolate mofetil.}\)

**Monoclonal antibodies**

The following fluorescein isothiocyanate (FITC), Phycocerythin (PE), phycoerythrin–cyamine-5 (PC5) and phycoerythrin–cyamine-7 (PC7)-conjugated mAbs were utilised: CD45-FITC, anti-HLA-DR-FTC, CD25-FETC, CD3-PETC, CD11b-PE, HLA-DR-PC5 and CD14-PC7 obtained from Beckman Coulter (Fullerton, CA). IgG1-FITC, CD4-FETC, CD3-PETC, CD14-PE, CD19-PE, CD56-PE and IgG1-PE were from BD Biosciences (San Jose, CA).

**Flow cytometric analysis**

For cell enumeration, LDF samples were incubated (20 min, 4°C) with the relevant mAbs and analysed on a Beckman Coulter FC500. For each blood sample, isotype controls and CD45 staining were used to correct for background staining and non-leucocytes, respectively.

DCs were enumerated with HLA-DR-PC7, CD11c-PE and FITC-p conjugated mAbs (CD3, CD14, CD19, CD56). Following gating on mix-PE-negative cells, plots of CD11c versus HLA-DR were used to determine the percentages of both total DC (HLA-DR\(_{\text{neg}}\)) and the DC subsets (CD11c\(_{\text{Pos}}\) and CD11c\(_{\text{Neg}}\)).

**Evaluation of MDSC function**

Monoclonal antibodies were performed in SILAC-RPMI (Sigma, St Louis, MO) supplemented with 100 \(\mu\)M arginine, 40 mg/L lysine, 50 mg/L leucine, 0.02 \(\mu\)M MnCl\(_2\) and 10% fetal calf serum (FCS) (Invitrogen, Auckland, New Zealand). T cells were isolated from the PBMC of NDs by immunomagnetic depletion using CD16, CD11b and HLA-DR mAb with goat anti-mouse
IgG Dynabeads according to the manufacturer protocols (Dynal AS, Oslo, Norway). T cells were incubated (5 min, RT) at 1 × 10^7/mL in 5% FCS/phosphate-buffered saline with 1 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) (Invitrogen) prior to washing.

MDSC were enriched from the LDF by immunomagnetic depletion using HLA-DR, CD3 and CD57 mAb with Dynabeads. MDSC frequencies in enriched preparations were 50–64% in RTRs and 5–7% in NDs.

Assays were performed in 96-well round-bottom plates precoated (5 μg/mL) with CD3 mAb (UCHT1; Beckman Coulter). CFSE-labelled T cells (1 × 10^5 per well) and 1 μg/mL CD28 mAb (28.2; Beckman Coulter) were cultured (37°C, 5% CO_2), with or without MDSC (1.5–2 × 10^5 per well) and/or formyl-methionyl-leucyl-phenylalanine (fMLP, 1 μM; Sigma). Following 72-h incubation, supernatants were harvested and T-cell proliferation determined by labelling cells with CD4-PC7 and CD8-PE and analysing the T-cell CFSE signal by flow cytometry. The level of IL-10 and IFN-γ in supernatants was determined by enzyme-linked immunosorbent assay (R&D systems, Minneapolis, MN).

Statistical analysis
Statistical analysis was performed by SPSS® for Windows version 17 (SPSS Inc., Chicago, IL). Categorical variables were compared by the chi-square or Fisher’s exact test. Correlations were analysed using Spearman rank correlations. The levels of continuous variables in NDs and patient groupings were compared using the Kurskal–Wallis test together with Dunn’s post test. Subgroups of patients were compared using Mann–whitney. A P-value < 0.05 was considered significant.

Results
Characteristics of study participants
Clinical and demographic characteristics of study participants are depicted in Table 1. ICIs attending hospital clinics for
removal of skin lesions were divided into SCC\textsuperscript{Neg} and SCC\textsuperscript{Pos} subsets based on the presence of histologically confirmed invasive SCC at the time of sample collection.

