Failure of Monocytes of Trauma Patients to Convert to Immature Dendritic Cells is Related to Preferential Macrophage-Colony-Stimulating Factor-Driven Macrophage Differentiation

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Failure of Monocytes of Trauma Patients to Convert to Immature Dendritic Cells is Related to Preferential Macrophage-Colony-Stimulating Factor-Driven Macrophage Differentiation

Asit K. De, Krzysztof Laudanski, and Carol L. Miller-Graziano

Following trauma, increased inflammatory monokine activation and depressed APC function can occur simultaneously. These contradictory monocyte (Mφ) dysfunctions could result if postinjury Mφ differentiation preferentially favored inflammatory macrophage (Mac) differentiation over development into the most potent APC, dendritic cells (DC). In this report, Mφ of trauma patients with a depressed MLR induction capacity are, for the first time, shown to be unable to differentiate in vitro to immature CD14+ DC under the influence of GM-CSF and IL-4. Trauma patient Mφ that retained MLR-inducing capacity had a nonsignificant reduction in DC differentiation capacity. Only patient Mφ populations with depressed differentiation to immature DC (iDC) demonstrated depressed IL-12 and IL-15 production and a continued reduced MLR induction capacity. Neither increased IL-10 production nor decreased CD11c (iDC) demonstrated depressed IL-12 and IL-15 production and a continued reduced MLR induction capacity. Neither increased IL-10 production nor decreased CD11c+ DC precursor numbers correlated with depressed Mφ-to-DC differentiation. Instead, these patients’ APC-dysfunctional Mφ populations had increased expression of inflammatory Mac phenotypes (CD64+, CD86low, HLA-DRlow) and up-regulated secretion of M-CSF. M-CSF combined with IL-6 inhibits Mφ-to-iDC differentiation and promotes Mφ-to-Mac differentiation by down-regulating GM-CSFR expression and increasing DC apoptosis. Both depressed GM-CSFR expression and increased Mφ iDC apoptosis, as well as increased expression of CD126 (IL-6R) and CD115 (M-CSFR), were detected in APC-defective patient Mφ. In vitro addition of anti-M-CSF enhanced the IL-4 plus GM-CSF-induced Mφ-to-DC differentiation of these patients. This suggests that, in trauma patients, enhanced Mφ-to-Mac differentiation with concomitant inhibited iDC development is partially due to increased circulating Mφ sensitivity to and production of M-CSF and contributes to postinjury immunobeamperations. The Journal of Immunology, 2003, 170: 6355–6362.

Any studies have demonstrated that monocyte (Mφ)/macrophage (Mac) dysfunctions are pivotal in the development of the dysregulated inflammatory cytokine production typical of trauma patients who develop multiple organ failure and infectious complications (1–6). Simultaneous with the often-exaggerated inflammatory response, these patients’ Mφ show depressed HLA-DR expression and a failure of Ag-presenting functions, which appears to be correlated with the development of T cell immunosuppression (7–13). The most potent APC is the dendritic cell (DC) (14–18). Consequently, a DC dysfunction might be causal or accompany some of these postinjury APC deficits. Human blood Mφ can differentiate to immature DC (iDC) under the influence of lymphokines like GM-CSF and IL-4 (17–20). These iDC require further maturation by T cell interactions or infectious stimuli to become potent immunostimulatory APC capable of activating naïve T cells and required for memory T cell reactivation and maintenance (19–22). Alternatively, under other cytokine influences and different in vivo environments, Mφ differentiate to Mac, which have higher potential for inflammatory cytokine production but lower expression of MHC class II and costimulatory molecules, are inefficient APC, and are unable to activate naïve T cells (19, 23–25). A failure of Mφ-to-iDC differentiation has been described in immunosuppressed Sezary syndrome and multiple myeloma patients and ascribed to their depressed levels of CD11c dendritic precursors (26–27). The dysregulated Mφ function seen in some trauma patients could also be related to depressed iDC differentiation and increased Mac differentiation. Mφ differentiation to iDC can be redirected to Mac differentiation by IFN-γ, IL-6, M-CSF, LPS, or IL-10 (20, 23–25, 28). LPS and IFN-γ are thought to mediate this Mφ-to-Mac differentiation switch by increasing Mφ IL-6 and M-CSF production, and down-regulating GM-CSFR expression overbalancing any IL-4 plus GM-CSF (4+GM) effect (25). In this study, Mφ of trauma patients with diminished APC function (defined as depressed MLR-inducing capacity and decreased IL-12 production) were assessed for in vitro differentiation to iDC under the influence of 4+GM. Up-regulated expression of CD14 and CD86, increased IL-12 and IL-15 production, as well as DC maturation and MLR induction in allogenic T lymphocyte cultures were also assessed. M-CSF production by MLR-dysfunctional patient Mφ was assessed and found to be elevated. Increased M-CSFR and CD64 expression appeared simultaneously with decreased Mφ GM-CSFR and IL-4R levels in APC-dysfunctional Mφ. Exogenous
addition of Ab to M-CSF during DC generation was assessed for improved patient Mφ-to-iDC differentiation. This study is the first investigation of APC-dysfunctional Mφ populations of trauma patients for an in vitro Mφ-to-iDC conversion defect.

