EXPERIMENTAL GENETICS

REGULATION OF INTERLEUKIN 2 FORMATION BY IMMUNOMODULATING PEPTIDES AND ITS POSSIBLE MECHANISM

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Trans- acting factors connected with DNA sequences of promoter and enhancer regions, and regulating gene expression, are no known for a whole range of genes. The possibility cannot be ruled out that peptides, which possess neuroimmunomodulating properties and which may perhaps be responsible for contact transmission of information between nervous and immune systems, may also belong to this group of factors. According to Chipens and co-workers [2], fragments of immunologically active proteins, synthesized in different brain structures and exerting an immunomodulating action, and also synthetic analogs of these fragments, are peptides of this kind.

In the investigation described below the effect of synthetic peptides possessing immunomodulating properties (SKD peptides, R tetrapeptide, and the shortened analog of luliberin — L) on synthesis of interleukin 2 (IL 2) in T lymphocytes and the possibility of their specific interaction with nucleotide sequences of the gene of this protein, was studied.

EXPERIMENTAL METHOD

The effect of regulatory peptides (SKD and R peptides were synthesized in the laboratory (head, G. I. Chipens) of the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR (Riga) on production of IL 2 by mouse spleen T lymphocytes, activated by concanavalin A (con A)) was investigated during incubation of the cells in the presence of different concentrations of the peptide for 24 h. The level of activity of IL 2 in the supernatant of the cultures was estimated by a biological method [1]. Recombinant human IL 2 ("Biogen" Inter-Institute Scientific–Technical Combine) was used as the standard for the calibration curve. To assess interaction between sequences of the IL 2 gene and peptides, HindIII-fragments of plasmid pUC190, containing the full-size gene of bovine IL 2 were used. (The plasmid pUC190 was obtained from Corresponding Member of the Academy of Sciences of the USSR E. D. Sverdlov), terminally labeled with ^{32}P dATP. The specificity of interaction was assessed by studying binding of the peptide–DNA complex on nitrocellulose filters [4].

EXPERIMENTAL RESULTS

Addition of the peptides to cultures of con A-activated (2 μg/ml) mouse splenic lymphocytes had opposite effects on IL 2 production by the cells. Peptide R induced a decrease in the formation of T-cell growth factor with an increase in concentration of the preparation. With peptides L and SKD a stimulating action on IL 2 production was discovered in a culture of mouse spleen cells, and the maximal effect, a 3-5-fold increase, was observed between concentrations of 55 and 110 ng/ml; in higher or lower concentrations the preparations had no effect (see Table 1).

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TABLE 1. Effect of Synthetic Peptides R, L, and SKD on IL 2 Production in a Culture of Con A-Activated Mouse Spleen Cells (n = 3-6)

Peptide	Concentration of peptide, ng/ml	IL 2 activity in culture medium, 10 IU IL 2/ml	% of control
Control (without peptide)	—	17,4	100
R	10	12,6	72
	60	11,6	67
	125	11,2	64
	250	11,3	65
	500	10,1	58
L	7,5	19,4	112
	37,5	17,6	101
	75	69,7	401
	150	20,0	115
SKD	11	19,4	112
	55	100,1	575
	110	53,9	310
	220	17,6	101

The effect produced, namely a change in IL 2 activity, could be due to modulation of expression of the IL 2 gene. In that case, there could be either direct interaction of peptide *trans*-factors with the regulatory sequences of the gene or a secondary indirect mechanism of regulation. Experiments were carried out to study the ability of peptides SKD, R, and L to form a complex with the nucleotide sequences of the DNA of IL 2. As a first step the DNA of pUC190 was mapped relative to the arrangement of HindIII sites. Five fragments, A, B, C, D, and E, were shown to be present, corresponding to 2.7, 2.68, 1.42, 1.88, and 0.6 kbp. According to the results of hybridization, the 3′- and 5′-flanking sequences of the gene correspond to fragments A and D. Binding of peptide-DNA complexes to nitrocellulose filters showed high affinity of peptides R and L to HindIII-fragments of pUC190 DNA, including regions of the bovine IL 2 gene. On the introduction of an equal number of labeled DNA fragments into each lane of the gel, complexes of peptides R and L with DNA were retained on the filters if the ratios of peptide to DNA were 0.002 and 0.005 respectively. The quantity of ^{32}P-DNA retained on the filter was reduced as the peptide concentration in the sample fell, in good agreement with data in the literature [4]. Thus, when the ratios of peptide R to DNA were 0.05, 0.02, 0.01, and 0.002, the fraction of DNA retained on the filter compared with the total quantity of ^{32}P-DNA in the sample was 42.2, 39.8, 31.6, and 18.6% respectively. With ratios of peptide L to DNA of 1.0, 0.2, 0.02, and 0.005, these values were 50.5, 46.7, 39.8, and 16.8% respectively (Fig. 1a). SKD bound with pUC190 DNA in trace amounts, only with ratios of peptide to DNA of 5 or more. Comparison of the experimental results and theoretical calculations shows that if the ratio of peptide to DNA is 0.002, for each fragment of DNA of the IL 2 gene measuring 2.7 and 1.88 kbp there are approximately 20-30 molecules of peptide R and 10-15 molecules of peptide L respectively. These peptides can evidently form stable complexes with HindIII-fragments of the IL 2 gene even with lower peptide to DNA ratios.

