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Innate Immunity in Multiple Sclerosis: Myeloid Dendritic Cells in Secondary Progressive Multiple Sclerosis Are Activated and Drive a Proinflammatory Immune Response[1]

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Multiple sclerosis (MS) is postulated to be a T cell-mediated autoimmune disease characterized clinically by a relapsing-remitting (RR) stage followed by a secondary progressive (SP) phase. The progressive phase is felt to be secondary to neuronal degenerative changes triggered by inflammation. The status of the innate immune system and its relationship to the stages of MS is not well understood. Dendritic cells (DCs) are professional APCs that are central cells of the innate immune system and have the unique capacity to induce primary immune responses. We investigated circulating myeloid DCs isolated directly from the blood to determine whether there were abnormalities in myeloid DCs in MS and whether they were related to disease stage. We found that SP-MS subjects had an increased percentage of DCs expressing CD80, a decreased percentage expressing PD-L1, and an increased percentage producing IL-12 and TNF-α compared with RR-MS or controls. A higher percentage of DCs from both RR and SP-MS patients expressed CD40 compared with controls. We then investigated the polarization effect of DCs from MS patients on naive T cells taken from cord blood using a MLR assay. Whereas DCs from RR-MS induced higher levels of Th1 (IFN-γ, TNF-α) and Th2 (IL-4, IL-13) cytokines compared with controls, DCs from SP-MS only induced a polarized Th1 response. These results demonstrate abnormalities of DCs in MS and may explain the immunologic basis for the different stages and clinical patterns of MS. ***The Journal of Immunology,*** **2006, 177: 4196–4202.**

Dendritic cells (DCs)[3] are professional APCs that have a unique capacity to induce primary T cell responses and to polarize naive T cells (1). DCs are important in regulating both immunity and tolerance (2–4). Therefore, DCs may play a central role in the induction, perpetuation, or prevention of autoimmune diseases (5).

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS white matter of putative cell-mediated autoimmune pathogenesis directed against myelin Ags (6, 7). In addition, a Th1-type bias exists in MS with increased production of IFN-γ, IL-12, IL-17, IL-18, and IL-23 (8–12). Clinically, MS usually begins as a relapsing-remitting (RR) illness after which it often then enters a chronic progressive stage. The reason for this change is not well understood. One contributing factor may be degenerative processes in the nervous system that are triggered by inflammation. In addition, studies have suggested that immune abnormalities are more prevalent in the progressive as opposed to the RR stage (8, 9, 11, 12). The basis for these immune abnormalities are unknown.

Previous studies of DCs in MS showed that patients with MS have higher levels of IFN-γ-, TNF-α-, and IL-6-secreting monocyte-derived DCs than healthy subjects (13) and have increased chemokine receptor CCR5 expression on blood myeloid DCs (14). To date, functional studies of myeloid DCs directly isolated from the peripheral blood of patients with MS have not been reported. Studies on directly isolated DCs provide the unique opportunity to directly characterize the status of a major component of the innate immune system in MS. In this study, we investigated the profile of myeloid ($CD11c^+$) DCs that were taken directly from the blood of patients at different stages of MS and their effect on the polarization of naive $CD4^+$ T cells.

Materials and Methods

Patients with MS from the Partners Multiple Sclerosis Center at the Brigham and Women's Hospital were included in the study. After informed consent, blood samples from 85 patients with MS and 26 healthy controls were studied. Among the patients, 62 had a course of RR and 23 of secondary progressive (SP). Blood was only taken from donors with no history or signs of infection and who had normal complete blood counts. We examined for potential gender bias by comparing results between males and females in all groups and found no difference between males and females in any of the groups tested. All patients were untreated. The untreated patients had not received steroid treatment for 3 mo before testing, IFN-β for 14 mo before testing, and had never been treated with glatiramer acetate, methotrexate, cyclophosphamide, or mitoxantrone (Table I). The RR-MS patients were primarily chosen from a pool of patients before the initiation of therapy.

Cell separation

PBMCs were isolated from heparinized venous blood by centrifugation over Ficoll-Hypaque (Pharmacia LKB Biotechnology). Cells were resuspended (2×10^6 cells/ml) in complete culture medium comprise of RPMI 1640 medium supplemented with 10% FBS, 4 mM L-glutamine, 25 mM

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[3] Abbreviations used in this paper: DC, dendritic cell; MS, multiple sclerosis; RR, relapsing-remitting; SP, secondary progressive; HC, healthy control; MFI, mean fluorescence intensity; PD-L1, programmed death ligand-1.

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Table I. *Subject population*

Group	No.	Female: Male	Age (years ± SD)	EDSS[a] (±SD)
RR-MS	62	52:10	41.3 ± 10.4	1.2 ± 1.1
SP-MS	23	15:8	53.2 ± 11.4	5.2 ± 2.1
Healthy controls	26	13:13	44.9 ± 12.5	

[a] EDSS, Expanded disability status scale.