Materials and Methods

Studied population

A total of 46 patients admitted to either University of Massachusetts Medical Center or University of Rochester Medical Center were enrolled in the study. There were 40 patients with serious mechanical trauma (injury severity score (ISS), >17; mean ISS, 38) and 6 patients with thermal trauma (total body surface burn area, >25%). There were 29 males and 17 females. The mean age was 52.3 years. Patient samples were first collected at least 2 days after trauma to reduce variables related to the initial treatment. Then, sample collection was continued one to two times per week until the day the patient was released or died. Appearance of MLR dysfunction for two consecutive assays was required for classification as APC dysfunctional. Patients (n = 6) whose Mφ had a one-time transient depression were included in the APC-responsive group. Some patients’ Mφ data appeared as APC functional at first, and then dysfunctional, as they progressed to a defect over time postinjury. No more than two samples for each patient were included. The exclusion criteria included whole-blood transfusion, immunosuppressive therapy, a history of immunoproliferative disease, or other immunological abnormalities. The clinical pathology of the patients was quantitatively compared with Mφ functions, using elevation in the Marshall multiple organ dysfunction syndrome (MODS) score during the 24-h period for which each blood sample was drawn (29). A Marshall MODS score of >6 represents significant organ dysfunction and clinical pathology (29). A normal control sample was tested along with each patient’s samples. The control samples were collected from a panel of 48 normal donors (volunteers from hospital and laboratory staff) who had been repeatedly assayed over a 12-mo period to establish their immune response levels and variation parameters. Their mean age was 41 years. Informed consent was obtained from all subjects. The study was approved by the University of Rochester Institutional Review Board.

Isolation of T lymphocytes and Mφ

Thirty milliliters of blood was drawn twice weekly from each patient using existing i.v. lines. Simultaneously, a similar amount of blood was drawn from the control’s antecubital vein. PBMC were isolated by centrifugation over a Ficoll-Hypaque gradient and then resuspended in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FBS (HyClone, Logan, UT), 50 U/ml penicillin G, 50 μg/ml streptomycin, 50 μg/ml gentamicin, 2.5 μg/ml fungizone, 4 mM L-glutamine, 1 mM/L sodium pyruvate, and 1% of MEM nonessential amino acids (complete medium). All media were supplemented with 20 U/ml polyomysin B (Calbiochem, La Jolla, CA). After 48-h incubation, the cell viability was >97%. T cells were isolated from PBMC using neuraminidase-treated SRBC as described (30). Mφ were isolated from T cell-depleted PBMC by negative magnetic separation with anti-CD19 beads and anti-CD2 beads according to the manufacturer’s instructions (Dynal, Lake Success, NY). A subsequent flow cytometric analysis revealed ~83% CD33+ cells in Mφ samples and 95% CD3+ positivity in separated T cells. Surface expression of CD126, CD33, IL-4R, and GM-CSFR was detected by fluorescence-activated cell sorting (FACS) analysis. CD11c (clone B-ly6; BD PharMingen), CD33 (clone P67.6; BD PharMingen), CD115 (clone 7-7A3-17; Lab Vision, Fremont, CA), and the endotoxin level was confirmed by the Limulus Ameba Coagulation (LAL) assay (to <0.1 endotoxin units/ml; Endosafe NE, Charles River, North America) before use. IL-12p70 plus p40, IL-10, and IFN-γ were quantified using CBA for 96-well plates (BD Pharmingen, San Diego, CA). IL-10, TNF-α, and IL-6 were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (PeproTech, Rocky Hill, NJ) or left unstimulated in 6-well plates (17, 19). In some experiments, neutralizing Ab for human h(M-CSF (1 μg/ml; goat polyclonal; R&D Systems, Minneapolis, MN) or a goat IgG control (Santa Cruz Biotechnology, Santa Cruz, CA) was added to DC cultures. Cells were incubated for 4–5 days at 37°C under 5% CO2. After 3 days of culture, 1.5 ml of supernatant was removed and replenished with fresh medium containing 10% FBS with 100 IU/ml IL-4 and GM-CSF. The cells were harvested on the fifth day. Supernatants were used for IL-10 evaluation, whereas cells were analyzed with flow cytometry by staining with anti-CD1a, anti-HLA-DR, anti-CD83, and anti-CD68. Apoptosis of DC was measured using Annexin V-FLUOS staining kits according to the manufacturer’s instructions (Roche Molecular Biochemicals, Indianapolis, IN). The Ag-presenting capabilities of cultured Mφ or DC were assessed by activity in MLR and production of immunostimulatory monokines. In the MLR, 1 × 10^5 Mφ or DC were cocultured with 1 × 10^5 cells/well allogenic T cells. The plates were incubated at 37°C under 5% CO2 for 5 days, and then pulsed with 1 μCi/well of [3H]thymidine for 24 h and harvested. The monokine production was evaluated with stimulation with 100 IU/ml IFN-γ and 0.5 μg/ml staphylococcal enterotoxin B (SEB) for 18 h. The supernatants were collected and assessed for IL-12 and IL-15 production by ELISA.

