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INTERFERON GAMMA IS ACTIVE ON HUMAN LYMPHOBLASTOID NAMALVA CELLS WITHOUT INDUCING AN ANTIVIRAL STATE

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Abstract—We demonstrate the presence of high affinity receptors specific for interferon-γ (IFN-γ) in human lymphoblastoid Namalva cells. The presence of these receptors, whose binding affinity and cross-linking characteristics were not distinguishable from those of the corresponding receptors in sensitive cells, was not consistent with the lack of responsiveness of Namalva cells to IFN-γ as regards growth inhibition, induction of 2′-5′ oligoadenylate synthetase activity and inhibition of virus multiplication. Nevertheless, IFN-γ enhanced the expression of two genes, HLA class II and c-myc. Although the mechanism of these IFN-γ-mediated modifications is not understood, these results provide evidence that the IFN-γ receptors present in Namalva cells are functional.

Interferons (IFNs) α, β and γ are a family of proteins defined by their ability to induce an antiviral state in sensitive cells [1]. In addition, they have many other biological activities in target cells, such as growth inhibition and immunomodulatory properties [1, 2]. IFN-γ is a lymphokine produced by activated T-lymphocytes, it is different from IFNs α and β in its cellular origin [3–5], its structure [6, 7] and also in some of its biological properties such as the regulation of immune responses by inducing the synthesis of class II histocompatibility antigens, activating macrophages and modifying the expression of specific genes [4, 8, 9]. The ability of IFNs to induce pleiotropic biological responses is generally admitted to begin by their initial interaction with specific receptors on the cell surface [10, 11]. It is now clear that the IFN-γ receptor is different from that of IFN-α and IFN-β [12–17].

Several studies have shown that IFN-γ receptors are widely distributed [15, 17, 18]. In this study, we demonstrate that, although IFN-γ is unable to induce the antiviral state in Namalva cells, highly specific receptors are present on these cells. The number of IFN-γ receptors was reduced when cells were treated with low concentrations of ligand. Furthermore, although IFN-γ did not induce cell growth inhibition or 2′-5′ oligoadenylate synthetase (2-5A synthetase) activity, the expression of *c-myc* and HLA class II genes was modified, suggesting that these receptors are indeed functional.

MATERIALS AND METHODS

Cells. Human lymphoblastoid Namalva cells (ATCC, CRL 1432) were cultured at 37° in 5% CO_2 in RPMI 1640 medium supplemented with 8% fetal calf serum (FCS). Only exponentially growing cells were used for binding experiments. Human amniotic Wish cells were cultured in Eagle's Minimal Essential Medium (MEM) (Boehringer) supplemented with non-essential amino acids and 10% fetal calf serum (FCS). These cells were used for IFN assay with vesicular stomatitis virus (VSV) as a challenge [19].

Interferons. Recombinant *E. coli*-derived HuIFN-γ (specific activity: 2×10^8 U/mg) was provided by Roussel-Uclaf. Recombinant *E. coli*-derived HuIFN-α2 (specific activity: 2×10^8 U/mg) was kindly provided by Shering–Plough.

Antigrowth effect. Namalva cells were cultured in RPMI 1640 medium (Boehringer) in the absence or presence of rHuIFN-γ and rHuIFN-α2 at the initial density of 5×10^5 cells/ml. Cell concentrations and viability were determined at 24, 48, 72 and 96 hr.

Antiviral effect. The antiviral effect of IFNs on Namalva cells was determined by testing the inhibitory effect on VSV yield. For this purpose, 1.5×10^6 Namalva cells in 1 ml of RPMI 1640 medium containing 8% FCS were seeded in Petri dishes in the absence or presence of various concentrations of rHuIFN-γ or rHuIFN-α2. After 18 hours at 37°, cells were washed, and the pelleted cells were infected with VSV as a multiplicity of infection of 0.1 and incubated for 1 hr at 37°. Cells were then washed again, resuspended in fresh medium and incubated for 18 additional hours. Cultures were then frozen and thawed before virus titration. The virus titer was determined on L929 cells. Titers are expressed in tissue culture infective dose ($TCID_{50}$) [20].

Assay of (2′-5′)oligo(A) synthetase. Exponentially-growing Namalva cells were incubated for 18 hr at 37° in the presence or absence of various concentrations of rHuIFN-γ and rHuIFN-α2. After washing, cells were lysed (1×10^7 cells/ml) and their 2-5A synthetase activity was assayed in the lysate supernatant, as described by Merlin *et al.* [21].