**Enumeration of MDSC**

MDSC share a common CD33\textsuperscript{Pos}, HLA-DR\textsuperscript{Neg} phenotype and can be further subdivided using CD14 expression into granulocytic- and monocytic-like subsets [4, 6]. MDSC were enumerated by firstly gating on the entire CD33\textsuperscript{Pos} myeloid population and then evaluating CD14 versus HLA-DR expression (Figure 1A). MDSC were defined as the HLA-DR\textsuperscript{Neg} cells and then subdivided into CD14\textsuperscript{Neg} and CD14\textsuperscript{Pos} subsets. Preliminary experiments confirmed that all MDSC expressed CD11b (data not shown).

The frequencies of CD33\textsuperscript{Pos} myeloid cells (Figure 1B, Table 1) present in the LDF were significantly elevated in RTRs, CKD patients and ICI-SCC\textsuperscript{Pos} relative to NDs. Analysis of total MDSC (CD33\textsuperscript{Pos}, HLA-DR\textsuperscript{Neg}) demonstrated that, relative to NDs, the frequencies of this population were significantly elevated (P < 0.01) in RTRs, CKD patients and ICI-SCC\textsuperscript{Pos} but not in ICI-SCC\textsuperscript{Neg} (Figure 2A, Table 1). The ratio was, however, significantly higher (P < 0.01) in RTRs than NDs and a significantly increased proportion (34 versus 12%, P = 0.028) had ratios >1.

Comparison of RTRs and ICI-SCC\textsuperscript{Pos} demonstrated that their frequencies of CD14\textsuperscript{Neg}-MDSC did not significantly differ while there was a significant difference (P < 0.05) in their frequencies of total MDSC, CD14\textsuperscript{Pos}-MDSC and the CD14\textsuperscript{Pos}/CD14\textsuperscript{Neg} ratio.

**Enumeration of DC subsets**

RTRs and CKD patients, but not ICI-SCC\textsuperscript{Pos} relative to NDs.

Analysis of total MDSC (CD33\textsuperscript{Pos}, HLA-DR\textsuperscript{Neg}) demonstrated that, relative to NDs, the frequencies of this population were significantly elevated (P < 0.01) in RTRs, CKD patients and ICI-SCC\textsuperscript{Pos} but not in ICI-SCC\textsuperscript{Neg} (Figure 2A, Table 1). A similar pattern was observed following analysis of the CD14\textsuperscript{Neg}-MDSC subset (Figure 2B, Table 1). In contrast, the frequencies of CD14\textsuperscript{Pos}-MDSC were, relative to NDs, significantly elevated (P < 0.001) only in RTRs (Figure 2C, Table 1).

Electronic back gating demonstrated that the majority of CD14\textsuperscript{Pos}-MDSC had the light scatter characteristics of normal monocytes, while the majority of CD14\textsuperscript{Neg}-MDSC had granulocytic light scatter characteristics (Figure 1A). Cells with polymorphonuclear morphology were also clearly visible following May-Grunwald-Giemsa staining of patient preparations with elevated CD14\textsuperscript{Neg}-MDSC frequencies (Figure 1C).

Analysis of the MDSC subset ratio (CD14\textsuperscript{Pos}/CD14\textsuperscript{Neg}) demonstrated that the majority of NDs, CKD patients and ICI\textsuperscript{Neg} had higher numbers of CD14\textsuperscript{Neg}-MDSC (Figure 2D, Table 1). A similar pattern was observed following analysis of the CD14\textsuperscript{Neg}/CD11c\textsuperscript{Pos} ratio in all patient groups (Table 1, Figure 3A–C). RTRs and ICI-SCC\textsuperscript{Neg} did not significantly differ with respect to any of the DC parameters.

**Ratio of MDSC and DC frequencies**

In NDs and ICI-SCC\textsuperscript{Neg}, most samples had higher frequencies of DC than MDSC and the MDSC/DC ratio was >2 in
only 6% of individuals (Figure 3D, Table 1). In contrast, the majority of ICI-SCC\textsuperscript{Pos} (60%), CKD patients (64%) and RTRs (91%) had MDSC/DC ratios >2 and overall, the MDSC/DC ratio of these groups was significantly higher than that of NDs.

