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Oligopeptides and phagocytosis in mice

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Thymic hormones and some of their synthetic fragments bind to specific surface receptor of cells in order to exert their regulatory effects. Distinct T-cell targeting has also been demonstrated on experimental models [1, 2].

Over 50 synthetic fragments of thymic hormones (Thymopoietin, Thymosin alpha 1 and Thymosin beta 4) were selected by combination of basic and acidic amino acids especially Lysine and Glutamine. No direct structure — effect relationship was found on inducing of immunoresponse differentiation. The naturally occuring low molecular weight oligopeptide tuftsin binds to T-cells, polimorphonuclear and mononuclear phagocytic cells. Multispecificity of TP-fragments was also demonstrated [3].

Materials and Methods

Separation of mononuclear cells

Ficoll-Uromiro (Pharmacia-Bracco) gradient was used to separate lymphocytes from blood donors. The cells adhered to glass surface were mainly mononuclear phagocytic cells.

Mice

18—24 g male CFLP (LATI) outbred and 3 month or 1 year old male NZB (SZBC) mice were used in experiments.

Immunosuppressed mice

Mice were injected for two days (80 mg/kg, p.o.) 6 hours before treatment of oligopeptide.

Phagocytic capacity

Phagocytic capacity was evaluated with the number of the engulfed, opsonised baker-yeasts [4].

Non-specific esterase enzyme activity

The non-specific esterase enzyme activity of resident PEC-cells from NZB mice treated with dipeptide mandelate were investigated with a semiquantitative gel-diffusion test developed by alpha-Naphtyl-propionate (Sigma) substrate.

Results

In vitro effect of TP-fragments and Glu-Lys-mandelate was tested on human monocytes from healthy volunters. The number of phagocyted opsonized baker-yeasts/100 cells was counted and results are demonstrated in Fig. 1.

TP5 moderately inhibited the phagocytic capability of cells, in contrast to that the dipeptides mandelate pro-

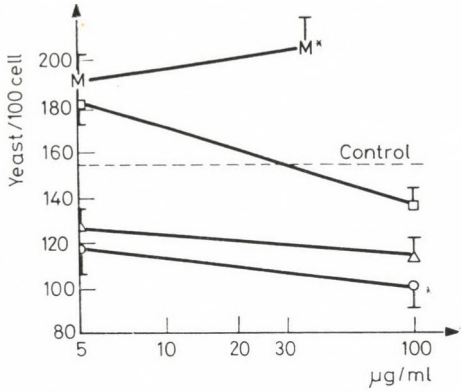

FIG. 1. *Effect of oligopeptides on human monocytes from blood donors (n = 8)* 30 mg/ml Glu-Lys NH_2. mandelate (M) produced a significant increasing (x = $P < 0.05$ Student's t-test compared to control value) effect on phagocytic capacity of monocytes. The effect of TP3 (△——△), TP4 (□——□) and TP5 (○——○) is demonstrated

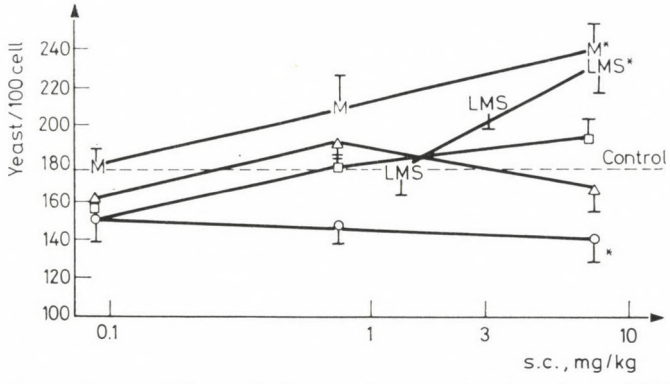

FIG. 2. *Effect of peptides and Levamisole on phagocytosis of resident PEC-cells in mice (n = 16)* CFLP male mice were treated with peptides (0.1, 1.0, 10 mg/kg s.c.) and Levamisole (1.5, 3.5, 10 mg/kg s.c.) two days. Baker-yeast was opsonized with sera of NZB mice. Data are signed with M (Glu-Lys. NH_2.mandelate) LMS (Levamisole), TP3 (△——△), TP4 (□——□) and TP5 (○——○). Results are means: $\bar{x} \pm$ S. E. M.

duced an increasing effect. The extent of stimulation of the phagocytic activity of mouse resident peritoneal macrophages with a single injection of oligopeptides is shown in Fig. 2.