Iodination of interferon. rHuIFN-γ was iodinated by the chloramine-T procedure, as previously

described [22]. The specific radioactivity of the ^{125}I-rHuIFN-γ was about 50 μCi/μg. Recovery of the antiviral activity of IFN-γ was 60–100%.

Binding assay. 1.5 to 2 × 10^6 Namalva cells were incubated at 4° for 2 hr with various concentrations of ^{125}I-rHuIFN-γ, in U-bottomed microtiter plates and in RPMI 1640 medium containing 5% FCS and tricin. Cells were then washed four times by centrifugation at 4° with PBS containing 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$ and 1% FCS. Cell-associated radioactivity was measured in a gamma counter. Non-specific radioactivity was determined in parallel experiments, in the presence of a 50–100-fold excess of unlabelled IFN-γ. Specific binding was determined by subtracting the nonspecific binding from total counts.

Cross-linking of ^{125}I-rHuIFN-γ. Namalva cells (2 × 10^7 cells/ml) were incubated with 1000 units/ml of ^{125}I-rHuIFN-γ in RPMI 1640 medium containing 5% FCS and tricin. After 2 hr incubation at 4°, cells were washed four times with serum-free cold phosphate-buffered saline (PBS) containing Ca^{2+} and Mg^{2+} to remove the unbound ^{125}I-rHuIFN-γ and then resuspended in Ca^{2+} and Mg^{2+}-free PBS. Cross-linking was carried out with a freshly prepared 50 mM solution of disuccinimidyl suberate (DSS) from Pierce Chemical Co (Rockford, IL) in DMSO which was added to the system at a final concentration of 1 mM. After incubation for 15 min at 4°, the reaction was stopped by addition of glycine at a 10 mM final concentration and the cell suspension was centrifuged. The cell pellets were then lysed by incubation for 30 min at 4° in PBS containing 1% Nonidet P40 and 1 mM phenyl methyl sulfonyl fluoride (PMSF). Cell lysates were centrifuged and the supernatant was analyzed by SDS–PAGE gel electrophoresis.

mRNA extractions and DNA probes. We have used four human cDNA probes: the 1600 bp ClaI-EcoRI genomic fragment containing the third exon of the human c-myc gene [23] provided by G. Brun, β-actin by M. Volovich (Institut Curie, Paris), the HLA-DPβ DNA (1,180 bp, SacI-PstI) fragment provided by C. Vaquero (Hôpital Cochin, Paris) and 2-5A synthetase cDNA for the 1.8 kb mRNA from J. Chebath (Weizmann Institute of Science, Rehovot, Israel). These probes were labeled by nick translation to specific radioactivities of 10^8 cpm/μg. Total RNA was extracted by the guanidinium thiocyanate procedure [24].

