# INTERFERON ALPHA2B IS THE PREDOMINANT SUBVARIANT DETECTED IN HUMAN GENOMIC DNAs

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**Summary.** – Chromosomal DNAs isolated from eight individuals from the Slovak population and from lymphoblastoid Namalwa cells were analyzed for the presence of genes coding for three subvariants of human interferon-alpha2 (IFN-alpha2), namely a, b, and c. The respective genes are regarded allelic, because they differ in the coding nucleotide sequence only at the position 137 (a:A, b/c:G) and/or at the position 171 (a/b:A, c:G). IFN-alpha2 sequences in genomes were selectively amplified using polymerase chain reaction (PCR). Resulting "consensus" PCR-product (the total mixture of PCR-derived clones) was sequenced and the subvariant-specific nucleotides at position 137 and 171 were determined. In one placental genomic DNA and in a mixture of genomic DNAs from leukocytes of seven donors only nucleotides specific for subvariant IFN-alpha2b could be detected. This suggests that the placental DNA contained only genes coding for IFN-alpha2b and these alleles were at least prevailing in donor' s genomes. On the other hand, the majority of genomic alpha2-sequences in Namalwa cells (from which IFN-alpha2c was originally derived), seems to be corresponding to subvariant IFN-alpha2c.

**Key words:** interferon-alpha2; subvariants; human genome

## Introduction

IFNs are proteins possessing antiviral, antiproliferative and immunomodulatory properties. The human IFN-alpha family is coded at least by 15 nonallelic genes located on chromosome 9 (Owerbach *et al.*, 1981; Henco *et al.*, 1985). Three IFN-alpha2 subvariants, designated 2a, 2b and 2c have been isolated as cDNAs from different libraries (Streuli *et al.*, 1980; Goeddel *et al.*, 1981; Dworkin-Rastl *et al.*, 1982). In the coding sequence their genes differ only at nucleotide positions 137 and/or 171 and therefore are regarded as allelic variants. The nucleotide differences result in one or two amino acid changes in the mature proteins (Table 1). The genes for IFN-alpha2a and IFN-alpha2c were derived from tumour cells, whereas IFN-alpha2b gene was isolated from leukocytes of blood donors. Therefore we analyzed at genomic level the presence of genes for three IFN-alpha2 subvariants in Slovak population. On the other hand, lymphoblastoid line Namalwa is the original source of the IFN-alpha2c cDNA. However, also cDNAs coding for IFN subvariant alpha2b were derived from these cells (G.R. Adolf, personal communication). This indicated that Namalwa cells are heterozygous in IFN-alpha2 locus and therefore we investigated whether both alleles 2b and 2c could be detected in the genome of Namalwa cells.

## Materials and Methods

*Preparation of genomic DNAs.* Genomic DNA derived from one human placenta was prepared according to Ausubel *et al.* (1987).

The genomic DNAs from human leukocytes were prepared as follows. The blood samples of 7 healthy volunteers were mixed. After lysis with 0.84% ammonium chloride (10 mins, 4 °C) the total white blood cells were collected by centrifugation. The pellet ($10^8$ leukocytes) was boiled in water for 5 mins and after centrifugation supernatant was directly used as template for PCR.

Genomic DNA from human lymphoblastoid cell line Namalwa was prepared similarly. Cells ($10^8$) growing in Dulbecco' s Minimal Essential Medium with 10% foetal calf serum were collected by centrifugation and processed as described for leukocytes.

*Isolation of IFN-alpha2 sequences by PCR.* The primers were a 24-mer, ATG GCC TTG ACC TTT GCT TTA CTG, the 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, the antisense oligonucleotide, corresponding to the end a region of 570 bp. The PCR primers and dNTPs were used at the final concentration of 0.1 μmol/l and 20 μmol/l, respectively. Amplification was carried out in Techne Programmable Dri-block using 2.5 U of *Taq* DNA polymerase (Promega) in a buffer recommended by the supplier. The following protocol was used: 95 °C/4 mins and 72 °C/3 mins; 32 cycles of 94 °C/1 min, 55 °C/1 min and 72 °C/1 min; final extension 72 °C/5 mins.

*Sequencing of PCR product.* The PCR product was purified using a LMP agarose gel and, after T4 polynucleotide kinase and Klenow enzyme treatment it was blunt-end ligated into the *Eco*RV-cleaved pBS(+) plasmid (Stratagene). In order to enrich recombinat plasmids the ligation mixture was recleaved with *Eco*RV prior to transformation into *E. coli* Sure cells (Stratagene). The *Eco*RV site is not contained in the amplified fragment and can only be restored in non-recombinat molecules (neither of PCR/primers complements the *Eco*RV site); only the latter can be linearized 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-GA) 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).

