Effect of Synthetic Fragments of HIV Protein Immunodominant Sites on Human Neutrophil Oxygen Metabolism

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The important role played by neutrophilic dysfunction in AIDS pathogenesis is now considered proven [1]. The purpose of our study was to reveal a possible influence of HIV glycoprotein gp 120 and gp 41 fragments on spontaneous and zymosan-induced chemiluminescence (CL) of neutrophils, which reflects the intensity of a "breathing burst". In addition, the results will help predict the *in vivo* side effects of the tested peptides when used as vaccine components.

MATERIALS AND METHODS

Blood samples were collected from healthy donors and from patients with various chronic allergic disorders. The neutrophils were isolated by isotonic lysis followed by differential centrifugation [2]; all the procedures were carried out at 4°C.

The studied peptides (Table 1) were synthesized by the solid-phase method and used in aqueous solutions. Zymosan (Reakhim, USSR) was opsonized by a mixture of blood sera from several donors. Luminol ($3\times\times10^{-5}$M) in Hanks solution (0.5 ml per cuvette) was used as a CL stimulant. The neutrophil concentration was 1 million/ml; CL was determined at 37°C.

TABLE 1. Peptides Used in the Study

No. of peptide	Correlation with natural fragment of HIV glycoprotein		
	HIV strain	qlycoprotein	amino acid fragment
337	BRU	gp120	252−272
480	BRU	gp120	307−329
24	MN	gp120	307−329
411	BRU	gp120	423−450
477	BRU	gp120	495−516
221	BRU	gp 41	573−593
551	BRU	gp 41	841−861

The investigation was carried out on an L-1251 luminometer (LKB, Finland) in the following variants: 1) the cells were added to the luminometer cuvette already containing peptide in the required concentration in Hanks solution; 2) the same as 1), but the cells were preincubated at 37°C for 20 min with subsequent cooling to 4°C to "prime" them; 3) the neutrophils were incubated for 20 min with the peptide in phosphate-buffered normal saline at 4°C, washed, and then added to the luminometer cuvette with Hanks solution and luminol; 4) the same as 3), but the cells were preheated at 37°C (see variant 2);

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TABLE 2. Neutrophil Chemiluminescence Primed by Heating (variant 4), $M \pm m$

Peptide concentration, M, and donor group	Preincubation with peptide									
	221		24		480		411		337	
	spont	control	spont	control	spont	control	spont	control	spont	control
Group 1, 10^{-5}	3.9±1.3	4.4±1.2	4.6±1.1	5.3±1.0	4.5±1.2	4.5±0.9	5.0±1.6	5.0±1.5	3.8±1.1	4.4±1.2
Group 2, 10^{-5}	6.0±2.5	4.2±2.1	4.7±2.2	4.2±2.1	11.7±4.6*	4.2±2.1	9.0±1.7	4.2±2.1	9.6±1.7	4.2±2.1

Note: spont. – spontaneous CL, asterisk – peptide effect is reliable in comparison with control for $p<0.05$.

5) the same as 1), but 1:2500 of dimethylsulfoxide (DMSO) was added to the cuvette. After a 20-min redistration of spontaneous luminescence, zymosan was added to the cuvette and the maximum level of induced CL was recorded. The results were statistically processed by Wilcoxson's method.

RESULTS

The experimental results in the first variant showed no influence of peptides in a concentration of 10^{-5} M on CL. Neutrophils primed by the heating-cooling cycle shoved a higher sensitivity to peptides, and suppression of spontaneous CL by peptides 480 and 24 was observed: 2.5±0.5 mV (control measurement [C] 3.6±0.6 mV, $p<0.05$) and 1.9±0.8 mV (C:2.9±1.1 mV, $p<0.05$), respectively.

In the experimental variant with peptide washing after cold incubation in a medium without bivalent cations, when endocytosis processes were supressed in contrast to variants 1,2, and 5, the detected effects were apparently not connected with internalization of the peptides bound to cell surface structures. In this case only an increase of spontaneous CL in the presence of peptide 221 was found: 6.5±2.0 mV versus 5.4±1.8 mV in the control ($p<0.05$, peptide concentration 10^{-5} M). Preheated neutrophils in the same experiment showed two types of reactivity to peptides, reliably different from each other for peptides 221, 480, 411, and 337 (Table 2). The cells of one group of donors proved insensitive to peptides, whereas others reacted by a sharp increase of the intensity of spontaneous CL in response to peptides 337, 480, 411, and 221. No correlation was revealed between donor health status and appurtenance to this or that group.

