Regulation of the Th1 immune response : the role of IL-23 and the influence of genetic variations

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Introduction

The immune system

The human immune system is crucial to our survival since among other functions it protects the integrity of the tissues against pathogens invading from our environment and helps limit damage exerted by our microbial adversaries. The intact skin and the mucosal membranes in the gut and lung provide a first physical barrier that effectively prevent pathogens such as bacteria from invading the human body. In case this first line of defence is breached, the pathogen is confronted by the immune system. The human immune system can be divided into an innate and an adaptive arm.

Innate immune system

The first line of defence against invading pathogens consists of the innate immune system. The innate immune response has evolved to recognize a broad spectrum of pathogens and defends the host from infection by pathogens in an apparent non-specific manner. Moreover, this part of the immune system provides immediate defense against infection (1). The system is composed of both humoral and cellular mechanisms. The complement system is key component of the humoral mechanism (2), whereas the cellular arm consists of many cell types, including granulocytes, monocytes, macrophages and natural killer (NK) cells. In contrast to the adaptive immune system, exposure of the innate immune system to a pathogen does not induce immunological memory, i.e., the ability to react more rapid and specific upon a second contact with the pathogen.

Phagocytosis of invading pathogens represents an early and crucial event in host defense. Phagocytic cells include granulocytes, monocytes, tissue macrophages and dendritic cells. Phagocytosis starts with the binding of a pathogen to cell surface receptors, and is followed by the uptake of the pathogen into a vesicle designated phagosome (3). Next, the phagosome fuses with lysosomes and subsequently matures into a phagolysosome (3). Pathogens contained within a phagolysosome are destroyed by lowered pH, enzymatic hydrolysis, and radical attack (3,4).

Recognition of pathogens is dependent on the binding of pathogen-derived structures to germline-encoded pathogen recognition receptors (PRRs) expressed by phagocytes (5,6). PRRs bind pathogen-associated molecular patterns (PAMPs) which are conserved between classes of pathogens (7,8). PRRs are involved in phagocytosis of pathogens and in the activation of pro-inflammatory pathways and recruitement of inflammatory cells (7). Phagocytes express membrane-bound as well as cytosolic PRRs. Of membrane-bound PRRs, perhaps the best known are the Toll-like receptors (TLRs) . The TLR family forms a class of PRRs consisting of at least 10 members that each recognize a different classe of pathogen-derived patterns (9). Apart from TLRs, membrane-bound C-type lectins and the cytosolic nucleotide binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid inducible gene (RIG)-I-like helicases (RLHs) play important roles in triggering innate immune responses (10-12). Members of the NLR family form the central

components of inflammasomes and act as intracellular sensors that detect cytosolic microbial components (10). The inflammasome is a multiprotein complex involved in activating caspase-1 and -5, leading to the processing and secretion of the pro-inflammatory cytokines IL-1β and IL-18. In addition, some of the PRRs are secreted into the bloodstream. Secreted PRRs include the C-type lectin mannan-binding lectin (MBL), collectins and ficolins (12). These PRRs bind to a wide range of bacteria, viruses, fungi and protozoa and serve to opsonise pathogens, help to activate complement and allow for enhanced recognition by phagocytic cells (12).

The early phase of an inflammatory response is characterised by an alteration of the local vascular permeability and influx of inflammatory cells. Upon recognition of pathogens via PRRs, cells of the innate immune system produce a wide range of inflammatory mediators such as cytokines, chemokines and interferons (IFN). These mediators orchestrate the development of the adaptive immune response.

Adaptive immune system

When pathogens successfully evade the innate immune system, a second line of protection is provided by the specific or adaptive immune system. The adaptive immune system provides a stronger and pathogen specific immune response and induces an immunological memory that enables it to react more promptly and specifically upon a second encounter with the same pathogen. Like the innate immune system, the adaptive immune system consists of a humoral and a cellular part. B cells and T cells are the major types of lymphocytes of the specific immune system (13). B cells are involved in the humoral part, whereas T cells are involved mainly in the cellular part, though intracellular signalling by soluble factors, interleukins or cytokines, plays a role in both arms.

B cells produce specific antibodies that specifically recognize and neutralize pathogens. Naïve B cells express a unique membrane bound B cell receptor (BCR). Ligation of the BCR on these cells by pathogen-derived structures induces the proliferation and differentiation of the cells into antibody secreting plasma cells (14). Generally, B cells require help from activated T helper cells to mature into fully activated plasma cells (15).

Two major subtypes of T cells can be distinguished: $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells, subtypes that play a distinct function. T cells express a membrane bound T cell receptor (TCR), which can recognize antigens bound to the major histocompatibility complex (MHC). There are two different classes of MHC molecules of importance here: MHC class I and MHC class II. MHC class I molecules are expressed on all cells and present intracellular-derived antigens to $CD8^+$ cytotoxic T cells, whereas MHC class II molecules are expressed only on professional antigen presenting cells (APC) like dendritic cells (DC) and macrophages, and present antigens of an extracellular origin to $CD4^+$ T cells. $CD4^+$ T helper cells help regulate the immune response, for instance to intracellular bacterial pathogens like *Salmonellae* and *Mycobacteria*, whereas $CD8^+$ cytotoxic T cells eliminate mainly virally-infected cells.

T helper cell immune responses: Th1, Th2 and Th17

An effective and specific immune response against pathogens that invade the human body is dependent on the differentiation of T lymphocytes into effector cells. Specific immune responses are driven by the emergence of pathogen-specific effector $CD4^+$ T-cell subsets: the Th1 cells, which primarily produce IFN-γ; Th2 cells, which produce IL-4, IL-5, IL-10 and IL-13 (16); and the recently discovered Th17 cells, producing IL-17.

Differentiation of naïve $CD4^+$ T cells into effector $CD4^+$ T cells is dependent on the type of pathogen and is guided by cells of the innate immune system. Th1 cells control intracellular pathogens, including viruses and bacteria, and are involved in autoimmune diseases (17). For example, host defense against intracellular bacterial pathogens such as *Salmonellae* and *Mycobacteria* is dependent on the type-1 IL-12/IL-23/IFN-γ pathway and deficiencies of components of this pathway render patients highly susceptible to infections with these pathogens (18). The Th2 cells are important in defence against helminths. In addition, the Th2 cell is thought to be the central effector cell in the pathogenesis of atopic allergic diseases (19). For example, IL-4 and IL-13 elicit antigen-specific IgE production in B cells, essential for the immediate hypersensitivity response, leading to mast cell degranulation and the subsequent release of histamine (20). The exact function of Th17 cells still has to be established. However, this T helper cell subset is thought to play an important role in the defense against bacterial, fungal, and viral infections at mucosal surfaces (21). In addition, Th17 cells are thought to be crucial for the development of various auto-immune diseases.

The activation and differentiation of naïve T helper cells is thought to be dependent on three signals (22). The first signal results from ligation of T-cell receptors (TCRs) by pathogen-derived peptides, presented by MHC class II molecules on the cell surface of APCs. This signal determines the antigen-specificity of the response. The second signal is provided by the co-stimulatory molecules CD80 and CD86 expressed by the APC, which bind and activate CD28 on the T cell. TCR triggering in the absence of this co-stimulatory signal leads to the induction of anergic T helper cells and might lead to tolerance. The third signal is the polarizing signal and depends on the cytokines activating the T cell. IL-12 and IL-4 are seen as the major polarizing cytokines for naïve T helper cells to differentiate into Th1 and Th2 cells, respectively (23,24). Both signals 2 and 3 are thought to be dependent on the binding of specific microbial products or endogenous danger signals on receptors expressed by APCs and the resultant cytokine production (22,25).

Cytokines

Innate and adaptive immunity are not just sequential mechanisms, but rather are interwoven and regulating each other in many reciprocal pathways. For instance, the innate immune system provides the cells of the adaptive system with information via the secretion of soluble mediators and

cell-cell contact. One of these soluble mediators is IFN-γ. IFN-γ is critical to many immune responses and regulates many processes.

Interferon-γ

Effective defence against different types of pathogens require different types of immune responses to be elicited. IFN-γ plays a central role in the type-1 immune response and is critical to innate and adaptive immunity against intracellular bacteria and parasites. IFN-γ and IFN-γR-deficient mice develop normally, however, these mice display selective defects in the immune response against pathogens, including *Salmonellae* and *Mycobacteria* (26-31). Humans with IFN-γR1 or IFN-γR2 deficiency show a clinical presentation similar to that observed in the mouse models; the patients have impaired Th1-immunity and suffer from unusually severe infections caused by weakly virulent *Mycobacteria* (32). On the other side, in both IFN-γ transgenic mice and in SOCS1-deficient mice, lacking negative feedback of the IFN-γ signal transduction pathway, fatal multi-system inflammation is observed (33,34).

Interferon-γ production

At early stages of infection, IFN-γ secretion by NK cells, NK-like T cells, NKT cells, γδT cells and $CD8^+$ T cells is crucial, whereas later on, T lymphocytes become the major source of IFN-γ when the adaptive immune response has evolved (35-39). IFN-γ produced in the early phases of an infection by the innate immune system skews the adaptive immune response towards a T helper 1 (Th1) phenotype (40-42). IFN-γ production is controlled by cytokines secreted by antigen presenting cells (APC). The IL-12 cytokine family (e.g. IL-12, IL-23 and IL-27) is thought to be of major importance in the induction of IFN-γ production. IL-18 and IL-1β are known to synergise with IL-12 and IL-23 in inducing IFN-γ production in various cell types (43,44). Together, these cytokines serve in the coupling of the innate and adaptive immune response (45).

Interferon-γ functions

IFN-γ is produced by Th1 cells, skews the immune response further towards a Th1 phenotype and inhibits Th2 cell populations. After binding of IFN-γ to the IFN-γR complex on its target cells, many effects are induced, including the following:

1) IFN-γ enhances the synthesis of components of the complement cascade and the acute phase response, thereby enhancing the humoral arm of innate immunity (46-49).

2) The availability of sufficient iron is essential for the growth of many pathogens. IFN-γ limits the availability of iron by reducing serum iron and increasing ferritin levels, thereby inhibiting growth of many extracellular pathogens (50). To inhibit the growth of intracellular

pathogens within infected macrophages, IFN-γ reduces the uptake of iron and increases iron efflux (51).

3) IFN-γ contributes to macrophage activation by increasing phagocytosis (52-54). IFN-γ increases the expression of the high-affinity IgG receptor, FcγRI (CD64) on the granulocyte, monocyte and macrophage cell surface. This receptor binds IgG, allowing for enhanced phagocytosis, binding of immune-complexes, and antibody-dependent cellular cytotoxicity (55,56).