HEPES buffer, 50 U/ml penicillin, and 50 μg/ml streptomycin (all from BioWhittaker).

Flow cytometry and ELISA

PBMCs were stained in U-bottom 96-well plates (Costar) with a mixture of FITC-conjugated mouse mAbs directed at human CD3, CD14, CD16, CD19, CD20, CD56 (lin 1), or appropriate isotype control Abs, and PE-conjugated mouse mAb directed at human CD11c or isotype control (both from BD Biosciences). The surface molecules HLA-DR, CD80, CD86, CD40, CD83, and their isotype controls were stained by CyChrome-conjugated mouse mAb directed at these molecules (BD Pharmingen). Staining for PD-L1 was conducted using a biotinylated mouse anti-human PD-L1 (provided by G. J. Freeman) or its isotype control and CyChrome-conjugated streptavidin (BD Pharmingen). Intracytoplasmic staining was done as described before (11), and PBMCs were incubated overnight in complete culture medium with monensin (3 μM; BD Pharmingen) alone or with 1, 10, or 100 ng/ml rIFN-γ (R&D Systems). The concentration and length of incubation time with monensin were titrated to allow maximum intracytoplasmic staining of the cytokines being studied without inducing cell death as measured by trypan blue staining.

After washing, the cells were stained to define the DCs as mentioned above, fixed with paraformaldehyde (Electron Microscopy Sciences), and stained by intracytoplasmic staining with allophycocyanin-conjugated anti-human IL-12 (p40/p70) or anti-human TNF-α mAb. Similarly, the intracytoplasmic detection of cytokines in naive $CD4^+$ T cells after MLR was done using PE-conjugated Abs directed to human IFN-γ, IL-4, and IL-13, and FITC-conjugated Abs directed to human CD4. In all intracytoplasmic staining, the cells were incubated in Perm/Wash buffer (BD Pharmingen). All ELISA reagents were from R&D Systems.

MLR and T cell cytokine measurement

Naive $CD4^+CD45RA^+$ T cells isolated from the umbilical cord blood of one donor (BioWhittaker) were divided into aliquots and kept frozen (−80°C, 10% DMSO in FCS). These cells (4×10^4/well) were cultured for 7 days with irradiated (3300 rad) fresh $CD11c^+$ DC (1×10^4/well) that were sorted using BD Vantage cell sorter (sorting purity, >95%). After 7 days, the cultured cells were counted and restimulated with plate-bound 1 mg/ml anti-CD3 mAb and 5 mg/ml anti-CD28 mAb in the presence of monensin (3 mM; BD Pharmingen) overnight for the single-cell cytokine analysis by flow cytometry, or without monensin for 72 h for analysis of cytokine secretion of IFN-γ, TNF-α, IL-4, IL-13, and IL-10 by array-based ELISA (15).

Calculations and statistical analysis

Results are presented as mean ± SD for each group. Statistical significance was calculated using Student's *t* test.

Results

Identification of $CD11c^+$ DCs

To identify $CD11c^+$ DCs for our investigations, we used a mixture of FITC-conjugated anti-human CD3, CD14, CD16, CD19, CD20, and CD56 (lin) plus a PE-conjugated anti-human CD11c Ab. As shown in Fig. 1*a*, the $CD11c^+$ DCs were identified as those cells that stained brightly for CD11c and were negative or dimly positive for the lin FITC Ab mixture. The $CD11c^+$ DCs were gated (box in *upper left corner* of Fig. 1*a*) and studied both for surface markers and expression of cytokine production. An example of staining for the surface expression of PD-L1 is shown in Fig. 1*b*. Intraindividual variability in the markers we studied were found to be <15% between consecutive measurements in three healthy controls and three patients with MS.

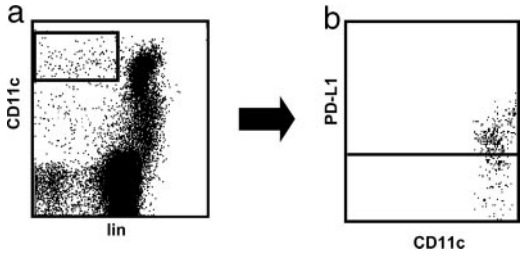

FIGURE 1. Flow cytometry analysis of myeloid DCs. *a*, PBMC were stained with a mixture of FITC-conjugated anti-human CD3, CD14, CD16, CD19, CD20, CD56 (lin), and PE-conjugated anti-human CD11c. After gating on live mononuclear cells in the forward- and side-scatter plot, the myeloid DCs were defined as bright for CD11c and negative or dimly positive for the mixture (gated on *upper left*). *b*, To measure expression of surface molecule such as HLA-DR, CD80, CD86, CD40, PD-L1, and/or the intracytoplasmic production of the cytokines IL-12 and TNF-α, these molecules were stained with a third flourochrome, and the level of expression/production was measured after gating only on the DC population. An example of staining for the surface expression of PD-L1 is shown.