Nonspecific peptide—DNA complexes are known to dissociate in a solution with ionic strength of 0.3 M [5]. To assess the specificity of binding of peptides R and L with IL 2 DNA, the NaCl concentration in the binding buffer was increased from 0.1 to 0.5 M. It was found that an increase of ionic strength to 0.5 M NaCl did not affect the ability of peptides R and L to bind with the DNA fragments (Fig. 1b). The specificity of complex formation was proved by experiments to study competition with unlabeled heterologous or homologous DNA. As the former we used ultrasonic fragments of phage λ DNA or restriction fragments of plasmid pST826, containing sequences some of which are present in the pUC190 vector. It will be clear from Fig. 2 that a 300-fold excess of phage λ DNA did not affect the stability of complexes of peptides R and L with fragments of the IL 2 gene. Similar results also were obtained when a tenfold excess of pST826 DNA was used. Conversely, a twofold excess of homologous DNA (unlabeled fragments of pUC190 DNA) was sufficient to reduce binding of peptides R and L with HindIII-fragments of pUC190 DNA by 1.7 times approximately (Fig. 2). An increase in the excess of homologous unlabeled DNA to tenfold led to total destruction of the complex of these peptides with B- and C-fragments of pUC190 DNA and reduced their binding with A- and D-fragments, containing respectively 3′- and 5′-flanking sequences of the IL 2 gene respectively, by about 3.7-4 times. At the same time, the results of these

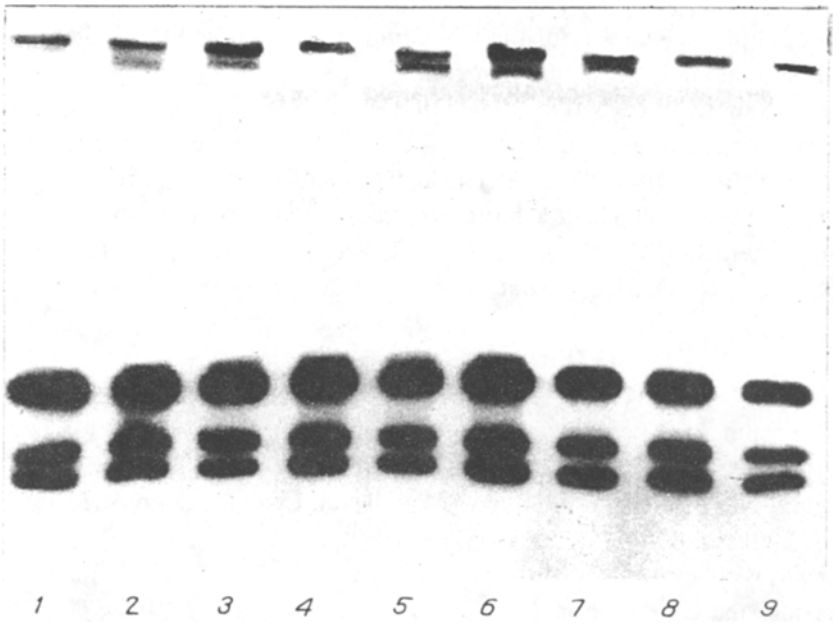

Fig. 1. Effect of NaCl concentration and peptide to DNA ratio on binding of HindIII-fragments of pUC190 DNA to nicrocellulose filters. 1) DNA of control fragments, NaCl concentration in binding buffer: 2) 0.01 M, 3) 0.3 M, 4) 0.4 M, 5) 0.5 M. Ratio of peptide R to DNA: 6) 0.05, 7) 0.02, 8) 0.01, 9) 0.002. Similar results were obtained for peptide L also.

