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The generation and properties of human M2-like macrophages: potential candidates for CNS repair?

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Abstract

Regulation of the immune response seems to be a promising strategy for a successful central nervous system (CNS) repair, and macrophages are considered to be prospective candidates for cell therapy. Using low serum conditions we generated human anti-inflammatory M2-like macrophages from peripheral blood monocytes and compared these cells (termed Mφ3) with "standard" pro-inflammatory Mφ1 and anti-inflammatory Mφ2, generated in the presence of GM-CSF and M-CSF. We focused primarily on the differences in T-cell stimulatory activity and production of various cytokines, chemokines, and growth factors. Low serum conditions had no negative impact on macrophage yield, the largest of which was for Mφ3. We showed that Mφ3 more closely resembled Mφ2 than Mφ1. Mφ2 and particularly Mφ3, but not Mφ1 expressed relatively low levels of CD86 and failed to stimulate T-cell proliferation. In contrast to pro-inflammatory Mφ1, unstimulated Mφ3 produced significantly lower levels of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-18, IL-12) and Th1/Th2-cytokines (IFN-γ, IL-2, IL-4) coupled with a higher IL-10 level. Moreover, concentrations of IL-1β and pro-inflammatory chemokines IL-8 and MCP-1 in Mφ-3 supernatants were lower not only when compared to Mφ1, but also to Mφ2 cultures. Like Mφ1 and Mφ2, Mφ3 was capable of producing neurotrophic- (BDNF, IGF-1), angiogenic- (VEGF), and other growth factors (EPO, G-CSF, FGF-basic, EGF) with neuroprotective and regenerative activity. In fact, IGF-1 production by Mφ-3 exceeds secretion of this factor by Mφ-1 and Mφ-2 by more than 25 fold. Thus, generated Mφ-3 represented M2-like macrophages with high regenerative potential.

Keywords: macrophage polarization, cytokines, chemokines, growth factors, CNS repair

Introduction

Following injury to the nervous system, the activation of the immune system profoundly affects the ability of neurons to survive and to regenerate damaged axons. The role of immune response is controversial. It has long been established that immune cells in the CNS can cause or augment tissue injury. However, recent investigations show that immune cells and their factors can contribute to neuroprotection and neuroregeneration. This dual role of the immune system is determined by the type and duration of the immune response and the balance between destructive and protective factors that ultimately define the net result of the neuro-immune interaction [5].

The immune system operates via innate (antigen-independent) and adaptive (antigen-specific) immunity. Inflammatory responses during traumatic injury or different CNS diseases are dominated by cells of the innate immune system, most importantly resident microglia and blood-borne macrophages. After phagocytosing cellular debris, microglia/macrophages present antigens to lymphocytes, thereby activating the antigen-specific immune response [33].

Unlike most other systems, the central nervous system has a limited capacity for regeneration. While the inhibitory effects of proteoglycans and myelin on axonal growth have been well established, the role of neuroinflammation in regeneration failure remains highly controversial [6]. Several studies have demonstrated the beneficial effects of macrophages (Mφ) following injury [23,25,27,37]; however, others revealed that macrophages promoted injury [9,19].

One of the possible explanations of these diverse macrophage effects could be connected with the differences between the macrophages used. Certainly, Mφ are remarkable for the heterogeneity and diverse biological activities [11]. There are at least two distinct functional Mφ subsets that are triggered in response to different stimuli: classical pro-inflammatory and nonclassical anti-inflammatory macrophages, also termed type 1 (M1) and type 2 (M2) macrophages. M1 are induced by IFN-γ, either alone or in concert with a microbial stimulus, possess high antigen-presented activity, and support Th1 response. These cells are involved in pro-inflammatory responses, mediate resistance to intracellular pathogens and anti-tumor resistance and are tissue destructive. In contrast, various forms of M2, generated in the presence IL-4 or IL-13, immune complexes, IL-10, etc., are not efficient at antigen presentation, suppress Th1 and/or favor Th2 response, and produce high levels of matrix-associated proteins. These cells are tolerogenic and generally oriented toward resistance to parasites, immunoregulation, tissue remodeling and repair, and tumor promotion [20,10,18]. It is important to note that macrophages can reversibly shift their functional phenotype in response to changes in their microenvironment. Sequential treatment of macrophages with multiple cytokines results in a progression through various functional phenotypes. That is, macrophages may progress from one functional phenotype to another [32,21].

