Interaction between M-CSF and IL-10 on productions of IL-12 and IL-18 and expressions of CD14, CD23, and CD64 by human monocytes[1]

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ABSTRACT

AIM: To Study the interaction of macrophage colony-stimulating factor (M-CSF) and interleukin-10 (IL-10) in productions of IL-12 and IL-18 and expressions of CD14, CD23, and CD64 by human monocytes. **METHODS:** Purified adherent human monocytes were cultured with M-CSF or IL-10 alone, or with M-CSF+IL-10 and 2-3d later, the culture supernatants and cells were separated and collected. IL-12P40 and IL-18 levels in the supernatants were determined by ELISA and the percentages of CD14, CD23, and CD64 positive cells were examined by flow cytometry. **RESULTS:** (1) IL-10 decreased M-CSF-induced IL-18 levels, while M-CSF further reduced IL-12P40 level in the culture supernatants of IL-10-treated monocytes; (2) IL-10 alone had no effect on the percentage of CD14-positive cells, but further increased the percentage of CD14-positive cells induced by M-CSF; M-CSF alone had no effect on the percentage of CD64-positive cells, but further increased the percentage of CD64-positive cells induced by IL-10; (3) IL-10 decreased the percentage of CD23-positive cells induced by M-CSF. **CONCLUSION:** Between M-CSF and IL-10, there were antagonistic effects on inducing IL-18 and CD23 expressions by monocytes; there were also synergistic effects on inhibiting IL-12P40 production and inducing CD14 and CD64 expressions by monocytes.

INTRODUCTION

Macrophage colony-stimulating factor (M-CSF) and interleukin-10 (IL-10) can be generated by monocyte-macrophages and related closely to the function of monocyte-macrophages. IL-10 is one of anti-in-flammatory cytokines, while M-CSF belongs to inflammatory cytokines. In our previous study, we demonstrated that M-CSF promoted TNF-α, IL-6, and IL-8 inductions of moncytes and increased the percentages of CD1lb, CD16, HLA-I, and HLA-II molecule positive monocytes[1]. In another study, we found between IFN-γ and IL-10, there were antagonistic effects on monocytes in many aspects, but there were also synergistic effects in some respects[2]. What is the interaction between M-CSF and IL-10 on cytokine productions and membrane surface molecule expressions of monocytes? This is an interesting question that is not yet illuminated

completely. We conjectured that M-CSF might be antagonistic to IL-10 in some aspects, or synergistic with IL-10 in some other aspects. In this paper, we tried to demonstrate M-CSF had antagonistic effects to IL-10 on some membrane molecule expressions and cytokine productions by monocytes, and also had synergistic effects with IL-10 on other membrane molecule expressions and cytokine productions.

MATERIALS AND METHODS

Cytokines Recombinant human macrophage colony-stimulating factor (rhM-CSF) expressed in silkworm was prepared by the Biochemistry Department of Nanjing University[1] and provided by Professor Junchuan QIN. The specific activity was 3.5×10^{10} CFU/g. Recombinant human interleukin-10 (rhIL-10) was purchased from Pepro Tech Co (Britain).

Monoclonal antibodies FITC-labelled antibodies, respectively to CD14 and CD64, PE-labelled antibodies to CD14 and CD23, and FITC-, PE-, and CY-labelled isotypic Igs were all purchased from Pharmingen Co (USA).

Cytokine detection kits Detection kits for human IL-12P40 and human IL-18 were purchased from Biosource Co (USA).

Other Reagents LPS was supplied by Sigma Co. The Ficoll-Hypaque lymphocyte separating medium was provided by Hematology Institute of Chinese Academy of Medical Sciences (Tianjin, China).