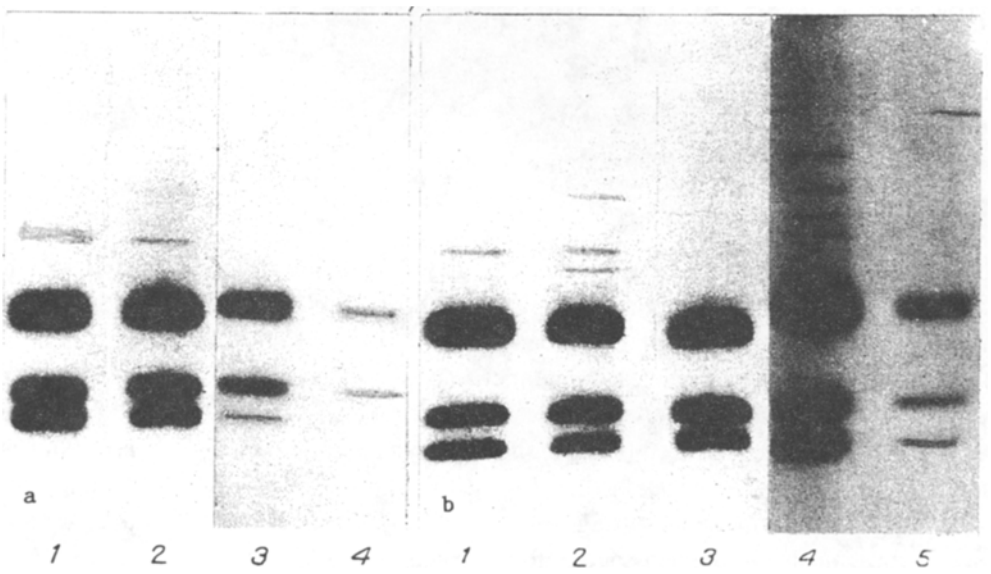

Fig. 2. Effect of unlabeled heterologous or homologous DNA on binding of HindIII-fragments of pUC190 DNA with peptides R (a) and L (b) on nitrocellulose filters. a: Ratio of peptide to DNA 0.05. 1) Interaction of pUC190 DNA fragments with peptide R in absence of competitive DNA; 2) 300-fold excess of DNA of phage λ; 3, 4) twofold and tenfold excess of unlabeled pUC190 DNA fragments. b: Peptide to DNA ratio 0.02. 1 and 4) Interaction of pUC190 DNA fragments with peptide L in absence of competitive DNA; 2 and 3) 100- and 300-fold excess of phage λ DNA; 5) tenfold excess of unlabeled pUC190 DNA fragments.

experiments indicate that affinity of peptides R and L for the DNA fragment containing the 3′-flanking region of the gene was greater than for the fragment containing the 5′-flanking region.

The results thus demonstrate specific interaction of peptides R and L with regulatory sequences of the IL 2 gene. The negative or positive character of regulation of IL 2 production by T lymphocytes may evidently be due to complex formation between specific nucleotide sequences of the IL 2 gene and the above-mentioned peptides. So far there has been only one indication in the literature of the existence of a protein nuclear factor AP-I [3], capable of regulating expression of the inducible IL 2 gene through interaction with its sequences. It is probable that peptide factors R and L may give an independent; regulatory effect, whereas peptide SKD may act indirectly through other protein factors.

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NEW HOST DNA SPECIFICITY SYSTEMS PAE 610 AND PAE 603

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The rapid development of genetic engineering has been largely due to the discovery and use of highly specific restriction endonucleases and modifying methylases, constituting the host specificity system (HSS) of DNA. Several hundreds of these enzymes are now known [13], but the broad front of research requires expansion of the arsenal of enzymes and identification of new restriction endonucleases and methylases. This task is linked, in turn, with the search for and study of new DNA restriction and modification (r-m) systems.

The aim of this investigation was to discover HSS among strains of *Pseudomonas aeruginosa* and to study them. Identification of RM systems in these microorganisms in the proposed research, by contrast with systems described previously [5, 9, 10, 11], is based on priority of biological testing, which enables a large number of strains to be tested and the distribution of HSS among microorganisms of a given species to be tested and, finally. the discovery of RM systems to be differentiated from the HSS already known.

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