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The Peptide Ala-Glu-Asp-Gly and Interferon Gamma: Their Role in Immune Response during Aging

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Abstract—The decrease in interferon gamma expression by lymphocytes during aging is one of the main mechanisms leading to an immunodeficiency state in the elderly. Cell-penetrating geroprotective peptide Ala-Glu-Asp-Gly has the ability to activate proliferation of lymphocytes in the thymus during its aging. The nucleotide sequence that complementary contacts with Ala-Glu-Asp-Gly has been found in the promoter region of interferon gamma gene. Thus, the immunoprotective effect of the peptide can be explained by activation of interferon gamma production in T cells.

Keywords: interferon gamma, lymphocytes, Ala-Glu-Asp-Gly, aging
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INTRODUCTION

The changes during age-related involution of the immune system are more pronounced compared to those of other organs and are caused by a decrease in T- and B-lymphocytes, as well as in thymic hormones and cytokines [7, 21, 22], the latter being involved in endocrine control of the immune and blood systems, and thymic hormones having many of the properties of neuropeptides [6, 19, 26].

At present, a series of reports have appeared on the fact that there is a decrease with aging in the formation, via mitogen-stimulated mononuclear cells, of interferon gamma (IFNγ), a key cytokine whose insufficiency plays a crucial role in the development of immunopathological states [16]. Moreover, F. Caytanot et al. demonstrated that the IFNγ level in the nocturnal primate *Microcebus murinus* correlates with the lifespan of animals [9]. From these experiments, the authors conclude that the IFNγ plasma level could be used to predict the life span of primates.

It should be noted that the level of cytokines, including that of interferons, in the blood depends on the intensity of the immune response to different foreign and self antigens [3, 4, 16, 17]. During aging, the number of antigens affecting our bodies increases and that affects the cytokine content. Therefore, a reduced interferon response to antigens with aging is adverse and can lead to the development of pathological states, even to a fatal outcome.

Interferon gamma is synthesized mainly by cytotoxic ($CD8^+$) mitogen- and antigen-stimulated killer cells, as well as by natural ones (NK cells $CD3^+CD16^+$ and $CD3^-$, $CD16^+$). IFNγ represents a family of glycoproteins with a molecular mass of 16–25 kDa. In the early stage of infection, IFNγ is almost absent or found in insignificant amounts. IFNγ production and secretion occur only after repeated encounter of earlier sensitized lymphocytes with antigens. This cytokine is not capable of immediately affecting an infectious agent. It exerts its effects mainly via monocytes, macrophages, and NK cells, which are strongly stimulated by it. Moreover, IFNγ enhances IFNα and IFNβ [5], increases the production of antibodies, leads to the production and secretion of anti-inflammatory cytokines, and activates NK cells and cytotoxic T-lymphocytes [16]. It also induces HLA class I and II expression in many cells, which promotes immune response; IFNγ enhances antigen presentation and facilitates its recognition by T-lymphocytes.

When released at the early stage of a pathological process by NK cells, IFNγ is directly involved in lymphocyte–endothelial cell adhesion in postcapillary veins. This effect is caused by the expression of adhesion molecules (ICAM-1), which results in increased adhesion of lymphocytes expressing the corresponding ligand, integrin LFA-1. IFNγ is able to rapidly increase vascular permeability to macromolecules and, together with TNFα, to induce the production and secretion of chemokines promoting leukocyte chemotaxis.

At the same time, investigations carried out at the St. Petersburg Institute of Bioregulation and Gerontology under the direction of V.Kh. Khavinson [8, 11–15] have revealed that small peptides, synthesized and constructed from the data obtained on the amino acid composition of the polypeptide complex isolated from different organs, are capable of modulating immune response and significantly increasing the life span of experimental animals [12, 15]. One such small peptide is epitalon (Ala-Glu-Asp-Gly) [8].