Recently, Kigerl et al has shown that in CNS injury rapidly induced M1 response than shift to M2 response. M1 were neurotoxic, whereas M2 promoted a regenerative growth response in adult sensory axons, even in the context of inhibitory substrates that dominated sites of CNS injury (e.g., proteoglycans and myelin). The authors concluded that switching macrophages toward an M2 phenotype could promote CNS repair while limiting secondary inflammatory-mediated injury [14]. Thus, boosting or modulating the immune response seems to be a promising strategy for successful CNS repair.

Since macrophages may be prospective candidates for cell therapy, the development of simple and reproducible technologies of M2-like macrophage generation seems to be a necessary step for the clinical application of this approach. For human monocytes GM-CSF treatment leads to the formation of Mφ1 macrophages with features of pro-inflammatory M1 cells, while the equivalent population following culture in M-CSF has been termed Mφ2 macrophages with features of M2 anti-inflammatory cells [34,35]. In addition, macrophages that ingest apoptotic cells are shown to decrease pro-inflammatory and acquire anti-inflammatory properties [8]. Utilizing of M2-like macrophages in experimental models and clinical trail was successfully demonstrated by the Michel Schwartz group [27,16]. Recently we developed a simple approach for generation of non-classical type2-like macrophages (Mφ3) in the presence of GM-CSF in serum-deficient conditions. The purpose of the current study was to compare the phenotype and functions of these Mφ3 with "standard" pro-inflammatory Mφ1 and anti-inflammatory Mφ2 subsets, generated in the presence of GM-CSF and M-CSF.

Materials and Methods

Isolation and generation of macrophages

Human blood samples were obtained from healthy donors with informed consent according to the policy approved by the local Ethical Committee. Human peripheral blood mononuclear cells (PBMCs) were obtained through density gradient centrifugation (Ficoll-Paque, Sigma-Aldrich) of heparinized whole blood samples. For monocyte separation PBMCs were plated at 3–5 x10^6/ml in tissue culture dishes (TPP, Switzerland) in RPMI-1640 (Sigma-Aldrich) with 5% FCS (Biolot, Russia) for 18 h and then washed to remove non-adherent residual lymphocytes. The percentage of CD14-positive cells was demonstrated by flow cytometry analysis to be greater than 90–93% of the total cells recovered.

Classical type-1 macrophages (Mφ1) were generated by culturing adherent cells in six-well tissue plates (Nunclon, Denmark) in RPMI-1640 supplemented with 5% autologous plasma, 2% FCS, 0.05 mM 2-mercaptoethanol, 2 mM sodium pyruvate, 0.3 mg/ml L-glutamine (all reagents of Sigma-Aldrich), 1% nonessential amino acids, 100 μg/ml gentamycin and 50 ng/ml recombinant human GM-CSF (R&D Systems) at 37°C with 5% CO2 for 7 days. Non-classical type 2 macrophages (Mφ2) were obtained in identical culture conditions in complete RPMI-1640 supplemented with rhM-CSF (50 ng/ml; R&D Systems). Non-classical type 3 macrophages (Mφ3) were generated by incubation of monocytes in serum growth factors deficiency conditions. Specifically, adherent cells were cultured for 7 days in complete RPMI-1640 supplemented with 2% autologous plasma (without FCS) and 50 ng/ml rhGM-CSF. Polarized Mφ (Mφ1, -2, -3) were harvested by using EDTA in Hanks' balanced salt solution, washed and counted.

