Molecular Immunology 47 (2010) 1023–1030

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Functional analysis of naturally occurring amino acid substitutions in human IFN-γR1

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ARTICLE INFO

Article history:
Received 2 September 2009
Received in revised form 9 November 2009
Accepted 13 November 2009
Available online 16 December 2009

Keywords:
IFN-γ
IFN-γR1
Signal transduction
Immune deficiency
Mutation

ABSTRACT

IFN-γ plays an essential role in the IL-12/IL-23/IFN-γ pathway that is required for the defense against intracellular pathogens. In the IFN-γR1 several amino acid substitutions have been reported that abrogate IFN-γ signaling. These substitutions can lead to a null phenotype and enhanced susceptibility to infection by poorly pathogenic mycobacteria, a disorder known as Mendelian Susceptibility to Mycobacterial Disease (MSMD). More common amino acid variations in the IFN-γR1 may also influence IFN-γR function, albeit more subtle. To determine the effect of various amino acid substitutions on IFN-γR1 expression and function we cloned two newly identified amino acid substitutions (S149L, I352M), four common variations (V14M, V61I, H335P, L467P), seven reported missense mutations (V61Q, V63G, Y66C, C77Y, C77F, C85Y, I87T) and the 818delTTAA mutation in a retroviral expression vector. IFN-γR1 expression was determined as well as responsiveness to IFN-γ stimulation. The two newly discovered variants, and the four common polymorphisms could be detected on the cell surface, however, the V14M, H335P and I352M variants were significantly lower expressed at the cell membrane, compared to the wild type receptor. Despite the variance in cell surface expression, these IFN-γR1 variants did not affect function. In contrast to literature, in our model the expression of the V63G variant was severely reduced and its function was severely impaired but not completely abrogated. In addition, we confirmed the severely reduced function of the I87T mutant receptor, the completely abrogated expression and function of the V61E, V61Q, C77F, C77Y and the C85Y mutations, as well as the overexpression pattern of the 818delTTAA mutant receptor. The Y66C mutation was expressed at the cell surface, it was however, not functional. We conclude that the V14M, V61I, S149L, H335P, I352M and L467P are functional polymorphisms. The other variants are deleterious mutations with V61E, V61Q, Y66C, C77F, C77Y and C85Y leading to complete IFN-γR1 deficiency, while V63G and I87T lead to partial IFN-γR1 deficiency.

1. Introduction

Interferon-γ (IFN-γ) is a pleiotropic cytokine playing a central role in type-1 immunity. Human host immunity against intracellular pathogens such as *Mycobacteria* and *Salmonellae* depends on an effective cell-mediated immune response mediated by type-1 cytokines (Ottenhoff et al., 2002). Upon encounter of these pathogens, antigen presenting cells (APC) produce cytokines such as IL-23, IL-1β, IL-18, IL-12 and TNF via the stimulation of pattern recognition receptors, including Toll-like receptors. Type-1 helper T (Th1) cells and natural killer (NK) cells express receptors for these cytokines and produce IFN-γ in response to IL-12 or IL-23 which can be enhanced by IL-1β and IL-18. IFN-γ, in turn, binds to the IFN-γ receptor (IFN-γR), present on nearly all cell types (Valente et al., 1992). APCs, including monocytes, macrophages and DCs, are activated by IFN-γ to produce increased levels of IL-12 and to enhance both antigen presentation and bactericidal activity (Leenen et al., 1994; Nathan and Hibbs, 1991). In addition IFN-γ can, depending on cell type, block proliferation, induce apoptosis and enhance expression of cell surface molecules such as HLA Class I and II, CD54 and CD64.