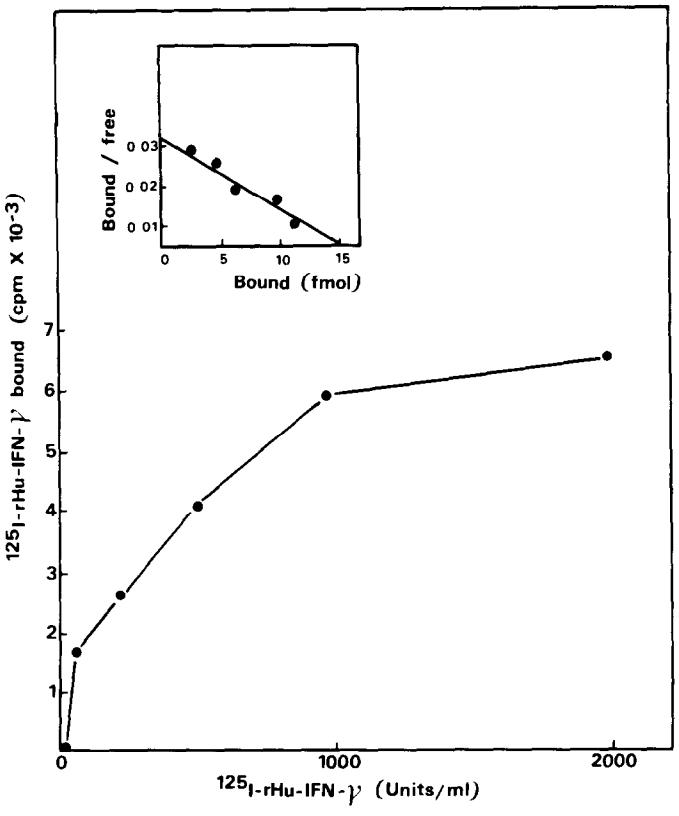

Fig. 1. Specific binding of ^{125}I-rHuIFN-γ to Namalva cells. Namalva cells at a density of 1.5 × 10^7 cells/ml were incubated for 2 hr with the indicated concentrations of ^{125}I-rHuIFN-γ. Curves represent the specific binding determined as described in Material and Methods. Non-specific binding was linear and unsaturable, and accounted for less than 30% of total binding at saturation. Points represent the means of triplicate cultures at each ligand concentration. Standard deviation was less than 10%. Inset is the Scatchard plot of the binding data. The ordinate indicates the ratio of bound to free ligand concentration, and the abscissa the fentomoles bound per 1.5 × 10^6 cells.

RESULTS

Binding of ^{125}I-rHuIFN-γ to Namalva cells and receptor modulation by the ligand

After iodination, the specific radioactivity of recombinant Hu-IFN-γ was about 50 μCi/μg. Analysis of the ^{125}I-rHuIFN-γ by SDS–PAGE, followed by autoradiography, showed a major radiolabeled band corresponding to the 17 kd monomer and a minor band corresponding to the 34 kd dimer (not shown). This ^{125}I-rHuIFN-γ bound to Namalva cells in a dose-dependent fashion and approached saturation at about 2000 units/ml (Fig. 1). This binding was inhibited by an excess of unlabeled rHuIFN-γ (not shown). Scatchard analysis of binding data indicated the presence of a homogeneous class of sites with an apparent equilibrium constant (K_D) between 10^{-9} and 10^{-10} M. The number of binding sites per cell was estimated at about 5000.

It was previously reported that HuIFN-α receptors are down-regulated in lymphoblastoid cell lines and tricholeucocytes [25, 26]. To ascertain whether IFN-γ receptors were also modulated by the ligand, Namalva cells were incubated for 18 hr at 37° with low concentrations of rHuIFN-γ. Cells were then washed extensively and assayed for their ability to bind ^{125}I-rHuIFN-γ. As shown in Fig. 2, the binding capacity of these cells decreased after pretreatment with unlabeled rHuIFN-γ. Reductions of 30 and 70% were observed in the number of receptors after preincubation with 50 and 200 U/ml of rHuIFN-γ, respectively. However, this treatment had no effect on the affinity of IFN-γ for its receptor. The concentration of rHuIFN-γ (200 units/ml) required to reduce the number of receptors by 70% was below the concentration required to saturate available receptors (< 2000 units/ml).

Cross-linking of ^{125}I-rHuIFN-γ to the Namalva cell surface

Cross-linking of ^{125}I-rHuIFN-γ to its receptor was carried out using the cross-linking reagent disuccinimidyl suberate (DSS). As shown in Fig. 3, a predominant radiolabeled rHuIFN-γ receptor complex was obtained with a M_r of 110,000 ± 5000 upon SDS–PAGE electrophoresis and autoradiography. The formation of this complex was prevented by an excess unlabeled rHuIFN-γ, but not by unlabeled rHuIFN-α2. This complex was not obtained in the absence of cross-linking reagent.

Biological effects of rHuIFN-γ

To correlate rHuIFN-γ binding to its receptor with the biological effects of rHuIFN-γ, we examined the action of rHuIFN-γ on Namalva cells. For this purpose, we studied three well known interferon-mediated effects: inhibition of cell growth, establishment of the antiviral state and induction of 2-5A