There was no significant difference between the ratios of the RTRs and ICI-SCC\textsuperscript{Pos}.

**MDSC and DC frequencies in RTR and CKD subgroups**

RTRs were subdivided into those with (RTR\textsuperscript{Pos}) or without (RTR\textsuperscript{Neg}) current or prior SCC at the time of sample collection. Both RTR subgroups had significantly higher (P < 0.05) MDSC subset frequencies and MDSC/DC ratios than NDs. The only significant difference between these subgroups was the higher levels of CD14\textsuperscript{Neg}-MDSC in RTR\textsuperscript{Pos} (Figure 4A). There was no significant difference between these subgroups with respect to DC frequencies (data not shown).

CKD patients were subdivided into those currently receiving no immunosuppression (CKD\textsuperscript{Nil}) and those receiving prednisone either alone or in combination with azathioprine or cyclophosphamide (CKD\textsuperscript{Plus}). The CKD\textsuperscript{Plus} subgroup had significantly higher CD14\textsuperscript{Neg}-MDSC frequencies and MDSC/DC ratios than both the CKD\textsuperscript{Neg} subgroup and NDs but did not differ significantly with respect to CD14\textsuperscript{Pos}-MDSC (Figure 4B). Although the CKD\textsuperscript{Nil} subgroup was not significantly different from NDs with respect to MDSC frequencies, both CKD subgroups had significantly lower DC frequencies than NDs (data not shown).

**Functional activity of MDSC**

MDSC were enriched (50–64% MDSC) by depletion of HLA-DR\textsuperscript{Pos}, CD57\textsuperscript{Pos} and CD3\textsuperscript{Pos} cells, which also removes all other immunoregulatory populations such as DC and Tregs. The effect of these preparations on normal T-cell responses was determined (Figure 5). Because the peptide fMLP can activate granulocytic suppressor activity [12, 26, 27], cultures were performed in both its absence and presence, except where MDSC numbers were limiting (Patient #4).

T-cell proliferation was either unchanged or increased by the addition of enriched MDSC preparations from all four RTRs (Figure 5A, B). In contrast, two out of three enriched MDSC preparations significantly inhibited T-cell proliferation in the presence of fMLP. Similar results were obtained for both the CD4\textsuperscript{Pos} and CD8\textsuperscript{Pos} T-cell populations (data not shown).

Analysis of proliferation assay supernatants demonstrated that all enriched MDSC preparations could significantly suppress IL-10 levels (P < 0.002, Figure 5C) and in three out of four preparations, this suppression occurred even in the absence of fMLP. The presence of fMLP alone had no effect on T-cell proliferation and IL-10 release.

A comparison of IFN-γ levels in the T cell and T cell + MDSC cultures was also able to be performed for Patients...
#1, #2, #3 and demonstrated that IFN-γ levels were not suppressed by MDSC (data not shown).

MDSC enrichment from NDs yielded preparations that, as expected, contained considerably lower frequencies of MDSC (5–7%). These enriched MDSC preparations, in contrast to those from RTRs, increased both proliferation and IL-10 release providing strong evidence that the suppression observed in RTR preparations is due to the higher numbers of MDSC present (Figure 5D). Activated granulocytes share many of the phenotypic and functional features of CD14\textsuperscript{Neg}-MDSC [12, 26, 27] and provide an \textit{in vitro} model of these cells. The observation that activated granulocytes inhibited both proliferation and IL-10 release (Figure 5D) provides further confirmation that the IL-10 suppression observed using RTR-derived preparations is a functional feature of the MDSC present.

**Fig. 4.** MDSC frequencies within subgroups of the RTRs and CKD patients are shown as scatter plots of CD14\textsuperscript{Neg}-MDSC frequencies, CD14\textsuperscript{Pos}-MDSC frequencies and MDSC/DC ratios. (A) Data for the RTRs subdivided into those with (RTR\textsuperscript{Pos}) or without (RTR\textsuperscript{Neg}) either current or prior SCC. (B) Data for the CKD patients subdivided into those receiving (CKD\textsuperscript{Plus}) or not receiving immunosuppression (CKD\textsuperscript{Nil}). Data from normal donors (NDs) are included where indicated and solid lines indicate median levels for each group of samples. Significant differences between patient subgroups are indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001).