$CD11c^+$ DCs in SP-MS have increased expression of CD40 and CD80 and decreased expression of PD-L1

$CD11c^+$ DCs from patients with MS and healthy controls were studied using flow cytometry to evaluate the state of activation as measured by MHC class II expression and the expression of costimulating molecules. We studied the surface expression of HLA-DR, costimulatory molecules: CD80, CD86, CD40, and PD-L1, and the DC maturation molecules, CD83 on $CD11C^+$ DCs. Fig. 2*a* demonstrates a representative profile of the surface expression of these molecules on circulating $CD11c^+$ DCs in a healthy individual, a patient with RR-MS and a patient with SP-MS. In the $CD11c^+$ DC population in healthy individuals, HLA-DR is expressed on a high proportion (>90%) of circulating $CD11c^+$ DCs, CD80, CD40, and PD-L1 is expressed on a low proportion (<20%) of $CD11c^+$ DCs, CD86 is experienced on a variable proportion (20–70%) of cells, and $CD11c^+$ DCs are negative for the maturation marker CD83. As shown in Fig. 2*b*, in MS subjects we found significantly higher percentages of $CD11c^+$ DCs expressing CD80 in patients with SP-MS (19.1 ± 8.6%) compared with patients with RR-MS (7.4 ± 1.7%; $p = 0.025$) and with healthy controls (9.9 ± 5.7%; $p = 0.041$). Similar mean fluorescence intensity (MFI) of positive CD80 signal was found between the controls, RR-MS, and SP-MS groups (54.8, 58.8, and 57.6, respectively). The percentages of $CD40^+$ DCs were significantly higher in SP-MS (16.4 ± 2.3%) and RR-MS (15.9 ± 2.4%) compared with healthy controls (6.6 ± 1.8%; $p = 0.036$ and $p = 0.032$, respectively). Similarly, there were significant differences in the MFI of positive CD40 between the RR-MS group (101.1 ± 41.0) or SP-MS group (116.6 ± 48.6) and the healthy controls group (53.7 ± 29.9; $p = 0.026$ and 0.022, respectively). Furthermore, significantly lower percentages of $CD11c^+$ DCs expressing PD-L1 were detected in patients with SP-MS (3.4 ± 1.1%) compared with patients with RR-MS (9.3 ± 2.4%; $p = 0.032$) and with healthy controls (12.2 ± 3.6%; $p = 0.022$). The differences in the MFI of positive PD-L1 were found to be reduced in patients with SP-MS (52.5±16.2) compared with patients with RR-MS (128.9 ± 58.5) and with healthy controls (119.8 ± 49.7), but these differences did not reach statistical significance ($p = 0.07$ and $p = 0.06$, respectively). No significant differences were found in the percentages or MFI of HLA-DR or CD86 expressing DCs. Thus, MS patients had increased expression of costimulating molecules associated with Th1-type responses (CD80, CD40) and decreased expression of