Human monocytes The Heparin-anticoagulated blood were obtained from healthy blood donors. Human mononuclear cells were separated by centrifugation with Ficoll-Hypaque lymphocyte separating medium. After being washed thrice with Hanks' solution, the mononuclear cells were adjusted to 1×10^{10}/L with RPMI 1640 medium supplemented with 10 % fetal calf serum (FCS). Then the cell suspensions were seeded into 96-well plates, 200 UI/well. After an overnight adherence step at 37 ℃ in 5 % CO_2, the wells were washed thrice with Hanks' solution to remove any nonadherent cells. The adherent cells left in the wells were monocytes and showed over 80 % CD14 positive by flow cytometry with the specific antibody.

Inductions and detections of cytokines In order to induce IL-12P40 and IL-18, monocytes in the wells were cultured with 10 % FCS RPMI 1640 medium supplemented with rhM-CSF, or rhIL-10, or rhM-CSF+IL-10 for 24 h and then switched over to being cultured with 10 % FCS RPMI 1640 medium supplemented with LPS for 48 h. The supernatants were harvested by centrifugation at $600 \times g$ for 15 min and then stored at -20 ℃ for cytokine detection. IL-12P40 and IL-18 concentrations of the culture supernatants were determined by double-antibodies sandwich ELISA. The assays were performed according to the kit protocol.

Test for membrane molecules by flow cytometry Monocytes in the wells were cultured with 10 % FCS RPMI 1640 medium supplemented with rhM-CSF, or rhIL-10, or rhM-CFS+rhIL-10 for 48 h. After being washed twice with cold PBS, the adherent monocytes were resuspended with RPMI 1640 medium. And then the medium was removed by centrifugation. The cell pellets were resuspended, respectively with the dilution of CD14, CD23, or CD64 antibody, or isotypic Igs labelled with fluorescein, and then incubated at 4 ℃ for 30 min. After two times of the centrifugation and washing step, the cells were resuspended and analyzed on a FACS Calibur(BD Biosciences). CD14 positive cells were gated, of which 1×10^4 cells were detected for CD23 and CD64. Data were analyzed using Cell Quest software from BD Biosciences.

Statistical analysis Data were presented as mean±SD. The statistical analysis was carried out using paired *t*-test. $P < 0.05$ was taken to be significant.

RESULTS

Interaction between M-CSF and IL-10 on IL-12P40 production by human monocytes Data were presented in Tab 1, which showed: (1) LPS stimulation significantly induced IL-12P40 production by human monocytes; (2) 5 µg/L, 25 µg/L and 50 µg/L IL-10 decreased IL-12P40 concentration by 66.85 %, 75.32 % and 84.35 %, respectively; (3) 0.5 U/L, 1.0 U /L, 5.0 U/L M-CSF also decreased IL-12P40 concentration by 66.77 %, 71.65 %, and 80.98 %, respectively; (4) 0.5 U/L, 1.0U /L, 5.0 U/L M-CSF combined with 25 µg/L IL-10 further decreased IL-12P40 concentration by 43.52 %, 61.23 %, and 75.53 %, compared with 25 µg/L IL-10 alone group. These data indicated that both M-CSF and IL-10 suppressed IL-12P40 production by human monocytes in a dose-dependent pattern, and that there was a synergistic effect between M-CSF and IL-10 on inhibiting IL-12P40 production.

Interaction between M-CSF and IL-10 on IL-18 production by human monocytes Data in Tab 1

also showed LPS induced IL-18 production. Under the stimulation of LPS combined with M-CSF, IL-18 in monocyte culture supernatants increased by 70.44 %, 89.25 %, or 126.32 %, respectively, at 0.5 U/L, 1.0 U/L, or 5.0 U/L M-CSF concentration correspondingly,compared with that under the stimulation of LPS only.While under LPS combined with IL-10, IL-18 reduced by 40.09 %,72.56 %, or 91.38 %, respectively, at 5 µg/L, 25 µg/L, or 50 µg/L IL-10 concentration corres-pondingly, compared with that under LPS only. Through adding M-CSF into the culture at the concentration of 0.5 U/L, 1.0 U/L, or 5.0 U/L, IL-18 concentration of the culture supernatants of monocytes treated with LPS plus 25 µg/L IL-10 increased by 1.02, 1.78, or 4.11 times correspondingly, compared with that treated only with LPS plus 25 µg/L IL-10. These results indicted that