4) IFN-γ induces antimicrobial effector function. After phagocytosis of a pathogen, IFN-γ enhances phagosome-lysosome fusion in infected macrophages and speeds up the maturation and acidification of the phagolysosome *(57-60).* IFN-γ enhances the expression of proteins involved in the subsequent destruction of pathogens in the phagolysosome*, including* immunity-related GTPases (IRG) (61), natural resistance-associated macrophage protein (NRAMP1) and proteins involved in the oxidative burst (62-64). The membrane-associated complex NADPH mediates the formation of superoxide anions (O_2^-). IFN-γ in synergy with TNF, enhances the transcription of genes encoding gp91-phox, p47-phox and p67-phox, all components of the NADPH complex, thereby promoting formation of this complex and the production of O_2^- anions and hydrogen peroxide (65). Furthermore, IFN-γ enhances the production of nitric oxide (NO), an unstable radical gas. IFN-γ maximises the expression of inducible nitric oxide synthase (iNOS), an enzyme that catalyzes the oxidation of one of the guanidino nitrogens of l-arginine to nitric oxide (NO) (52).

5) In addition, IFN-γ enhances antigen processing and the expression of the MHC class I and II molecules, thereby increasing antigen presentation and promoting the induction of cell-mediated immunity (66,67). The IFN-γ induced up-regulation of class I MHC increases the potential for cytotoxic T cells to recognize infected cells. On the other hand, via the up-regulation of class II MHC molecules, IFN-γ promotes the activation of $CD4^+$ T helper cells.

6) Immunoglobulines are important for the opsonisation and neutralisation of many pathogens. IFN-γ stimulates immunoglobulin (Ig) production and matures the antibody response by the induction of class switching in B cells (68).

7) IFN-γ primes cells for more rapid and heightened production of pro-inflammatory cytokines upon TLR stimulation (69-71). For example, in the course of an immune response, the IFN-γ produced by innate immune cells provides a strong positive feedback loop to enhance IL-12, IL-23 and TNF production by monocytes and macrophages (69-71), thereby enhancing Th1 immunity.

Interferon-γ signal transduction

The IFN-γR is comprised of two ligand-binding IFN-γR1 chains associated with two signal-transducing IFN-γR2 chains (Fig. 1) (66). Binding of IFN-γ as a non-covalently linked homodimer to its receptor induces receptor oligomerization and activation of the receptor-associated Janus kinases (JAK)1 and JAK2 by trans-phosphorylation. JAK1 and JAK2 associate with the IFN-γR1 and IFN-γR2, respectively. JAK1 phosphorylates the functionally critical tyrosine residue on position 440 (Y440) in the intracellular domain of each IFN-γR1 chain. The Y440 serves as the docking site for signal transducer and activator of transcription (STAT)1. After docking to the Y440, STAT1 is phosphorylated

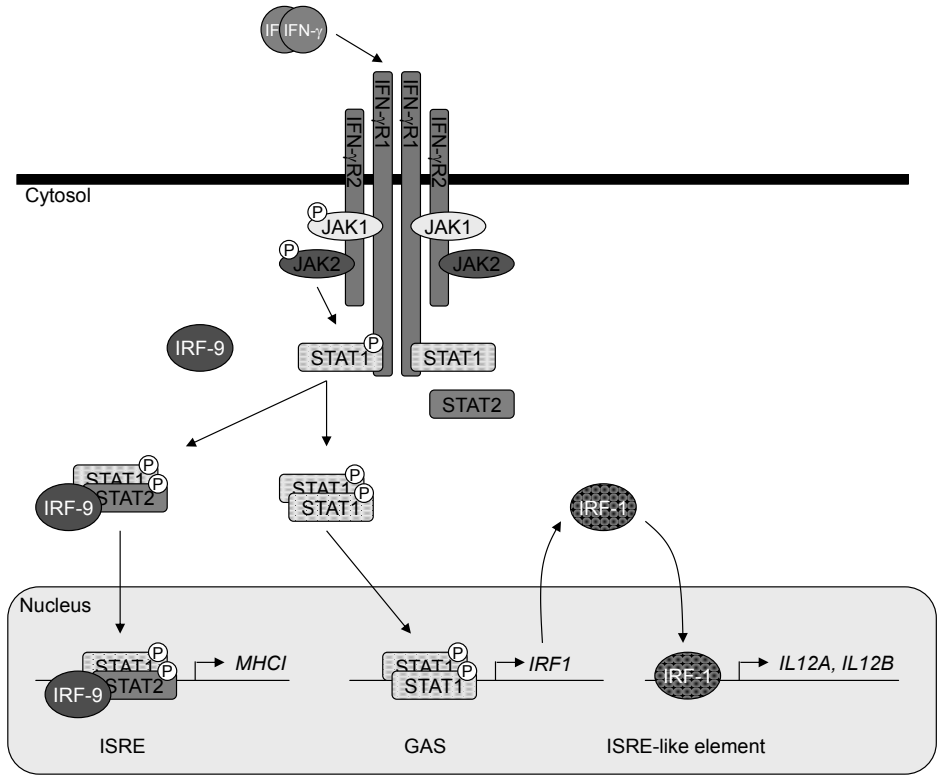

Figure 1. IFN-γ signal-transduction. IFN-γ oligomerization of the IFN-γ-receptor subunits IFN-γR1 and IFN-γR2 leads to the phosphorylation and activation of JAK1, JAK2 and the tyrosine 440 of the IFN-γR1. Subsequently STAT1 docks to the phosporylated IFN-γR1 to become phosphorylated itself. Phosphorylated STAT1 dimerizes and translocates to the nucleus where it binds to GAS-elements. Among the primary response genes are a number of transcription factors including IRF-1. IRF-1 plays a role in regulating the expression of secondary response genes through promoters with IRSE-like motifs. To a lesser extent, IFN-γ signalling also results in the formation of STAT1:STAT1:IRF-9 and STAT1:STAT2:IRF-9 (ISGF3) complexes

on its tyrosine 701 (Y701) and serine 727 (S727) residues (66). Phosphorylation of the Y701 is essential for subsequent dimerisation of STAT1 molecules, while the phosphorylation of the S727 is

needed for full transcriptional activity. After being phosphorylated, STAT1 dissociates from the receptor, dimerizes and translocates to the nucleus, where it directly regulates the expression of IFN-γ responsive genes (e.g. *ICAM1*) (72), or indirectly activates genes, via the induction of transcription factors such as interferon regulatory factor (*IRF)1, IRF8* and *CIITA* (e.g. *IL12B, IL12A, B2M* and *HLA*) (73-76). Apart from STAT1 homodimers, IFN-γ induces, although to a lesser extent, STAT1/STAT2/IRF-9 complexes (also known as IFN-stimulated gene factor 3 (ISGF3)). ISGF3 induces target genes containing an interferon-stimulated response element (ISRE) element. Via the induction of these transcription factors, IFN-γ regulates a wide range of distinct cellular programs via the activation of multiple genes.

The IL-12 cytokine family

Cytokines can be classified based on size, gene organization, sequence homology and structural motifs, such as the common four-helix bundle present in type I cytokines (77). Type I cytokines regulate development, differentiation, and activation of immune cells and cells of the inflammatory system (77). The cytokines IL-12, IL-23, IL-27 and IL-35 are members of the IL-12 cytokine subfamily, which is part of the type I cytokine superfamily. A specific feature of the members of the IL-12 cytokine family is that they are formed by heterodimerization of specific subunits. IL-12p40 can be secreted as a monomer or as a disulfide-linked heterodimer, linked to IL-12p35 or IL-23p19 (78). When IL-12p40 is bound to IL-12p35, it forms IL-12. While the IL-12p40 is homologous to the α-chain of the soluble IL-6 receptor, the IL-12p35 subunit is structurally related to the type I cytokines. The complex of IL-23p19 and IL-12p40 forms IL-23. IL-23p19 was identified on the basis of its homology with IL-6 and the IL-12p35 chain (78). The discovery of IL-23 led to a re-evaluation of the function of IL-12 in experiments based on the neutralization or deletion of IL-12p40. In mice, IL-12p40 can form a homodimer named IL-12p80, in humans however, IL-12p80 is not reported (79,80).

Similar to IL-12 and IL-23, IL-27 is a heterodimeric cytokine consisting of Epstein-Barr virus-induced molecule 3 (EBI3), an IL-12p40 homologue, noncovalently bound to p28, an IL-12p35 homologue (81). EBI3 has also been reported to bind to IL-12p35, forming IL-35 (82,83). The major cellular source of IL-12, IL-23 and IL-27 are APCs. By contrast, murine IL-35 is produced by regulatory T cells (83). Human regulatory T cells do not constitutively express IL-35 (84). However, human rhinovirus stimulated DCs induce IL-35 production in FOXP3 negative regulatory T cells (85). The IL-12 cytokine family members are thought to be crucial in the development and maintenance of a Th1 response and in mice deficiency in either IL-23 or IL-12 significantly compromises the host's ability to eliminate pathogens (18,86).

Regulation of IL-12 and IL-23 production

IL-12 and IL-23 share the common IL-12p40 subunit. For the production of IL-12, both IL-12p40 and IL-12p35 must be co-expressed, whereas for the expression of IL-23 IL-12p40 and IL-23p19 must be co-expressed. The expression of the p40, p35 and IL-23p19 genes is independently regulated. IL-12p40 is produced in large excess over the IL-12 and IL-23 hetero-dimers; a role of this excess IL-12p40 remains unknown. Prior to secretion, the various subunits are assembled to form the different hetero-dimeric cytokines. After dimerisation, N-linked glycosylation of IL-12p35 is a required step in the secretion of IL-12 (87).

A variety of stimuli can induce IL-12 and IL-23 production. For example, monocytes, macrophages and dendritic cells (DCs) produce IL-23 following exposure to PAMPs that act through TLRs (88). In contrast to IL-23, an extra stimulus such as IFN-γ is needed for the induction of IL-12 (40,89). In line, in whole blood assays with bloodcells obtained from patients with complete IFN-γR deficiency, no IL-12 production can be detected in response to M. *bovis* BCG infection *in vitro* (90). IFN-γ primes for IL-12 production and enhances IL-23 production in human monocytes and macrophages (71,89,91). In contrast, IFN-γ is reported to inhibit IL-23 production in human DCs (89), indicating a dual role for IFN-γ in the regulation of IL-23 production, depending on the cell type.

The balance between IL-12 and IL-23 production is dependent on the specific TLR being stimulated. In human DCs TLR2 ligands increase IL-23, but at the same time inhibit IL-12 production (91). In addition, activation of human myeloid DCs by TLR4 ligands, like lipopolysaccharide (LPS), leads to the expression of p19, p35 and p40 the building blocks of both IL-23 and IL-12. TLR2 activation by peptidoglycan (PGN), however, induces high levels of IL-23, but not of p35 expression or IL-12 synthesis (92,93). Not all TLR2 agonists are equally good IL-23 inducers: for instance, PGN is a more potent IL-23 inducer than PAM_3CSK_4 (94), which can be explained by the fact that PGN not only stimulates IL-23 production via TLR2 but also via NOD2 stimulation by the intracellular PGN metabolite muramyl dipeptide (MDP) (93). These data indicate that synergy between TLR2 and NOD2 agonists favour the induction of IL-23. In addition, several combinations of TLR agonists induce both IL-12 and IL-23. TLR3 and TLR4 ligands synergize with TLR8 ligands in the induction of both IL-12 and IL-23 (88,95,96). Other PRRs influence the balance between IL-12 and IL-23 production. The dectin-1 agonist β-glucan curdlan induces IL-23 synthesis by DCs without inducing IL-12 production (97), indicating that signalling through C-type lectins also favours IL-23 production.