Flow cytometry analysis

For evaluation of the Mφ phenotype, cell suspensions were incubated for 20 min at 4°C with fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated antibodies specific for human CD14, CD86, CD90, and HLA-DR or isotype controls. All monoclonal antibodies were obtained from BD Biosciences (USA). After incubation with antibodies, cells were washed with PBS containing 0.1% sodium azide (Sigma-Aldrich) and 0.1% bovine serum albumin, and were then analyzed with a FACSCalibur using CellQuest software (BD Biosciences).

T-cell proliferation assays

The antigen-presenting and allostimulatory activity of Mφ was determined by measuring T-cell proliferation in the mixed lymphocyte culture (MLC). Different types of Mφ

were collected after generation and $1x10^5$ cells were then plated in RPMI-1640 supplemented with 0.3 mg/ml L-glutamine, 5 mM HEPES buffer, 100 µg/ml gentamycin and 10% inactivated donor serum (AB (IV) group), and added to $1x10^6$ allogeneic responder PBMCs. All cultures were carried out in triplicate in round-bottom 96-well tissue culture plates, in a final volume of 150 µl of RPMI complete medium. T-cell proliferation was assessed after 5 days by adding [^{3}H]thymidine (1 µCi/well) for 18 h. Cells were then harvested and thymidine incorporation was measured in a liquid scintillation counter SL-30 (Intertechnic, France). The stimulatory capacity of Mφ in MLC was expressed by the stimulation index (SI) = cpm in MLC (PBMCs+Mφ) / cpm in control culture (PBMCs alone).

Cytokines, chemokines, and growth factor measurements

Culture supernatants of generated Mφ (Mφ1, -2, -3) were collected and stored at –80°C prior to measurement. The concentration of secreted cytokines/chemokines was determined by using the Bio-Plex Protein Array System (kits and equipment of Bio-Rad, USA based on Luminex xMAP technology; sensitivity 2 pg/ml) in the case of TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, IFN-γ, IL-8, MCP-1, and MIP-1β, and by using ELISAs from Diagnostic System Laboratories for insulin-like growth factor-I (IGF-I, sensitivity 0.01 ng/ml); from BioSource for basic fibroblast growth factor (FGF-basic, sensitivity 7 pg/ml); from R&D Systems for brain-derived neurotrophic factor (BDNF, sensitivity 20 pg/ml); from Invitrogen Corp. for vascular endothelial growth factor (VEGF, sensitivity 5 pg/ml); from Protein Contour (St-Petersburg, Russia) for erythropoietin (EPO, sensitivity 4 pg/ml) and epidermal growth factor (EGF, sensitivity 2 pg/ml); and from Vector-Best (Novosibirsk, Russia) for IL-18 (sensitivity 5 pg/ml).

Statistical analysis

Statistical analysis was performed using the STATISTICA software version 6.0 (StatSoft. Inc., USA). The Mann-Whitney non-parametric two-tailed U test was used to determine the significance of data, which are presented as median and inter-quartile range (IQR). Values of $p < 0.05$ were considered statistically significant.

Results

Characterization of generated Mφ

We generated three distinct Mφ subsets in vitro from peripheral blood monocytes and performed a series of parallel comparisons between them. As a first step, we measured cell yield and their phenotype. The number of Mφ1 and Mφ2 obtained from $1x10^6$ PBMCs was $3.35x10^4$ (IQR $2.2–7.4x10^4$) and $2.50x10^4$ (IQR $1.4–4.5x10^4$), whereas Mφ3 yield was significantly higher — $5.0x10^4$ (IQR $3.3– 0.4x10^4$, pU<0.01), indicating that a low serum condition increased the quantity of macrophages generated in the presence of GM-CSF.