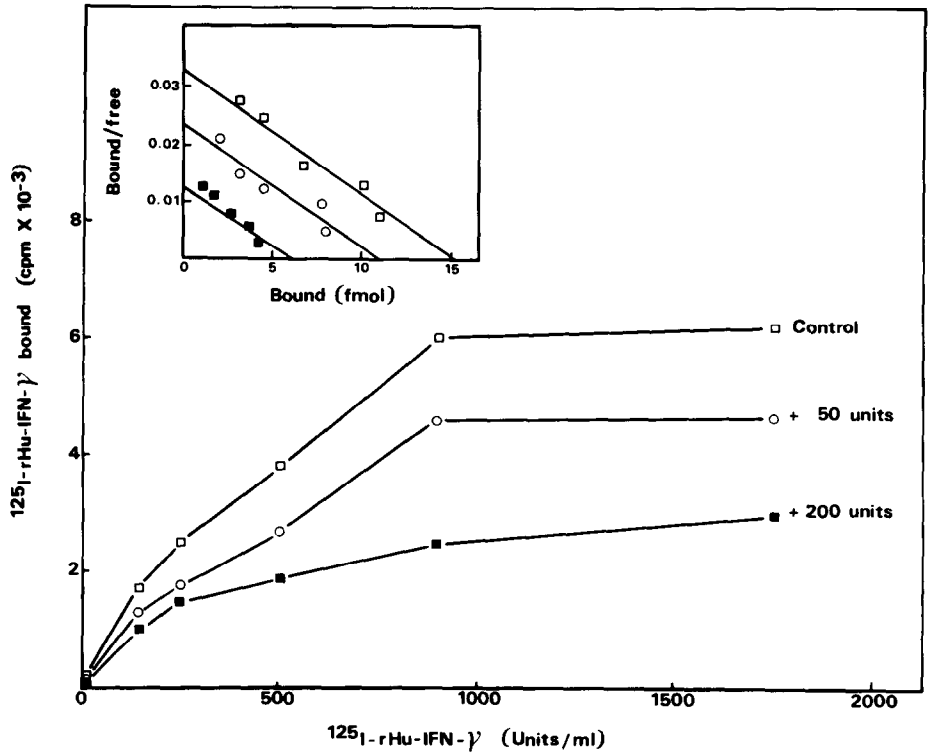

Fig. 2. Modulation of HuIFN-γ receptors in Namalva cells. Namalva cells were incubated in culture medium containing unlabeled rHuIFN-γ. After 16 hr at 37°, each culture was washed with PBS containing Ca^{2+}, Mg^{2+} and incubated for 2 hr at 4° with the indicated concentrations of ^{125}I-rHuIFN-γ for specific binding determination, as described in Fig. 1. Specific binding to untreated control cells (□—□) and to cells pretreated with either 50 units/ml (○—○) or 200 units/ml (■—■) of rHuIFN-γ.

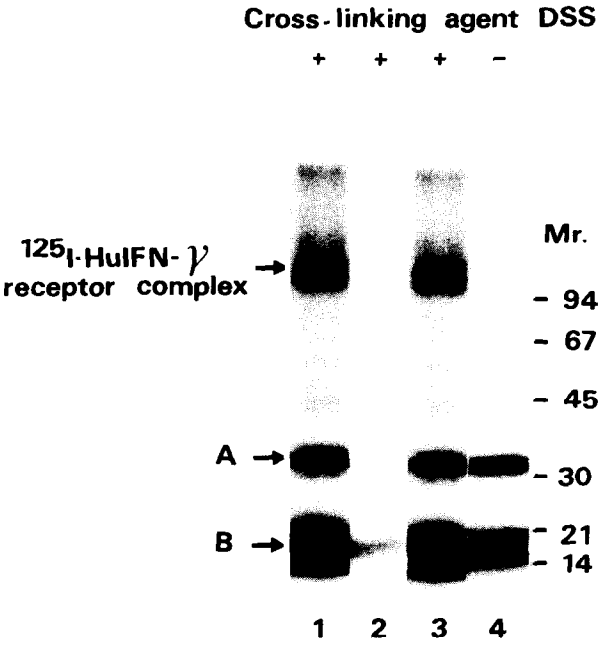

Fig. 3. Cross-linking of ^{125}I-labeled rHuIFN-γ bound to Namalva cells and SDS–PAGE analysis. Namalva cells (2×10^7 cells) were incubated for 2 hr at 4°, either with ^{125}I-rHuIFN-γ (2000 units/ml) only (lanes 1 and 4), or with ^{125}I-rHuIFN-γ plus 5×10^4 units/ml of unlabeled rHuIFN-γ (lane 2) or 5×10^4 units/ml of unlabeled rHuIFN-α2 (lane 3). Cells were then washed and either treated with 1 mM DSS for 20 min at 4° (lanes 1, 2 and 3) or left without DSS, also for 15 min at 4° (lane 4). Their lysates were prepared and analyzed by SDS–PAGE electrophoresis on 5–15% slab gels. The figure shows an autoradiograph of a dried gel. A and B indicate non-cross-linked ^{125}I-IFN-γ dimer (A) and monomer (B).