Flow cytometric analysis

Mouse mAb recognizing human CD1a (clone BL6; Coulter, Brea, CA), CD11c (clone B-ly6; BD PharMingen), CD33 (clone P67.6; BD PharMingen), CD83 (clone H115e; BD PharMingen), CD86 (clone 23B1; BD PharMingen), HLA-DR (clone G46-6; BD PharMingen), and CD126 (clone M91; Immunotech, Marseille, France) were used. A rat anti-human CD115 (clone 7-7A3-17; Lab Vision, Fremont, CA) was detected by secondary anti-rat PE-conjugated Ab-defined CD115 (Serotec, Raleigh, NC). A total of 1 × 10^5 cells incubated with conjugated mAb or the appropriate isotype controls in recommended dilutions or in pretested amounts (CD115, CD126) was washed twice with PBS containing 2% FBS and resuspended in 500 μl for two- or four-color analysis after appropriate compensation adjustments. Mφ IL-4R and GM-CSFRe expression was tested by using specific fluorochrome kits (R&D Systems) and analyzed on a Coulter XL or FACS Calibur.

RNase protection assay (RPA)

A total of 1 × 10^5 4+GM-treated DC from controls and patients were treated for 8–10 h with SEB (0.5 μg/ml) plus IFN-γ (100 IU/ml). Total cytoplasmic RNA was isolated using Tri-reagent (Molecular Research Center, Cincinnati, OH). Antisense probes for quantification of RNA for hIL-10, hIL-12 p35 and p40, hIL-15, and hIFN-γ were labeled with [32P]UTP using Riboquant in vitro transcription kit (BD Pharmingen). The band intensities of RPA gels were quantified using NIH Image software and adjusted for loading irregularities by comparison to the ribosomal gene product L32.

ELISA-based cytokine detection assays

Cytokine levels in culture supernatants were measured using cytokine-specific ELISA according to the manufacturer’s guidelines. The lower limits of detection for the assays were as follows: IL-12p70 plus p40, <5 pg/ml; IFN-γ, <2 pg/ml; IL-10, <3 pg/ml (all from Endogen, Woburn, MA); IL-4, <1.25 pg/ml; GM-CSF, <0.27 pg/ml (BioSource International, Camarillo, CA); and M-CSF, <25 pg/ml (R&D Systems).

Statistical analysis

Due to the nonparametric distribution of the studied variables, the primary tests performed were the Kruskal-Wallis H and Mann-Whitney U tests for comparisons of the nonparametric variables. One-way ANOVA with least significant differences test and Student’s t test were performed when the effect of neutralizing Ab on DC generation was studied due to the parametric distribution of the studied variables confirmed by the Lavene test. A regression analysis was computed by the stepwise method. Statistical significance was set at a two-tailed value of p < 0.05. SPSS version 8.0 software was used for all calculations.

