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INTERFERON ALPHA2B BUT NOT INTERFERON ALPHA2A DETECTED IN HUMAN GENOMIC DNA

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Summary. – The presence of the genes for human interferon (IFN)-alpha2a and IFN-alpha2b subvariants in human genomic DNA was studied using polymerase chain reaction (PCR). The respective genes differ in the coding sequence only at the position 137, adenine (2A) being substituted by guanine (2B). IFN-alpha2-sequences were selectively amplified from the placental genomic DNA using specific primers. When sequencing the mixture of PCR-derived clones, at the position 137 guanine, specific for subvariant 2b could be detected. This indicates that, at least in the genome analyzed, only sequence coding for IFN-alpha2b was found. Our finding supports the view, that genes for IFN-alpha2a and IFN-alpha2b seem to be rather allelic than occuring at distinct loci.

Key words: interferon-alpha2; subvariants; human genome

Introduction

Proteins of the human IFN-alpha family are coded at least by 15 nonallelic genes located on chromosome 9 (Owerbach *et al.*, 1981; Henco *et al.*, 1985). The three IFN-alpha2 subvariants – a, b and c – were isolated as cDNAs from different libraries (Streuli *et al.*, 1980; Goeddel *et al.*, 1981; Dworkin-Rastl *et al.*, 1982). In the coding sequence they differ less than 1 % from each other. Therefore they are often regarded as allelic variants, although evidence that at least IFN-alpha2a and IFN-alpha2b may be derived from different genes was presented (Hotta *et al.*, 1988).

To contribute to elucidation of discrepancy regarding allelic or nonallelic nature of subvariant 2a and 2b, we performed the study which aimed at detection of both genes at the level of human genomic DNA. For examination of genomic DNA the PCR was used.

Materials and Methods

Isolation of IFN-alpha2-sequences by PCR. The primers used in the PCR were 24-mer, ATG GCC TTG ACC TTT GCT TTA CTG, sense oligonucleotide corresponding to the beginning of the signal sequence of human IFN-alpha2 and a 27-mer, TCA TTC CTT ACT TCT TAA ACT TTC TTG, antisense oligonucleotide, corresponding to the end of human IFN-alpha2 gene (Henco *et al.*, 1985). These primers span a fragment of 570 bp. The PCR-primers and dNTPs were used at the final concentration of 0.1 μmol/l and 20 μmol/l, respectively. Genomic DNA (1 μg) isolated from human placenta (Ausubel *et al.*, 1987) was used as a template. Amplification was carried out in a Techne Programmable Dri-block using 2.5 U of Taq DNA polymerase (Promega) in buffer recommended by the supplier. The following protocol was used: 95 °C for 4 min, 72 °C for 3 min; then 32 cycles: 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min; final extension 72 °C for 5 min.

Sequencing of the PCR-product. The PCR-product was purified using a LMP agarose gel and, after phosphorylation of 5'-ends with T4 polynucleotide kinase and making the protruding ends blunt with Klenow enzyme it was then blunt-end ligated into the *Eco*RV-cleaved pBSK (+) plasmid (Stratagene). In order to enrich for recombinant plasmids the ligation mixture was recleaved with *Eco*RV prior to transformation into Sure (Stratagene) competent cells. The *Eco*RV site does not occur in the amplified fragment, but it can occur only in non-recombinant molecules (neither of PCR-primers complements the *Eco*RV site); only the latter can be linearized by *Eco*RV and therefore they will be unable to transform competent cells. NEB ligation buffer was used in all steps. An aliquot of the transformation mixture was plated on selective media (AMP, IPTG, X-Gal) to check the efficiency of ligation and selection against non-recombinant plasmids. The rest was cultivated overnight and the total plasmid DNA isolated according to a standard procedure (Ausubel *et al.*, 1987) served as a template for double stranded sequencing using a 20-mer, TGT GAT CTG CCT CAG ACC CA (consensus sequence of human alpha-IFNs, corresponding to the beginning of the mature sequence (Henco *et al.*, 1985)) as sequencing primer and the T7 sequencing kit (Pharmacia).

Results and Discussion

Human IFN-alpha2a and IFN-alpha2b subvariants differ in one amino acid at the position 23 of the mature protein: lysine (2a) or arginine (2b). The IFN-alpha 2a cDNA was originaly cloned from virus-induced myeloblast cell line (Goedell *et al.*, 1981), whereas IFN-alpha2b cDNA was cloned from virus – induced human leukocytes (Streuli *et al.*, 1980). Owing to the minimal nucleotide differences, IFN-alpha2a and IFN-alpha2b have been considered to be allelic (Henco *et al.*, 1985). However, to determine if these genes represent polymorphic variants of a single gene or distinct loci can be a difficult task, especially when dealing with cDNA-derived sequences. The only study of Hotta *et al.* (1988) analyzing the IFN-alpha2 subvariants at the level of human genome claimed that at least IFN-alpha2a and IFN-alpha2b may represent distinct genes. They used method based on hybridization of the subvariant-specific oligonucleotides with DNA from human leukocytes. To avoid a possible nonspecific hybridization of probes, we chose an alternative approach, in which the high selectivity of PCR was utilized.