The IFN-γR is comprised of two ligand-binding IFN-γR1 chains associated with two signal-transducing IFN-γR2 chains (Boehm et al., 1997). Binding of IFN-γ to its receptor induces receptor oligomerization and activation of the receptor-associated Janus kinases JAK1 and JAK2 by trans-phosphorylation. The JAKs phosphorylate the tyrosine 440 that is part of the STAT1 docking site in the intracellular domain of the IFN-γR1, allowing for subsequent STAT1 phosphorylation (Boehm et al., 1997). Phosphorylated STAT1 dissociates from the receptor, dimerizes and translocates to the nucleus, where it regulates the expression of IFN-γ responsive genes directly (e.g. *CD54*) (Ramana et al., 2002), or indirectly via the induction of other transcription factors such as IRF1, IRF7 and CIITA

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doi:10.1016/j.molimm.2009.11.016

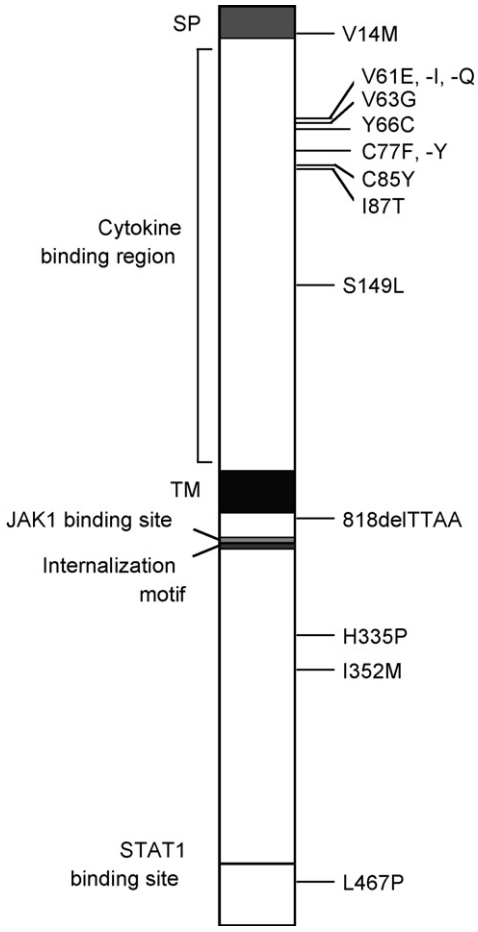

Fig. 1. Schematic presentation of the IFN-γR1. On the left the various domains are indicated, on the right the variations that were analyzed are indicated. TM = Transmembrane domain, SP = signal peptide.

(e.g. *B2M* and *HLA*) (Boehm et al., 1997). Although STAT1 is the main mediator of IFN-γ responses, IFN-γ has also been reported to induce STAT3 or STAT5 phosphorylation in a few other cell types (van Boxel-Dezaire and Stark, 2007). In Fig. 1 a schematic representation of the IFN-γR1 is provided.

Polymorphisms and mutations in the IFN-γR1 chain influence IFN-γ responses. For example, patients with Mendelian Susceptibility to Mycobacterial Disease (MSMD) due to IFN-γR1 deficiency, have impaired Th1-immunity and suffer from unusually severe infections caused by weakly virulent *Mycobacteria* (van de Vosse et al., 2004). Most recessive IFN-γR1 deficiencies result in complete loss of cellular responsiveness to IFN-γ, due to mutations that preclude the expression of IFN-γR1 on the cell surface (Pierre-Audigier et al., 1997; Rosenzweig et al., 2002; Newport et al., 1996; Jouanguy et al., 1996; Roesler et al., 1999). Another group of IFN-γR1 deficiencies is due to missense mutations which result in normal expression of IFN-γR1 at the cell surface, however, the resulting receptors show no or diminished binding of IFN-γ (Allende et al., 2001; Jouanguy et al., 2000). Dominant-negative defects such as the 818delTTAA mutation in the intracellular domain of the receptor result in milder forms of MSMD. The 818delTTAA mutation product lacks the JAK1 and STAT docking site as well as the receptor recycling domain which leads to accumulation of aberrant receptor complexes on the cell surface (Jouanguy et al., 1999; Glosli et al., 2008). In addition, several polymorphisms of the IFN-γR1 have been found that may have an effect on IFN-γ responses. The H335P and L467P variants of the IFN-γR1 have been associated with the production of high antibody titers against *Helicobacter pylori* (Thye et al., 2003) and susceptibility to allergic disease and the production of high IgE titers (Aoki et al., 2003). The frequency of the V14M allele in SLE patients is significantly higher than that of the healthy control population and the presence of a V14M allele correlated with an altered Th1/Th2 balance in favor of Th2 (Tanaka et al., 1999; Nakashima et al., 1999).

