Peptides 32 (2011) 929–937

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Functional modulation of the pathway between dendritic cells (DCs) and CD4+T cells by the neuropeptide: Methionine enkephalin (MENK)☆

Fengping Shan[a,b,*], Yanjie Xia[a], Ning Wang[a], Jingjuan Meng[c], Changlong Lu[a], Yiming Meng[a], Nicolas P. Plotnikoff[d]

[a] Department of Immunology, School of Basic Medical Science, China Medical University, No. 92, North Second Road, Heping District, Shenyang 110001, PR China
[b] Institute of Pathology and Pathophysiology, China Medical University, No. 92, North Second Road, Heping District, Shenyang 110001, PR China
[c] Central Laboratory, China Medical University, No. 92, North Second Road, Heping District, Shenyang 110001, PR China
[d] TNI Pharmaceutical Company, 25 Harbor Road, Ocean City, NJ 08226, USA

ARTICLE INFO

Article history:
Received 9 December 2010
Received in revised form 29 January 2011
Accepted 31 January 2011
Available online 16 February 2011

Keywords:
Methionine enkephalin
Dendritic cell
CD4+T cell
Interleukin-2
Interferon-γ
Modulation

ABSTRACT

MENK, the endogenous neuropeptide, is suggested to be involved in the regulatory loop between the immune and neuroendocrine systems, with modulation of various functions of cells related to both the innate and adaptive immune systems. Our present research findings show that MENK serves as an immune modulator to the pathway between DCs and CD4+T cells. We studied changes of DCs in key surface molecules, the activity of acid phosphatases (ACPs), the production of IL-12, and the effects on murine CD4+T cell expansion and their cytokine production by MENK alone, and in combination with interkeukin-2 (IL-2) or interferon-γ (IFN-γ). In fact, we found that MENK could markedly induce the maturation of DCs through the addition of surface molecules such as MHC class II, CD86, and CD40 on murine DCs, the production of IL-12, and the down-regulation of ACP inside DCs, (which occurs when phagocytosis of DCs is decreased, and antigen presentation increased with maturation). We also found that MENK alone or in combination with IL-2 or IFN-γ, could markedly up-regulate both CD4+T cell expansion and the CD4 molecule expression *in vivo* and *in vitro* and that MENK alone, or MENK + IL-2, could enhance the production of interferon-γ from CD4+T cells. Moreover, MENK alone, or MENK + IFN-γ, could enhance the production of IL-2 from CD4+T cells. It is therefore concluded that MENK can exert positive modulation to the pathway between dendtritic cells and CD4+T cells.

1. Introduction

MENK, the endogenous neuropeptide, is suggested to be involved in the regulatory loop between the immune and neuroendocrine systems, with modulation of various functions of cells related to both innate and adaptive immune systems in a naloxone reversible manner [4]. Since the endogenous opioid ligand might interact with more than one type of delta receptors which have been detected in various immune cells, such as T cell, NK, DCs and macrophage [3,10,16,5] at a moderate range of concentrations, MENK may work as a tonic activation agent that plays a role in immune modulation and cell proliferation [33]. We recently demonstrated that MENK could activate the CD4+T cells by increasing expression of delta receptors on the surface of the CD4+T cells (under separate paper).

Both IL-2 and IFN-γ are T cell activators via either autocrine or paracrine signaling, and play important roles in the T cell mediated immune responses (especially in CD4+T cell response) [19].

Dendritic cells (DCs), originally identified by Steinman (1972), represent the pacemakers of the immune response. DCs are potent antigen presenting cells (APCs) that possess the ability to stimulate naïve T cells. They are crucial to the presentation of peptides and proteins to T cells, and are therefore critical for the induction of the T cell responses which result in cell-mediated immunity [8].

Due to the great importance of the pathway between the DCs and the CD4+T cells in maintaining the balanced immunity in the body and its role in fighting cancers and other human-threatening diseases, like AIDS and since the precise mechanisms of the pathway between DCs and CD4+T cell on which MENK acts remain unclear We endeavored to investigate the direct effect application

Abbreviations: MENK, methionine enkephalin; OGF, opioid growth factor; OGFr, opioid growth factor receptor; DCs, dendritic cells; ACP, acid phosphatase; LPS, lipopolysaccharides; FCM, flow cytometry; NX, naltrexone.

☆ This work was supported financially by National Natural Science Foundation of China. No. 30771990 (to Fengping Shan).

* Corresponding author at: Department of Immunology, Basic School of Medicine, China Medical University, No. 92, North Second Road, Shenyang 110001, PR China. Tel.: +86 24 133 224 38530; fax: +86 24 8660 4452.
E-mail address: fpshan@mail.cmu.edu.cn (F. Shan).

0196-9781/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2011.01.033

of MENK has on this pathway. As such, we conducted the following studies to try to answer these questions.

2. Materials and methods

2.1. Reagents

MENK was provided by Penta Biotech Inc. USA (≥97% purity). In our previous pre-experiments we tested the effect of a range of MENK concentrations from 10^{-1} M to 10^{-14} M on the proliferation of murine splenocytes *in vitro* and demonstrated that the optimal concentration is 10^{-1} M. Based on these studies, we used this optimal concentration in current study. Recombinant murine IFN-γ (Catalog No.: 315-05), and recombinant murine IL-2 (Catalog No.: 212-12) were purchased from PEPROTECH Inc. The mAbs used in this study include FITC-anti-CD4, FITC-conjugated anti-CD40, PE-anti-MHC-II and PE-anti-CD86 which were purchased from eBioscience (San Diego, CA) and BD Pharmingen (San Jose, CA). The ELISA assay kits for IFN-γ, IL-2 and IL-12 analysis were all purchased from eBioscience (San Diego, CA). Mouse CD4+T Lymphocyte Enrichment Set-DM was purchased from BD PharMingen. RT-PCR kits were purchased from Takra (Japan). RT-PCR primer was prepared by Nanking Kinsit Gene Tech. Co., China. Trizol was a product of Invitrogen (Carlsbad, CA). Lipopolysaccharide (LPS) was a product of Sigma–Aldrich. Other chemicals frequently used in our laboratory were all products from Sigma–Aldrich or BD Pharmingen.

