Production of Lymphocyte-Activating Factors by Mouse Macrophages during Aging and under the Effect of Short Peptides

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Age-specific characteristics of production of lymphocyte-activating factor by mouse peritoneal macrophages and modulation of this production by short synthetic peptides (Vilon, Epithalon, and Cortagen) were studied. The production of lymphocyte-activating factors by macrophages stimulated with lipopolysaccharides *in vitro* was lower in old animals. The opposite modulating effects of short peptides on the production of lymphocyte-activating factors by resident and lipopolysaccharide-stimulated macrophages in young and old mice were demonstrated for the first time. This is a possible mechanism of immune system dysfunction during aging, which opens new vistas for its correction with short synthetic peptides.

Key Words: *lymphocyte-activating factors; peptides; aging*

Aging is largely determined by impairment of the defense functions of the body, including mechanisms of congenital and acquired immunity and their regulation [1,2,7,13]. Purposeful modulation of these mechanisms opens new vistas for inhibition of aging and treatment of age-specific diseases. The use of drugs modulating functional activity of the immune system cells is regarded as a promising approach to the correction of age-related immune system dysfunction. These immunomodulating drugs include natural and synthetic peptides developed on the basis of endogenous regulators of defense functions and characterized by high biological activity [3,4,6,8,10,11].

Changed production of lymphocyte-activating factors (LAF) by mononuclear phagocytes is an informative parameter characterizing activity of the immune system. LAF is a complex indicator including several immunomodulating cytokines, such as IL-1, IL-6, and TNF-α [5,9].

We studied LAF production by peritoneal macrophages (PM) of young and old mice and modulating effect of short synthetic peptides on this production.

MATERIALS AND METHODS

Experiments were carried out on male (CBA× C57Bl/6)F_1 hybrid mice: 64 young (2 months, 18-22 g) and 52 old (19-20 months, 24-30 g).

Isolated resident PM were activated and LAF production was induced by *E. coli* LPS (Sigma) in a concentration of 200 μg/ml cell suspension.

The lymphocyte-activating characteristics of incubated PM were evaluated by their comitogenic effects on the proliferation of mouse thymocytes stimulated with concanavalin A (ConA) in the suboptimal dose [12]. The concentration of LAF pro-

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ducing a 50% stimulating effect on thymocyte proliferation from maximum stimulating effect of ConA in the suboptimal dose was taken for one unit of LAF activity.

Synthetic peptides Vilon (Lys-Glu), Epithalon (Ala-Glu-Asp-Gly), and Cortagen (Ala-Glu-Asp-Pro) created on the basis of amino acid analysis of endogenous peptide bioregulators from the thymus, pineal gland, and brain cortex, respectively (St. Petersburg Institute of Bioregulation and Gerontology) were added to mouse macrophage suspension in final concentrations of 0.0025, 0.025, and 0.25 ng/ml.

The results were statistically processed using Student's *t* test.

RESULTS

Resident PM of young and old mice did not produce LAF without stimulation, while after *in vitro* LPS stimulation LAF release was weaker by 62.5% compared to the corresponding parameter in young mice (Figs. 1-3).

Vilon stimulated the production of LAF by macrophages of young and old mice without extra activation of cells with LPS (Fig. 1). Stimulation of PM isolated from animals of both age groups with LPS together with Vilon in the same doses (except 0.0025 ng/ml for young mice) increased LAF production by macrophages in comparison