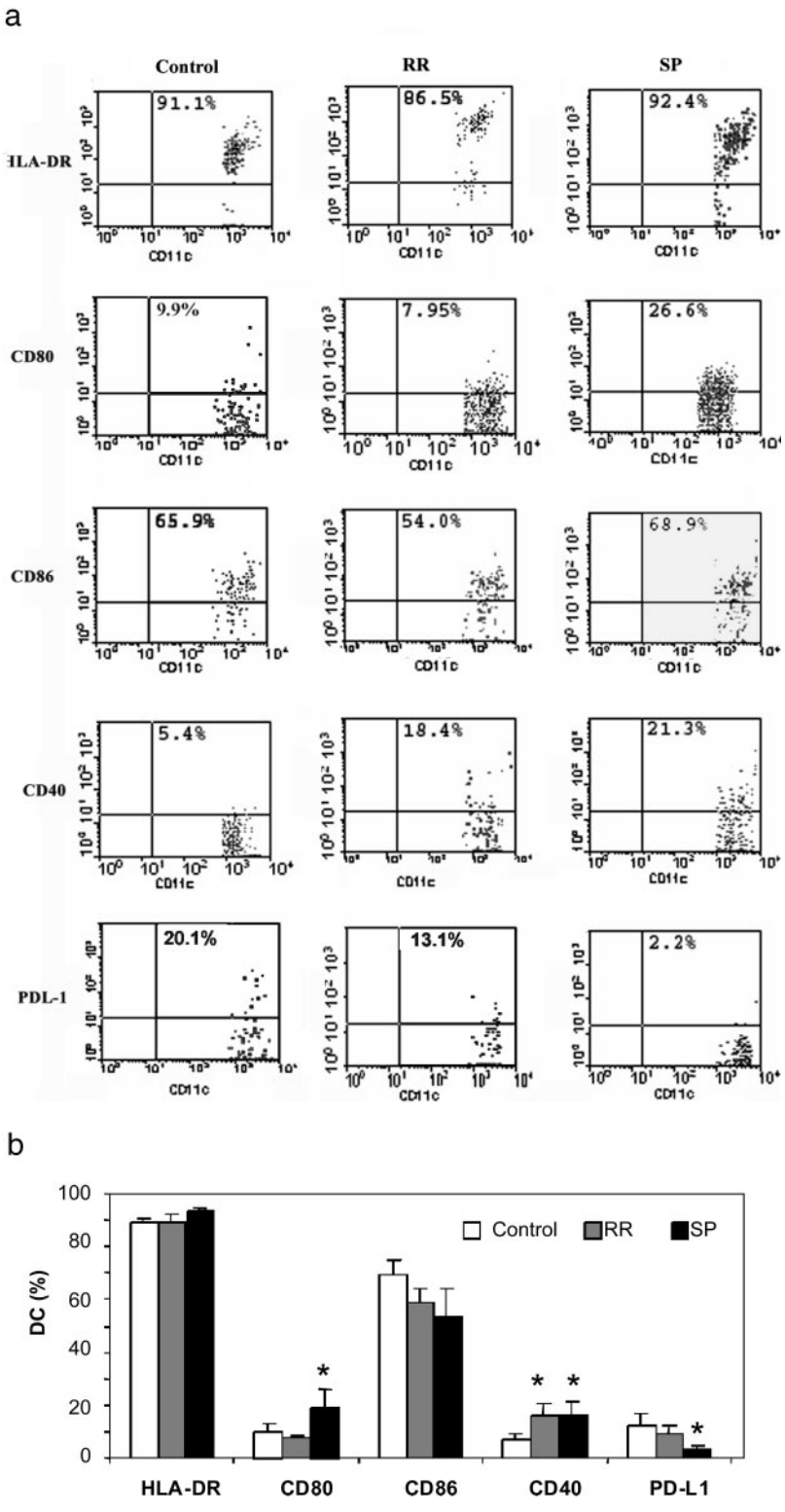

FIGURE 2. Expression of HLA-DR, CD80, CD86, CD40, PD-L1, and CD83 on CD11c$^+$ DCs in healthy controls and MS subjects. *a*, Representative expression of HLA-DR, CD80, CD86, CD40, PD-L1, and CD83 on CD11c$^+$ DCs in a healthy individual, a patient with RR-MS, and a patient with SP-MS as measured by flow cytometry. *b*, DCs from patients with SP-MS express significantly higher levels of CD80 and lower levels of PD-L1 compared with DCs from RR-MS and healthy controls: ∗, (SP) CD80 vs RR, 0.025; (SP) CD80 vs control, 0.041; (SP) PD-L1 vs RR, 0.032; (SP) PD-L1 vs control, 0.022. CD40 was expressed at significantly higher levels on DCs from both RR-MS and SP-MS vs healthy controls: ∗, RR CD40 vs control, 0.032; SP CD40 vs control, 0.036.

PD-L1, an inhibitory costimulating molecule. Of note, there was no expression of CD83 on CD11c$^+$ cells in either MS patients or healthy controls. In addition, there were no differences in the absolute number of CD11c$^+$ DCs per 1 μl of blood between the groups (healthy controls, 1.32 ± 0.59; RR-MS, 1.61 ± 0.76; SP-MS, 1.39 ± 0.055), or in the percentage of CD11c$^+$ DCs in the total PMBC population between groups (healthy controls, 0.56 ± 0.18%; RR-MS, 0.55 ± 0.18%; SP-MS, 0.60 ± 0.021%).

Increased percentage of IL-12- and TNF-α-producing DCs in SP-MS

To investigate the functional characteristics of CD11c$^+$ DCs in MS, we measured cytokine production using single-cell cytokine analysis by flow cytometry. We investigated IL-10, IL-12, TNF-α, and IFN-γ production by unstimulated CD11c$^+$ DCs taken directly from the bloodstream. Fig. 3*a* demonstrates a representative

