EXPERIMENTAL GENETICS

Activation of Interleukin-2 Gene Transcription by Nuclear Factors from the Spleen and Brain of Immunized Rats

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The nuclear protein factors which stimulate synthesis of mRNA of interleukin-2 (IL-2) in the system of surviving murine T lymphocytes are isolated from cells of the spleen and brain of immunized rats. The active fractions of spleen and brain (13-19 and 25 kD, respectively), which interact with the promoter-enhancer sequences of the IL-2 gene localized in the region from +39 to -141 base pairs, are isolated by the method of high performance liquid chromatography from the total nuclear extract. The induction of luciferase synthesis is shown for protein factor-treated Jurkat cells transformed with p-IL-2LUC recombinant plasmid.

Key Words: *interleukin-2 gene; protein factors; regulation*

Regulation of the expression of individual genes with transacting factors is an issue at the forefront of molecular biology. This problem involves both an understanding of the nature of these factors and identification of the regulatory sequences of DNA, which are the binding sites of the factors per se. Another aspect concerns the mechanisms of competition between transacting proteins and histones for DNA binding sites, which cause a rearrangement of the nucleosomal structure of chromatin of the controlled gene and activation of its transcription [2]. A typical example of an inducible gene is the interleukin-2 (IL-2) gene. It is well known that expression of this lymphokine is a crucial factor in the functions of the immune system [4]. To date, several protein factors interacting with regulatory sequences of the IL-2 gene and affecting its expression have been identified [6]. However, the factors themselves have not been studied in depth, and little is known about their total amount, hierarchy, and universality. In our previous study [3] protein factors which differ considerably from earlier known factors with respect to molecular weight were isolated from cell nuclei of the spleen and brain of immunized rats. These nuclear proteins form stable specific complexes with the regulatory sequences of the IL-2 gene and stimulate the production of an IL-2-like factor in the system of surviving murine splenic lymphocytes.

In the present study we further analyzed the effect of protein factors on the production of IL-2 mRNA in the model system and on the expression of the luciferase (LUC) marker gene under the promoter of the IL-2 gene in order to verify the presence of regulatory proteins common to the nervous and immune systems which act at the gene level. In this study we also identified the region of the

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IL-2 gene promoter where the binding sites of these proteins and HI histone are localized.

MATERIALS AND METHODS

p-IL-2-LUC recombinant plasmid containing 587 base pairs (b.p.) of the 5'-flanking region of the human IL-2 gene (from +38 to -548 b.p.) was used. The regulatory zone of the IL-2 gene is attached to the LUC gene at the Hind III site and contains a single Hinf I restriction site (-141 b.p.). Regulatory DNA of IL-2 gene was obtained by digestion of the plasmid DNAs with Hind III restriction enzyme and by isolation of the Hind III fragment of DNA by preparative electrophoresis in 1% low-gelling agarose. The Hind III fragments were either labeled by [α^{32}P]^{d}ATP-nick-translation or restricted using Hinf I endonuclease for subsequent isolation of the proximal and distal fragments of the promoter. A standart Boehringer Mannheim kit was used for nonradioactive labeling of these fragments with digoxigenin. Male rats were immunized with sheep erythrocytes ($1\text{-}5\times10^9$ cells in 1 ml sterile physiological saline) during 7 days. The protein factors from cell nuclei of the spleen and brain were prepared by selective extraction followed by salting out with ammonium sulfate. Polypeptides found in the nuclear extracts were characterized by discontinuous electrophoresis (DISC-PAGE) in 15% polyacrylamide gel in the presence of sodium dodecylsulfate (SDS), using protein markers with a known Mr (molecular weight). HI histone was extracted from the hepatocyte nuclei with 0.74 N $HClO_4$ and separated from other proteins by gel-chromatography on P-60 Bio-Gel. The protein factors were fractionated by gel-chromatography on a column with Superose 12. The estimated Mr of the proteins comprising separate chromatographic zones was determined by calibrating the column with the marker proteins. For detection of DNA-binding proteins, the nuclear extracts were separated by SDS-DISC-PAGE into components which were transferred by electroblotting onto Hybond Amersham nitrocellulose membranes and hybridized with ^{32}P- or digoxigenin-labeled DNA. The above-mentioned methods were described in detail previously [3]. The human T-cell leukemia line Jurkat (subclone J32) was used for transfection with DNA of p-IL-2-LUC recombinant plasmid. Transfection was performed by the DEAE-dextran method [7]. The proteins of the nuclear extract or their chromatographic fractions (20 ng/ml) were added to the medium and the cells were incubated for 16-18 h. For recombinant gene induction ConA (2.0 ng/ml,