Commensal gram-negative bacteria as well as gram-positive bacteria are able to induce p19 expression in DCs. Intact gram-positive bacteria, however, preferentially stimulate production of IL-12 over IL-23 (98). Gram-negative bacteria induce more IL-23 compared to gram-positive bacteria, indicating that a component of gram-negative bacteria synergizes with PGN, a cell-wall component of both gram-negative and gram-positive bacteria, in the induction of IL-23.

A variety of cytokines is known to modulate IL-12 and IL-23 production. Harris et al. showed that IL-1β induces IL-23 production in human monocytes in response to gliadin (99). GM-

CSF primes monocytes for enhanced IL-23 production in response to a variety of TLR agonists (88). Moreover, macrophages generated in the presence of GM-CSF (type 1 macrophages) produce IL-23 in response to various stimulations, whereas macrophages generated under pressure of M-CSF (type 2 macrophages) do not (89). Furthermore, GM-CSF is known to amplify the IL-23 inducing activity of prostaglandin E_2 (PGE_2) in a synergistic manner (100). In combination with GM-CSF, IL-4 is used for the in vitro generation of DCs and known to enhance IL-12 production (101,102). IL-4 downregulates IL-23p19 mRNA, IL-12p40 mRNA and IL-23 protein expression in virally infected macrophages (103) and inhibits TLR agonist induced IL-23 production in DCs (104). These results suggest that IL-4 can shift the balance between IL-23 and IL-12 production towards IL-12. In contrast to IL-4, PGE_2 *inhibits IL*-12 production, but stimulates IL-23 expression in LPS-treated DC in mice (100,105,106), thereby inducing a shift in the IL-23/IL-12 balance in favour of IL-23. The effect of PGE_2 is dependent on the induction of cAMP (107). The nucleotide adenosine triphosphate (ATP) also influences the balance between IL-12 and IL-23 in DCs, favouring a shift towards IL-23 (107). IL-10 is a potent negative regulator of many pro-inflammatory cytokines, including IL-12 and IL-23. IL-$10^{-/-}$ mice produce more IL-23 in response to LPS, compared to wild-type mice (108). Both IL-12 and IL-23 can induce IL-10 production (109-111), which may provide a negative feedback signal. Finally, IL-12 and IL-23 seem to cross-regulate each other. IL-23 deficient mice produce higher levels of IL-12 in a colitis model and IL-23 suppresses IL-12 production in murine DCs upon TLR stimulation (112), suggesting an inhibiting role of IL-23 on IL-12 synthesis.

Activated T cells regulate IL-12 and IL-23 production by cell-cell contact, via a mechanism in which CD40L expressed on activated T cells after activation binds CD40 on APCs. Apart from IFN-γ, CD40-CD40L interactions seem to be crucial for the induction of IL-12. CD40 or CD40L deficient mice fail to produce IL-12 in response to *Leishmania* infection (113). In monocyte-derived DCs, both IL-12 and IL-23 production are upregulated after CD40 ligation (78,98,104,114-117). Furthermore, ligation of CD40L by CD40 enhances the IFN-γ inducing capacities of IL-12 on T cells (118).

Summarizing, regulation of IL-12 and IL-23 production during infection is dependent on a complex network of mediators; the TLRs stimulated by the infective agent, the cytokines induced and contact dependent T-cell regulation. Importantly, IL-12 production is dependent on IFN-γ and CD40-CD40L interactions, whereas these stimulations are not needed for the induction of IL-23. Moreover, IL-23 production is preceding IL-12 production during infections (89,119,120).

Biological functions of IL-12 and IL-23: induction of IFN-γ and the Th1 (and Th17) response

Generally, IL-12 is thought to be the primary trigger that initiates Th1 differentiation and IFN-γ production (Fig. 2). As mentioned earlier, paradoxically, for the induction of IL-12 production, the Th1 cytokine IFN-γ is needed (40). Moreover, Abdi et al. showed that Th1 polarization is critically dependent on IFN-γ. This means that for the initiation of Th1 differentiation, IFN-γ is needed to

complement this “third signal”. Naïve T cells and APCs are unable to produce this cytokine, indicating that other cells producing IFN-γ are needed for the initiation of Th1 differentiation. As mentioned, the primary sources of IFN-γ include effector cells of the innate immune system like NK cells, NKT cells, NK-like T cells and γδT cells, therefore these cells may provide this third signal (41,121,122). NK and NKT cells constitutively express IFN-γ mRNA, allowing rapid induction and secretion of IFN-γ (123). Moreover, NK derived IFN-γ is known to push differentiation of T helper cells into the Th1 subclass (41,42). However, the stimuli leading to the initial IFN-γ production in these cells are not well characterized.

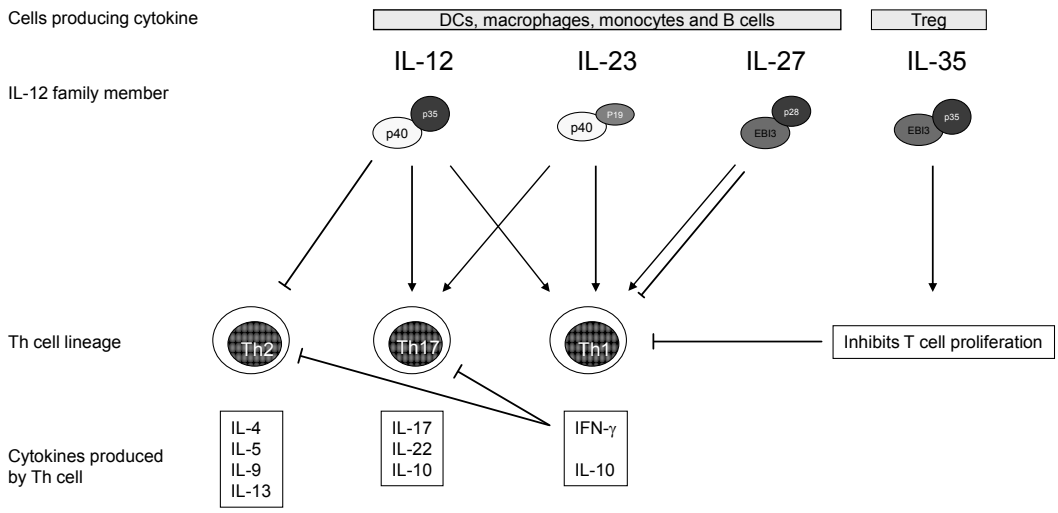

Figure 2: IL-12 family members and T helper cell subsets. All IL-12 family members are composed of two subunits. IL-12, IL-23 and IL-27 are produced by dendritic cells, macrophages, monocytes and B cells. IL-12 promotes Th1 cell differentiation. IL-23 can induce both IFN-γ and IL-17 and is involved in both Th1 and Th17 cell differentiation. IL-27 promotes Th1-cell differentiation, an effect that is most prominent in the absence of IL-12, whereas at later stages of the immune response IL27 inhibits Th1 cells. IFN-γ inhibits both Th2 and Th17 cells. In contrast to the other IL-12 family members IL-35 is produced by regulatory T cells and inhibits proliferation of all T cell lines.

IL-23 is produced early in infections, without the need of IFN-γ. Moreover, this cytokine is capable of inducing IFN-γ production (43,78,88,103,124). IL-23 is among the first cytokines secreted by APCs in response to stimulation with PAMPs such as LPS (89). Thus, early APC-derived IL-23 may be an initial trigger of IFN-γ production. Experimental data in favour of this hypothesis comes from the observation that mice deficient in the p40 subunit of IL-12/23 are far more impaired in their ability to generate Th1 responses to pathogens as *Salmonellae* and *Mycobacteria* than are mice lacking the p35 subunit of IL-12 (125-128), suggesting a role for IL-23 in the induction of IFN-γ production. Also the fact that IL-23 induces the expression of substance P (129), a peptide reported to enhance IFN-γ

production by Th1 cells, in macrophages points to a role of IL-23 in the initiation of IFN-γ production (130).

Other cytokines, including the IL-12 family member IL-27, are reported to regulate Th1 differentiation early in infection, independently of IL-12 (131-133) (Fig. 2). Although several lines of evidence indicate that IL-23 and IL-27 have a role in the induction of IFN-γ, it should be noted that none of these cytokines is indispensable for the induction of a Th1 response (134-136). This suggests that several factors, including IL-23, IL-27 can induce IFN-γ production, without the need of all these factors being produced. Apart from IL-23 and IL-27, IL-1β and IL-18, both members of the IL-1 family of cytokines, are likely to be involved in the induction of innate IFN-γ. Both IL-1β and IL-18 are released soon after the first contact between phagocytes and pathogens and both these cytokines promote IFN-γ production by NK cells and T cells (44,137). Moreover, IL-18 is essential in the innate immune activation of CD4 T cells in *Salmonella*-infected mice (137).

Concluding, IL-12 is likely to be involved in the maintenance of an ongoing Th1 response, while other cytokines like IL-23 are involved in the initiation of a Th1 immune response.

IL-12 and IL-23 receptor

Structure and expression

IL-23 and IL-12 signal through a common IL-12Rβ1 chain complemented by the IL-23R and the IL-12Rβ2, respectively (Fig. 3). While IL-12Rβ1 is constitutively expressed in activated $CD4^+$ T cells, NK cells and NK-like T cells (17,138), IL-23R and IL-12Rβ2 expression are critical for the ability to respond to IL-23 and IL-12, respectively. To generate a high affinity binding site for IL-12, both the IL-12Rβ1 and IL-12Rβ2 chains need to be co-expressed (139). The IL-12Rβ2 expression is highly regulated on T cells. Resting T cells do not express IL-12Rβ1 or IL-12Rβ2 receptors, but these subunits are induced upon T-cell activation, and receptor expression correlates with IL-12 responsiveness (140). Furthermore, the IL-12Rβ2 is expressed on Th1 cells, but not on Th2 cells (141). In Th1 cells, the expression of the IL-12Rβ2 chain is upregulated by IL-12 itself, IFN-γ, IL-18 and IL-27 (124,142).

The IL-23R protein is very similar to IL-12Rβ2, in particular with respect to the two cytokine binding domains and the immunoglobulin domain. IL-23R expression patterns are not well defined, because a specific antibody for FACS staining is not available (109). Expression of the *IL23R* mRNA has been reported in a broad range of cells, including memory T cells, NK-like T cells, Th1, Th2 as well in Th17 clones, γδT cells, NKT cells, NK cells, DCs and in activated macrophages (43,78,143-148). Apart from lymphocytes, also microglia, a type of glial cell that acts as the first and main form of active immune defense in the central nervous system, are reported to express *IL23R* mRNA and to respond to IL-23 (149). The Th1 associated transcription factor T-bet is involved in the induction of both the *IL-23R* mRNA and the IL-12Rβ2 (150).