After 7 days of culture, the majority of Mφ1, Mφ2, and Mφ3 were adherent cells with a classical "fried egg" morphology (data not shown) that expressed CD14 on their cell surface (Table 1). A small number of adherent cells had a stretched, spindle-like morphology (fibroblast-like cells). The average number of these cells in Mφ1 (n=8) and Mφ2 (n=8) populations was similar and constituted 25% (IQR 22–45 and 16.5–33.5%, respectively), and was slightly higher (Median 32.5%, IQR 17–43%, n=6) in the Mφ3 subset. However, the expression of CD90 antigen (a typical marker for a fibroblasts and mesenchymal stem cells) in all Mφ populations was low and the percentage of CD90+ cells did not exceed 2–3%.

Percentage of positive cells						
Marker	Mφ1		Mφ2		Mφ3	
	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N
CD14	78 (70–84)	17	87 (78–91)	9	82 (67–92)	25
HLA-DR	97 (91–98)	21	96 (96–98)	9	87 (73–97)	17
CD86	37 (23–53)	18	27 (15–39)	13	23 (11–58)	17
CD90	2.5 (0–5.0)	10	2.0 (0–5.0)	13	3 (0.6–5.0)	8

Table 1: Phenotype Mφ1, Mφ2 and Mφ3 subsets

All three Mφ populations strongly expressed the HLA-DR antigen, though the percentage of HLA-DR positive cells in the Mφ3 cultures was lower than in the Mφ1 and Mφ2. All types of monocyte-derived macrophages also expressed the CD86 antigen. The mean number of CD86+ cells in Mφ2 and Mφ3 was lower than in Mφ1, though not significantly.

The ability of Mφ to induce T-cell proliferation

The revealed differences of HLA-DR and CD86 expression in distinct Mφ populations could influence their antigen-presenting function. To determine whether Mφ1, Mφ2, and Mφ3 differed quantitatively in their capacity to present antigen, we tested and compared their ability to induce an allogeneic T-cell response. For this purpose distinct Mφ subsets derived from the same donor were cocultured with allogeneic PBMCs over a period of 5 days, and the T-cell proliferation was determined (Table 2).

Culture		Mφ1 (n=24)	Mφ2 (n=24)	Mφ3 (n=24)
PBMCs alone	Median	330	140	370
	IQR	105–720	105–410	70–1300
PBMCs + Mφ (10:1)	Median	7380	3130 **	2070 ** #
HLA-DR	IQR	3500–13220	1600–3680	330–3230
Stimulation index	Median	19.6	14.8	3.4 ** ##
CD90	IQR	14.9–74.5	6.2–35.3	1.4–13.7

*Mφ (1x105 cells) were cultured with 1x106 allogeneic PBMCs over 5 days. 3[H]-thymidine (1 µCi/well) was added 18 h before harvesting to measure T-cell proliferation (cpm). The stimulation index is expressed in calculated units (cpm in MLC (PBMCs+Mφ) / cpm in control culture (PBMCs alone). ** pU < 0.01 vs Mφ1; # pU < 0.05 and ## pU < 0.01 vs Mφ2.*

Table 2: The stimulatory effect of Mφ1, Mφ2 and Mφ3 subsets on allogeneic T-cell proliferation

Analysis of [^{3}H]thymidine incorporation revealed a strong proliferative response in PBMCs cocultured with Mφ1, whereas weak proliferation could be observed in PBMCs cocultured with Mφ2 or Mφ3. Remarkably, the T-cell stimulatory capacity of Mφ3 expressed by the stimulation index (SI) was significantly lower than that of Mφ1 and Mφ2.