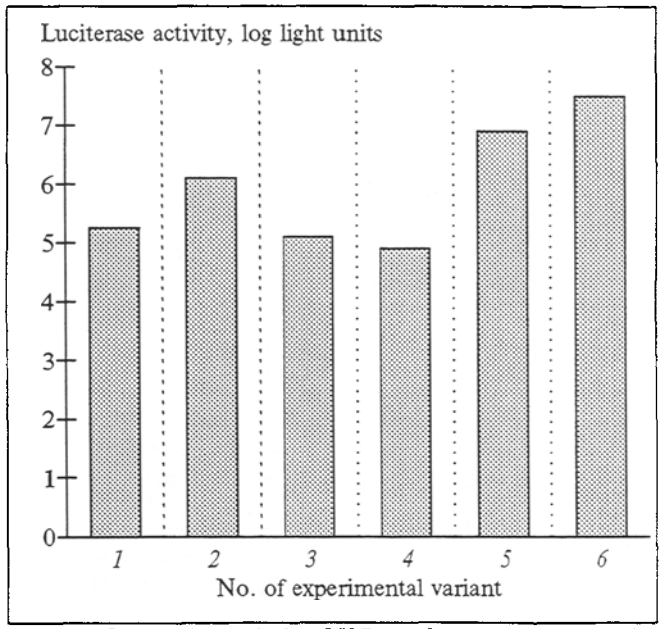

Fig. 1. Expression of the LUC marker gene under the influence of the promoter of the IL-2 gene (p-IL-2-LUC), transfected in Jurkat cells. *1*) T lymphocytes incubated for 48 h without treatment with p-IL-2-LUC DNA and protein factors; *2*) the same in the presence of p-IL-2-LUC; *3–6*) cells transformed with p-IL-2-LUC, incubated in the presence of NPS with molecular weights of 60, <5, and 13–19 kD (*3–5*, respectively) and of NPB with a molecular weight of 25 kD (*6*).

Sigma) was introduced to the system, after which the cells were additionally incubated for 24 h. Expression of the LUC gene was assessed by measuring the activity of luciferase in the sample obtained from transfected cells after Williams [8]. The synthesis of IL-2 mRNA in T lymphocytes incubated with protein factors (20 ng per cell) was analyzed by isolation of total RNA from the cells [1] followed by its spot-hybridization with digoxigenin-labeled cDNA. The intensity of staining of immunoprecipitate containing antibodies to digoxigenin was assessed on an LKB Ultraskan XL Laser densitometer.

RESULTS

In the previous study [3] we obtained preparations of nuclear proteins from cells of the spleen and brain (NPS and NPB, respectively) of immunized rats which were characterized by their molecular weight (SDS-PAGE) and by their ability to form stable complexes with the regulatory DNA of the human IL-2 gene. Electroblotting of proteins onto nitrocellulose membranes followed by hybridization with ^{32}P-DNA showed that *in vitro*, in the presence of 0.5 M NaCl and a 500-fold excess of heterologous DNA, NPS with 13-19 kD and HI histone form stable complexes with the promoter