It is interesting that in variant 5 just these same peptides, except for 337, proved to be able to dramatically enhance spontaneous CL in all donors (Table 3). Under such conditions peptide 480 significantly lowered the level of zymosan-induced CL. All the effects shown were dose-dependent. A slight suppresion of stimulated CL by all peptides in concentrations of 5×10^{-5} M was evidently nonspecific.

Peptide effects were found to be significantly changed in the presence of low DMSO concentrations (under 1:2500) virtually not stimulating CL; a dose-dependent DMSO influence on the peptide effect was observed. A possible explanation of this could be increased membrane permeability in the presence of DMSO, which potentiated spontaneous CL caused by peptide 480 when added to the cells of all donors in experimental variants 3 and 4 with incubation, although in this case suppression of zymosan-induced CL was not observed (the data are not presented). One possible interpretation of the results is as follows: apparently peptides 480, 411, 337, and 221 are able to cause a marked activation of spontaneous luminescence of neutrophils only penetrating into the cell, or else in the presemce of increased membrane permeability. Since suppression of peptide 480-stimulated CL was observed only at 37°C, it may be assumed to depend on the functional activity of the cells. Peptide 24, differing from 480 only in a few amino acids, failed to intensify spontaneous CL in any of the experimental variants, although, like its

TABLE 3. Neutrophil Chemiluminescence (in mV) in the Presence of DMSO (Variant 5), $M \pm m$

Concentration, M	Incubation with peptide											
	221		477		480		411		337		551	
	spont.	stim.	spont.	stim.	spont.	stim.	spont.	stim.	spont.	stim.	spont.	stim.
10^{-7}	5.5±1.4	37±13	8.5±3.3	46±11	7.4±1.8	46±9.8	8.1±2.1	51±9.9	7.6±1.8	50±10	9.6±2.6	44±9.8
10^{-6}	5.7±1.2	42±12	9.0±3.4	42±7.3	11±2.5*	46±10	11±2.9*	52±9.7	9.6±2.1*	45±9.8	11±3.6	39±7.8
10^{-5}	5.6±0.8	50±11	10±2.8	49±8.9	19±2.5*	40±9.4*	12±3.3*	42±8.9	6.6±1.5	46±9.8	10±2.8	41±9.0
Control	5.7±1.1	54±9.1	9.9±2.9	48±7.4	7.1±1.3	47±9.9	7.7±2.4	48±8.6	7.1±1.3	46±9.1	9.5±2.4	42±9.3
$5\cdot10^{-5}$	19.4±2.5*	43±9.3*	20±3.1	36±4.8*	33±2.9*	14±1.5*	18±1.0*	33±5.5*	8.4±2.0	40±6.8*	15±5.4	52±15*
Control	11±2.0	62±9.6	18±4.6	56±7.8	11±2.2	66±10	14±2.5	50±8.5	11±2.2	66±10	14±5.5	65±12

Note: spont. – spontaneous, stim. – stimulated CL, asterisk – peptide effect is reliable in comparison with control for $p<0.05$.

analog, it suppressed spontaneous CL in variant 3 (but not in the presence of DMSO).

Hence, some of the immunodominant fragments of the surface HIV glycoproteins were found to disturb the normal CL kinetics of neutrophils, which is known to reflect their production of active oxygen derivatives - significant factors of the organism's nonspecific protection. The data obtained attest to the need for a careful selection of peptides suitable for the creation of vaccine preparations with the aim of preventing possible side effects on neutrophilic function, including the side effects which may occur during DMSO therapy. Furthermore, the effects observed with some peptides suggest that the detected disturbances of CL response and superoxide production [3,4] in neutrophils of AIDS patients may be the result of a direct influence of HIV glycoproteins on phagocytosing cells.

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EXPERIMENTAL GENETICS

Peculiarities of Expression of the Plasmid pAP42 Genetic Region Determining "Sex" Pili Synthesis and the Surface Exclusion System in Different Cells of *Escherichia coli*

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The functional specificity of "sex" pili synthesized by bacteria under the genetic control of plasmids, and of the surface exclusion systems (Sfx systems) determines the efficiency of conjugational transfer of F-like plasmids between cells of different bacterial populations [3,4,6,9,10]. Meanwhile, the patterns of expression of the genetic region represented in the genomes of different plasmids and allowing for the formation of a distinct functional type of pili and group affiliation of the Sfx system in different bacterial cells, e.g., serologically nontyped and typed variants, require scrutinized study.

The goal of this study was an analysis of the expression of this region as a constituent of the F-

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