Generated Mφ differ in cytokine and chemokine production

To further characterize the secretory profile of generated Mφ subsets, we measured the production of Th1/pro-inflammatory (IFN-γ, IL-2, IL-1β, TNF-α, IL-12, IL-17, IL-18, IL-6) and Th2/anti-inflammatory cytokines (IL-4, IL-10, IL-13). Cytokine levels were measured in supernatants of 7-day cultures of Mφ1, Mφ2 and Mφ3. Mφ1 spontaneously produced considerable levels of IL-1β, IL-6, TNF-α, IFN- γ, IL-4, and IL-17 (Table 3). This finding confirms the pro-inflammatory nature of Mφ1 and their capacity for T-cell activation. Mφ2 were characterized by lower secretory activity for some of these cytokines, though the differences were significant only for IL-4 and IL-18. In contrast, Mφ3 displayed remarkably decreased basal levels of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-18), Th1-cytokines (IFN-γ, IL-2), and IL-4. Mφ3 also differed from Mφ1 by a 2-fold lower IL-12 production and more pronounced production of IL-10, though not significantly. In addition to cytokines, we measured the levels of various inflammatory chemokines in the supernatants of unstimulated macrophages. Generated Mφ constitutively produced high levels of IL-8, MCP-1, and MIP-1β. Mφ1 and Mφ2 demonstrated similar levels in their production. In contrast, secretion of neutrophil-attracting IL-8 and monocyte-attracting MCP-1 by Mφ3 was significantly lower than by Mφ1 and Mφ2. However, the production of T-cell attracting MIP-1β by Mφ3 did not differ from that by Mφ1 and Mφ2. Together, these data confirm the pro-inflammatory nature of Mφ1 and significantly less pro-inflammatory activity of Mφ3.

Production of growth factors by generated Mφ

All three types of unstimulated macrophages secreted detectable concentrations of erythropoietin, G-CSF, FGF-basic, BDNF, and IGF-1 (Table 4). Mφ1 and Mφ2 produced analogous levels of these growth factors, although there was a strong tendency to higher production of EPO by Mφ2. Despite the decreased production of pro-inflammatory cytokines, Mφ3 secreted concentrations of G-CSF, EPO, FGF-basic and

Cytokines& chemokines (pg/ml)	Mφ1 (n=10) Median	IQR	Mφ2(n=10) Median	IQR	Mφ3 (n=24) Median	IQR
IFN-γ	872	734–995	839	539–1010	626 * ↓	440–830
IL-2	154	115–154	115	70–155	72 *↓	47–115
IL-1β	405	246–670	313	150–790	195 * # ↓	68–290
TNF-α	175	124–282	148	55–224	99 * ↓	51–156
IL-12	28	20–29	19	7–25	14	3–33
IL-17	308	245–483	257	177–448	214	112–427
IL-18	33	29–51	27 * ↓	16.5–31.2	19 * ↓	15.7–35.8
IL-6	21340	13430–27340	20350	8380–25060	10900 * ↓	4110–21770
IL-4	215	198–246	119 ** ↓	79–141	106 ** ↓	53–190
IL-10	5	2–10	2	2–2	15	2–60
IL-13	78	37–113	48	37–78	78	42–112
IL-8	90380	74280–93340	67400	57940–94430	44320 ** ## ↓	29150–59000
MCP-1	11140	5680–14000	11910	4160–17660	3345 ** ## ↓	1100–4460
MIP-1β	1 960	1250–5590	1 560	930–2700	2220	790–7620

** $p_U < 0.05$ and ** $p_U < 0.01$ vs Mφ1; # $p_U < 0.05$ and ## $p_U < 0.01$ vs Mφ2.*

Table 3: Cytokine/chemokine concentrations secreted by Mφ1, Mφ2, and Mφ3

Growth factors (pg/ml)	Mφ1 (n=10) Median	IQR	Mφ2(n=10) Median	IQR	Mφ3 (n=24) Median	IQR
G-CSF	670	505–1610	730	315–2310	430	180–1050
EPO	19.2	1.7–36.9	46.5	33.8–81.1	34.9	21.5–56.5
FGF-basic	104	57–124	150	87–180	109	45–126
EGF	207	148–331	283	245–420	138	38–310
BDNF	392	187–705	438	215–739	131 * # ↓	78–235
IGF-1	322	170–8560	152	116–459	8310 * ## ↑	520–9500
VEGF (n=6)	5.0	5.0–97	92.8 * ↑	59.2–298	422.4 * # ↑	107.7–524.7

** $p_U < 0.05$ and ** $p_U < 0.01$ vs Mφ1; # $p_U < 0.05$ and ## $p_U < 0.01$ vs Mφ2.*

Wilcoxon matched non-parametric paris test was used to determine the significance of VEGF..