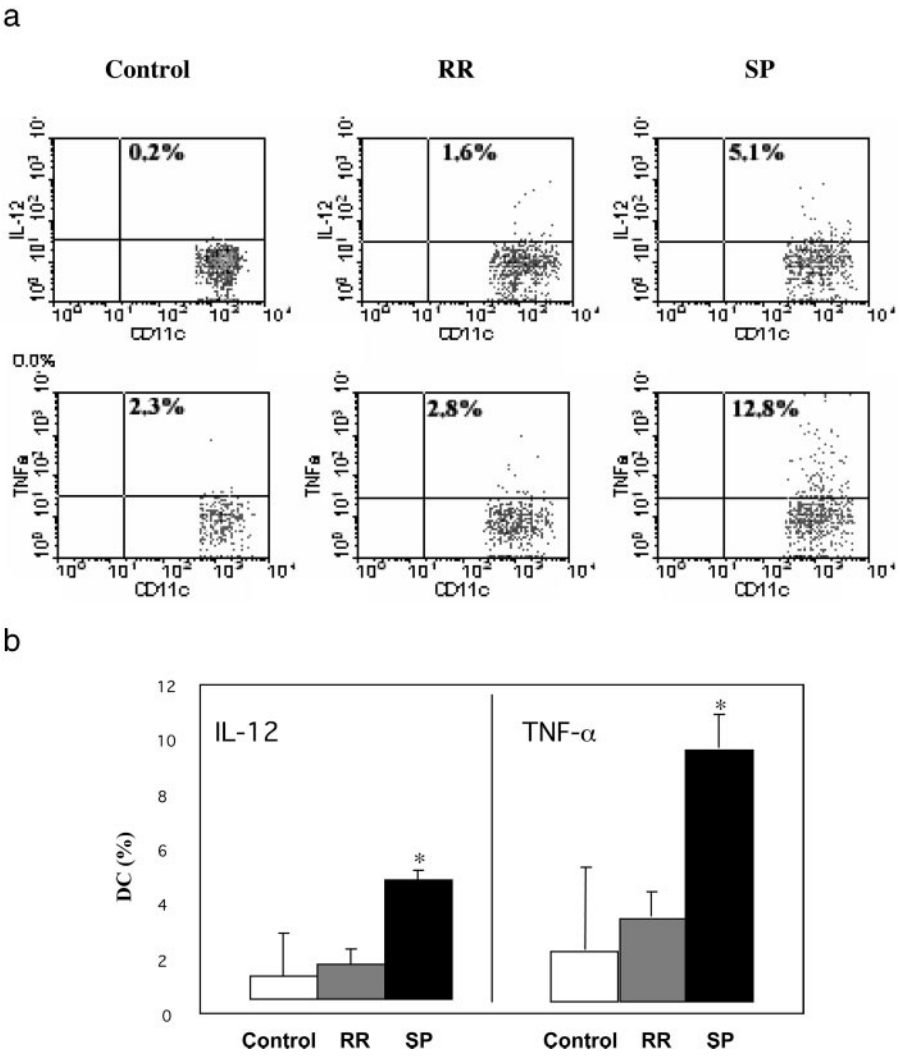

FIGURE 3. Percentage of IL-12- and TNF-α-producing CD11c$^+$ DCs in healthy controls and MS subjects. CD11c$^+$ DCs that were positive for CD11c$^+$ and negative or dimly positive for lineage markers (see Fig. 1) were analyzed by the intracytoplasmic staining for detection of IL-12 or TNF-α after overnight culture with monensin without stimulation. *a*, Representative intracytoplasmic staining of IL-12 and TNF-α in CD11c$^+$ DCs in a healthy individual patient with RR-MS and a patient with SP-MS as measured by flow cytometry. *b*, There were increased percentages of DCs, which expressed IL-12 and TNF-α in SP-MS compared with RR-MS or controls: for IL-12: SP-MS vs RR, $p = 0.037$; vs control, $p = 0.045$; for TNF-α, SP-MS vs RR, $p = 0.014$; vs control, $p = 0.021$.

profile of cytokine staining for circulating CD11c$^+$ DCs in a healthy individual, a patient with RR-MS, and a patient with SP-MS. We found that the percentage of unstimulated CD11c$^+$ DCs that produced IL-12 and TNF-α was higher in patients with SP-MS (4.4 ± 0.5 and 9.2 ± 1.2%, respectively) compared with patients with RR-MS (1.3 ± 0.7%, $p = 0.037$; and 3.05 ± 1.0%, $p = 0.014$, respectively) and with healthy controls (0.8 ± 1.7%, $p = 0.045$; and 1.8 ± 1.5%, $p = 0.021$) (Fig. 3*b*). IL-10 and IFN-γ could not be detected in CD11c$^+$ DCs even after stimulation with LPS and IFN-γ.