Results

Segregation of trauma patient Mφ data on the basis of altered Mφ functions

Our data indicated that the Mφ of severely injured patients, as a group, have depressed MLR levels compared with Mφ of normal...
controls. However, the Mφ responses of the severely injured patient group could be segregated into those with essentially normal MLR induction capacity (high MLR inducing) and those with a dramatically reduced MLR immunostimulatory capacity which was <33% of paired normal control (Fig. 1A). Patient Mφ with low MLR-inducing capacity also had significantly reduced IL-12 production (Fig. 1A) as described previously (7–9). However, patient Mφ retaining high MLR-inducing capacity also maintained IL-12 production (Fig. 1A). Patients whose Mφ had low MLR-inducing capacity also developed T cell anergy as evidenced by a failure of their simultaneously isolated T lymphocytes to proliferate in response to direct TCR stimulation (immobilized anti-CD3 plus anti-CD4) (Table I). As previously described, this T cell anergy was also reflected in a failure to produce IL-4, IFN-γ, or GM-CSF (Table I) (30). Finally, the patients with APC-dysfunctional MLR did not show a significant correlation with their initial ISS. However, patients with low MLR-stimulatory Mφ had significantly increased incidence of clinical pathology as indicated by a mean Marshall MODS score of 8.5, where a score of >6 indicates significant organ failure (p < 0.05; Table II) (29). This suggested that postinjury Mφ AP dysfunction may contribute to trauma patient pathology. Using these Mφ MLR-based segregation categories, patient Mφ with low or high MLR were assessed for Mφ-to-iDC conversion.

Patient Mφ differentiation to iDC

Well-established Mφ AP dysfunction in trauma patients could partially result from failed Mφ-to-iDC differentiation. Other studies of immunosuppressed patients with AP defects identified a reduction in Mφ CD11c+ DC precursors numbers concurrent with increases in more Mac-like Mφ but normal in vitro 4+GM-induced differentiation of Mφ to iDC (26, 27). As seen in Fig. 1B, no decrease in CD11c precursor numbers was detected in populations of trauma patient Mφ that were identified as having low MLR-inducing capacity (APC defect), indicating a normal level of released precursors. However, these low MLR-inducing Mφ did show increased expression of the Mac phenotype marker CD64 (Fig. 1B). This more Mac phenotype of circulating Mφ could indicate an altered Mφ differentiation potential. 4+GM-induced Mφ-to-iDC differentiation was compared among Mφ of trauma patients with low MLR induction, Mφ of trauma patients with high MLR induction, and control Mφ. The differentiation of Mφ to iDC indicated by the acquisition of CD1a expression was significantly depressed only in the low APC-capacity patient Mφ (Fig. 2A).

Development of mDC AP capacity during interaction with allogeneic T cells in MLR

Many of the described normal characteristics of in vitro Mφ-to-iDC differentiation were evident in our trauma patient Mφ culture systems. Five-day in vitro culture of human Mφ with 4+GM induces differentiation only to iDC, not to mDC (18–22). Our 4+GM-cultured Mφ populations showed in controls an iDC expression of CD83 (DC maturation marker) of 1.37% positive (mean) after 5 days of culture, whereas patient 4+GM-cultured Mφ were 5.36% CD83+ positive in MLR-high populations and 2% CD83+ positive in MLR-low populations. Human iDC in allogenic MLR cultures are stimulated by the control allogenic T cells to mature to potent

<table>
<thead>
<tr>
<th>T Cell Function</th>
<th>Control</th>
<th>High Mφ MLR</th>
<th>Low Mφ MLR</th>
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</thead>
<tbody>
<tr>
<td>T cell proliferation (DPM, ×10^3)</td>
<td>17.85</td>
<td>17.69</td>
<td>1.18</td>
</tr>
<tr>
<td>IL-4 production (pg/ml)</td>
<td>12.4</td>
<td>9.3</td>
<td>2.2</td>
</tr>
<tr>
<td>IFN production (pg/ml)</td>
<td>2304.0</td>
<td>1508.0</td>
<td>104.0</td>
</tr>
<tr>
<td>GM-CSF production (pg/ml)</td>
<td>355.5</td>
<td>553.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*The cutoff point for patients’ samples was set up as 33% of the control Mφ MLR values.

Table I. Isolated T cell functions of lymphocytes assayed simultaneously with their Mφ MLR

<table>
<thead>
<tr>
<th>MODS score in patients</th>
<th>High Mφ MLR</th>
<th>Low Mφ MLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODS</td>
<td>5.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

*High MLR vs low MLR, >33% or <33% of control values, respectively.