Tab 1. Effects and interaction of M-CSF and IL-10 on LPS-induced IL-12P40 and IL-18 productions by human monocytes. *n*=6. Mean±SD. [a]$P>0.05$, [b]$P<0.05$ *vs* medium control. [d]$P>0.05$, [e]$P<0.05$ *vs* group 2. [g]$P>0.05$, [h]$P<0.05$ *vs* group 4.

| Group | Treatment | | | IL-12P40 | IL-18 |
| | LPS | IL-10 | M-CSF | | |
No	/mg·L^{-1}	/µg·L^{-1}	/U·L^{-1}	/ng·L^{-1}	/ng·L^{-1}
1	0	0	0	2.74±2.46	No detection
2	10	0	0	66.57±10.70[b]	8.93±0.75
3	10	5	0	22.07±3.69[e]	5.35±0.72[e]
4	10	25	0	16.43±2.80[e]	2.45±0.84[e]
5	10	50	0	10.42±3.30[e]	0.77±0.50[e]
6	10	0	0.5	22.12±2.04[e]	15.22±0.73[e]
7	10	0	1.0	18.87±2.05[e]	16.90±0.57[e]
8	10	0	5.0	12.66±3.73[e]	20.21±2.53[e]
9	10	25	0.5	9.28±5.40[h]	4.96±0.71[h]
10	10	25	1.0	6.37±3.31[h]	6.80±0.63[h]
11	10	25	5.0	4.02±2.87[h]	12.53±1.14[h]

IL-10 suppressed, while M-CSF enhanced, LPS-induced IL-8 production by monocytes, moreover, M-CSF antagonized the inhibitory effect of IL-10 on IL-18 production.

Interaction between M-CSF and IL-10 on CD14, CD23 and CD64 expressions by monocytes Because we proved that the IL-10 at 25 µg/L or the M-CSF at 5.0 U/L concentration showed effectually biological activity of affecting cytokine productions, here 25 µg/L or 5.0 U/L was chosen as the experimental concentration of IL-10 or M-CSF, respectively. The experimental results of membrane molecule expressions were shown in Tab 2, which were as follows: (1) M-CSF slightly induced CD14 expression by monocytes, IL-10 alone did not affect CD14 expression, but had a synergetic effect with M-CSF on inducing CD14 expression; (2) M-CSF enhanced, while IL-10 inhibited CD23 expression, there was an antagonistic effect of M-CSF to IL-10 on CD23 expression; (3) IL-10 enhanced CD64 expression by monocytes, M-CSF alone did not significantly affect CD64 expression, but had a synergistic effect with IL-10 on inducing CD64 expression.

DISCUSSION

A lot of information about effects of M-CSF and IL-10 on immunomolecule expressions of monocytes had been reported. However, the reports on interaction between M-CSF and IL-10 in cytokine productions and membrane molecule expressions by monocytes are quite few. IL-10 is being tested in treating some autoimmune diseases such as autoimmune diabetes[3], autoimmune thyroiditis[4], rheumatoid arthritis (RA) and multiple sclerosis (MS). Simultaneously, M-CSF increases in the patients with these diseases. In the patients with systemic lupus erythematosus (SLE), IL-10 levels increase generally and increasing M-CSF are involved in

Tab 2. Effects and interaction of M-CSF and IL-10 on membrane molecule expressions of monocytes. *n*=6. Mean±SD. [b]$P<0.05$ *vs* medium control. [e]$P<0.05$ *vs* IL-10 alone group. [h]$P<0.05$ *vs* M-CSF alone group.

| Membrane molecule | Percentages of expression-positive cells under different treatment | | | |
	Medium control	IL-10 (25 µg/L)	M-CSF (5.0 U/L)	IL-10 (25 µg/L)+ M-CSF (5.0 U/L)
CD14	80.15±12.59	80.39±14.95	86.93±7.25[b]	89.99±6.98[beh]
CD23	42.68±3.39	35.78±5.16[b]	51.34±8.37[b]	47.93±4.90[eh]
CD64	1.60±0.73	3.87±1.91[b]	2.29±0.79	6.54±4.64[beh]

autoimmune renal injury[5,6]. Both M-CSF and IL-10 are suggested to be used to treat atherosclerosis, because the former protects macrophages from lipoperoxidative injury[7] and the latter inhibits endarteritis. So it is important to study the interaction of M-CSF and IL-10 on immune system, especially on immune molecule expressions.