Short cell penetrating peptides (CPPs) of natural and synthetic origin are involved in the activation of cell proliferation and differentiation by transcription factors. CPPs represent a group of peptides containing no more than 20 amino acid residues with a molecular mass of up to 4 kDa [10]. Under physiological conditions, CPPs are multiply charged ions. They have the ability to form noncovalent bonds with nucleic acids, amino acids, peptides and to transport them to the destination within cell, e.g. to chromatin in the nucleus [10, 25]. CPPs group contains the natural short peptides (R-PTD$_4$, bt-NLS, Lig1-PBD-F, F(Ahx)-TAT), as well as synthetic ones, e.g., Ala-Glu-Asp-Gly made at the St. Petersburg Institute of Bioregulation and Gerontology. It is still unclear how highly hydrophilic CPPs penetrate a cell and genome; current hypotheses (direct membrane penetration, endocytosis, inverted micelle formation) are under debate [24]. Anyway, hydrophilic short peptides, in contrast to steroid hormones, can bind to hydrophilic groups of phospholipids outside the plasma membrane, cluster, and enter the cell using a pinocytic-like mechanism [11, 24].

The nuclear membrane has a transport system of nuclear pore complexes formed by protein complexes, nucleoporins, that regulate the traffic of nucleoprotein complexes in and out of the nucleus. The internal diameter of a nuclear pore is 42 nm; the external diameter is 50 nm. Therefore, they are permeable to diffusing molecules with a molecular mass of up to 5 kDa; the sizes of the Ala-Glu-Asp-Gly peptide under study are 1.3–1.4 nm in length and about 0.6–1 nm in diameter (Fig. 1) and their molecular masses are 0.27 and 0.38 kDa, respectively. Thus, the peptides Lys-Glu and Ala-Glu-Asp-Gly due to their steric characteristics are able to enter the nucleus through nuclear pores, which was mentioned in [11]; as well, the FITC-labeled Ala-Glu-Asp-Gly peptide has been shown to penetrate the cytoplasm, nucleus, and nucleolus of HeLa cells [11]. The penetration mechanism of Ala-Glu-Asp-Gly into the nucleus could be similar to that described for natural CPPs [23]; however, there is the possibility that Ala-Glu-Asp-Gly could be delivered to a nucleus by large natural CPPs.

With microchip technology, it has been established that Ala-Glu-Asp-Gly regulates expression of genes functionally related to different cell systems, including

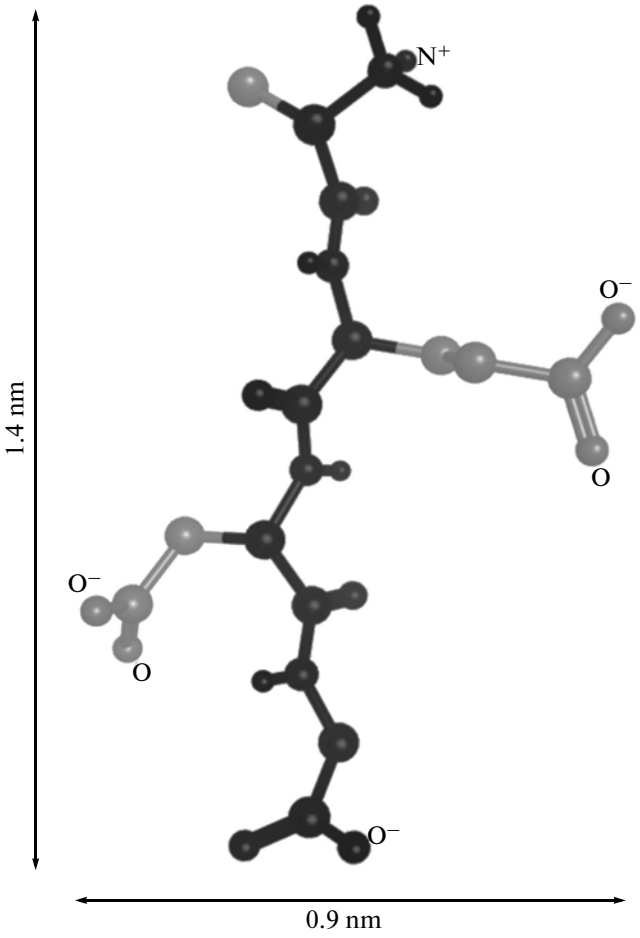

Fig. 1. Conformation of Ala-Glu-Asp-Gly peptide with optimal minimization energy. Dark gray, carbon atoms (C); light gray, oxygen atoms (O); black, nitrogen atoms (N).