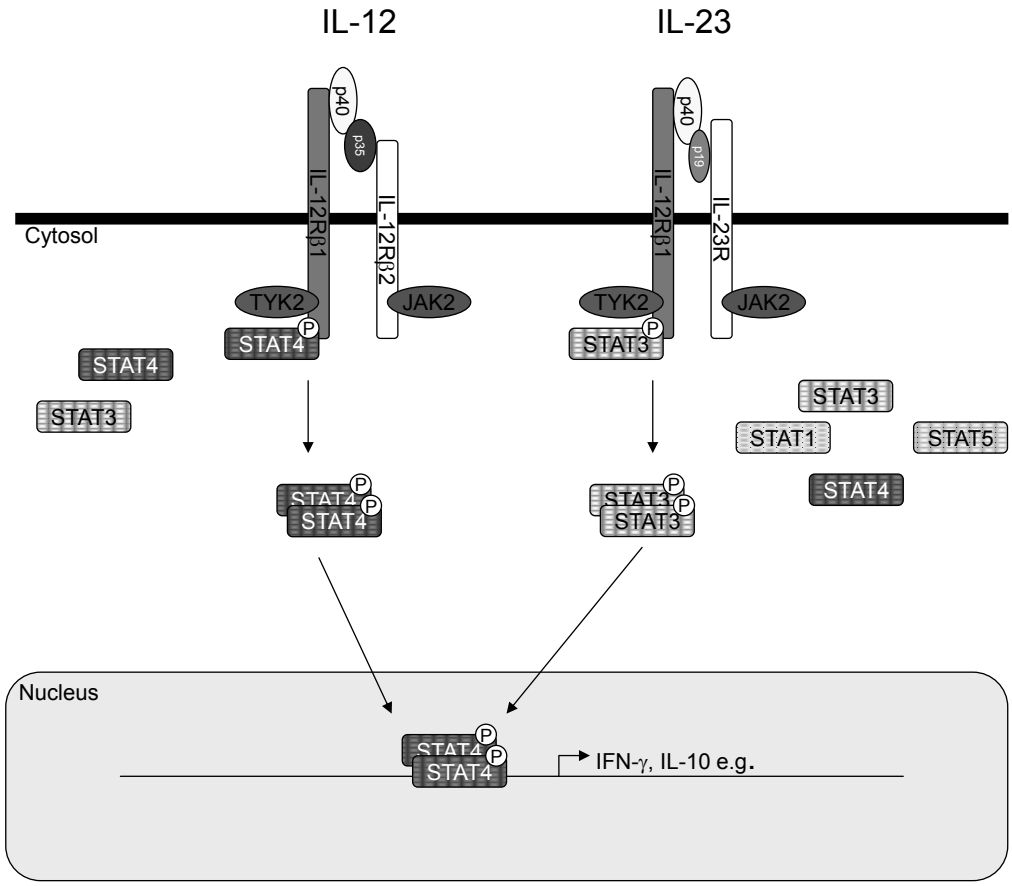

Figure 3. IL-12 and IL-23 signal transduction. IL-12 and IL-23 activate the JAK/STAT pathway. IL-12 is composed of IL-12p40 and p35 subunits that bind to IL-12Rβ1 and β2, respectively. Upon binding of IL-12 to its receptor, TYK2 and JAK2 associate with the IL-12Rβ1 and β2, respectively, followed by JAK transphosphorylation and subsequent phosphorylation of the receptor chains by activated JAKs. Subsequently, the IL-12Rβ2 is phosphorylated and serves as a docking site for STAT molecules. STAT4 binds to the receptor chain and is phosphorylated. Next STAT4 homodimers are formed and translocate to the nucleus where they bind to STAT-binding elements, like in the IFN-γ promoter, and regulate transcription. IL-23 is a heterodimeric cytokine composed of p40 and p19 that bind to the IL-12Rβ1 and the IL-23 receptor (IL-23R), respectively. Like IL-12, IL-23 stimulation induces JAK2 and TYK2 phosphorylation. Subsequently, mainly STAT3 is phosporylated.

Signal transduction

The IL-23R associates with JAK2, whereas the IL-12Rβ1 associates with TYK2 (Fig. 3) (144). Upon binding of IL-23 to the IL-23 receptor complex, JAK2 and TYK2 are phosphorylated. Subsequently, STAT1, STAT3, STAT4 and STAT5 can dock to the receptor and are reported to be phosphorylated, however, the most prominent STAT induced by IL-23 is STAT3 (43,109,144,151). The tyrosine residues of the IL-23R responsible for STAT recruitment have not yet been established. The human

IL-23R contains seven tyrosine residues, three of which represent putative Src Homology 2(SH2)-binding sites which may be involved in binding the SH2-sites of STAT meolecules (144,152).

Similar to the IL-23R, the IL-12Rβ2 associates with JAK2, which is phosporylated upon binding of IL-12 to the IL-12 receptor complex. IL-12Rβ1 bound TYK2 is also phosphorylated after binding of IL-12 to the receptor complex. During IL-12 signal transduction, STAT4 docks to the tyrosine 800 of the IL-12Rβ2 chain and this tyrosine is critical for IL-12 signal transduction (153). In contrast to IL-23, IL-12 mainly induces STAT4 phosphorylation.

After phosphorylation of STAT molecules in response to either IL-12 or IL-23, the phosporylated STAT molecules dimerize to form homo- and heterodimers and subsequently translocate to the nucleus to regulate their target genes. STAT3 is critical in IL-23-dependent induction of IL-17 (151,154-156). Experiments eluding the role of STAT4 in the development of Th17 cells are contradictive (154,157,158). IL-23 and IL-12 are both inducers of IFN-γ and IL-10 (78,109). STAT4 is essential for IL-12 induced IFN-γ (159). STAT4 is likely to be important in IL-23 induced IFN-γ, however, this is not yet elucidated.

Mendelian Susceptibility to Mycobacterial Disease.

Patients have been described with unusually severe infections caused by otherwise poorly pathogenic *Mycobacteria* and *Salmonellae*, a condition also known as Mendelian Susceptibility to Mycobacterial Disease (MSMD [MIM 209950 [OMIM]) (18). MSMD is a heterogeneous disorder that can be caused by mutations in genes involved in the Th1 - IL-12/-23/IFN-γ - cytokine signalling cascade. In these patients, genetic defects have been identified in the genes encoding IL-12p40, IL-12Rβ1, TYK2, IFN-γR1, IFN-γR2, and STAT1 proteins (32).

Deficiencies in the IL-12 and IL-23 pathways

IL-12p40

In total 20 cases of IL-12p40 deficiency have been reported and five different mutations in the *IL12B* gene have been found (160). All known *IL12B* mutations are recessive and result in complete IL-12p40 deficiency with a lack of detectable IL-12p40 secretion by cells of the patients (161,162). These patients lack active IL-12 and IL-23. As a result, these patients produce severely reduced amounts of IFN-γ *in vitro* (162).

In three children (one Pakistani and two Indian), a large homozygous deletion causing a frameshift in the *IL12B* gene has been identified, resulting in a protein of 184 amino acids including only 139 out of the 328 original amino acids in the N-terminal region and 45 novel amino acids in the C-terminal region (161-163). This protein cannot be detected and the stability is unknown. Interestingly, Pulickal et al report one female sibling homozygous for this mutation who was not clinically affected, suggesting a multifactorial basis for the observed phenotype in the clinically

affected sibling. Four kindreds from Saudi Arabia were found to have a small insertion (316insA) causing a frameshift, resulting in a short protein lacking the amino acids essential for dimerization with IL-12p35 (161). Small frameshift deletions (528del2 and 297del8) resulting in a premature stop-codon were detected in four patients (age between 3 months-28 years) who presented with Bacillus Calmette-Guérin (BCG)-itis (164,165). Interestingly, all patients with IL-12p40 deficiencies who were BCG vaccinated developed BCG-itis (161,162,164,165). In about half of the cases, salmonellosis was reported (161,162).

IL-12Rβ1

Since IL-12Rβ1 is an essential component of IL-12R as well as IL-23R, deficiencies of this receptor chain will affect both IL-12R and IL-23R dependent signalling. Over eighty individuals have now been reported with complete IL-12Rβ1 deficiency (165-177), resulting in deficient IFN-γ production. IL-12Rβ1 deficiency is the most frequent known genetic cause of MSMD. Mycobacterial infection and salmonellosis are the infectious diseases most frequent found in these patients.

Most reported IL-12Rβ1 mutant alleles cause recessive complete IL-12Rβ1 deficiency. In most cases IL-12Rβ1 expression is abrogated due to a diverse array of mutations, including nonsense (167), missense (169-171,173,178-180), and splice mutations (165,169,175,181), microinsertions (182), microdeletions (165,169), microduplications (182) and large deletions (167,174,183,184). Fieschi et al. reported a patient with a large in-frame deletion in the *IL12RB1,* resulting in the surface expression of nonfunctional IL-12Rβ1 (183). This same deletion was found in an unrelated 6 year old boy (184). In all IL-12Rβ1-deficient patients both IL-12 and IL-23 responses are abrogated and IL-12 and IL-23-dependent production of IFN-γ is severely impaired (90,183,185). One patient with a partial IL-12Rβ1 deficiency has been reported (169). In this patient, IL-12 response was diminished, but not abrogated.

Several siblings of IL-12Rβ1 deficient patients have been identified that were homozygous for the same *IL12RB1* mutation as the index patient, however, they remained asymptomatic with regard to unusual infections with intracellular bacteria (172,173). The clinical penetrance of IL-12Rβ1 deficiency is estimated between 66 (10-15) and 91% (49 of 54) (32,186), suggesting more factors play a role in the increased susceptibility to mycobacterial and salmonella infections in these patients. Patients with a deficiency of the IL-12p40 subunit or the IL-12Rβ1 have defects in both the IL-12 and IL-23 cascade, however, the relative roles of IL-12 and IL-23 are not known.

Infections with BCG in patients with IL-12p40 deficiency or IL-12Rβ1 deficiency apparently protect against subsequent illness due to nontuberculous environmental *Mycobacteria*, but not against disease due to *Salmonellae*. Unlike salmonellosis, recurrent mycobacterial infections are rare in these patients (173). Moreover, patients with a defect in the IL-12Rβ1 are reported who controlled BCG after receiving vaccinations, indicating that in some patients IL-12 and IL-23 are redundant for mounting an effective immune response against BCG (187).

TYK2

TYK2 is involved in multiple signalling pathways, including IL-23 and IL-12 (173).

Minegishi et al. identified a homozygous TYK2 mutation in a patient who had been clinically diagnosed with hyper-IgE syndrome (188). This patient suffered a BCG lymphadenitis and a non-typhi *Salmonella* infection. Furthermore, this patient showed unusual susceptibility to various microorganisms including virus and fungi (188). Apart from IL-23 and IL-12, the IL-6, IL-10 and type I interferon pathways were impaired in cells from this patient, demonstrating that TYK2 plays obligatory roles in multiple cytokine signals involved in innate and adaptive immunity. These data suggest that TYK2 deficiency is characterised by a broad spectrum of clinical features, including both autosomal recessive hyper-IgE syndrome (AR-HIES) and MSMD.

Deficiencies in the IFN-γ signalling pathway

IFN-γR deficiencies selectively predispose to severe infections due to poorly pathogenic mycobacteria, but by contrast with IL-12/IL-23 system defects, only rarely leads to non-typhoid *Salmonellae* infection. IFN-γR deficiency is a heterogeneous disorder with different clinical, genetic, immunological, and histopathological types. In addition, various STAT1 deficiencies have been described, all impairing IFN-γ signal transduction.