Table 4: Growth factors production by Mφ1, Mφ2 and Mφ3

EGF comparable with Mφ2, though significantly lower concentration of BDNF. But the most prominent difference was revealed for the production of IGF-1, which was much higher in Mφ3 in comparison with Mφ1 and Mφ2 cultures. Concerning VEGF, its detectable concentrations in 7-day cultures were determined only in a quarter of tested donors. Among these cultures VEGF was predominantly produced by Mφ2, and especially by Mφ-3, but not Mφ1.

Discussion

Over the last decade, there has been an increasing interest in the role of the inflammatory reaction in CNS injury. Moreover, this interest has focused on the dominant cell type observed during inflammation, the macrophage. However, in the CNS the contribution of these cells to the healing process remains questionable [6].

The contradictory data regarding the contribution of Mφ to CNS recovery could be explained by diverse macrophage activities, many of which appear to be oppositional in nature. The destructive potential of macrophages in CNS pathology may be caused by pro-inflammatory activity, whereas their regenerative capacity may be linked with anti-inflammatory features [12].

In the search for macrophages with potential regenerative activity we developed a simple method for the generation of macrophages in growth factor deficient conditions and analyzed the phenotype and functional activity of these macrophages, termed Mφ3, with pro-inflammatory Mφ1 and anti-inflammatory Mφ2. We speculated that the deficiency of growth factors in low serum conditions may be one of the key factors capable of activating regenerative properties of macrophages. Particularly, low serum conditions during macrophage cultivation could stimulate deprivation-induced apoptosis of culturing cells (including admixture of non-adherent cells), and the ingestion of apoptotic cells may change the functional activity of macrophages toward an anti-inflammatory phenotype.

The received data demonstrated that low serum conditions did not influence the efficacy of Mφ3 generation. Moreover, the yield of Mφ3 significantly exceeded the number of Mφ1 and Mφ2. These data are correspondent with Plesner's study, who showed an enhanced yield of M-CSF treated macrophages in cultures with 1% fetal calf serum [22].

According to study of Verreck et al, anti-inflammatory Mφ2 have a lower expression of HLA-DR and CD86 molecules after LPS stimulation, though unstimulated macrophages expressed similar levels of these molecules [34]. We have shown that as compared to Mφ1 and Mφ2, Mφ3 cultures contained lower numbers of HLA-DR and CD86-positive cells. These differences, though not statistically significant, were important for the association with the decreased capacity of Mφ3 to stimulate allogeneic T cell proliferation. Type-2 anti-inflammatory macrophages are known to have a lower ability to stimulate T-cell proliferation in MLC [11]. This is in agreement with our data, and pointed to the lower allostimulatory activity of Mφ2 in comparison with Mφ1. Notably, Mφ3 virtually failed to stimulate lymphocyte proliferation in MLC. The medium value of the Mφ3 stimulation index was more than 6-fold lower than that of Mφ1. This fact strongly suggests that generated Mφ3 are not immunogenic and in this respect resemble anti-inflammatory M2 macrophages.

To further evaluate the pro- and anti-inflammatory activity of generated macrophages we compared their capacity to spontaneous production of Th1/pro- and Th2/anti-inflammatory cytokines. In contrast to Mφ1, Mφ3 produced significantly (2-fold) lower concentrations of pro-inflammatory (IL-1β, TNF-α, IL-6, IL-18) and Th1/Th2-cytokines (IFN-γ, IL-2, IL-4). Mφ3 supernatants also contained 2-fold lower concentrations of IL-12 and higher levels of IL-10, though these differences were not statistically significant.