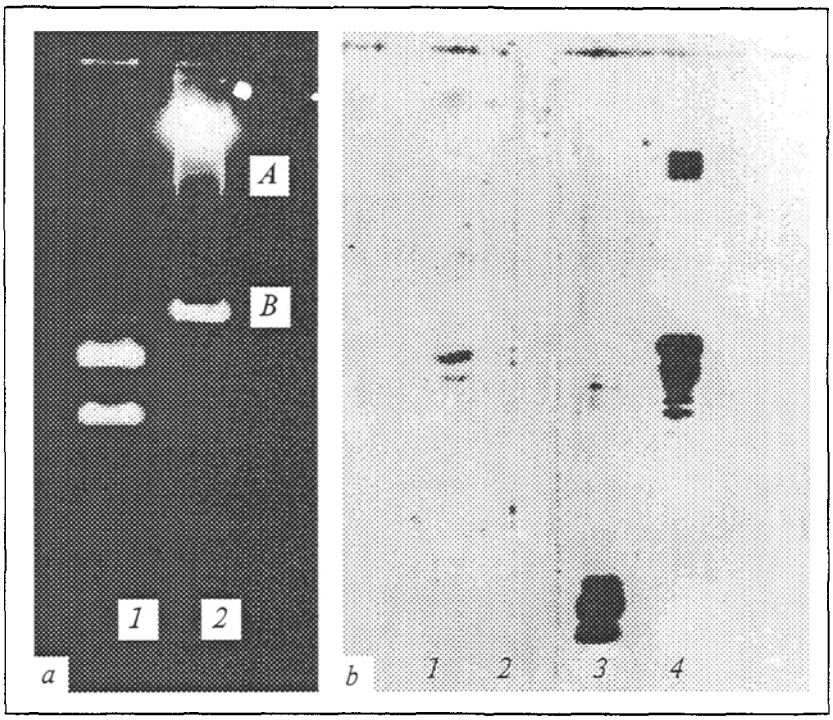

Fig. 2. Localization of binding sites of protein factors of the spleen in nucleotide sequences of the regulatory region of the IL-2 gene. *a*) restriction of DNA of p-IL-2-LUC with Hind III endonuclease (*2*) and restriction of B fragment with Hinf I endonuclease (*1*): *b*) immunodetection of DNA-protein complexes: *A*) incubation with fragment of regulatory region of IL-2 gene from -142 to -548 b.p.; *B*) incubation of fragment from +39 to -141 b.p. with NPS (*1* and *3*) and histone HI (*2* and *4*).

region (from +39 to -548 b.p.) of the IL-2 gene. Similar results were also obtained for the fractions of NPS isolated by fast-performance liquid chromatography. In this case specific binding of the proteins with Mr of 8-16 kD with the promoter DNA of the IL-2 gene was observed. A discordance between the total number of polypeptides identified by the two methods and the molecular weight of the proteins interacting with the regulatory DNA of the IL-2 gene may be attributed to the different resolving power of these methods and different error of Mr determination. Nevertheless, the molecular weights of DNA-binding polypeptides determined by the two methods (8-16 and 13-19 kD) are quite close. This correlation and the capability of specific binding with the promoter DNA of the IL-2 gene suggests that in both cases we are dealing with the same proteins. As for the preparations of NPB, out of the great number of polypeptides present there, only polypeptide with Mr of about 25 kD formed a stable complex with the regulatory DNA of the IL-2 gene. Thus, the molecular weight of the NPS and NPB recognizing the regulatory sites of the promoter DNA of the IL-2 gene differs markedly from that of such well-known transfactors as AP-1 or NF kB (47 and 75, 50 and 65 kD, respectively). This inevitably raises the question as to whether these proteins are the transacting factors. An indirect answer to this question was obtained in the experiments on blast-transformation of T lymphocytes. The total NPB proved to exhibit the strongest immunomodulating effect; the effect of the fraction of NPS with Mr 8-16 kD was 2-2.5 times weaker, and of the total NPS weaker still.

More convincing results were obtained by us in studies of the effect of NPB and individual fractions of NPS on the expression of the recombinant p-IL-2-LUC gene in transfected J32 Jurkat T cells. The results of these experiments are presented in Fig. 1. We see that the treatment of transfected cells with the mitogen ConA revealed the luciferase activity, which was due both to activation of the IL-2 promoter and to an increase in the number of cells carrying the recombinant gene. Addition of total NPB and of the fraction of NPS with a Mr of 8-16 kD elevated the luciferase gene expression almost 24- and 7-fold, respectively, as compared to its expression in the cells treated with mitogen alone. The fractions with a Mr of 60 and 5 kD exerted a pronounced negative effect on the expression of the reporter gene. The data on the binding of brain polypeptides with a Mr of 25 kD and of polypeptides of the spleen with a Mr of 13-19 kD to the regulatory DNA of the IL-2 gene lead to the conclusion that the activating effect of these proteins is due to their interaction with certain regions of the 5'-flanking DNA of the IL-2 gene. In other words, these polypeptides may be regarded as transacting factors.

For NPS with Mr 13-19 kD this region of the promoter DNA was identified by digestion of the Hind III fragment of plasmid DNA with Hinf I restriction endonuclease. Only one Hinf I restriction site (-141 b.p.) is present in the regulatory region of the IL-2 gene; as a result, two fragments of DNA are formed: the proximal (from +39 to -141 b.p.) and distal (from -142 to -548 b.p.) regions of the promoter (Fig. 2, *a*). Reportedly [6], either of these sites can form complexes with regulatory proteins. Hinf I-fragments were isolated by preparative electrophoresis in 1.5% low-gelling agarose and were labeled with digoxigenin-d-inosine triphosphate. Formation of the DNA-protein complexes was detected in electroblots by

immunoassay. As is seen from Fig. 2, *b*, HI histone and NPS with a Mr of 19, 18, and 13 kD form complexes only with the DNA of the proximal region of the promoter. Only HI histone shows a minor binding to the distal region. The presence of a binding site common to the transacting proteins from the spleen nuclei and to HI histone in the IL-2 gene promoter indicates the possibility of competition between them during activation of this gene. This is corroborated by the fact that an additional hypersensitive site does not appear in this region until after stimulation of T lymphocytes [7].