FIGURE 1. Characteristics of low MLR-inducing Mφ from trauma patients. A, Mφ data from normal controls and trauma patients were segregated based on their ability to induce proliferation of allogenic T cells in an MLR setup, as described in Materials and Methods. (Low MLR-inducing were ≤33% of control value, whereas high MLR inducing were >33% of control value.) The low MLR-inducing Mφ (n = 30; skewness, 2.57 ± 0.43) and high MLR-inducing Mφ (n = 58; skewness, 1.61 ± 0.3) data were compared with control normal Mφ (n = 64; skewness, 1.97 ± 0.3). Mφ from control normals (n = 23; skewness, 2.61 ± 0.48) and trauma patients (n = 12; skewness, 0.3 ± 0.61 for low MLR-inducing Mφ; and n = 16; skewness, 2.31 ± 0.56 for high MLR-inducing samples) were also stimulated with SEB (0.5 μg/ml) plus IFN-γ (100 IU/ml) for 18 h, assessing IL-12 production (tested by ELISA). Median and range are presented. B, Mφ were also assessed for surface expression of CD64 (n = 30 (normal), 19 (high MLR), and 20 (low MLR)) and CD11c (n = 27 (normal control) and 6 and 23 for low MLR-inducing and high MLR-inducing patient Mφ samples, respectively) by flow cytometry and the data are individually expressed as the percentage of positive cells with the median indicated by the bar. *p < 0.05, and **p < 0.001, compared with normal control values. $, p < 0.05, compared with immunostimulatory patient samples.
Mφ OF TRAUMA PATIENTS FAIL TO DIFFERENTIATE TO iDC

Figure 2. Posttrauma low-MLR capacity Mφ fail to differentiate into iDC. A. Fresh Mφ of patients and controls were stimulated with 4+GM (100 ng/ml each) for 5 days and assessed for CD1a expression (n = 66, 61 (high MLR), and 31 (low MLR)) and for subsequent induction of allogeneic T cell proliferation in an MLR (n = 60, 53 (high MLR), and 30 (low MLR)), as described in Materials and Methods. The Mφ of patients were again segregated into low MLR-inducing and high MLR-inducing Mφ based on their ability to stimulate an MLR (low MLR were ≤33% of control value, whereas immunostimulatory were >33% of control value). Then, the 4+GM-treated Mφ were subsequently analyzed by MLR to induce allogeneic T cells proliferation. Median is indicated by the bar. **, p < 0.001, compared with control. #, p < 0.05, compared with immunostimulatory patient samples. B. Correlation of induction of MLR by Mφ to induction by subsequently differentiated iDC. Variance analysis was performed between MLR capacity of fresh Mφ and that of 4+GM-treated Mφ (iDC).

APC (34). Similarly, DC of controls induced a median T cell proliferation in the MLR of 22,845 dpm compared with 1,506 dpm for the MLR cultures of undifferentiated Mφ (Fig. 1A). The high MLR-inducing Mφ of patients that differentiated to iDC showed a similar allogeneic MLR-induced maturation, stimulating 16,057 dpm of T cell proliferation vs 1,208 for the Mφ. In contrast, patient Mφ with low-MLR capacity not only failed to differentiate to CD1a+ iDC but also showed only minimal increases in MLR-inducing capacity when cultured with allogeneic T cells (Fig. 2A). Although the MLR-inducing capacity of patient Mφ was highly correlated with the ability of their 4+GM-cultured Mφ to mature from iDC to highly competent APC in a subsequent allogeneic MLR, there was a greater degree of MLR depression than reduced CD1a expression (Fig. 2B).

Characteristics of APC-dysfunctional patient 4+GM-cultured Mφ

Because the MLR defect of 4+GM-cultured Mφ was greater than the depressed CD1a expression of 4+GM-cultured Mφ, we reevaluated the patient Mφ data on the basis of MLR dysfunction after culture with 4+GM. This data analysis included both Mφ with a dysfunctional differentiation to iDC and those with iDC maturation defects. Using this more inclusive criteria, the difference in CD1a differentiation between the low- and high-MLR-inducing cells of patients was even more striking (Fig. 3A compared with Fig. 2A). A variation in 4+GM-induced CD1a differentiation was observed in the high MLR patient Mφ population, ranging from a low of 50% of control CD1a conversion (illustrated by the Fig. 3A flow cytometry profile) to a high of 110% of control. In contrast, CD1a levels of low MLR-inducing 4+GM-cultured Mφ were uniformly depressed, often almost negative (Fig. 3A). Similarly, the HLA-DR expression of low MLR-inducing iDC failed to increase (Fig. 3B), and the up-regulation of the costimulatory molecule CD86 by these cells was depressed (Fig. 3C) in both the total percentage of positive cells and the ligand expression density (mean fluorescence intensity (MFI)). These data explain some of the reduced MLR capacity of these 4+GM-cultured patient Mφ.