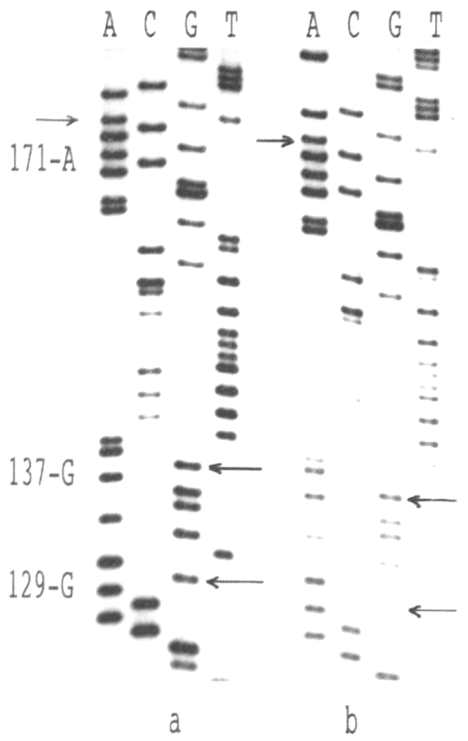


**Fig. 1**
**Part of "consensus" IFN-alpha2 coding sequences**
DNAs isolated from human placenta (a) and mixture of donor's leukocytes (b). Nucleotides at the positions 129 (G), 137 (G) and 171 (A) are indicated by arrows.

## Results

The coding sequences of three genes for IFN-alpha2 subvariants are identical, except for one- or two-nucleotide differences: G in the alpha2b/c genes at position 137 becomes A in the alpha2a gene, and A at position 171 in alpha2a/b genes is replaced by G in alpha2c gene. Our study was based on the identification of these subvariant-specific nucleotides in IFN-alpha2 sequences derived from different genomic DNAs. To analyze the presence of all three subvariants simultaneously during one screening, the high selectivity of PCR was utilized. Our strategy was as follows.

**Table 1. Differences between the three IFN-alpha2 subvariants at nucleotide and amino acid level IFN**

| | Nucleotides at positions | | | | | | Amino acids at positions | |
|---|---|---|---|---|---|---|---|---|
| | 136 | 137 | 138 | 170 | 171 | 172 | 23 | 34 |
| alpha2a | A | A | A | C | A | T | Lys | His |
| alpha2b | A | G | A | C | A | T | Arg | His |
| alpha2c | A | G | A | C | G | T | Arg | Arg |

In the first step, the IFN-alpha2 sequences in genomic DNA were PCR-derived using IFN-alpha2-specific primers with 100% homology between three subvariants. The resulting PCR-product corresponded according to its molecular weight to the segment of human IFN-alpha gene (data not shown). The approach relies on the amplification of IFN-alpha2 sequences only, whereas related genes of other alpha/subtypes were not amplified. This presumption was verified by the presence of a single alpha2-subtype–specific nucleotide G at the position 129 in the sequenced "consensus" PCR-product (Fig. 1).

In the second step, instead of sequencing several individual PCR-derived clones, the total mixture of PCR-derived sequences ("consensus" PCR-product) was analyzed and screened for the subvariant–specific nucleotides at the positions 137 and 171.

Sequencing of the "consensus" IFN-alpha2 derived from placental DNA or a mixture of 7 genomic DNAs showed at

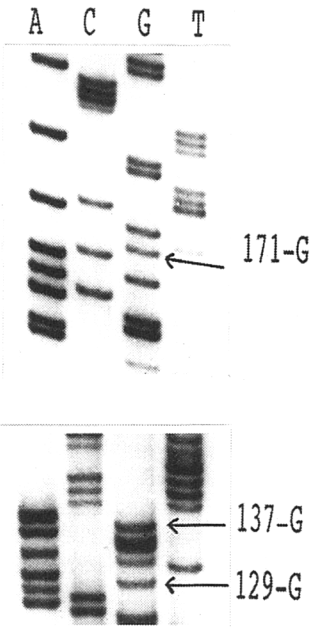


**Fig. 2**
**Part of "consensus" IFN-alpha2 coding sequences derived from DNA isolated from Namalwa cells**
Nucleotides G at the positions 129, 137 and 171 are indicated by arrows.

position 137 only G specific for subvariants 2b/c, whereas at position 171 only A specific for subvariants 2a/b was found (Fig. 1). This indicated unequivocally that in the genomes examined only genes for subvariant IFN-alpha2b could be identified.

Surprisingly, when the "consensus" IFN-alpha2 sequence isolated from the genomic DNA of Namalwa cells was characterized, only G at both positions 137 and 171, specific for subvariant 2c, could be demonstrated (Fig. 2).