In this paper, we demonstrated that IL-10 had an antagonistic effect to M-CSF on inducing CD23 expression and enhancing LPS-induced IL-18 production, but had a synergistic effect with M-CSF on inhibiting IL-12P40 production. We also found that IL-10 alone did not affect CD14 expression on monocytes, but had a synergistic effect with M-CSF on inducing CD14 expression; and that M-CSF alone did not affect CD64 expression, but had a synergistic effect with IL-10 on inducing CD64 expression.

IL-12 is one of the most important cytokines which induce ThO cell differentiation to Thl cell. IFN-γ has similar effect. IL-18 is an important inductor of IFN-γ, and moreover, has a synergistic effect with IL-12 on inducing IFN-γ production. While IL-10 inhibits IL-12 productions by dendritic cells and macrophages[8,9] and IFN-γ production by Thl cells. According to the results from the present experiments, it is reasonable to infer that inhibiting IL-12 and IL-l8 is one of the mechanisms, by which IL-10 suppresses activation and differentiation of Thl cells. M-CSF enhances IL-18 expression and antagonizing inhibitory effect of IL-10, which is favorable for IFN-γ production and T cell activation. But M-CSF inhibits the expression of IL-12P40, which is unfavorable for differentiating to Thl cell.

M-CSF plays an important role in mediating inflammation. In addition, according to our results, M-CSF strengthens the function of inflammatory monocytes as immunological effector cells by increasing CD23 and synergistically enhancing CD64 expressions. Because CD23 is FcεRII and takes part in phagocytosis and IgE-dependent ADCC of monocyte-macrophages, meanwhile, CD64 is FcγRI and takes part in phagocytosis, bacteriolysis and IgG-dependent ADCC of monocyte-macrophages. The present results also suggest that as an important member of Th2 cytokines, IL-10 promotes not only antibody production, but also antibody effect by enhancing CD64 (FcγRI) expression and sequentially promoting IgG-mediated immunological effects. In this aspect, there is a synergistic effect between IL-10 and M-CSF. On the other hand,

M-CSF might strengthen IgE-mediated immunological effect through inducing CD23 expression, but IL-10 might suppress IgE-induced immunological effect through inhibiting CD23 expression and antagonizing the effect of inducing CD23 of M-CSF.

CD14 is a membrane molecule on the surface of monocyte-macrophages, which serves as LPS receptor. The synergistic effect between M-CSF and IL-10 on inducing CD14 expression is very important for the regulation of inflammation. Bergamini *et al* found LPS induced a strong IL-10 production in M-CSF-treated macrophages and M-CSF increased the IL-10 response of macrophages to LPS by enhancing both the expression of membrane-bound CD14 and the sensibility of CD14-expressing cells to LPS stimulation[10]. This IL-10 response acts as a regulatory mechanism of negative feedback in inflammation. The synergistic effect between M-CSF and IL-10 on inducing CD14 expression should further increased the IL-10 response of macrophages to LPS.

As for the mechanism of interaction between M-CSF and IL-10, they might be related to the following: (1) M-CSF induces IL-10 production by monocytes[9-11] and increases the IL-10 response of macrophages to stimulation[10]; (2) IL-10 increases the expression of M-CSF receptor in macrophages and enhances the sensibility of macrophages to M-CSF stimulation[12]; (3) M-CSF and IL-10 use similar Jak-STAT pathways of signal transduction[13], on the other hand, IL-10 inhibits activation of Ras gene[14], which codes for Ras protein, a signaling molecule in the dominant pathway of M-CSF signal transduction.

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