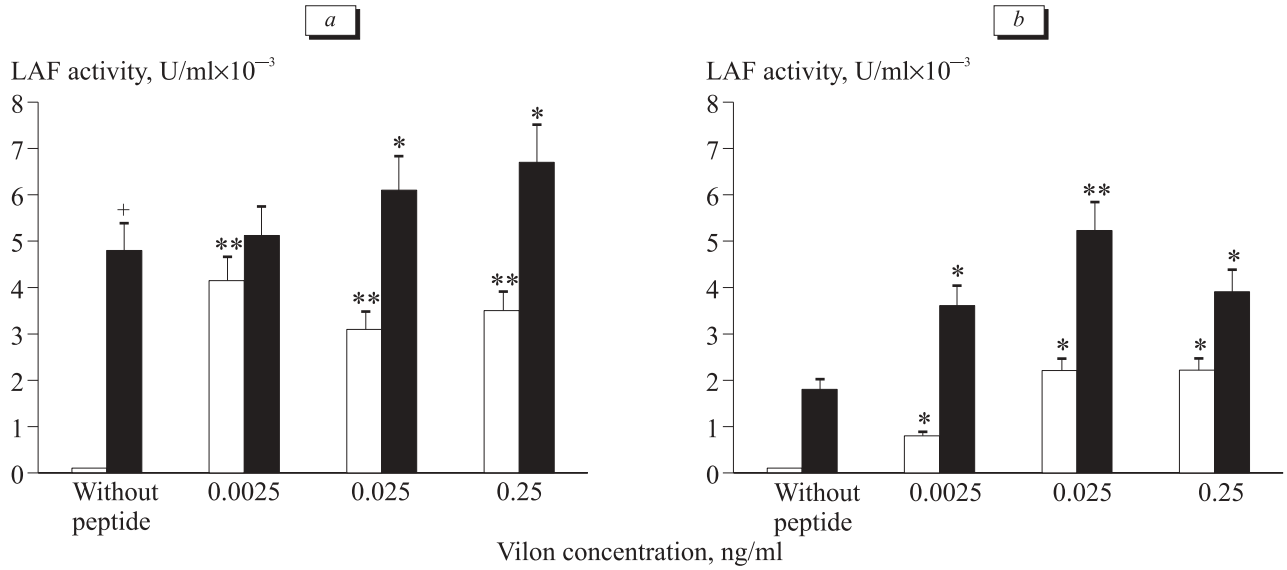

Fig. 1. Effect of Vilon on LAF production by PM of young (*a*) and old (*b*) mice. Here and in Figs. 2, 3: light bars: no additional stimulation of cells *in vitro*; dark bars: LPS stimulation. $^{+}p<0.01$ compared to old mice; $^{*}p<0.05$, $^{**}p<0.01$ compared to LAF production without addition of the peptide.

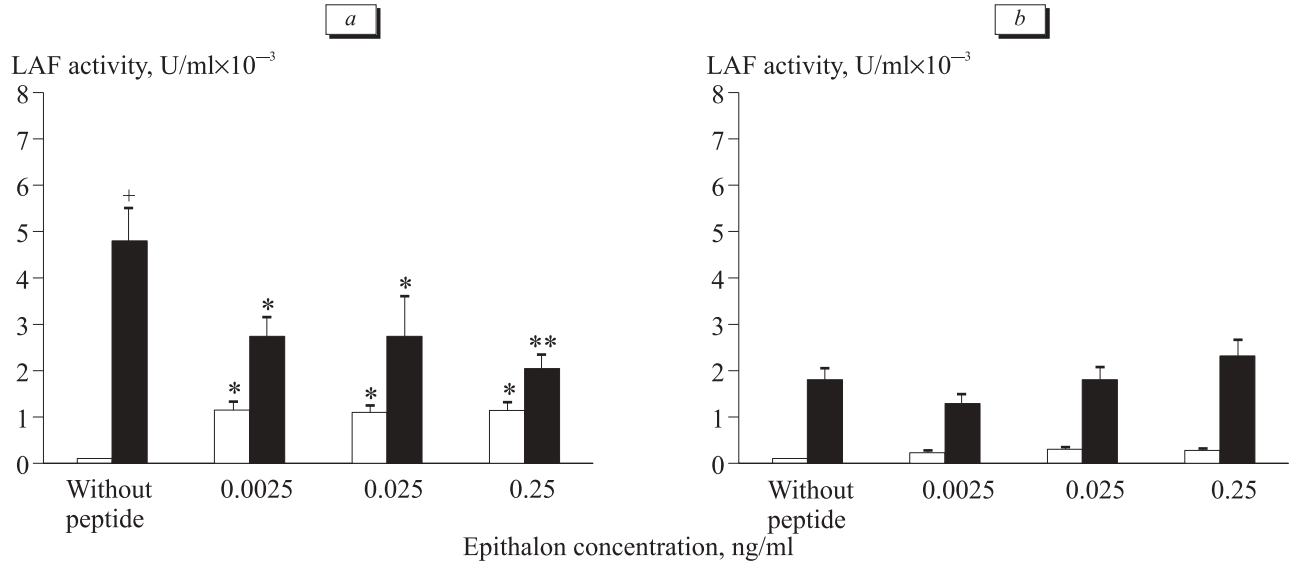

Fig. 2. Effect of Epithalon on LAF production by PM of young (*a*) and old (*b*) mice.