## Discussion

The genes coding for three human IFN-alpha2 subvariants were originally isolated as cDNAs from different libraries. IFN-alpha2a was cloned from virus-induced myeloblast cell line KG-1 (Goeddel *et al.*, 1981), IFN-alpha2b was cloned from virus-induced human leukocytes (Streuli *et al.*, 1980), and IFN-alpha2c was prepared from virus-induced lymphoblastoid Namalwa cells (Dworkin-Rastl *et al.*, 1982). Owing to the minimal nucleotide differences, these three genes have been considered to be allelic (Henco *et al.*, 1985). There are apparent differences in the frequency of the three subvariants in human population. Until now, only single genes for IFN-alpha2a and IFN-alpha2c have been isolated from continuous cell lines. The majority of obtained IFN-alpha2 genes represent the subvariant 2b and were prepared from normal cells (Lawn *et al.*, 1981; Ovchinnikov *et al.*, 1984; Kúdela *et al.*, 1992; Kaluz *et al.*, 1993). Adolf *et al.* (1990) analyzed the amino acid sequence of the natural IFN-alpha2 purified from human leukocyte IFN-alpha of donors from Finnish population. At the position 23 only arginine (subvariants 2b/c) but not lysine (subvariant 2a) was identified. It was not possible to determine, whether the 2b and/or 2c subvariants were present in natural IFN-alpha2, because the amino acid at the position 34 was not identified.

Therefore we decided to test the presence of genes coding for these IFN-alpha2 subvariants in genomic DNAs obtained from leukocytes or placenta of individuals from Slovak population. In our study we chose the approach based on the high selectivity of PCR. This attitude allowed to analyze the presence of all three IFN-alpha2 subvariants simultaneously in one experiment. After analysis of genomic DNAs of eight individuals, only the sequence of genes coding for subvariant IFN-alpha2b could be identified.

Taken into account our findings and literature data the subvariant alpha2b appears to be the most frequent IFN-alpha2 allele at least in European population. The higher immunogenicity of subvariant 2a over 2b in the treated patients (Dianzani *et al.*, 1991) might also reflect this situation, since the immune system could recognize IFN-alpha2a as "non/self".

The identification of IFN-alpha2c genes only in the genome of Namalwa cells was rather surprising. Because of demonstration of IFN-alpha2b and IFN-alpha2c at both cDNA and protein levels in Namalwa cells (Allen, 1992; Adolf *et al.*, 1991) we considered these cells as heterozygous in locus for IFN-alpha2. Therefore we expected at the nucleoside position 171 in the "consensus" PCR-product A and G with approximately the same frequency. In contrast, obtained data indicated that the majority of genomic alpha2 sequences represented the 2c subvariant. We speculated that analyzed cells might lose one copy of chromosome 9, which carried the allele for IFN-alpha2b. However, karyological analysis of Namalwa cells found two copies of the chromosome 9 (data not shown). There is also another possibility, namely that the population of Namalwa cells is heterogeneous, with the majority of cells homozygous for allele 2c, whereas only small portion of cells is homozygous for allele 2b. But this is only a hypothesis and for elucidation of the frequencies of IFN-alpha2c genes in population of Namalwa cells an additional study seems to be necessary.

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## References

Adolf, G.R., Kalsner, I., Ahorn, H., Maurer-Fogy, I., and Cantell, K. (1991): Natural human interferon-alpha2 is O-glycosylated. *Biochem. J.* **276**, 511–518.

Allen, G. (1982): Structure and properties of human interferon-alpha from Namalwa lymphoblastoid cells. *Biochem. J.* **207**, 397–408.

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

Dianzani, F., Antonelli, G., Currenti, M., Turriziani, O., and Riva, E. (1991): Neutralizing and nonneutralizing antibodies to IFN in hepatitis patients treated with different IFN alpha preparations. *J. Interferon Res.* **11**, suppl. 1, S79.

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., Hichstadt, J., Kovacic, T., Pasek, M., Schambeck, A., Schmid, J., Todokoro, K., Walchli, M., Nagata, S., and Weissman, C. (1985): Structural relationship of human interferon alpha genes and pseudogenes. *J. mol. Biol.* **185**, 227–260.

Kúdela,O., Zelnik, V., Kaluz, Š., and Pastorek, J. (1992): The use of modified oligonucleotides for amplification and oriented cloning of human alpha interferon gene. *Acta virol.* **36**, 488–490.

Kaluz, Š., Gibadulinová, A., and Kontsek, P. (1993): Interferon alpha 2b but not interferon alpha 2a detected in human genomic DNA. *Acta virol.* **37**, 97–100.

Lawn, R.M., Gross, M., Houck, C.M., Franke, A.E., Gray, P.W., and Goeddel, D.V. (1981): Dna sequence of a major human leukocyte interferon gene. *Proc. Natl. Acad. Sci. USA* **78**, 5435–5439.

Ovchinnikov, Yu.A., Sverdlov, E.D., Monastyrskaya, G.S., Tsarev, S.A., Zaytseva, E.M., Arsenyan, S.G., Chakhmakcheva, O.G., Novokhatsky, A.S., Aspetov, R.D., and Kuznetsov, V.P. (1984): Expression in *Escherichia coli* of a mutant human alpha2-interferon. *Molek. Biol.* **18**, 48–59 (in Russian).

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 chromosome 9. *Proc. Natl. Acad. Sci. USA* **78**, 3123–3127.

Streuli, M., Nagata, S., and Weissmann, C. (1980): At least three human type alpha interferons: Structure of alpha 2. *Science* **209**, 1343–1347.