cytokine IL-2 [12]. In experiments on mice, the effect of epitalon (Ala-Glu-Asp-Gly) at a dose of 50 pg/mL and 5, 50 and 100 ng/mL on IL-2 gene expression was studied in spleen lymphocytes in vitro. Ala-Glu-Asp-Gly was demonstrated to promote synthesis of messenger RNA of IL-2 in lymphocytes, the effect depending on the concentration and duration of use [12]. Maximum expression of IL-2 was observed at 5 h after exposure to the peptide at the lowest two concentrations, while higher concentrations and a time interval of up to 20 h resulted in a reduced effect. Therefore, Ala-Glu-Asp-Gly has a selectivity for binding sites in promoter regions of genes. Amino acid residues of natural CPPs are believed to form a hydrogen bond network with functional groups in the major groove of DNA double helix. CPPs of Ala-Glu-Asp-Gly seem to have similar effect, which may cause its immunoprotective properties.

It should be noted that IL-2 promotes IFNγ synthesis via cytotoxic lymphocytes and NK cells. Moreover, NK cells begin to intensively produce IFNγ only

Possible binding sites for epitalon in promoter region of *IFN*γ gene

mRNA	Sequence (5' → 3')
*IFN*γ	1 cacattgttc tgatcatctg aagatcagct attagaaga**g aaag**atcagt taagtc**cttt**
(NM_000619.2)	61 **g**gacctgatc agcttgatac aagaactact g**atttc**aact t**ctttg**gctt aattctctcg 121 **gaaac**g

Note: Boldface denotes expected gene binding sites for peptides. Promoter regions of genes are brought from GenBank (NCBI), numerical symbols denotes ordinal numbers of nucleotide in the gene, and the number of the sequence in the database GenBank [2] is shown in parentheses.

after interaction with cancer and virus-infected cells; this effect is enhanced by IL-12. Herewith, as was shown by a number of authors [1,2], the IL-2 and IL-12 concentrations are significantly decreased in elderly people.

Then, it can be suggested that the decrease in IFNγ could be caused by the decrease in IL-2 synthesis. Ala-Glu-Asp-Gly normalizing the IL-2 level should inevitably lead to an increase in the IFNγ level in the blood [18]. However, it is possible that IFNγ can interact with the DNA binding site in the promoter of the corresponding gene.

To prove the hypothesis, we determined the location of binding sites complementary to Ala-Glu-Asp-Gly in the promoter region of *IFN*γ gene (table, Fig. 2). Our investigations revealed ATTTC, ATTTG, GTTTG, and CTTTC sequences in the promoter regions. Thus, it can be concluded that Ala-Glu-Asp-Gly binds to *IFN*γ genes in the promoter region and activates gene expression or acts as a cofactor in DNA transcription.

Thus, this research demonstrated that *IFN*γ gene sequences found contain the DNA fragment, ATTTG, which is potential binding site to Ala-Glu-Asp-Gly. Domains of transcription factors interacting with DNA have an α-helical structure. In α-helix