IFN-γR1

Patients with partial and complete IFN-γR1 deficiencies have been described. All patients with autosomal recessive complete IFN-γR1 deficiency present with symptoms of BCG-itis or environmental mycobacteria infection within the first 5 years of life (189). Complete IFN-γR1 deficiency is characterised by a complete lack of functional IFN-γR1. This lack of functional IFN-γR1 can be due to mutations that preclude expression of IFN-γR1 on the cell surface (190-194). Mutations leading to the abrogation of cell surface expression identified to date, include nonsense and splice mutations and frameshift deletions and insertions, all resulting in a premature stop codon upstream from the segment encoding the transmembrane domain. In several patients, missense mutations or a small deletion in the region encoding the ligand binding domain of the IFN-γR1 have been identified. The resulting mutant receptors (C77Y, V61Q, 295del12 and 652del3) show no binding of IFN-γ (195).

In contrast to complete deficiency, partial IFN-γR1 deficiency is associated with milder course of infections and patients with partial IFN-γR1 deficiency may remain asymptomatic for much longer periods of time, possibly even until late adulthood (189). Partial IFN-γR1 deficiencies are mainly due to truncations in the cytoplasmic domain of the receptor. In exon 6, a small deletion hotspot is found around nucleotide 818, favouring mispairing events during replication (196-198). A frameshift due to the deletion of four nucleotides (818del4) leads to a premature stop codon. The

truncated receptor lacks the signalling domains as well as the receptor recycling domain, resulting in accumulation of non-functional IFN-γR1 proteins that are thought to exert a dominant-negative effect over normal IFN-γR1 molecules encoded by the wild-type allele. In a patient suffering recurrent *Mycobacterium avium* osteomyelitis, a mutation at nucleotide 832 (designated E278X) was identified (199). This mutation causes a premature stop codon and results in truncated IFN-γR1 lacking the recycling domain, leading to accumulation of the non-functional receptor on the cell membrane. This mutant receptor, like the 818del4 mutant receptor, exerts a dominant-negative effect. In a Japanese girl suffering BCG lymphadenitis after BCG vaccination, another dominant negative mutation (774del4) was detected (200). Like the 818del4, this mutation produces a truncated form of the IFN-γR1 which lacks the recycling domain, resulting in overexpression of the mutant receptor and a dominant-negative effect on IFN-γ signalling. Storgaard et al. report a patient with a nucleotide deletion at position 794, resulting in an overexpressed negative dominant receptor as well (201). Two children of healthy heterozygous parents have been reported with a homozygous variant (I87T) IFN-γR1, affecting the extracellular part of the receptor (202). When homozygous, this mutation leads to a severely reduced, but not completely abrogated, response to IFN-γ. In addition, in one patient a missense mutation (V63G) in the region encoding the ligand binding domain of the IFN-γR1 has been identified, severely impairing cellular response to IFN-γ (203).

IFN-γR2

IFN-γR2 deficiency is a rare form of MSMD. Mutations can lead to partial and complete IFN-γR2 deficiencies. Four mutations leading to complete IFN-γR2 deficiencies have been reported. In a child with disseminated *Mycobacterium fortuitum* and *M. avium* complex infections, a two-base pair homozygous recessive frameshift deletion has been found in the *IFNGR2* coding region (204). This deletion results in a premature stop in the extracellular region and the abrogation of IFN-γ responsiveness. The second form of complete IFN-γR1 deficiency was detected in three children from two families. In these children a T168N missense mutation was detected in the IFN-γR2. This missense mutation resulted in a receptor with an extra consensus site for N-glycosylation, resulting in a extra polysaccharide chain being added to the IFN-γR2. The addition of this polysaccharide chain resulted in the expression of a non-functional IFN-γR2 on the cell surface (205). In another patient, an in-frame microinsertion in the *IFNGR2* was discovered, leading to an receptor of abnormally high molecular weight and most of the protein remained intracellular (206). The IFN-γR2 expressed on the cell surface was non-functional. In two siblings, a deletion in the IFN-γR2 transmembrane domain (791delG) causing a frameshift and premature stop codon 8 triplets downstream was found (207). This deletion in the transmembrane domain disables golgi processing and subsequent cell surface expression. The two siblings were homozygous for this mutation and

their cells were not responsive to IFN-γ. Their heterozygous parents were healthy and showed a diminished IFN-γ responsiveness.

One partial IFN-γR2 deficiency has been reported so far. In a person with a history of recurrent infections with BCG and *Mycobacterium abscessus,* a homozygous nucleotide substitution in *IFNGR2* was detected (208). This nucleotide substitution resulted in an amino acid substitution (A114C) in the extracellular region of the encoded receptor. The resulting receptor was expressed on the cell surface. Cellular responses to IFN-γ however, were impaired but not abolished, indicating a partial deficiency.

STAT1

Signal transducer and activator of transcription 1 (STAT1) is critical for cellular responses to type I (IFN-α/β) and type II (IFN-γ) IFNs (67). In 2001, the first human germline mutation of STAT1 was found and associated with susceptibility to mycobacterial disease (209). In cells from a heterozygous patient, a missense mutation (L706S) resulted in impaired STAT1 Tyr-701 phosphorylation and affects the IFN-γ activation factor (GAF) mediated IFN-γ response, but not the interferon-stimulated gene factor 3 (ISGF3) mediated IFN-γ response, in a dominant negative manner (209). Two siblings homozygous for the STAT1 missense mutation P696S, displayed severely impaired but not abolished responses to IFN-γ, IFN-α/β, IFN-λ1 and IL-27 (210). The mutation impaired splicing of the mRNA The misspliced forms lacking exon 23 were not translated into a mature protein and residual full-length mRNA resulted in low but detectable normal functional STAT1 protein. The affected patients suffered severe but curable intracellular bacterial and viral infection. Two recessive complete mutations of the STAT1-binding domain have been described as well (211). The resulting STAT molecules are normally phosphorylated but the nuclear-translocated STAT1-dimers do not bind correctly to IFN-γ activation site (GAS) elements. The patients having these mutations were highly susceptible to mycobacterial infections. Both patients died of overwhelming viral infections (212), indicating both the type I and type II interferon pathways were affected by these mutations. In a patient suffering disseminated BCG infection, abrogation of STAT1 expression was reported (213). Complete STAT1 deficiency resulted from a frameshift due to a homozygous insertion (1928insA), causing a premature stop codon and thereby abrogation of STAT1 expression. The lack of STAT1 resulted in unresponsiveness of cells to IFN-α and IFN-γ. This patient died after bone marrow transplantation, from a fulminant EBV infection.

IFN-γ

No patients have been described with genetic IFN-γ deficiency. Despite the fact that IFN-γ deficient mice develop normally (29), in humans IFN-γ deficiency may be incompatible with life. However, acquired IFN-γ deficiencies secondary to neutralizing auto-antibodies have been described in over ten patients (214-217). These patients were susceptible primarily to mycobacterial infections.

Treatment options

For all patients, early diagnosis of infection and appropriate antibiotic therapy, based on the sensitivity of the (myco-) bacterial species identified, is crucial. Patients with IL- 12p40, IL-12Rβ1, and partial IFN-γR defects usually respond well to antibiotic treatment.

Patients with mutations in the IL-12Rβ1 or IL-12p40 lack the effects of IL-12 and IL-23 and therefore produce little IFN-γ in response to infections. Apart from the appropriate antibiotics, these patients can benefit from treatment with recombinant IFN-γ (163,184,218,219). Studies of IFN-γ therapy in patients with partial IFN-γR1 or partial IFN-γR2 deficiencies have not been performed. However, patients suffering partial IFN-γR deficiency can benefit from treatment with high dose recombinant IFN-γ (189). By contrast, patients with complete IFN-γR1, complete IFN-γR2 or STAT1 deficiencies are not able to respond to IFN-γ and thus will not benefit from treatment with recombinant IFN-γ. Moreover, these patients are less responsive to antimicrobial treatment (220). Hematopoietic stem cell transplantation (HSCT) is theoretically a curative treatment for these patients, however, a poor outcome is reported in most of the patients who received HSCT (221). To prevent infections in these patients, prophylactic life-long anti-microbial therapy is indicated. In contrast to patients with complete IFN-γR or complete STAT1 deficiency, mycobacterial infection in patients with other deficiencies usually can be controlled and the need for prophylactic treatment is controversial. Patients doing well without prophylaxis have been reported (208,222). In addition, in patients with defects in the IL-12p40 subunit or in the IL-12Rβ1 chain the use of prophylactic treatment after the clearance of the first infection, to prevent the recurrence of mycobacterial infections is unclear, while in these patients new episodes of mycobacterial infections generally do not develop (172). However, recurrent infections with *Salmonellae* are common.

In two patients, with complete IFN-γR1 and complete IFN-γR2 deficiency respectively, suffering from disseminated infection with *Mycobacterium avium* complex, treatment with IFN-α as additional therapy has been described (223,224), however, the effect of this treatment remains unclear. Hematopoietic stem cell transplantation is the only available curative treatment of complete IFN-γR and STAT1 deficiencies, however, the overall success rate of stem cell transplantation is low (221).

Outline of Thesis

Part 1**: The role of IL-23 in inducing IFN-γ production and in the initiation of a Th1 response.**
IL-12 is thought to be the classical IFN-γ inducing cytokine. However, for APCs to produce IL-12 in response to PAMPs like LPS, IFN-γ itself is needed as an additional stimulus. This indicates that another factor is needed to drive early IFN-γ production, allowing for IL-12 production and subsequent amplification of IFN-γ production. IL-23 and IL-18 are among the first cytokines secreted by APC in response LPS, without the need of IFN-γ. We hypothesized that early APC-derived IL-23 may be an initial trigger of IFN-γ production in NK and NK-like T cells and in **Chapter 1**, we evaluated the IFN-γ inducing capacities of IL-23 and IL-18 on $CD56^+$ NK and NK-like T cells.

IL-23 is produced by APC like monocytes and macrophages. In **Chapter 2,** the effect of a variety of TLR agonists, as well as infection with live *Salmonella*, on the production of IL-23, IL-18 and IL-1β by monocytes and macrophages were tested. Next, the role of these cytokines in the induction of IFN-γ was elucidated. Finally, we evaluated the role of IL-23 induced IFN-γ in the priming for subsequent IL-12 production by monocytes.

Part 2: **Genetic variations in the type-1 cytokine pathway.**
The effects of IL-23 on its target cells are mediated via a receptor complex consisting of an IL-12Rβ1 and a specific IL-23R chain. The R381Q and P310L variants of the IL-23R have been reported to be associated with autoimmune diseases, suggesting they have an effect on IL-23R function. In **chapter 3,** these variants and a newly discovered IL-23R variant, namely Y173H, were functional characterized.