DCs from subjects with MS polarize naive T cells toward proinflammatory T cells

To characterize the functional properties of DCs from MS subjects, we examined the differentiation of naive T cells in an allogeneic MLR using DCs from MS patients. Naive CD4$^+$CD45RA$^+$ T cells isolated from human umbilical cord blood were cultured for 7 days with irradiated DCs from patients with MS or from healthy controls. After 7 days, T cells were stimulated with anti-CD3 and anti-CD28 and the DC polarized CD4$^+$ T cells were analyzed for single-cell cytokine production by flow cytometry. As shown in Table II, there was an increased percentage of cells producing IFN-γ and a decreased percentage of cells producing IL-13 in CD4$^+$ T cells that were primed with DCs from SP-MS patients (TDC-SP) vs healthy controls (TDC-HC). In CD4$^+$ T cells that were primed with DCs from patients with RR-MS, like SP-MS, there was an increased percentage of cells producing IFN-γ vs controls, but unlike SP-MS, there was also an increase of T cells producing IL-13. If we compared SP-MS with RR-MS, there was an increased percentage of IFN-γ-producing T cells and lower percentages of IL-4 and IL-13.

Based on the differential effects we observed in Table II, we calculated the ratio of the percentages of cells that produced the

Table II. *Percentage of cytokine-producing T cells following priming of naive T cells by DCs from patients with MS*[a]

DC Used for Priming	Percentage of Cytokine-Producing T Cells		
	IFN-γ	IL-13	IL-4
Control	10.0 ± 1.0	8.4 ± 1.9	2.4 ± 2.4
RR	18.2 ± 2.1[b]	13.9 ± 2.7[c]	7.6 ± 1.4[d]
SP	27.6 ± 2.6[e]	2.9 ± 1.3[f]	2.1 ± 1.2

[a] Naive CD4$^+$CD45RA$^+$ T cells isolated from human umbilical cord blood were cultured for 7 days with irradiated DCs from patients with MS or from healthy controls. After 7 days, T cells were stimulated with anti-CD3 and anti-CD28, and the DC polarized CD4$^+$ T cells were analyzed for single-cell cytokine production by flow cytometry. Results are expressed as percentage of cytokine-producing T cells ± SD. Subjects studied were RR-MS ($n = 14$), SP-MS ($n = 7$), and controls ($n = 8$).

[b] $p = 0.037$ vs control.

[c] $p = 0.033$ vs control.

[d] $p = 0.047$ vs control, $p = 0.039$ vs SP-MS.

[e] $p = 0.011$ vs control, $p = 0.042$ vs RR-MS.

[f] $p = 0.038$ vs control, $p = 0.019$ vs RR-MS.

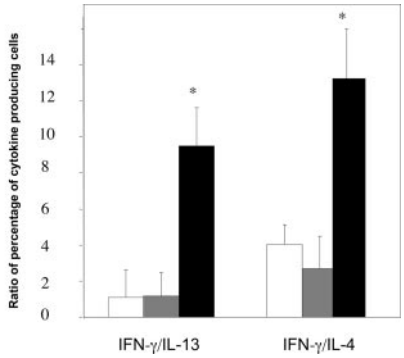

FIGURE 4. Ratio of percentage of cytokine-producing cells and cytokine secretion following priming of naive T cells by DCs from patients with MS. Cord blood naive ($CD4^+CD45RA^+$) T cells from the same donor were incubated (primed) with irradiated DCs from patients with MS or from healthy controls in a MLR for 7 days. $CD4^+$ T cells were then stimulated with anti-CD3/anti-CD28 Abs, and the production or secretion of cytokines was measured using flow cytometry and intracytoplasmic staining for cytokine production. The ratio between the percentages of T cells producing IFN-γ (a Th type-1 cytokine) vs IL-13 and vs IL-4 (Th type-2 cytokines) is higher after priming with DCs from SP-MS compared with those that were primed with DCs from RR-MS and from healthy controls.

proinflammatory cytokine (IFN-γ) vs those that produced anti-inflammatory cytokines (IL-13 and IL-4) in our groups. As shown in Fig. 4, there was a marked increase in the ratio of cells producing IFN-γ vs IL-13 and in the ratio of cells producing IFN-γ vs IL-4 in SP-MS vs controls. For IFN-γ:IL-13 ratios, SP = 9.5 ± 2.1, HC = 1.1 ± 1.5, and RR = 1.2 ± 1.3 ($p = 0.01$, SP vs HC and RR). For IFN-γ:IL-4 ratios, SP = 13.2 ± 2.4, HC = 4.1 ± 0.6, and RR = 2.4 ± 0.9 ($p = 0.03$, SP vs HC, and $p = 0.02$, SP vs RR). There was no difference between RR and controls in these ratios.