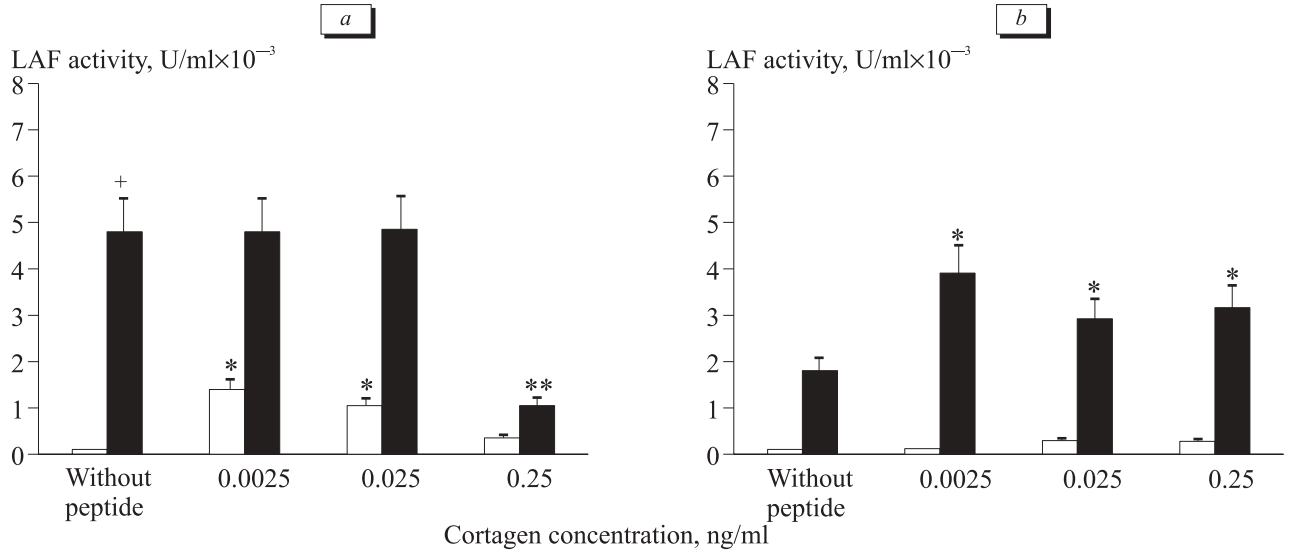

Fig. 3. Effect of Cortagen on LAF production by PM of young (*a*) and old (*b*) mice.

with the cells stimulated with LPS without peptide (Fig. 1).

Young mouse macrophages also responded by LAF production to the addition of Epithalon in all the studied doses into incubation medium (Fig. 2, *a*) and to Cortagen in doses of 0.0025 and 0.025 ng/ml (Fig. 3, *a*). In contrast to Vilon, these peptides did not stimulate LAF release by macrophages of old mice in any of the doses (Figs. 2, 3).

The intensity and vector of changes in LPS-induced production of LAF by macrophages of young and old mice under the effect of peptides were different. Stimulation of macrophages from young mice with LPS in combination with Epithalon in all the studied doses decreased LAF production (Fig. 2, *a*), while Cortagen reduced LAF production only in a dose of 0.25 ng/ml (Fig. 3, *a*). Addition of Epithalon together with LPS to macrophage suspension from old mice did not change LAF activity of incubated cells (Fig. 2, *b*), while addition of Cortagen to the medium increased it (Fig. 3, *b*).

Hence, LAF release by *in vitro* LPS-stimulated macrophages is less pronounced in old animals, which attests to decreased of PM functional activity reserve during aging. These results are in line with the data on the development of quantitative deficiency of immunocompetent cells (primarily the lymphoid cells) and decrease in their functional activity with age [2,7,13].

The strategy of leveling the changes in functional activity of immunocompetent cells with aging can be based, among other things, on the use of short synthetic peptides. All the three studied peptides induce the production of LAF by macrophages of young mice, but only Vilon exhibited the same effect on old animal macrophages. The most significant result of the study is the increase in LPS sensitivity of old mouse macrophages under the effect of Vilon and Cortagen; hence, these peptides restore functional activity of PM, reduced during aging.

We conclude that modification of LAF production by macrophages is a possile mechanism of immune dysfunctions during aging and suggests correction of this dysfunction with short peptide bioregulators.

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