2.2. Mice

Male C57BL/6 mice with 6–8 weeks old and weighing 18–20 g were purchased from Shanghai SIPPR/BK Experimental Animal Co. Ltd., a pathogen-free animal supplier. All experimental procedures were conducted per the guidelines of Institutional Animal Care.

2.3. Cell culture

The murine DCs 2.4 cell line was an immature one established through the transfection of GM-CSF, myc and raf genes into the C57BL/6 mouse [25]. It was kindly donated by Dr. Feili GONG from Hubei Medical University, China, and was passaged several times in our laboratory.

The DCs were grown in RPMI 1640 supplemented with 10% fetal calf serum, 2 ml L-glutamine, and 1.2% sodium bicarbonate. All media contained antibiotics (100 Units/ml penicillin, 100 μg/ml streptomycin, and 100 μg/ml kanamycin). Unless otherwise indicated, all cells including DCs and CD4+T cells were grown in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cells were plated and counted 24 h later to determine the seeding efficiency before experimentation.

2.4. Acid phosphatase (ACP) activity detection

The concentration of DCs was adjusted to 1×10^6/ml. The ACP activity inside the DCs after treatment with 10^{-12} M MENK for 24 h was measured at OD 520 nm by the phenol-4-AAP (amino antipyrine) method in conjunction with ACP testing kit (Jiancheng Bio-Engineering Institute of the South).

2.5. Analysis by flow cytometry (FCM)

2.5.1. Analysis of DCs by FCM

The cultured DCs served as both testing group (after treatment with 10^{-12} M MENK for 24 h) and control group. They were collected and stained with anti-CD40, anti-CD86 and anti-MHC-II antibodies for 30 min for optimal staining, and then washed with 2% FACS-PBS twice. The cells were harvested and immediately fixed in 4% paraformaldehyde, and were subsequently collected using FACS Calibur (Becton Dickinson, San Diego, CA). The data, obtained from the analysis of the fixed cells by FCM, were then analyzed using Flow Jo Software (Tree Star, Ashland, OR).

Table 1
RT-PCR reaction of the CD4+T cells at mRNA level.

Primer	Denaturation	Annealing	Extension
β-Actin	94 °C 30 s	55 °C 1 min	72 °C 1 min
CD4+T cell	94 °C 30 s	55 °C 30 s	72 °C 30 s

Primer	Sequences	Size
The primer sequences are as follows:		
β-Actin	Sense 5′TCAGAAGGACTCCTATGTGG 3′ Antisense 5′TCTCTTTGATGTCACGCACG 3′	500 bp
CD4+T cell	Sense 5′GGCAACTTGGTGTGAATGAC 3′ Antisense 5′CCTGTGTTCCATGTAGTGGC 3′	110 bp

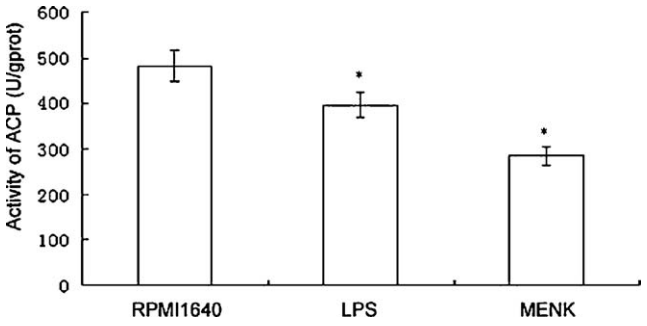

Fig. 1. ACP activity of the DCs after treatment with 10^{-12} M MENK. ACP activity of the DCs was down-regulated after immature DCs matured due to decreasing phagocytosis and increasing antigen presentation ($p < 0.01$) vs. ACP activity in the RPMI 1640 group.

2.5.2. Analysis of CD4+T cells by FCM

The CD4+T cells, collected as previously described, were stained with anti-CD4 antibody for 30 min, and then washed twice with 2% FBS-PBS. These cells were then harvested and immediately fixed in 4% paraformaldehyde. Samples of the cells were collected using a FACS Calibur (Becton Dickinson, San Diego, CA). The data, collected from the analysis of the samples by FCM, were analyzed using Flow Jo Software (Tree Star, Ashland, OR).

2.6. Mice grouping and studying in vivo and in vitro

2.6.1. Mice grouping

With 6 mice in each group, C57BL/6 mice were divided into groups of six as follows: MENK, IL-2, MENK + IL-2, IFN-γ, MENK + IFN-γ, NX + MENK, and normal saline.

2.6.2. Studying in vivo

In vivo, we treated the C57BL/6 mice by injecting with only MENK in one group, and with MENK and IL-2 in another group, and with MENK and IFN-γ in the last group. The mice were treated by i.p. with 20 mg/kg MENK, IL-2 10^4 U, IFN-γ 10^5 U, and 10 mg/kg NX (administration of MENK 0.5 h after NX administration) once a day for 7 successive days. The mice were then sacrificed 7 days later. The splenocytes of the mice of each group were separated and proliferation of CD4+T cells in the splenocytes was determined with FCM and re-checked by RT-PCR analysis of the CD4 molecule RNA. Some portion of the separated mice splenocytes were purified with magnetic beads. Its purity (90%) was confirmed with FCM. The purified CD4+T cells were adjusted to 3×10^6/ml and then allowed