We then measured the pattern of secreted cytokines from T cells that were differentiated by MS DCs. This allowed us to assess TNF-γ, IL-2, and IL-10 in addition to IFN-γ, IL-4, and IL-13. As shown in Table III, both TDC-SP and TDC-RR secreted increased amount of IFN-γ, TNF-α, and IL-2 compared with TDC-HC, and there was an increased secretion of IFN-γ and TNF-α from TDC-SP vs TDC-RR. In terms of the Th2 cytokines IL-4 and IL-13, the secretion of IL-13 was increased in TDC-RR compared with TDC-HC and with TDC-SP, and IL-13 from TDC-SP was lower than from control. TDC-RR secreted increased levels of IL-4 compared with TDC-HC and TDC-SP. Interestingly, the secretion of IL-10, which is considered a regulatory cytokine but in humans may be associated with IFN-γ-secreting cells, was increased both in TDC-RR (92.3 ± 21.2 pg/ml, $p = 0.002$) and TDC-SP (62.3 ± 15.3 pg/ml, $p = 0.013$) compared with TDC-HC (8.3 ± 3.6 pg/ml) (Table III).

When we compared the ratio of secreted proinflammatory cytokines (IFN-γ and TNF-α) vs anti-inflammatory cytokines (IL-4 or IL-13), similar results were observed as was found in Fig. 4 with a bias toward Th1-type responses especially in SP-MS and in RR-MS only for the ratio of IFN-γ:IL-4 (data not shown).

Discussion

We investigated the profile and function of myeloid DCs directly isolated from the peripheral blood of MS patients. We found changes in both RR- and SP-MS compared with healthy controls and different immune profiles in SP- vs RR-MS subjects with more pronounced changes in SP patients. Whereas both RR and SP patients had increased expression of CD40, DCs from SP-MS had an increased percentage of DCs expressing CD80 and a lower percentage expressing PD-L1 compared with RR-MS. In addition, a higher percentage of DCs from SP-MS isolated directly from the blood produced IL-12 and TNF-α compared with RR or healthy controls.

In functional studies, we found that naive T cells primed with DCs from both RR and SP patients produced increased amounts of IFN-γ, TNF-α, and IL-2, although there was more IFN-γ produced in SP- than RR-MS. Thus, DCs from both RR and SP patients polarized T cells to produce more Th1-type cytokines. However, in contrast, the production of IL-4 and IL-13 by naive T cells primed by DCs from MS patients markedly differed between RR and SP patients. We found that RR patients produced significantly increased amounts of IL-13 and IL-4 compared with both healthy controls and SP patients.

The costimulatory molecules CD40 and CD80 are implicated in the induction of immunity mediated by type 1 Th cells (Th1) (16, 17). Furthermore, CD40 is known to provide a major signal for production of IL-12 and TNF-α in DCs and cell lines (17, 18) and in MS CD40 was found to be related to the increased production of IL-12 and IL-18 (11, 12). CD80 can be involved in the exacerbation of experimental autoimmune encephalomyelitis, the animal model of MS (19) and is reported to correlate with disease

Table III. *Cytokine secretion patterns of T cells following primary of naive T cells by DCs from patients with MS*[a]

DC Used for Priming	IFN-γ	TNF-α	IL-2	IL-4	IL-10	IL-13
Control	10.2 ± 33	22.7 ± 45	7.7 ± 5	24.2 ± 5.1	8.3 ± 3.6	454.6 ± 81
RR	148.4 ± 55[b]	314.1 ± 77[c]	37.9 ± 8[d]	45.3 ± 9.1[a]	92.3 ± 21[f]	1181.5 ± 111[g]
SP	243.5 ± 76[h]	425 ± 92[i]	36.1 ± 13[d]	22.7 ± 5.3	62.3 ± 15[j]	183 ± 102[k]

[a] Naive $CD4^+CD45RA^+$ T cells isolated from human umbilical cord blood were cultured for 7 days with irradiated DCs from patients with MS or from healthy controls. After 7 days, T cells were stimulated with anti-CD3 and anti-CD28, and the DC polarized $CD4^+$ T cells were analyzed for cytokine production by ELISA. Results are expressed as picograms per milliliter ± SD. Subjects studied were RR-MS ($n = 14$), SP-MS ($n = 7$), and controls ($n = 8$). Values are expressed as picograms per milliliter.

[b] $p = 0.002$ vs control.
[c] $p = 0.001$ vs control.
[d] $p = 0.022$ RR MS vs. control; 0.026 SP-MS vs control.
[e] $p = 0.036$ vs control; 0.033 vs SP.
[f] $p = 0.002$ vs control.
[g] $p = 0.001$ vs control; $p = 0.001$ vs SP-MS.
[h] $p = 0.001$ vs control; $p = 0.039$ vs RR-MS.
[i] $p = 0.001$ vs control.
[j] $p = 0.013$ vs control.
[k] $p = 0.042$ vs control.