It is clear from the data in the figure that dipeptide mandelate is a definitive inducer of macrophages also compared to the effect of Levamisole reference compound. Phagocytic activity of resident macrophages was inhibited with injection of TP5 but no effect was found with TP3 and TP4. Figure 3 depicts the similarity of dipeptide mandelate stimulation in immunosuppressed mice. Mice were pretreated with suboptimal inhibitory doses (ED_{40}) of cyclophopshamide. Dose-dependent enhancement was observed with mandelate, but not with TP3

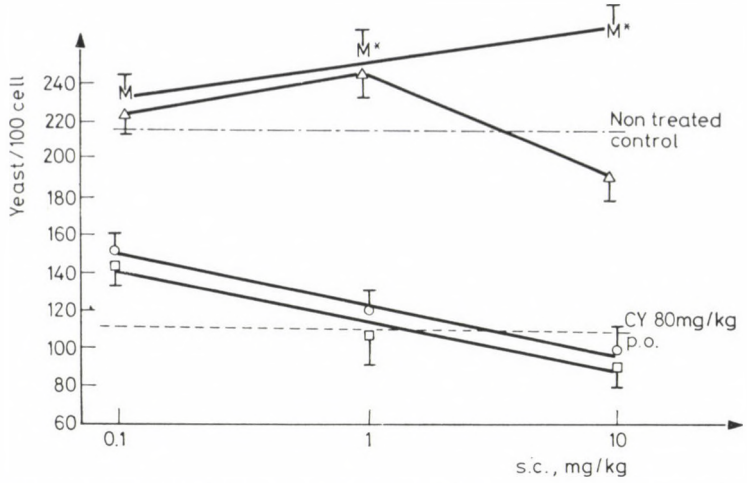

FIG. 3. *Restoration of phagocytosis in cyclophosphamide treated mice (n = 8).* Restoration on immunosuppressed phagocytic capacity of resident PEC macrophages was elicited by treatment of dipeptide. mandelate (M) and TP3 (△——△). TP4 (□——□) and TP5 (○——○) produced only a moderate effect on cell activation

TABLE I

Enhancement of nonbalanced immunoresponse

Peptide	Stimulation in percentage		
	Maturation of immune response[1]		CY inhibited mice[2]
	anti-SRBC titer	PFC	anti-SRBC titer
TP3	34	50	I. A.
TP4	42	48	100
TP5	39	47	81
Glu-Lys. NH_2 Mandelate	20	I. A.	80

[1] New-born rat were injected with peptide (1 mg/kg, i.p.), two weeks later immunized and at 3th week determined the anti-SRBC antibody titers as well as the number of PFC

[2] CFLP mice were immunosuppressed with cyclophamide (CY/100 mg/kg, i.p.) 6 hours later immunized (with 1% SRBC suspension) and the determination of anti-SRBC antibody was performed at 4th day of experiment

treatment. No restorative effect was detected with TP4 and TP5.

The effect of oligopeptides on maturation of immunoresponse was compared (Table I) to the effect of TP-s.

TP-fragments increased the immunoresponse in new-born rats measured by anti-SRBC antibody and the number of plaque forming cells (PFC). Mandelate could produce a small amount increased titer of antibody but the effect was slightly higher if mice were preliminary immunosuppressed.

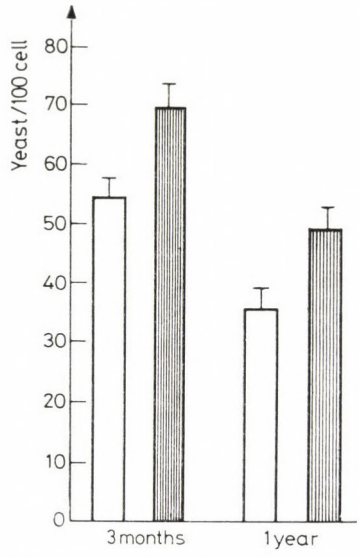

FIG. 4. *Phagocytic activity of PEC macrophages from NZB mice (n = 8).* The down regulated phagocytic activity of NZB mice was enhanced with 4 days injection of Glu-Lys NH_2. Mandelate (1 mg/kg, s.c.). The muber of engulfed yeast particles is shown from nontreated (□) and treated (▥) mice

CONCLUSION

These studies provide the evidence that TP-fragments develope a modest stimulatory effect on phagocytic capacity of macrophages.

No augmentation of the inducing effect was observed with TP4, but indirectly with TP3 and only in vitro with TP5. However, Glu-Lys.NH_2. mandelate is responsible to augment both in vitro and in vivo stimulatory effect on macrophages. Dipeptide shows multifunctional effect. Phagocytic capacity parallel with nonspecific-esterase activity were 4 times increased by treatment of dipeptide mandelate in NZB mice.

The ability of dipeptide to regulate macrophage phagocytosis and enhancement of lysosomal function may be important in diseases. The investigation of the efficacy dipeptide is in progress.

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