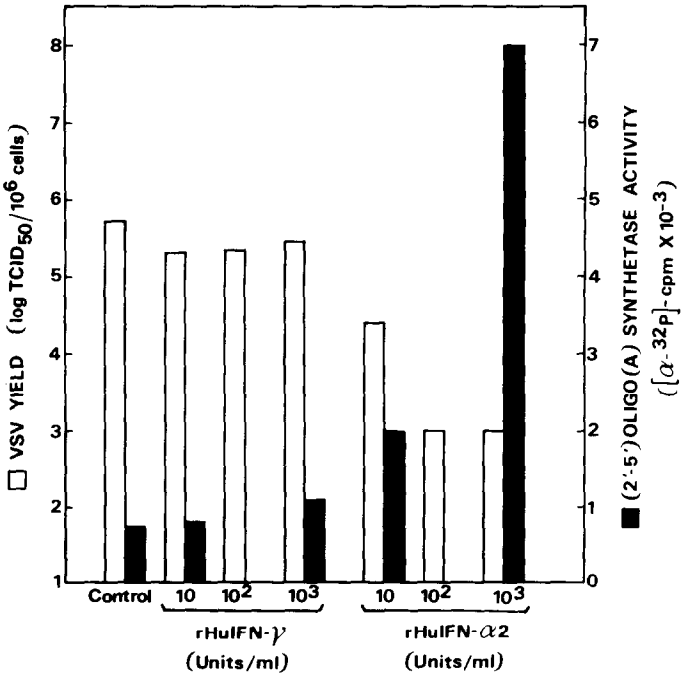

Fig. 4. Measurement of VSV multiplication and 2-5A synthetase activity in Namalva cells treated with rHuIFN-γ and rHuIFN-α2. Namalva cells were incubated in the presence or absence of the indicated concentrations of rHuIFN-γ or rHuIFN-α2. After 16 hr incubation at 37°, VSV multiplication and 2-5A synthetase activity were determined as described under Materials and Methods.

synthetase. Treatment of Namalva cells for 72 hr with as much as 10,000 U/ml of rHuIFN-γ did not affect cell growth whereas 100 U/ml of IFN-α inhibited this growth by more than 70% (not shown).

The antiviral state and 2-5A synthetase activity induced were measured in Namalva cells after treatment with IFN-γ doses ranging from 10 to 10,000 U/ml. As shown in Fig. 4, no antiviral effect or increase in 2-5A synthetase activity was observed, while only 10 U/ml of IFN-α induced both these effects. Although IFN-γ was not active in Namalva cells, it did induce an antiviral state and the 2-5A synthetase activity in other cells such as Wish cells (our unpublished observation and Ref. 14).

Since Namalva cells exhibited high affinity-specific receptors for IFN-γ without expressing the usual IFN-mediated biological properties, we attempted to detect changes in the expression of genes known to be modulated by IFN-γ in sensitive cell, such as c-myc and HLA class II genes [9]. Figure 5 shows a Northern blot analysis of the mRNA which was extracted from Namalva cells pretreated or not with IFN-α or IFN-γ at doses known to saturate the specific receptors, and hybridized with a specific probe corresponding to the third c-myc exon. Treatment with IFN-γ enhanced the levels of c-myc mRNA expression whereas, as already reported [27], IFN-α lowered it. In addition, we observed a slight increase, from 2.4 kb to 2.6 kb, in the M_r of the c-myc transcript. When the same mRNAs were hybridized with an HLA class II probe, the specific band at 1.6 kb was strongly amplified in the cells pretreated with rHuIFN-γ but not in those treated with IFN-α.

Since some of the IFN-γ-mediated activities may be blocked at the post-transcriptional level, Northern blots were rehybridized with the 2-5A synthetase probe. No enhanced amount of 2-5A synthetase mRNA was observed in IFN-γ-treated cells in contrast to IFN-α (not shown). These results indicated that expression of the 2-5A synthetase gene was not induced in Namalva cells by IFN-γ. No significant differences in either IFN-γ or IFN-α-treated cells compared to untreated cells were detected when the same blots were rehybridized with a probe of a constitutively-expressed gene: β-actin (not shown), indicating that the differences obtained with the other probes were relevant.