Lastly, in the final series of experiments it was shown that NPB and NPS directly stimulate IL-2 gene transcription. It is known that in resting T lymphocytes the IL-2 gene is inactive, and only stimulation with mitogens or phorbol esters induces the synthesis of IL-2 mRNA [5]. In this case the maximum synthesis of mRNA is observed after 8 h of incubation of stimulated cells. In our experiments stimulation of surviving T lymphocytes of mouse spleen was performed with ConA, ConA + p-IL-2, TPA (12-O-tetradecanoylphorbol 13-acetate), or TPA + PHA. The protein factors were added to the incubation medium in combination with or without the above-mentioned stimulators. The results show that the protein factors of nuclei from the brain and spleen largely contribute to mitogen- and phorbol ester-induced stimulation of the synthesis of IL-2 mRNA. This contribution markedly surpasses the effect of the esters and mitogens themselves and is equal to the increase of mRNA synthesis resulting from the activation of the IL-2 gene under the influence of protein factors alone (Fig. 3). As a matter of fact, however, the stimulating effect of NPS and NPB on mRNA synthesis proves to be reversed as compared to their activating effect on the promoter of the IL-2 gene. Total NPS prove to be more powerful activators of IL-2 mRNA synthesis in T lymphocytes than total NPB. This may be due to different mechanisms of transacting protein-induced activation. In the one case this activation results from the direct interaction of transfactors with the IL-2 gene promoter, whereas in the other case additional mechanisms of transduction of the activating signal are triggered.

Thus, the nuclear protein factors isolated by us from cells of the spleen and brain of immunized rats had molecular weights which differed from those of earlier known factors, formed complexes with nucleotide sequences of the promoter region of the IL-2 gene, and stimulated its expression. Our findings lend weight to our suggestion that there are bioregulators common to the nervous and immune systems which act at the level of the IL-2 gene.

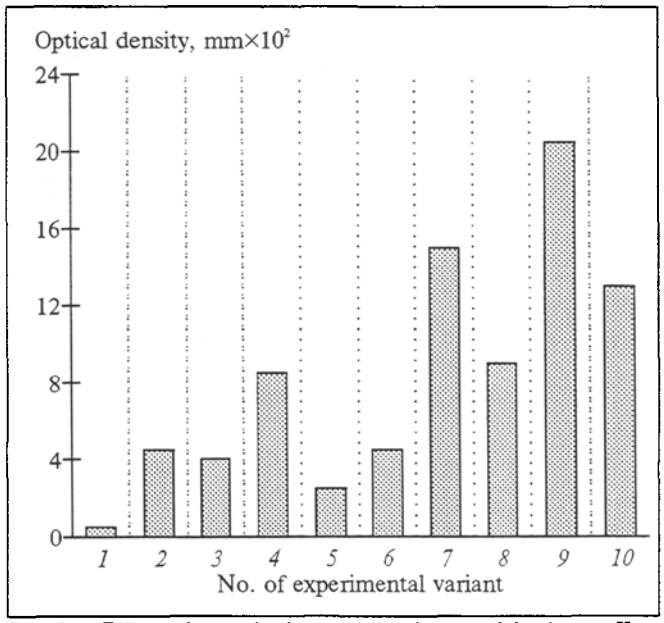

Fig. 3. Effect of protein factors of spleen and brain on IL-2 mRNA synthesis in surviving T lymphocytes of murine spleen. *1*) in the absence of effectors; *2*) in the presence of ConA; *3*) the same with p-IL-2; *4*) the same with ConA + p-IL-2; *5*) the same with TPA + PHA; *6*) the same with TPA; *7*) in the presence of NPS; *8*) in the presence of NPB; *9*) in the presence of TPA + PHA + NPS; *10*) in the presence of TPA + PHA + NPB.

REFERENCES

1. P. Chomczynski and N. Sacchi, *Analyt. Biochem.*, **162**, № 1, 156-159 (1987).
2. G. Felsenfeld, *Nature*, **355**, № 6375, 219-224 (1992).
3. T. B. Kazakova, O. D. Dolgiy, O. I. Golovko, *et al.*, *Biotechnology Therapeutics*, **4**, № 1,2, 63-76 (1993).
4. D. A. Morgan, F. W. Ruscetti, and R. C. Gallo, *Science*, **193**, 1007-1008 (1976).
5. A. Mouzaki, R. Weil, L. Muster, *et al.*, *EMBO J.*, **10**, № 8, 1399-1406 (1991).
6. T. J. Novak, P. N. White, and E. V. Roytenberg, *Nucleic Acids Res.*, **18**, № 15, 4523-4533 (1990).
7. U. Siebenlist, D. B. Durand, P. Bressler, *et al.*, *Molec. Cell. Biol.*, **6**, № 9, 3042-3049 (1986).
8. T. U. Williams, J. E. Buzlein, S. Ogden, *et al.*, *Analyt. Biochem.*, **176**, № 1, 26-30 (1989).