In the IFN-γR1, one of the IFN-γ receptor chains, several amino acid substitutions have been reported that abrogate IFN-γ signalling. These substitutions lead to enhanced susceptibility to infection with weakly pathogenic mycobacteria and salmonellae, as described above. More common amino acid variations in the IFN-γR1 may also have an effect on IFN-γR function, albeit more subtle. In **chapter 4** we describe two new variants of the IFN-γR1, namely S149L and 352M. To compare the effect of the variations at a molecular level, we cloned the IFN-γR1 and the newly discovered *IFNGR1* variants, as well as four known polymorphisms (V14M, V61I, H335P, L467P, all six reported missense mutations (V61Q, V63G, C85Y, C77Y, C77F, I87T) and the 818del4 mutant. For all these variants, 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.

Part 3: **Treatment options for a genetic deficiency in the type-1 cytokine pathway.**

Patients with complete deficiency of the IFN-γR1 are not responsive to IFN-γ and therefore adding IFN-γ to traditional treatment of infections with antibiotics is not an option in these patients. A severe clinical course is seen in IFN-γR deficient patients and these patients often succumb to mycobacterial infections very early in life. In **chapter 5** we evaluated the ability of recombinant IFN-γ to compensate for the absence of IFN-γ effects in cells obtained from an IFN-γR1 deficient patient.

In the final section the results of the previous chapters will be summarized and discussed.

References

1. Tosi, M. F. 2005. Innate immune responses to infection. *J. Allergy Clin. Immunol.* 116: 241-249.
2. Rus, H., C. Cudrici, and F. Niculescu. 2005. The role of the complement system in innate immunity. *Immunol. Res.* 33: 103-112.
3. Stuart, L. M., and R. A. Ezekowitz. 2005. Phagocytosis: elegant complexity. *Immunity.* 22: 539-550.
4. Nauseef, W. M. 2007. How human neutrophils kill and degrade microbes: an integrated view. *Immunol. Rev.* 219: 88-102.
5. Linehan, S. A., L. Martinez-Pomares, and S. Gordon. 2000. Macrophage lectins in host defence. *Microbes. Infect.* 2: 279-288.
6. Kumagai, Y., O. Takeuchi, and S. Akira. 2008. Pathogen recognition by innate receptors. *J. Infect. Chemother.* 14: 86-92.
7. Robinson, M. J., D. Sancho, E. C. Slack, S. LeibundGut-Landmann, and Reis e Sousa. 2006. Myeloid C-type lectins in innate immunity. *Nat. Immunol.* 7: 1258-1265.
8. Trinchieri, G., and A. Sher. 2007. Cooperation of Toll-like receptor signals in innate immune defence. *Nat. Rev. Immunol.* 7: 179-190.
9. Takeda, K., and S. Akira. 2007. Toll-like receptors. *Curr. Protoc. Immunol.* Chapter 14: Unit.
10. Yu, H. B., and B. B. Finlay. 2008. The caspase-1 inflammasome: a pilot of innate immune responses. *Cell Host. Microbe* 4: 198-208.
11. Rietdijk, S. T., T. Burwell, J. Bertin, and A. J. Coyle. 2008. Sensing intracellular pathogens-NOD-like receptors. *Curr. Opin. Pharmacol.* 8: 261-266.
12. van de Wetering, J. K., L. M. van Golde, and J. J. Batenburg. 2004. Collectins: players of the innate immune system. *Eur. J. Biochem.* 271: 1229-1249.
13. Raff, M. C. 1973. T and B lymphocytes and immune responses. *Nature* 242: 19-23.
14. Rajewsky, K. 1996. Clonal selection and learning in the antibody system. *Nature* 381: 751-758.
15. Smith, K. M., L. Pottage, E. R. Thomas, A. J. Leishman, T. N. Doig, D. Xu, F. Y. Liew, and P. Garside. 2000. Th1 and Th2 CD4+ T cells provide help for B cell clonal expansion and antibody synthesis in a similar manner in vivo. *J. Immunol.* 165: 3136-3144.
16. Bousquet, J., P. K. Jeffery, W. W. Busse, M. Johnson, and A. M. Vignola. 2000. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am. J. Respir. Crit Care Med.* 161: 1720-1745.
17. Gately, M. K., L. M. Renzetti, J. Magram, A. S. Stern, L. Adorini, U. Gubler, and D. H. Presky. 1998. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu. Rev. Immunol.* 16: 495-521.
18. van de Vosse, E., and T. H. Ottenhoff. 2006. Human host genetic factors in mycobacterial and Salmonella infection: lessons from single gene disorders in IL-12/IL-23-dependent signaling that affect innate and adaptive immunity. *Microbes. Infect.* 8: 1167-1173.
19. Barrett, N. A., and K. F. Austen. 2009. Innate cells and T helper 2 cell immunity in airway inflammation. *Immunity.* 31: 425-437.
20. Busse, W. W., and R. F. Lemanske, Jr. 2001. Asthma. *N. Engl. J. Med.* 344: 350-362.
21. Peck, A., and E. D. Mellins. 2010. Precarious balance: Th17 cells in host defense. *Infect. Immun.* 78: 32-38.
22. Kapsenberg, M. L. 2003. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* 3: 984-993.
23. O'Garra, A. 1998. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity.* 8: 275-283.
24. Hsieh, C. S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy. 1993. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260: 547-549.
25. Kapsenberg, M. L., C. M. Hilkens, E. A. Wierenga, and P. Kalinski. 1998. The role of antigen-presenting cells in the regulation of allergen-specific T cell responses. *Curr. Opin. Immunol.* 10: 607-613.
26. Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo, J. Vilcek, R. M. Zinkernagel, and M. Aguet. 1993. Immune response in mice that lack the interferon-gamma receptor. *Science* 259: 1742-1745.
27. Cooper, A. M., D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme. 1993. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J. Exp. Med.* 178: 2243-2247.
28. Bao, S., K. W. Beagley, M. P. France, J. Shen, and A. J. Husband. 2000. Interferon-gamma plays a critical role in intestinal immunity against Salmonella typhimurium infection. *Immunology* 99: 464-472.
29. Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science* 259: 1739-1742.

30. Flynn, J. L., J. Chan, K. J. Triebold, D. K. Dalton, T. A. Stewart, and B. R. Bloom. 1993. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. *J. Exp. Med.* 178: 2249-2254.
31. Jouanguy, E., R. Doffinger, S. Dupuis, A. Pallier, F. Altare, and J. L. Casanova. 1999. IL-12 and IFN-gamma in host defense against mycobacteria and salmonella in mice and men. *Curr. Opin. Immunol.* 11: 346-351.
32. van de Vosse, E., M. A. Hoeve, and T. H. Ottenhoff. 2004. Human genetics of intracellular infectious diseases: molecular and cellular immunity against mycobacteria and salmonellae. *Lancet Infect. Dis.* 4: 739-749.
33. Chong, M. M., D. Metcalf, E. Jamieson, W. S. Alexander, and T. W. Kay. 2005. Suppressor of cytokine signaling-1 in T cells and macrophages is critical for preventing lethal inflammation. *Blood* 106: 1668-1675.
34. Young, H. A., and K. J. Hardy. 1995. Role of interferon-gamma in immune cell regulation. *J. Leukoc. Biol.* 58: 373-381.
35. Cui, J., T. Shin, T. Kawano, H. Sato, E. Kondo, I. Toura, Y. Kaneko, H. Koseki, M. Kanno, and M. Taniguchi. 1997. Requirement for Valpha14 NKT cells in IL-12-mediated rejection of tumors. *Science* 278: 1623-1626.
36. Lertmemongkolchai, G., G. Cai, C. A. Hunter, and G. J. Bancroft. 2001. Bystander activation of CD8+ T cells contributes to the rapid production of IFN-gamma in response to bacterial pathogens. *J. Immunol.* 166: 1097-1105.
37. Rowland, C. A., G. Lertmemongkolchai, A. Bancroft, A. Haque, M. S. Lever, K. F. Griffin, M. C. Jackson, M. Nelson, A. O'Garra, R. Grencis, G. J. Bancroft, and R. A. Lukaszewski. 2006. Critical role of type 1 cytokines in controlling initial infection with Burkholderia mallei. *Infect. Immun.* 74: 5333-5340.
38. Ferrick, D. A., M. D. Schrenzel, T. Mulvania, B. Hsieh, W. G. Ferlin, and H. Lepper. 1995. Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells in vivo. *Nature* 373: 255-257.
39. D'Ombrain, M. C., D. S. Hansen, K. M. Simpson, and L. Schofield. 2007. gammadelta-T cells expressing NK receptors predominate over NK cells and conventional T cells in the innate IFN-gamma response to Plasmodium falciparum malaria. *Eur. J. Immunol.* 37: 1864-1873.
40. Abdi, K., N. Singh, and P. Matzinger. 2006. T-cell control of IL-12p75 production. *Scand. J. Immunol.* 64: 83-92.
41. Byrne, P., P. McGuirk, S. Todryk, and K. H. Mills. 2004. Depletion of NK cells results in disseminating lethal infection with Bordetella pertussis associated with a reduction of antigen-specific Th1 and enhancement of Th2, but not Tr1 cells. *Eur. J. Immunol.* 34: 2579-2588.
42. Martin-Fontecha, A., L. L. Thomsen, S. Brett, C. Gerard, M. Lipp, A. Lanzavecchia, and F. Sallusto. 2004. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nat. Immunol.* 5: 1260-1265.
43. van de Wetering, D., R. A. de Paus, J. T. Van Dissel, and E. van de Vosse. 2009. IL-23 modulates CD56+/CD3- NK cell and CD56+/CD3+ NK-like T cell function differentially from IL-12. *Int. Immunol.* 21: 145-153.
44. Tominaga, K., T. Yoshimoto, K. Torigoe, M. Kurimoto, K. Matsui, T. Hada, H. Okamura, and K. Nakanishi. 2000. IL-12 synergizes with IL-18 or IL-1beta for IFN-gamma production from human T cells. *Int. Immunol.* 12: 151-160.
45. Beadling, C., and M. K. Slifka. 2006. Regulation of innate and adaptive immune responses by the related cytokines IL-12, IL-23, and IL-27. *Arch. Immunol. Ther. Exp. (Warsz.)* 54: 15-24.
46. Sacks, S., W. Zhou, R. D. Campbell, and J. Martin. 1993. C3 and C4 gene expression and interferon-gamma-mediated regulation in human glomerular mesangial cells. *Clin. Exp. Immunol.* 93: 411-417.
47. Raz, R., J. E. Durbin, and D. E. Levy. 1994. Acute phase response factor and additional members of the interferon-stimulated gene factor 3 family integrate diverse signals from cytokines, interferons, and growth factors. *J. Biol. Chem.* 269: 24391-24395.
48. Nakagomi, A., S. B. Freedman, and C. L. Geczy. 2000. Interferon-gamma and lipopolysaccharide potentiate monocyte tissue factor induction by C-reactive protein: relationship with age, sex, and hormone replacement treatment. *Circulation* 101: 1785-1791.
49. Terui, T., K. Ishii, M. Ozawa, N. Tabata, T. Kato, and H. Tagami. 1997. C3 production of cultured human epidermal keratinocytes is enhanced by IFNgamma and TNFalpha through different pathways. *J. Invest Dermatol.* 108: 62-67.
50. Feelders, R. A., G. Vreugdenhil, A. M. Eggermont, P. A. Kuiper-Kramer, H. G. van Eijk, and A. J. Swaak. 1998. Regulation of iron metabolism in the acute-phase response: interferon gamma and tumour necrosis factor alpha induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. *Eur. J. Clin. Invest* 28: 520-527.