Gordon and coworkers [11] have described alternatively activated macrophages after treatment with IL-4 or IL-13, which produce IL-10 without microbial stimulation. At the same time the study of Verreck demonstrated that unlike alternatively activated Mφ, M-CSF polarized Mφ2 failed to release IL-10 without activation, but effectively secreted IL-10 after mycobacterial activation. However, activated Mφ-2 produced no or relatively low levels of IL-12, IL-1β, IL-6, TNF-α [34]. We also did not reveal any significant concentrations of IL-10 in the supernatants of unstimulated Mφ2. In contrast to Mφ-2, Mφ-3 spontaneously produced IL-10 and displayed significantly less pro-inflammatory phenotype (as compare with Mφ1) without any additional stimulation.

Our results are also in agreement with findings suggesting a high ability of M-CSF polarized Mφ2 to secrete pro-inflammatory chemokines [35]. Mφ3 were also shown to secrete MIP-1β levels comparable with Mφ1 and Mφ2, but lower levels of IL-8 and MCP-1. This indicated that unlike Mφ1 and Mφ2 subsets, Mφ3 has less capacity to attract neutrophils and monocytes and therefore is less effective in supporting inflammation, whereas they could recruit effector Th1 cells and modify their functions.

One possible mechanism underlying the beneficial role of macrophages in CNS repair is connected with their capacity to produce a wide range of growth factors that can promote neuroprotection and regeneration [30,17,6]. The comparative analysis of some growth factors in the supernatants of generated macrophages revealed that all three Mφ subsets spontaneously produced detectable levels of EPO, G-CSF, IGF-1, FGF-basic, EGF, and BDNF. Mφ3 secreted concentrations of G-CSF, FGF-basic and EGF similar to Mφ1 and Mφ2, EPO comparable with Mφ2, and a lower level of BDNF, but more than 25-fold higher level of IGF-1. As for VEGF, this growth factor, identified only in quarter of patients, was produced by both Mφ2 and Mφ3, but not Mφ-1 and was significantly higher in Mφ3- than in Mφ2 cultures.

Production of classical neurotrophic factors including CNTF, IGF, HGF, PDGF, NGF, BDNF, GDNF, and NT-3 by macrophages have been shown in numerous studies [3,7,13]. Evaluation of two of these factors (BDNF and IGF-1) in cultures of distinct macrophage subtypes in our study supported previous data and demonstrated comparable production of these

factors by inflammatory Mφ1 and anti-inflammatory Mφ2. Moreover we have shown for the first time that in spite of a lower level of BDNF, Mφ3 were characterized with exclusively high secretion of IGF-1.

IGF-1 is a potent neurotrophic factor. Its pleiotropic effects range from classical trophic actions on neurons such as housekeeping or anti-apoptotic/pro-survival effects to modulation of brain-barrier permeability, neuronal excitability, or new neuron formation. IGF-1 is also known to significantly improve axon growth and remyelination [2,4]. The finding that IGF-1 is secreted abundantly by Mφ3 may point toward an important potential role for these macrophages in neuroprotection and regeneration.

In addition to neurotrophic factors, generated macrophages produced significant levels of VEGF. Detection of VEGF (in 7-day macrophage supernatants) only in part of the tested donors could be connected with an earlier peak of VEGF production. Nevertheless, in detectable cases VEGF was predominantly produced by both Mφ2 and Mφ3. VEGF has direct neuroprotective effects on motoneurons, induces neurogenesis and angiogenesis and its reduced levels cause neurodegeneration in part by impairing neural tissue perfusion [31,38].

Other factors, such as EPO, G-CSF, FGF-β, and EGF, produced by Mφ-3 and Mφ1/Mφ2 subsets could also underlay the neuro-regenerative macrophage potential. Erythropoietin functions as a tissue-protective cytokine in addition to its crucial hormonal role in red cell production. This cytokine promotes both neuroprotection and neuroregeneration in various models of CNS injury and disease and is considered to be a promising candidate as neuroprotective agent [29,15]. G-CSF appears to have anti-apoptotic effect and stimulate differentiation of adult neural stem cells [26]. EGF is a motility factor for microglial cells and is shown to enhance the differentiation, maturation and survival of a variety of neurons in the central nervous system [36]. FGF-basic promotes the survival and neurite growth of brain neurons in vitro and in vivo, suggesting that it functions as a neurotrophic factor. In addition FGF acutely modulates synaptic transmission in the hippocampus, suggesting that it has a role similar to a neurotransmitter or neuromodulator [1].