At the beginning of our study, we prepared genomic DNA from human placenta. The respective IFN-alpha2-sequences were PCR-derived using human IFN-alpha2-specific primers with 100 % homology between subvariants. This approach relies on the selective amplification of IFN-alpha2-sequences as

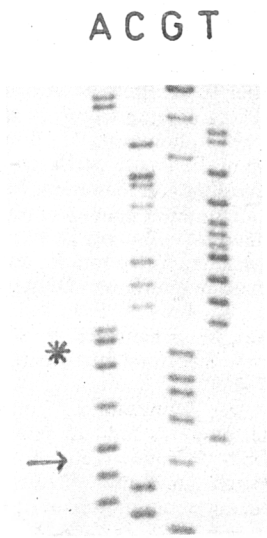

Fig. 1
Part of IFN-alpha2-coding sequence
The asterisk indicates nucleotide at the position 137, the arrow indicates nucleotide at the position 130.

confirmed by presence of a single G at the position 130 (indicated by arrow in Fig. 1) in the mixture of PCR-isolated sequences. In human IFN-alpha family only subtype alpha2 has G at this position. Provided that genes for IFN-alpha2a and IFN-alpha2b are nonallelic, both sequences should be obtained with approximately the same frequency. The resulting PCR-product corresponding according to its molecular weight to the segment of human IFN-alpha2 gene (not shown) was then cloned and the single IFN-alpha2 clones were analyzed. However, after sequencing four randomly selected clones, only IFN-alpha2b genes were detected. The coding sequences of genes for subvariants 2a and 2b are identical except for a single difference at the position 137, where in the IFN-alpha2a gene is A and in the IFN-alpha2b gene is G. To elucidate the question of the presence of IFN-alpha2a gene in the analyzed human genomic DNA, the respective region of the mixture of PCR-derived clones was sequenced and screened for nucleotide A at the position 137. The sequence of interest is shown in Fig. 1 and it unmabiguously demonstrates, that at this decisive position (indicated by asterisk) only nucleotide G specific for subvariant INF-alpha2b is present.

In conclusion, our findings demonstrate that at least in human genome analyzed, only gene coding for INF- alpha2b could be detected. This supports the view that subvariants 2a and 2b could be rather allelic variants then nonallelic genes. Our data are supported also by Diaz *et al.* (1991), who mapped only one IFN-alpha2-gene (without subvariant-determination) on the human chromosome 9. Further studies are needed for a definite answer to a question of genetic determination of IFN-alpha2-subvariants.

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References

Ausubel, F. M., Brent, R., Kingston, R. F., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987): *Current protocols in molecular biology*. J. Wiley and Sons, New York.

Diaz, M. O., Pomykala, H., Bohlader, S., Maltepe, E., and Olopade, O. (1991): A complete physical map of the type-I interferon gene cluster. *J. Interferon Res.* **11**, suppl. 1, 885.

Dworkin-Rastl, E., Dworkin, M. B., and Swetly, P. (1982): Molecular cloning of human alpha and beta interferon genes from Namalwa cells. *J. Interferon Res.* **2**, 575–585.

Goeddel, D. V., Leung, D. W., Dull, T. J., Gross, M., Lawn, R. M., McCandliss, R., Seeburg, P. H., Ullrich, A., Yelverton, E., and Gray, P. W. (1981): The structure of eight distinct cloned human leukocyte interferon cDNAs. *Nature* **290**, 20–26.

Henco, K., Brosius, F. J., Fujisawa, A., Fujisawa, J.-I., Haynes, J. R., Hochstadt, J., Kovacic, T., Pasek, M., Schambeck, A., Schmidt, J., Todokoro, K., Wälchli, M., Nagata, S., and Weissmann, C. (1985): Structural relationship of human interferon alpha genes and pseudogenes. *J. mol. Biol.* **185**, 227–260.

Hotta, K., Monahan, J., Collier, K. J., and Pestka, S. (1988): Detection of human leukocyte interferon-αA and -α2 genes in genomic DNAs by the use of deoxyoctadecyloligonucleotide probes. *J. Interferon Res.* **8**, 51–60.

Owerbach, D., Rutter, W. J., Shows, T. B., Gray, P., Goeddel, D. V., and Lawn, R. M. (1981): Leukocytes and fibroblast interferon genes are located on human chomosome 9. *Proc. natn. Acad. Sci. U. S. A.* **78**, 3123–3127.

Streuli, M., Nagata, S., and Weissmann, C. (1980): At least three human type α interferons: Structure of α2. *Science* **209**, 1343–1347.