Other immunostimulatory defects of these 4+GM-cultured patient Mφ included a reduced production of IL-12 even after additional stimulation with SEB plus IFN-γ, which should have matured any DC through Toll-like receptor 2 activation (Fig. 4A). IL-15 levels were also reduced in this system (data not shown). This reduced IL-12 and IL-15 production was also detectable at the mRNA level and was not the result of reduced viability, because the housekeeping ribosomal protein gene L32 was unaffected (Fig. 4B).

Possible contributors to the failure of trauma patients to develop DC with potent APC function

4+GM-induced Mφ-to-DC differentiation can be altered by numerous microenvironmental changes (28). Although increased IL-10 production inhibits Mφ-to-DC differentiation, low-MLR capacity 4+GM-cultured patient Mφ had reduced IL-10 at both the mRNA (Fig. 4B) and protein levels (Fig. 4C). Increased DC apoptosis during differentiation could contribute to a DC differentiation defect. In fact, the low MLR-inducing 4+GM-cultured Mφ of patients did have significantly increased apoptosis compared with controls after a 5-day culture period. However, the patient Mφ with high MLR-inducing capacity also showed increased apoptosis during 5-day culture (Fig. 4C). Decreased GM-CSF stimulation can decrease Mφ-to-iDC differentiation (25). Although all cultures had similar exogenous GM-CSF added, the expression of the GM-CSFR on patient Mφ with low-MLR capacity was significantly reduced, perhaps reducing efficiency of the GM-CSF induction (Fig. 5). IL-4 is necessary to drive Mφ to iDC (19). A reduced ratio of IL-4 to GM-CSF can result in a failure of Mφ to convert to iDC in a timely fashion (25, 35, 36, 37). Again, although added IL-4 concentrations were constant, the levels of Mφ IL-4R expression were significantly reduced on low MLR-capacity Mφ.

Finally, the differentiation of human Mφ to iDC is finely balanced against their differentiation to activated Mac (21, 23–25). M-CSF drives Mφ differentiation to Mac and inhibits GM-CSFR expression and iDC differentiation (19, 23–25). Other cytokines like IL-6, which up-regulate the M-CSFR (CD115), cooperate with M-CSF to increase Mφ-to-Mac differentiation (23–25). Patient Mφ with low or high DC MLR induction capacity were compared with control Mφ for production of M-CSF and expression of both the IL-6R (CD126) and the M-CSFR (CD115). The median expression of CD115 of control Mφ was 5.1% positive (Fig. 6A and Table III). Patients whose 4+GM Mφ had induced higher levels of proliferation in the MLR had a median CD115 expression of 3.8% positive. In contrast, 40% of low DC MLR Mφ were CD115 positive. Interestingly, the Mφ that showed low DC MLR function had similar CD126 expression (44.1% positive) to that of control.
Mφ (33.4% positive), but those trauma patient Mφ that could differentiate into iDC with high MLR-inducing capacity had down-regulated their CD126 expression (19.5% positive). The difference in the Mφ M-CSF production of the two patient groups was the most dramatic. Normal and patient Mφ that could differentiate to potent DC APC produced significantly lower levels of M-CSF than did the patient Mφ that failed to differentiate to MLR-inducing DC (Fig. 6B). Mφ production of M-CSF and their response to M-CSF seemed to highly influence eventual DC differentiation. Addition of Ab to M-CSF into 4+GM patient Mφ cultures improved both their differentiation to CD1a⁺ iDC and their subsequent maturation to APC in allogenic MLR (Fig. 6C).