51. Nairz, M., G. Fritsche, P. Brunner, H. Talasz, K. Hantke, and G. Weiss. 2008. Interferon-gamma limits the availability of iron for intramacrophage Salmonella typhimurium. *Eur. J. Immunol.* 38: 1923-1936.
52. Nathan, C. F., and J. B. Hibbs, Jr. 1991. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr. Opin. Immunol.* 3: 65-70.
53. Leenen, P. J., B. P. Canono, D. A. Drevets, J. S. Voerman, and P. A. Campbell. 1994. TNF-alpha and IFN-gamma stimulate a macrophage precursor cell line to kill Listeria monocytogenes in a nitric oxide-independent manner. *J. Immunol.* 153: 5141-5147.
54. Flesch, I., and S. H. Kaufmann. 1987. Mycobacterial growth inhibition by interferon-gamma-activated bone marrow macrophages and differential susceptibility among strains of Mycobacterium tuberculosis. *J. Immunol.* 138: 4408-4413.
55. Ioan-Facsinay, A., S. J. de Kimpe, S. M. Hellwig, P. L. van Lent, F. M. Hofhuis, H. H. van Ojik, C. Sedlik, S. A. da Silveira, J. Gerber, Y. F. de Jong, R. Roozendaal, L. A. Aarden, W. B. van den Berg, T. Saito, D. Mosser, S. Amigorena, S. Izui, G. J. van Ommen, M. van Vugt, J. G. van de Winkel, and J. S. Verbeek. 2002. FcgammaRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. *Immunity.* 16: 391-402.
56. Petroni, K. C., L. Shen, and P. M. Guyre. 1988. Modulation of human polymorphonuclear leukocyte IgG Fc receptors and Fc receptor-mediated functions by IFN-gamma and glucocorticoids. *J. Immunol.* 140: 3467-3472.
57. Ishibashi, Y., and T. Arai. 1990. Effect of gamma-interferon on phagosome-lysosome fusion in Salmonella typhimurium-infected murine macrophages. *FEMS Microbiol. Immunol.* 2: 75-82.
58. Santic, M., M. Molmeret, and K. Y. Abu. 2005. Modulation of biogenesis of the Francisella tularensis subsp. novicida-containing phagosome in quiescent human macrophages and its maturation into a phagolysosome upon activation by IFN-gamma. *Cell Microbiol.* 7: 957-967.
59. Ghigo, E., C. Capo, C. H. Tung, D. Raoult, J. P. Gorvel, and J. L. Mege. 2002. Coxiella burnetii survival in THP-1 monocytes involves the impairment of phagosome maturation: IFN-gamma mediates its restoration and bacterial killing. *J. Immunol.* 169: 4488-4495.
60. Schaible, U. E., S. Sturgill-Koszycki, P. H. Schlesinger, and D. G. Russell. 1998. Cytokine activation leads to acidification and increases maturation of Mycobacterium avium-containing phagosomes in murine macrophages. *J. Immunol.* 160: 1290-1296.
61. Taylor, G. A., C. G. Feng, and A. Sher. 2007. Control of IFN-gamma-mediated host resistance to intracellular pathogens by immunity-related GTPases (p47 GTPases). *Microbes. Infect.* 9: 1644-1651.
62. Hackam, D. J., O. D. Rotstein, W. Zhang, S. Gruenheid, P. Gros, and S. Grinstein. 1998. Host resistance to intracellular infection: mutation of natural resistance-associated macrophage protein 1 (Nramp1) impairs phagosomal acidification. *J. Exp. Med.* 188: 351-364.
63. Govoni, G., S. Vidal, M. Cellier, P. Lepage, D. Malo, and P. Gros. 1995. Genomic structure, promoter sequence, and induction of expression of the mouse Nramp1 gene in macrophages. *Genomics* 27: 9-19.
64. Karhumaki, E., and H. Helin. 1987. Regulation of oxidative metabolism by interferon-gamma during human monocyte to macrophage differentiation. *Med. Biol.* 65: 261-266.
65. Gupta, J. W., M. Kubin, L. Hartman, M. Cassatella, and G. Trinchieri. 1992. Induction of expression of genes encoding components of the respiratory burst oxidase during differentiation of human myeloid cell lines induced by tumor necrosis factor and gamma-interferon. *Cancer Res.* 52: 2530-2537.
66. Boehm, U., T. Klamp, M. Groot, and J. C. Howard. 1997. Cellular responses to interferon-gamma. *Annu. Rev. Immunol.* 15: 749-795.
67. Schroder, K., P. J. Hertzog, T. Ravasi, and D. A. Hume. 2004. Interferon-gamma: an overview of signals, mechanisms and functions. *J. Leukoc. Biol.* 75: 163-189.
68. Snapper, C. M., and W. E. Paul. 1987. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 236: 944-947.
69. Ma, X., J. M. Chow, G. Gri, G. Carra, F. Gerosa, S. F. Wolf, R. Dzialo, and G. Trinchieri. 1996. The interleukin 12 p40 gene promoter is primed by interferon gamma in monocytic cells. *J. Exp. Med.* 183: 147-157.
70. Hayes, M. P., J. Wang, and M. A. Norcross. 1995. Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-gamma of lipopolysaccharide-inducible p35 and p40 genes. *Blood* 86: 646-650.
71. Butchar, J. P., K. V. Parsa, C. B. Marsh, and S. Tridandapani. 2008. IFNgamma enhances IL-23 production during Francisella infection of human monocytes. *FEBS Lett.* 582: 1044-1048.
72. Ramana, C. V., M. P. Gil, R. D. Schreiber, and G. R. Stark. 2002. Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. *Trends Immunol.* 23: 96-101.
73. Masumi, A., S. Tamaoki, I. M. Wang, K. Ozato, and K. Komuro. 2002. IRF-8/ICSBP and IRF-1 cooperatively stimulate mouse IL-12 promoter activity in macrophages. *FEBS Lett.* 531: 348-353.

74. Wang, I. M., C. Contursi, A. Masumi, X. Ma, G. Trinchieri, and K. Ozato. 2000. An IFN-gamma-inducible transcription factor, IFN consensus sequence binding protein (ICSBP), stimulates IL-12 p40 expression in macrophages. *J. Immunol.* 165: 271-279.
75. Maruyama, S., K. Sumita, H. Shen, M. Kanoh, X. Xu, M. Sato, M. Matsumoto, H. Shinomiya, and Y. Asano. 2003. Identification of IFN regulatory factor-1 binding site in IL-12 p40 gene promoter. *J. Immunol.* 170: 997-1001.
76. Turcotte, K., S. Gauthier, D. Malo, M. Tam, M. M. Stevenson, and P. Gros. 2007. Icsbp1/IRF-8 is required for innate and adaptive immune responses against intracellular pathogens. *J. Immunol.* 179: 2467-2476.
77. Boulay, J. L., and W. E. Paul. 1993. Hematopoietin sub-family classification based on size, gene organization and sequence homology. *Curr. Biol.* 3: 573-581.
78. Oppmann, B., R. Lesley, B. Blom, J. C. Timans, Y. Xu, B. Hunte, F. Vega, N. Yu, J. Wang, K. Singh, F. Zonin, E. Vaisberg, T. Churakova, M. Liu, D. Gorman, J. Wagner, S. Zurawski, Y. Liu, J. S. Abrams, K. W. Moore, D. Rennick, R. de Waal-Malefyt, C. Hannum, J. F. Bazan, and R. A. Kastelein. 2000. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity.* 13: 715-725.
79. Heinzel, F. P., A. M. Hujer, F. N. Ahmed, and R. M. Rerko. 1997. In vivo production and function of IL-12 p40 homodimers. *J. Immunol.* 158: 4381-4388.
80. Walter, M. J., N. Kajiwara, P. Karanja, M. Castro, and M. J. Holtzman. 2001. Interleukin 12 p40 production by barrier epithelial cells during airway inflammation. *J. Exp. Med.* 193: 339-351.
81. Pflanz, S., J. C. Timans, J. Cheung, R. Rosales, H. Kanzler, J. Gilbert, L. Hibbert, T. Churakova, M. Travis, E. Vaisberg, W. M. Blumenschein, J. D. Mattson, J. L. Wagner, W. To, S. Zurawski, T. K. McClanahan, D. M. Gorman, J. F. Bazan, M. R. de Waal, D. Rennick, and R. A. Kastelein. 2002. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity.* 16: 779-790.
82. Devergne, O., M. Birkenbach, and E. Kieff. 1997. Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. *Proc. Natl. Acad. Sci. U. S. A* 94: 12041-12046.
83. Collison, L. W., C. J. Workman, T. T. Kuo, K. Boyd, Y. Wang, K. M. Vignali, R. Cross, D. Sehy, R. S. Blumberg, and D. A. Vignali. 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450: 566-569.
84. Bardel, E., F. Larousserie, P. Charlot-Rabiega, A. Coulomb-L'Hermine, and O. Devergne. 2008. Human CD4+ CD25+ Foxp3+ regulatory T cells do not constitutively express IL-35. *J. Immunol.* 181: 6898-6905.
85. Seyerl, M., S. Kirchberger, O. Majdic, J. Seipelt, C. Jindra, C. Schrauf, and J. Stockl. 2010. Human rhinoviruses induce IL-35-producing Treg via induction of B7-H1 (CD274) and sialoadhesin (CD169) on DC. *Eur. J. Immunol.* 40: 321-329.
86. Langrish, C. L., B. S. McKenzie, N. J. Wilson, M. R. de Waal, R. A. Kastelein, and D. J. Cua. 2004. IL-12 and IL-23: master regulators of innate and adaptive immunity. *Immunol. Rev.* 202: 96-105.
87. Carra, G., F. Gerosa, and G. Trinchieri. 2000. Biosynthesis and posttranslational regulation of human IL-12. *J Immunol* 164: 4752-4761.
88. van de Wetering, D., R. A. de Paus, J. T. Van Dissel, and E. van de Vosse. 2009. Salmonella Induced IL-23 and IL-1beta Allow for IL-12 Production by Monocytes and Mj1 through Induction of IFN-gamma in CD56 NK/NK-Like T Cells. *PLoS. One.* 4: e8396.
89. Verreck, F. A., T. de Boer, D. M. Langenberg, M. A. Hoeve, M. Kramer, E. Vaisberg, R. Kastelein, A. Kolk, R. de Waal-Malefyt, and T. H. Ottenhoff. 2004. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. *Proc. Natl. Acad. Sci. U. S. A* 101: 4560-4565.
90. Feinberg, J., C. Fieschi, R. Doffinger, M. Feinberg, T. Leclerc, S. Boisson-Dupuis, C. Picard, J. Bustamante, A. Chapgier, O. Filipe-Santos, C. L. Ku, L. de Beaucoudrey, J. Reichenbach, G. Antoni, R. Balde, A. Alcais, and J. L. Casanova. 2004. Bacillus Calmette Guerin triggers the IL-12/IFN-gamma axis by an IRAK-4- and NEMO-dependent, non-cognate interaction between monocytes, NK, and T lymphocytes. *Eur. J. Immunol.* 34: 3276-3284.
91. Gerosa, F., B. Baldani-Guerra, L. A. Lyakh, G. Batoni, S. Esin, R. T. Winkler-Pickett, M. R. Consolaro, M. De Marchi, D. Giachino, A. Robbiano, M. Astegiano, A. Sambataro, R. A. Kastelein, G. Carra, and G. Trinchieri. 2008. Differential regulation of interleukin 12 and interleukin 23 production in human dendritic cells. *J. Exp. Med.* 205: 1447-1461.
92. Re, F., and J. L. Strominger. 2001. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J. Biol. Chem.* 276: 37692-37699.
93. van Beelen, A. J., Z. Zelinkova, E. W. Taanman-Kueter, F. J. Muller, D. W. Hommes, S. A. Zaat, M. L. Kapsenberg, and E. C. de Jong. 2007. Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. *Immunity.* 27: 660-669.