There was no significant difference between males and females with respect to the frequencies/ratios of either DC or MDSC subsets and there was no significant correlation between age and either DC or MDSC frequencies/ratios.

In analysis of RTRs, there was a moderate negative correlation ($R = -0.435$, $P = 0.016$) between time from transplant and MDSC/DC ratio. Although patient numbers precluded statistical analysis, patients with elevated frequencies of both MDSC subsets were observed in all of the immunosuppressive regimen groups. There was no significant correlation between prednisone dose and MDSC frequencies in RTRs although a significant negative correlation ($R = -0.46$, $P = 0.02$) between prednisone dose and CD11c\textsuperscript{Pos}-DC frequencies was observed.

**Discussion**

This study demonstrates, for the first time, that RTRs and SCC patients have significantly elevated circulating levels of functional MDSC and a systemic increase in the circulating MDSC/DC ratio.

DCs are powerful immunoregulators [19, 20] and, as reported previously [21, 22, 24, 28], we found RTRs had significantly decreased DC numbers. SCC tumour tissue has been reported to contain decreased DC numbers [29, 30]. However, we observed no significant changes in the circulating DC frequencies of ICI-SCC\textsuperscript{Pos}, which suggests the reduced DC frequencies in RTRs reflect transplant rather than SCC-associated events. This is supported by the observation, in both this study and previous reports [21], that DC frequencies decreased in association with increasing corticosteroid levels and CKD.

MDSC are powerful suppressors of immune responses [4, 5] and although increased MDSC numbers have been reported in some cancer patients [6–15], their frequencies in either RTRs or SCC patients have not previously been analysed.

In contrast to the majority of MDSC studies, the current study simultaneously enumerated both MDSC subsets. The MDSC frequencies in NDs were both similar to previous reports [6–15] and those of ICI-SCC\textsuperscript{Neg}. In contrast, RTRs, CKD patients and ICI-SCC\textsuperscript{Pos} had significantly elevated levels of CD14\textsuperscript{Neg}-MDSC. However, only RTRs had a concomitant increase in CD14\textsuperscript{Pos}-MDSC. These results suggest that the MDSC subsets are mobilised independently and that, in ICIs, the development of SCC is primarily associated with mobilisation of CD14\textsuperscript{Neg}-MDSC. Comparison of the SCC\textsuperscript{Neg} and SCC\textsuperscript{Pos} subgroups of RTRs, although limited by low numbers, also indicated that differences in SCC status are reflected predominantly by changes in CD14\textsuperscript{Neg} MDSC frequencies. All RTRs received prednisone as part of their immunosuppressive therapy raising the possibility it may mobilise MDSC. However, the observation in RTRs that MDSC frequencies, unlike those of DC, were not significantly correlated with prednisone dosage suggests that changes in MDSC frequencies are not solely the result of differences in corticosteroid levels. The analysis of CKD patients suggests that the presence of reduced renal function does not, by itself, markedly modulate MDSC mobilisation, despite its effect on DC frequencies. It does, however, suggest that exposure to immunosuppressive drugs increases...
mobilisation of CD14\textsuperscript{Neg}-MDSC, although the relative contribution of each drug remains unknown. This question and the possibility that exposure to immunosuppressive therapies is merely a surrogate marker of those CKD patients whose underlying disease has increased MDSC mobilising activity cannot be addressed without performing large prospective studies. Overall, these data suggests that CD14\textsuperscript{Neg}-MDSC mobilisation can occur with both immunosuppressive therapy and SCC development, while CD14\textsuperscript{Pos}-MDSC mobilisation appears to be predominantly induced by transplant-related therapies and/or inflammation [4, 17, 18]. The relative importance of CD14\textsuperscript{Neg}-MDSC and CD14\textsuperscript{Pos}-MDSC in cancer development is not currently known.
Studies on the functional activity of human MDSC have focused predominantly on their ability to suppress autologous T-cell responses [7, 9, 11–13, 16]. In the current study, we demonstrate that, despite their in vivo exposure to differing combinations of immunosuppressive agents and other therapies, all MDSC preparations tested showed some level of immunosuppressive activity. The results also provide the first evidence that human MDSC can suppress IL-10 release. Although commonly regarded as being immunosuppressive, there is now considerable evidence that, depending on the immunological context, inhibition of IL-10 release may not only suppress antitumour responses [31, 32] but also increase MDSC activity [32, 33]. Although these IL-10 results contrast with a previous report [7], that study differed in that it utilised patient-derived T cells and analysed the effect of CD14<sup>pos</sup>-MDSC. The observation in this study that activated granulocytes, which provide an in vitro model of purified CD14<sup>Neg</sup>-MDSC [12, 26, 27], also suppressed IL-10 release further supports IL-10 suppression as a functional feature of granulocytic MDSC.