**Discussion**

These data represent the first reexamination of the Ag-presenting function of Mφ of posttrauma patients in several years and the first identification of a DC differentiation defect in trauma patients. Our data indicate that trauma patient Mφ that are unable to support allogenic T cell proliferation in an MLR response are unable to differentiate into CD1a⁺ iDC under the influence of exogenous 4+GM. Unlike some previously detected patient DC defects, the posttrauma iDC defect does not result from a loss of circulating CD11c⁺ DC precursors, nor is it a universal result of injury. Patients whose Mφ can function as APC in allogenic MLR also possess the capacity to differentiate to iDC. The CD1a⁺ iDC phenotype was not developed in 4+GM-cultured Mφ of patients identified as MLR dysfunctional, nor was increased Mφ CD86 or HLA-DR expression seen. The 4+GM-cultured patient DC maturation in response to subsequent SEB (assessed by increased IL-12 and IL-15 production) also failed to occur in the MLR-dysfunctional Mφ group, as did further DC maturation in an allogenic MLR subsequent to 4+GM cultures. Elevated in vivo IL-6 levels combined with high M-CSF levels favor Mφ differentiation to Mac over Mφ differentiation to DC (23, 25, 27, 38). In vitro culture of human Mφ with M-CSF differentiates them to Mac (19, 25, 39). The APC-competent and APC-dysfunctional trauma patient Mφ groups did not differ in their IL-6 levels (data not shown).

However, elevated IL-6 levels are produced by numerous cell types in the postinjury microenvironment and would influence Mφ-to-Mac differentiation in APC-dysfunctional patient Mφ that do not down-regulate their CD126 (IL-6R) expression (40, 41). The failure of APC-dysfunctional Mφ to decrease CD126 expression would increase their sensitivity to elevated circulating IL-6 levels postinjury.

In this study, patient Mφ with low immunostimulatory capacity had increased Mac-like phenotype characteristics, such as increased CD64 expression, increased production of M-CSF, increased expression of the M-CSFR (CD115), and down-regulated GM-CSFR expression (31, 42, 43). A postinjury increase in myeloid M-CSFR expression has also been recently reported in a
FIGURE 4. Altered functions of the 4+GM-treated low MLR Mφ of patients. A, 4+GM-induced Mφ from patients (n = 14, low-MLR capacity, and n = 21, high-MLR capacity samples) and control samples (n = 36; two outliers are >2500) were assessed for IL-12 production (tested by ELISA) after stimulation with SEB (0.5 μg/ml) plus IFN-γ (100 IU/ml) for 18 h. Data represent individual patient values with the median shown as a bar. *, p < 0.05, compared with control. B, 4+GM-treated Mφ (1 × 10^6 cells) were stimulated for 8–10 h in the presence of SEB (0.5 μg/ml) plus IFN-γ (100 IU/ml). Total cytoplasmic RNA was isolated and mRNA levels for IL-12, IL-15, and IL-32 (loading control) were assayed by multiprobe RPA. The data are representative of three experiments. C, Supernatants of 4+GM-cultured Mφ of patients (n = 18 (low MLR-inducing samples) and 28 (high MLR-inducing samples)) and controls (n = 47) were tested for 10-IL levels by ELISA, while the cells were assessed for apoptosis by staining with Annexin V-FITC and analyzed by flow cytometry. ***, p < 0.001, compared with normal control median value. Ranges are indicated. Skewness in low MLR IL-10 data: 2.41 ± 0.54; skewness in Annexin-FITC samples, 1.14 ± 0.69.

mumine burn model (2). Increased M-CSFR and decreased GM-CSFR expression are also characteristics of CD34+ stem cells that shifted from DC to Mac differentiation after M-CSF plus IL-6 culture (23). Increased circulating CD64+ inflammatory Macs have been previously described in injury, and these more inflammatory Mac have altered function (41, 44–46). We have previously reported that trauma patient Mφ with high CD64 expression produce exaggerated amounts of the 26-kDa, cell-associated form of TNF-α with increased TNF-α mRNA stability (1, 47). A recent report describes hM-CSF-differentiated PBMC Mac as producing higher TNF levels than Mφ because of their increased TNF mRNA stability. These data additionally suggest a shift toward a more Mac-like phenotype for circulating, aberrant Mφ populations of trauma patients (48). Finally, both Mφ-to-iDC differentiation and the subsequent MLR-inducing capacity were improved if anti-M-CSF was added to patient Mφ 4+GM cultures, supporting the contribution of M-CSF to the DC defect of the patients. However, additional M-CSF effects on DC function, besides blocking Mφ-to-iDC differentiation, have been described. Appropriate DC MHC class II expression is also altered by M-CSF exposure (38). Consequently, the iDC MLR increases after anti-M-CSF treatment could reflect improved MHC class II levels as well as improved Mφ-to-iDC differentiation.

