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## The research of the relationship among experimental allergic encephalomyelitis guinea pig serum TNF- $\alpha$ , IFN- $\gamma$ , NPY

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### ABSTRACT

To investigate the effect of neuropeptide Y on guinea pig serum Th1 cytokines TNF- $\alpha$  and IFN- $\gamma$  content, and study the effect of NPY on EAE and its possible immune mechanism. Method: 30 guinea pigs were randomly divided into normal control group, EAE control group, NPY intervention group (inject NPY into lateral ventricle), the levels of TNF- $\alpha$  and IFN- $\gamma$  in guinea pigs serum were observed. Results: Compared to the EAE control group, the levels of TNF- $\alpha$  and IFN- $\gamma$  in the serum of the NPY intervention group were significantly lower ( $P < 0.05$ )

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### KEYWORDS

Neuropeptide Y;  
Animal model of multiple sclerosis;  
Th1 cytokines.

### INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is used to study the multiple sclerosis, which is worldwide recognized animal model, over the years; its pathogenesis has always been the hot spot that scholars study. Neuropeptide Y (NPY) is a kind of peptide substance that has biological activity and can participate in neural information transfer; it is for immunity to have obvious regulative effect. This experiment adopts the lateral ventricle injected NPY as intervention, the morbid effects of NPY on EAE guinea pigs was observed, as well as on the serum Th1 cytokines TNF- $\alpha$ , IFN influence of content, the purpose is to study immune mechanism of NPY on EAE morbid effects.

### MATERIAL AND METHODS

#### Material

Thirty healthy female guinea pigs (age 8 to 12 weeks);

ten rats (age three months). Main laboratory reagents: (1) whole Freund adjuvant (containing BCG vaccine 10 mg/ml) is the product of American Sigma company. (2) the neuropeptide Y is the product of American Sigma company. (3) IFN- $\gamma$  detection kit was bought from Shanghai Senxiong science and technology industrial co., LTD. (4) the TNF- $\alpha$  detection reagent is provided by Beijing Huaying biotechnology institute.

#### Methods

Intervention in respective group, thirty guinea pigs were randomly divided into three groups: normal control group: (1) lateral ventricle was injected into ten microliters normal saline once, after one virtual module. 2 EAE model group: lateral ventricle was injected into ten microliters normal saline once, after one week preparing EAE model. (3) NPY intervention group: lateral ventricle was injected into ten microliters NPY once, after one week preparing EAE model.

The lateral ventricle was injected into trace NPY,

after anesthetizing abdominal cavity of guinea pig, which should be fixed on the stereotactic instrument. Take the injection site with herringbone stitch sagittal suture junction thickness 1.2mm, right midline 2mm, subdural 4mm, using a Hamilton syringe vertical piercing, cerebrospinal fluid reflux. Withdrawing injection NPY 10 $\mu$ L, then injected methylene blue to identify liquid is injected into the lateral ventricle.

The establishment of EAE model; the subcutaneous tissue of hindlimb of guinea pigs were injected into crude antigen MBP, each side of every rat was injected into 0.2ml and manufactured EAE model. After modeling appear specific neurological deficits, and finally confirmed EAE model by brain tissue pathological examination.

The activity assay of TNF- $\alpha$  and IFN- $\gamma$ ; the radio-immunoassay is adopted to detect the serum level TNF- $\alpha$  levels, using double antibody sandwich enzyme linked immunosorbent assay to detect IFN- $\gamma$  levels.

Statistical analysis; the data is expressed as ( $\bar{x} \pm s$ ), the comparison of samples mean between the two groups adopted t-test, samples mean among multi-group were conducted one-way anova, pairwise comparisons adopted LSD test (adopting t' test when variance is nonhomogeneity). Statistical processing was performed by the SPSS 11.5 software.

## RESULTS

### The morbid condition of NPY intervention group, EAE control group and the normal control group

Normal control group guinea pigs were not taken bad, EAE control group all guinea pigs were taken bad, NPY intervention group except that one sample was not taken bad, the rest were taken bad. Morbid animal manifested as hair loss, loss of appetite and even refusing foods, body weight was significantly decreased, appear limb weakness, ataxia and paralysis of different degree.

### The serum TNF- $\alpha$ , IFN- $\gamma$ level comparisons of NPY intervention group, EAE control group and the normal control group,

TABLE 1: The peak serum of the guinea pig TNF- $\alpha$ , IFN- $\gamma$  level comparison ( $\bar{x} \pm s$ )

Grouping	N	TNF- $\alpha$ (ng/ml)	IFN- $\gamma$ (ng/ml)
NPY intervention group (1)	10	1.76 $\pm$ 0.53	12.45 $\pm$ 3.49
EAE control group (2)	10	2.63 $\pm$ 0.80	25.51 $\pm$ 0.84
Normal control group (3)	10	1.18 $\pm$ 0.16	1.87 $\pm$ 0.22

## DISCUSSION

The immunological pathogenesis of experimental allergic encephalomyelitis (EAE) is disproportion or dysfunction of T-cell subsets. Th1 cell subsets secrete IL-2, IFN- $\gamma$ , and TNF- $\alpha$  and so on, and trigger cellular immunity; Th2 cell subsets secrete IL-4, IL-10 and TGF- $\beta$  and so on, inhibit Th1 cytokine production. Under normal circumstances, two kinds of cytokines are in a state of dynamic balance inside body, in order to maintain the body's normal immune function. Under disease states, when Th0 cells was stimulated by specific antigen, which can differentiate into Th1 and Th2 direction, resulting in dysequilibrium of Th1 / Th2, then generate immune drift phenomenon.

Neuropeptide Y (neuropeptide Y, NPY) is the one of the peptide neurotransmitter that most widely distributed in the central nervous system<sup>[2,3]</sup>, and which is closely connected to immunity, it can affect differentiation of Th cell. It was found in experiment, NPY intervention group guinea pig serum Th1 cytokine IFN- $\gamma$ , TNF- $\alpha$  level significantly lower than EAE control group, indicating that NPY can inhibit Th1 cytokine IFN- $\gamma$ , TNF- $\alpha$  secretion. Several studies found that, IFN- $\gamma$  and TNF- $\alpha$  in the promotion EAE morbidity and increase EAE disease that may play an important role, causing demyelination<sup>[4,5]</sup>. It was found in experiment, EAE control group guinea pig serum IFN- $\gamma$ , TNF- $\alpha$  value were significantly higher than the normal group, guinea pigs, and the higher the serum IFN- $\gamma$ , TNF- $\alpha$  level, the shorter the morbidity latency of guinea pigs, the more severer neurological dysfunction, further shows that they are the key pathogenic factor of EAE morbidity. The NPY can inhibit Th1-type cytokines IFN- $\gamma$ , TNF- $\alpha$  secretion, and thus can inhibit the activation of the MPC-like cells, inhibit monocyte-macrophage cells to produce inflammatory mediators, reducing demyelination, so it was found from experiment that NPY intervention group guinea pig EAE morbid condition was significantly lighter than the control group, showing that NPY has a protective effect on the morbidity and symptoms of EAE model.

Therefore, inhibiting Th1 cytokines by immunomodulator, which should be an effective way that treated EAE and MS. IFN- $\gamma$ , TNF- $\alpha$  is an important inflammatory mediator between EAE and MS,

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which can prevent its occurrence, development, and provide demyelinating disease with ideal therapeutic target, show a bright future for the treatment of EAE and MS.

### CONCLUSION

The lateral ventricle was injected into NPY can take protective effects on EAE, the protective mechanism is probably by reducing TNF- $\alpha$ , IFN- $\gamma$  levels and play a role.

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