The MDSC analysed in this study had a variable effect on T-cell proliferation. Other human MDSC studies have also reported similar variation [7, 9, 11–13, 34] possibly reflecting differences in patient exposure to immunomodulatory factors such as medications. In the current study, the MDSC were obtained from patients receiving differing combinations of (i) immunosuppressive therapies which can impair myeloid cell function [35] and (ii) drugs such as antihypertensives which have been shown to inhibit the ability of human MDSC to suppress T-cell proliferation [36]. Although these therapies may impact on in vitro T-cell proliferation assays, it does not preclude the MDSC from affecting other aspects of antitumour immunity or having different activity at a tumour site. Recent data suggests that exposure to the tumour microenvironment may in fact be required to fully up-regulate the suppressor mechanisms and activity of circulating human MDSC [37]. Further larger studies are, therefore, required to determine the in vivo triggers of maximal MDSC activity and the effect of different therapeutic and immunosuppressive protocols on both MDSC numbers and function.

It is well established that RTRs and ICI-SCC have a significantly increased cancer risk [1, 2, 38]. There is also some evidence that CKD patients have an increased cancer risk [39] and, based on studies of other patient groups [40], it is likely that this risk is highest in the subset receiving immunosuppressive therapies. There is now considerable evidence that the recruitment of immunosuppressive cell types promotes tumour progression [3]. The finding in this study that, in RTRs, ICI-SCC<sup>pos</sup> and immunosuppressed CKD patients, there is a significant increase in immunosuppressive MDSC raises the possibility that they may play a role in the increased cancer risk of these patients [1, 2, 38]. Immunosuppressive agents such as cyclosporine do not completely suppress T-cell function [41, 42], and, therefore, the MDSC-mediated inhibition of these activities still has potential functional relevance in RTRs with respect not only to cancer immunosurveillance but also transplant tolerance. The probable involvement of immunosuppressive therapy in at least some aspects of the MDSC mobilisation occurring in RTRs and CKD patients does not diminish the significance of this study’s findings as such therapies remain the cornerstone of these patients’ treatment. It does, however, underline the importance of performing further larger studies to determine the influence of different therapies on both MDSC mobilisation and function.

There is currently no means available for identifying individual RTRs at increased risk of SCC or those whose immunosuppressive treatments could be safely tapered to reduce cancer occurrence and drug toxicities. A recent study has indicated that differences in the frequencies of some circulating immune populations can identify patients with increased SCC risk [43]. The results of the current study raise the possibility that increased MDSC numbers may provide a useful marker of those RTRs with a higher level of functional immune suppression and who are therefore, at increased risk of cancer, but lower risk of transplant rejection.

In summary, the results of this study demonstrate that MDSC numbers are significantly elevated in RTRs and raise the possibility that MDSC may play an important role in suppressing systemic immune responses. Further studies, in larger patient groups, are required to evaluate both the prognostic value and clinical implications of increased MDSC numbers in these patients.

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