The data in this study do not address the full component of DC APC defects or mechanisms inducing APC dysfunction that could be occurring postinjury. The 4+GM Mφ-derived iDC population represents both future interstitial DC and future Langerhans DC depending on received maturation signals (15, 19). Although human PBMC Mφ-derived DC appear to be potent APC, the subsets may have different capacities for T lymphocyte activation (15, 18, 31, 34). Consequently, the detected difference in MLR-inducing capacity may be related to undetected shifts in patient DC subsets. TGFβ matures Langerhans like DC. Mφ TGFβ levels are known to be increased in these immunoberrant trauma patients, possibly increasing Langerhans like DC (46). In two preliminary experiments, no increase in CD207 (the Langerhans marker) was detected on low MLR-capacity patient Mφ, but these data are far from conclusive. A posttrauma increase in plasmacytoid or lymphoid-derived DC precursors could greatly affect overall DC APC capacity (15, 16, 49). The plasmacytoid DC population is suggested to preferably induce regulatory T cells (15, 16). However, our isolation technique selects only for myeloid DC, and therefore,
FIGURE 6. The posttrauma DC differentiation defect in low MLR-inducing Mφ correlates with increased sensitivity to and production of M-CSF. A, Low and high MLR-inducing Mφ from patients and controls were assessed for expression of CD115 (M-CSFR) and CD126 (IL-6R) by flow cytometry. Flow cytometry gates were set according to the isotype controls. The data are representative of 10 experiments. B, Fresh Mφ from patients (n = 12 (low MLR-inducing) and 11 (high MLR-inducing samples)) and controls (n = 25) were stimulated with IL-4 plus GM-CSF (100 ng/ml each) for 5 days and the cells were assessed for M-CSF production (tested by ELISA) after stimulation with SEB (0.5 μg/ml) plus IFN-γ (100 IU/ml) for 18 h. C, Neutralizing Ab to M-CSF (1 μg/ml) or isotype control was added to patient Mφ (n = 12) at the beginning of 4+GM treatment (5 days) followed by assessment of CD1a expression as well as MLR activity. Data are expressed as mean percent increases in MLR activity or CD1a expression. *, p < 0.01, compared with normal control value.

the detected posttrauma defect is in the myeloid Mφ DC population. Our examination of isolated patient Mφ also does not address the in vivo T lymphocyte contribution to intensifying or prolonging a postinjury APC dysfunction. Diminished T cell IL-4 and/or IL-13 production due to postinjury T cell apoptosis would further intensify any in vivo Mφ-to-iDC defect.

A defect in iDC-to-mDC development may also be occurring. There was a small (<10%) population of CD1a+ DC generated in the 4+GM-cultured Mφ of some APC-dysfunctional patients. The APC defect was more apparent in the allogenic T cell MLR cultures of these patients’ iDC (Fig. 2B). Consequently, a DC maturation defect may also contribute to postinjury APC dysfunction. A recent report showed that septic but not simple trauma patients have increased in vivo levels of DC apoptosis in their lymph nodes, suggesting that the interdigitating DC may fail to respond to T cell maturation and/or survival signals (50). No difference in apoptosis levels between APC-competent and APC-dysfunctional patient Mφ during 4+GM cultures was detected. Both were increased. However, we did not assess apoptosis during the subsequent MLR. Increased apoptosis of the low MLR-capacity iDC populations during the MLR could have contributed to the more significant level of APC defects detected in this second culture period (Fig. 2B).

The most significant difference seen in the low MLR-inducing patient Mφ population and the high MLR-inducing patient Mφ was their production of M-CSF and expression of M-CSFR. The failure of low MLR-inducing patient Mφ to differentiate to iDC most closely paralleled their expression of more Mac-like characteristics such as increased CD64*, high M-CSFR expression, reduced GM-CSFR expression, low APC function, low IL-12 production, and high M-CSF production (31, 42). Our previous publications, as well as those of others, suggest that posttrauma immunoaberrant Mφ have increased Mac-like characteristics (44–47). The partial amelioration of the Mφ-to-iDC differentiation defects in these low APC-capacity Mφ of trauma patients after anti-M-CSF treatment suggest preferential Mac differentiation. The known posttrauma microenvironment contains increased circulating IL-6, PGE2, and gut-released LPS that can alter Mφ receptor expression and preferentially induce M-CSF, thereby interfering with Mφ-to-iDC differentiation while favoring inflammatory Mac development.

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References


production, but interleukin-12 therapy after injury restores resistance to infection. 