In this report we compare the effect of two novel variations, identified in patients suffering mycobacterial infections (S149L, I352M unpublished data), four known polymorphisms (V14M (Nakao et al., 2001; Tanaka et al., 1999), V61I (SNP database, 2006), H335P, L467P (Thye et al., 2003; Aoki et al., 2003), all seven reported missense mutations (V61Q (Jouanguy et al., 2000), V63G (Allende et al., 2001), Y66C (Dorman et al., 2004), C77Y (Jouanguy et al., 2000), C77F (Chantrain et al., 2006), C85Y (Noordzij et al., 2007), I87T (Jouanguy et al., 1997; Remiszewski et al., 2006)) and the 818delTTAA mutation (Jouanguy et al., 1999) on the expression and function of IFN-γR1 in the same genetic background. In addition, because the mutation at nucleotide 182 (g**T**A → g**A**A) reported by Jouanguy et al. (2000) may have been aberrantly designated as V61Q (Val → Gln), instead of V61E (Val → Glu), we analyzed both variations. For this purpose we cloned wild type *IFNGR1* and the *IFNGR1* variants (see also Fig. 1), into a retroviral expression vector and transduced the constructs into the IFN-γR1 deficient cell line SKLC-7. We analyzed the signal transduction, the regulation of CD54, CD64, HLA-DR and HLA class I expression and the cytokine production in response to IFN-γ.

2. Materials and methods

2.1. Cloning IFN-γR1 variants into a retroviral expression vector

The full-length *IFNGR1* coding sequence was PCR amplified from cDNA of a healthy control with the sense primer 5′-AATTGGATCCGGTAGCAGCATGGCTCTCCT-3′ and the anti-sense primer 5′-AAGGCTCGAGTCATGAAAATTCTTTGGAATCT-3′ and cloned into the retroviral vector pLZRS-IRES-GFP (Heemskerk et al., 1997) after digestion with the enzymes *BamH*I and *Xho*I (Fermentas). Variations were introduced by site directed mutagenesis (Higuchi et al., 1988). All constructs were sequence verified and were transfected in the Phoenix-A packaging cell line using calciumphosphate (Invitrogen). Supernatants with retroviral particles carrying the expression construct were generated as described before (de Paus et al., 2008).

2.2. Cells, culture conditions and retroviral transduction

The human IFN-γR1$^{-/-}$ cell-line SKLC-7 (Kaplan et al., 1998) and the human monocytic cell-line THP-1 (ATCC TIB-202) were cultured in RPMI1640 medium supplemented with 10% FCS, 20 mM GlutaMax, 100 U/ml Penicillin and 100 μg/ml Streptomycin (Gibco/Invitrogen). 0.25×10^6 cells were retrovirally transduced by overnight incubation on a CH-296 (RetroNectin^{TM}, Takara Shuzo) coated 48 wells plate in the presence of 1 ml of virus containing supernatant. Cells were washed and cultured for at least four days before analysis in further assays. All subsequent FACS measurements were performed on cells gated for equal GFP expression.

2.3. Analysis of IFN-γR1 expression

To detect IFN-γR1 membrane expression cells were labeled with IRγ2 (Watzka et al., 1998) and 177.10 (Novick et al., 1989) antibodies (kindly provided by Heiner Böttinger and Daniela Novick respectively) and PE conjugated monoclonal antibodies GIR94, GIR208, and as an isotype control IgG1 (BD Biosciences). After labeling with the γR99 antibody (Garotta et al., 1990) (kindly provided by Francesco Novelli) the cells were counterstained with