Several groups have confirmed the therapeutic potential of activated microglia and monocyte derived macrophages in the injured spinal cord [3,23-25]. The success of these preclinical models prompted a Phase I clinical trial that was completed without any adverse effects. Implantation of macrophages preincubated with dermis was well tolerated. Of the eight patients with complete spinal cord injury, three recovered clinically significant neurological motor and sensory function [16].

Recent study of this group showed that augmenting the naive monocyte pool by either adoptive transfer or CNS-specific vaccination resulted in a higher number of spontaneously recruited cells and improved recovery. Notably, the enhancement of motor functions was associated with anti-inflammatory activity of infiltrating macrophages, mediated by interleukin 10 [28].

In this aspect, the Mφ3 subset described in our study is characterized by low pro-inflammatory/immunogenic properties and high regenerative potential and therefore may represent new candidates for cell therapy in CNS injuries.

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М2-подобные макрофаги у человека: потенциальные кандидаты для стимуляции репаративных процессов в ЦНС?

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Резюме

Регуляция иммунного ответа представляется перспективной стратегией в области восстановления повреждений центральной нервной системы (ЦНС). При этом важная роль в качестве кандидатов для клеточной терапии отводится макрофагам. Используя культуральные условия с низким содержанием сыворотки, мы разработали протокол генерации противовоспалительных, М2-подобных, макрофагов из моноцитов периферической крови и сравнили эти клетки (обозначенные как М3) со «стандартными» провоспалительными (Мφ1) и противовоспалительными (Мφ2) макрофагами, генерированными, соответственно, в присутствии GM-CSF и M-CSF.

Основное внимание было прежде всего сосредоточено на способности макрофагов стимулировать пролиферацию Т-клеток, а также продукцию макрофагами различных цитокинов, хемокинов и ростовых факторов. Дефицит сывороточных факторов не сказывался негативным образом на количестве генерированных макрофагов. Напротив, наибольший выход клеток наблюдался в культурах М3. По своим свойствам М3 макрофаги больше походили на Мφ2, чем на Мφ1. Так, в отличие от Мφ1, макрофаги Мφ2 и, особенно, М3 отличались относительно низким уровнем экспрессии CD86 и не стимулировали пролиферативный ответ Т-клеток. В противоположность провоспалительным Мφ1 нестимулированные М3 продуцировали гораздо меньшие уровни провоспалительных (IL-1β, TNF-α, IL-6, IL-18, IL-12) и Th1/Th2 цитокинов (IFN-γ, IL-2, IL-4), вместе с тем - более высокий уровень IL-10. Более того, концентрации IL-1β и провоспалительных хемокинов IL-8 и MCP-1 в супернатантах М3 были снижены не только по сравнению с Мφ1, но также и с Мφ2 культурами. Подобно Мφ1 и Мφ2, М3 обладали способностью продуцировать нейротрофические (BDNF, IGF-1), ангиогенные (VEGF) и другие ростовые факторы с нейропротективной и регенераторной активностью (EPO, G-CSF, FGF-basic, EGF). При этом уровень продукции IGF-1 макрофагами 3-его типа превышал секрецию этого фактора Мφ1 и Мφ2 более чем в 25 раз.

Суммируя полученные данные, можно заключить, что генерируемые М3 клетки представляют М2-подобные макрофаги с высоким регенераторным потенциалом.

Ключевые слова: поляризация макрофагов, цитокины, хемокины, ростовые факторы, восстановление повреждений ЦНС

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