DISCUSSION

The action of IFN-γ is initiated by its binding to specific cell surface receptors. Here, we demonstrated the presence of high affinity-specific receptors for IFN-γ in Namalva cells. However, the presence of these receptors was not consistent with the lack of responsiveness of the cells to IFN-γ as regards growth inhibition, induction of 2-5A synthetase activity or inhibition of virus multiplication. Thus, Namalva cells were apparently resistant to IFN-γ since none of the usual interferon-mediated biological activities was expressed in these cells.

The properties of IFN-γ receptors in Namalva cells were not dintinguishable from those of the corresponding receptors in sensitive cell lines like Wish cells [14] and sensitive Daudi cells [12, 15, 25], as regards binding affinity and cross-linking characteristics. We therefore tried to establish whether IFN-γ exerted other biological effects on Namalva cells, particularly with respect to the modification of gene expression. The results obtained show that IFN-γ indeed enhances the expression of two genes, HLA class II and c-myc. Although the mechanism of these IFN-γ-mediated modifications of gene expression is not yet understood, these results provide evidence that the IFN-γ receptor is functional.

Several possible explanations may be considered. First, receptor heterogeneity might account for the differential effects of IFN-γ on a variety of cell lines [18]. Although the biochemical properties of the receptors we studied seem to be similar to those already reported to be present in cells like Wish cells [14], which are sensitive to the antiviral action of IFN-γ, it cannot be concluded that the structure of the receptor in Namalva cells is identical to that of Wish cells. This point will only become clear once the

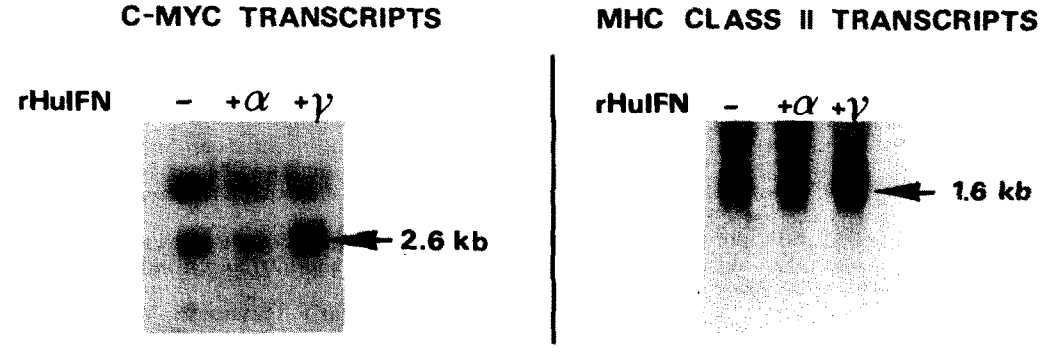

Fig. 5. Identification of *c-myc* and MHC class II transcripts in mRNA from Namalva cells treated with rHuIFN-γ or rHuIFN-α2. Total RNA (25 μg) from untreated Namalva cells or Namalva cells treated with 1 nM (2000 U/ml) rHuIFN-α2 and 1 nM (2000 U/ml) rHuIFN-α was fractionated on agarose gel and transferred to nitrocellulose. Blots were hybridized with either a ^{32}P-labeled nick-translated HLA-DPβ cDNA probe (1180 bP SacI PstI fragment) or a ^{32}P nick-translated c-myc probe corresponding to the third genomic exon.

receptors have been isolated and the corresponding genes cloned.

Another explanation for the present results might be the existence of more than one signal transduction pathway. One pathway, absent in Namalva cells, might lead to the antiviral and anti-growth activity and 2-5A synthetase induction. Another pathway might account for the modifications observed in gene expression, which might not necessarily be related to the activities usually mediated by IFN-γ. Different post-receptor controls may also be involved in the biological effects mediated by IFN-γ.

Although Namalva cells appeared as a resistant cell line as regards the establishment of the antiviral state and the inhibition of cell growth by IFN-γ, expression of some genes was specifically modified by this IFN.

The use of cellular models in which high affinity receptors are present but in which only certain properties of IFN-γ are expressed would be useful in understanding the mechanism of signal transduction, which mediates the different biological effects of this IFN.

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