severity and duration of MS (20). CD80 can indirectly induce secretion of cytokines such as IL-12 and TNF-α via induction of CD40L expression on T cells, which in turn will further activate the DCs to produce these cytokines (21). Programmed death ligand-1 (PD-L1) is a recently discovered inhibitory costimulatory molecule and blockade of the programmed death 1-programmed death ligand pathway worsens experimental autoimmune encephalomyelitis (22, 23). We found decreased percentages of DCs expressing PD-L1 in patients with SP-MS. All together, DCs from SP-MS exhibit a proinflammatory profile of costimulatory molecules. Furthermore, we found that a higher percentage of DCs from SP-MS spontaneously produce IL-12 and TNF-α. This is the first report of increased IL-12 production in MS from unstimulated cells. This finding most likely relates to the increased expression of CD80 and decreased expression of PD-L1 on DCs of SP-MS (21).

MS is a chronic disease with a postulated autoimmune pathogenesis. The reason for its onset and continuation is not completely clear, but it is felt that one of the major mechanisms is an immune-mediated attack directed against brain white matter tissue (24). The existence of autoreactive T cells against brain white matter molecules in healthy individuals renders the hypothesis of defective central tolerance (negative selection) less likely than a hypothesis that involves dysregulation of peripheral tolerance (25–28). We show here that DCs from patients with MS are more mature and polarize naive T cells to secrete higher levels of cytokines compared with DCs from healthy controls. In RR-MS, the cytokine profile includes both proinflammatory and anti-inflammatory cytokines, which may explain the immunological substrate that leads to a RR clinical course. Thus, even though DCs from RR-MS patients have increased expression of CD40 and induce naive T cells to secrete increased amounts of IFN-γ, there is a compensatory increase in the production of IL-4, IL-13, and IL-10. This increase disappears when patients enter the progressive stage.

MS begins as a RR disease that involves clinical attacks followed by recovery. In over half of patients, the RR pattern transitions into a progressive course, with progressive worsening in the absence of attacks. The factors associated with this change are not well understood. One of the major hypotheses related to this change is that inflammation in the nervous system that occurs during the RR phase triggers degenerative changes that affect axons and that the SP phase is related to immune-independent degenerative processes with the CNS (29). Our observations demonstrate that major changes occur in the peripheral immune compartment of MS patients when the disease transitions to the progressive stage and provide a new explanation for the progressive phase of the disease. Thus, the transition to the progressive phase is associated with a change in the phenotype and functional properties of myeloid DCs, which contributes to the progressive nonremitting course of this phase of the disease. This may be associated with a more diffuse inflammatory process in the brain and activation of microglial cells (30). We did not find a subset of RR patients that had the DC phenotype of SP-MS from the beginning of their disease. Thus, it does not appear that those who switch from RR to SP have a different DC population right from the beginning of their disease. However, ours was a cross-sectional study. To definitively establish that the transition from RR to SP is associated with a change in DC will require a longitudinal immunological analysis of individual RR patients over a 5- to 15-year period, Such a study is underway at our MS Center as part of our CLIMB natural history study (31).

There are different types of human DCs defined by cytokine-driven conditions in vitro (32). In addition to myeloid DCs that we describe here, plasmacytoid DCs may be relevant in MS. Although they express much lower levels of MHC and costimulatory molecules and are less effective simulators of T cells, they have been reported to skew toward Th2 responses in the presence of IL-3 (33, 34) and to induce $CD4^+CD25^+$ regulatory T cells (35) after being activated by CpG oligodeoxynucleotides. Furthermore, plasmacytoid DCs are the most abundant source of type I IFN after activation of viruses (36, 37). This may be relevant to MS given the evidence that viral activation may trigger MS relapses (38) as does systemic administration of IFN-γ (39).

In experimental autoimmune encephalomyelitis, it is known that there is intracerebral recruitment and maturation of DCs both at the onset and during the progression of the disease (40) and that DCs permit immune invasion of the CNS (41). Epitope spreading in both an autoimmune and viral model of MS appears to be initiated in the CNS (42) and may contribute to local activation and expansion of pathogenic cells (43). Our observation of activated myeloid DCs in the blood of SP-MS patients demonstrates that chronic DC activation occurs both in the periphery and CNS in MS, and because these cells most likely migrate to the CNS, they reflect chronic CNS inflammation and can play a role in both peripheral and central activation of the immune system in MS.

In summary, our findings provide new information regarding the innate immune system in MS patients. It is known that T cells play a central role in the earlier stages of the disease process, because blocking of T cell entry into the brain in RR-MS with anti-VLA-4 Ab markedly decreases MS attacks and CNS inflammation as measured by gadolinium enhancing lesions (38). Our results suggest that the SP form of MS may have a T cell-independent component and that therapies that target myeloid DCs and the innate immune system may be effective in this stage of the disease.

Disclosures

The authors have no financial conflict of interest.

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