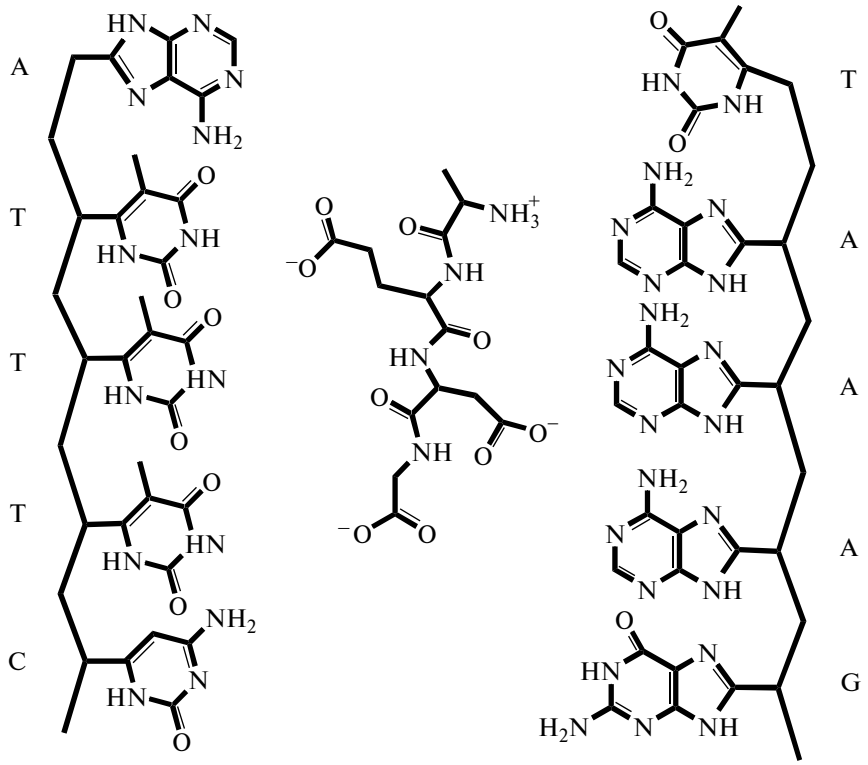

Fig. 2. Ala-Glu-Asp-Gly peptide localization in major groove of DNA double helix in nucleotide sequence, ATTTC (5'-3'), and one complementary to it, TAAAC (3'-5'). Bold lines designate bonds between carbon atoms' letters denote nitrogen atoms (*N*) and oxygen atoms (O).

structure of proteins one helical turn contains 3.61 residues, i.e. tetrapeptide is a minimal fragment capable of forming α-helix structure. Ala-Glu-Asp-Gly is related to such tetrapeptides. Under physiological conditions, the distance between the first and the last carbon atoms of the Ala-Glu-Asp-Gly backbone is 5.43 Å, which corresponds exactly to the pitch of the α-helix in the protein molecule [12]. Therefore, the tetrapeptide Ala-Glu-Asp-Gly could interact with the major groove of DNA.

It has been found that Ala-Glu-Asp-Gly is capable of increasing the size of different thymus cell subpopulations in an organotypic cell culture of the thymus of old rats. Thus, upon exposure of the peptide, there is an increase by a factor of 2–4 in $CD5^+$ thymocytes, $CD8^+$ cells (cytotoxic T lymphocytes), and $CD20^+$ cells (B-lymphocytes), while the expression of proapoptotic transcription factor p53 is decreased.

In addition, Ala-Glu-Asp-Gly inhibits thymic epithelial cells and activates thymocytes: on the one hand, it reduces the expression of molecular markers for activation of thymic epithelial cells (CD54, CD69 and HLA-DR) and, on the other hand, it enhances the expression of thymocyte-activating molecules (CD54 and HLA-DR) [20].

Thus, the immunoprotective effect of Ala-Glu-Asp-Gly could be associated with thymocyte and end-stage lymphocyte activation, as well as with enhancement of IFNγ synthesis in them. By influencing *IL*-2 and *IFN*γ expression, the tetrapeptide normalizes cell-mediated immunity functions (through $CD8^+$ and NK cells) and that of the humoral one (through the T helper 2 clone) and thus results in a decrease in morbidity and mortality among elderly people. Epitalon is sure to have other effects such as normalization of antioxidant protection [8], the hemostasis system [3, 4], etc.

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