94. Carmody, R. J., Q. Ruan, H. C. Liou, and Y. H. Chen. 2007. Essential roles of c-Rel in TLR-induced IL-23 p19 gene expression in dendritic cells. *J. Immunol.* 178: 186-191.
95. Napolitani, G., A. Rinaldi, F. Bertoni, F. Sallusto, and A. Lanzavecchia. 2005. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat. Immunol.* 6: 769-776.
96. Bekeredjian-Ding, I., S. I. Roth, S. Gilles, T. Giese, A. Ablasser, V. Hornung, S. Endres, and G. Hartmann. 2006. T cell-independent, TLR-induced IL-12p70 production in primary human monocytes. *J. Immunol.* 176: 7438-7446.
97. LeibundGut-Landmann, S., O. Gross, M. J. Robinson, F. Osorio, E. C. Slack, S. V. Tsoni, E. Schweighoffer, V. Tybulewicz, G. D. Brown, J. Ruland, and Reis e Sousa. 2007. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat. Immunol.* 8: 630-638.
98. Smits, H. H., A. J. van Beelen, C. Hessle, R. Westland, E. de Jong, E. Soeteman, A. Wold, E. A. Wierenga, and M. L. Kapsenberg. 2004. Commensal Gram-negative bacteria prime human dendritic cells for enhanced IL-23 and IL-27 expression and enhanced Th1 development. *Eur. J. Immunol.* 34: 1371-1380.
99. Harris, K. M., A. Fasano, and D. L. Mann. 2008. Cutting Edge: IL-1 Controls the IL-23 Response Induced by Gliadin, the Etiologic Agent in Celiac Disease. *J. Immunol.* 181: 4457-4460.
100. Sheibanie, A. F., I. Tadmori, H. Jing, E. Vassiliou, and D. Ganea. 2004. Prostaglandin E2 induces IL-23 production in bone marrow-derived dendritic cells. *FASEB J.* 18: 1318-1320.
101. Naik, S. H. 2008. Demystifying the development of dendritic cell subtypes, a little. *Immunol. Cell Biol.* 86: 439-452.
102. Zou, G. M., and Y. K. Tam. 2002. Cytokines in the generation and maturation of dendritic cells: recent advances. *Eur. Cytokine Netw.* 13: 186-199.
103. Pirhonen, J., S. Matikainen, and I. Julkunen. 2002. Regulation of virus-induced IL-12 and IL-23 expression in human macrophages. *J. Immunol.* 169: 5673-5678.
104. Roses, R. E., S. Xu, M. Xu, U. Koldovsky, G. Koski, and B. J. Czerniecki. 2008. Differential Production of IL-23 and IL-12 by Myeloid-Derived Dendritic Cells in Response to TLR Agonists. *J. Immunol.* 181: 5120-5127.
105. Sheibanie, A. F., J. H. Yen, T. Khayrullina, F. Emig, M. Zhang, R. Tuma, and D. Ganea. 2007. The proinflammatory effect of prostaglandin E2 in experimental inflammatory bowel disease is mediated through the IL-23-->IL-17 axis. *J. Immunol.* 178: 8138-8147.
106. van der Pouw Kraan TC, L. C. Boeije, R. J. Smeenk, J. Wijdenes, and L. A. Aarden. 1995. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J. Exp. Med.* 181: 775-779.
107. Schnurr, M., T. Toy, A. Shin, M. Wagner, J. Cebon, and E. Maraskovsky. 2005. Extracellular nucleotide signaling by P2 receptors inhibits IL-12 and enhances IL-23 expression in human dendritic cells: a novel role for the cAMP pathway. *Blood* 105: 1582-1589.
108. Muhlbauer, M., P. M. Chilton, T. C. Mitchell, and C. Jobin. 2008. Impaired Bcl3 Up-regulation Leads to Enhanced Lipopolysaccharide-induced Interleukin (IL)-23P19 Gene Expression in IL-10-/- Mice. *J. Biol. Chem.* 283: 14182-14189.
109. de Paus, R. A., D. van de Wetering, J. T. Van Dissel, and E. van de Vosse. 2008. IL-23 and IL-12 responses in activated human T cells retrovirally transduced with IL-23 receptor variants. *Mol. Immunol.* 45: 3889-3895.
110. Peritt, D., M. Aste-Amezaga, F. Gerosa, C. Paganin, and G. Trinchieri. 1996. Interleukin-10 induction by IL-12: a possible modulatory mechanism? *Ann. N. Y. Acad. Sci.* 795: 387-389.
111. Vanden Eijnden, S., S. Goriely, D. De Wit, F. Willems, and M. Goldman. 2005. IL-23 up-regulates IL-10 and induces IL-17 synthesis by polyclonally activated naive T cells in human. *Eur. J. Immunol.* 35: 469-475.
112. Becker, C., H. Dornhoff, C. Neufert, M. C. Fantini, S. Wirtz, S. Huebner, A. Nikolaev, H. A. Lehr, A. J. Murphy, D. M. Valenzuela, G. D. Yancopoulos, P. R. Galle, M. Karow, and M. F. Neurath. 2006. Cutting Edge: IL-23 Cross-Regulates IL-12 Production in T Cell-Dependent Experimental Colitis. *J. Immunol.* 177: 2760-2764.
113. Grewal, I. S., P. Borrow, E. G. Pamer, M. B. Oldstone, and R. A. Flavell. 1997. The CD40-CD154 system in anti-infective host defense. *Curr. Opin. Immunol.* 9: 491-497.
114. Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med.* 184: 747-752.
115. Jefford, M., M. Schnurr, T. Toy, K. A. Masterman, A. Shin, T. Beecroft, T. Y. Tai, K. Shortman, M. Shackleton, I. D. Davis, P. Parente, T. Luft, W. Chen, J. Cebon, and E. Maraskovsky. 2003. Functional comparison of DCs generated in vivo with Flt3 ligand or in vitro from blood monocytes: differential regulation of function by specific classes of physiologic stimuli. *Blood* 102: 1753-1763.

116. Uhlig, H. H., B. S. McKenzie, S. Hue, C. Thompson, B. Joyce-Shaikh, R. Stepankova, N. Robinson, S. Buonocore, H. Tlaskalova-Hogenova, D. J. Cua, and F. Powrie. 2006. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. *Immunity.* 25: 309-318.
117. Jasny, E., M. Eisenblatter, K. Matz-Rensing, K. Tenner-Racz, M. Tenbusch, A. Schrod, C. Stahl-Hennig, V. Moos, T. Schneider, P. Racz, K. Uberla, F. J. Kaup, and R. Ignatius. 2008. IL-12-impaired and IL-12-secreting dendritic cells produce IL-23 upon CD154 restimulation. *J. Immunol.* 180: 6629-6639.
118. Peng, X., A. Kasran, P. A. Warmerdam, M. de Boer, and J. L. Ceuppens. 1996. Accessory signaling by CD40 for T cell activation: induction of Th1 and Th2 cytokines and synergy with interleukin-12 for interferon-gamma production. *Eur. J. Immunol.* 26: 1621-1627.
119. Happel, K. I., P. J. Dubin, M. Zheng, N. Ghilardi, C. Lockhart, L. J. Quinton, A. R. Odden, J. E. Shellito, G. J. Bagby, S. Nelson, and J. K. Kolls. 2005. Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. *J. Exp. Med.* 202: 761-769.
120. Schuetze, N., S. Schoeneberger, U. Mueller, M. A. Freudenberg, G. Alber, and R. K. Straubinger. 2005. IL-12 family members: differential kinetics of their TLR4-mediated induction by Salmonella enteritidis and the impact of IL-10 in bone marrow-derived macrophages. *Int. Immunol.* 17: 649-659.
121. Scharton, T. M., and P. Scott. 1993. Natural killer cells are a source of interferon gamma that drives differentiation of CD4+ T cell subsets and induces early resistance to Leishmania major in mice. *J. Exp. Med.* 178: 567-577.
122. Biron, C. A., K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather. 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* 17:189-220.: 189-220.
123. Stetson, D. B., M. Mohrs, R. L. Reinhardt, J. L. Baron, Z. E. Wang, L. Gapin, M. Kronenberg, and R. M. Locksley. 2003. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* 198: 1069-1076.
124. Becskei, A., and M. J. Grusby. 2007. Contribution of IL-12R mediated feedback loop to Th1 cell differentiation. *FEBS Lett.* 581: 5199-5206.
125. Holscher, C., R. A. Atkinson, B. Arendse, N. Brown, E. Myburgh, G. Alber, and F. Brombacher. 2001. A protective and agonistic function of IL-12p40 in mycobacterial infection. *J. Immunol.* 167: 6957-6966.
126. Lehmann, J., S. Bellmann, C. Werner, R. Schroder, N. Schutze, and G. Alber. 2001. IL-12p40-dependent agonistic effects on the development of protective innate and adaptive immunity against Salmonella enteritidis. *J. Immunol.* 167: 5304-5315.
127. Cooper, A. M., A. Kipnis, J. Turner, J. Magram, J. Ferrante, and I. M. Orme. 2002. Mice lacking bioactive IL-12 can generate protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present. *J. Immunol.* 168: 1322-1327.
128. Khader, S. A., J. E. Pearl, K. Sakamoto, L. Gilmartin, G. K. Bell, D. M. Jelley-Gibbs, N. Ghilardi, F. de Sauvage, and A. M. Cooper. 2005. IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-gamma responses if IL-12p70 is available. *J. Immunol.* 175: 788-795.
129. Blum, A., T. Setiawan, L. Hang, K. Stoyanoff, and J. V. Weinstock. 2008. Interleukin-12 (IL-12) and IL-23 induction of substance p synthesis in murine T cells and macrophages is subject to IL-10 and transforming growth factor beta regulation. *Infect. Immun.* 76: 3651-3656.
130. Blum, A. M., A. Metwali, G. Cook, R. C. Mathew, D. Elliott, and J. V. Weinstock. 1993. Substance P modulates antigen-induced, IFN-gamma production in murine Schistosomiasis mansoni. *J. Immunol.* 151: 225-233.
131. Yoshida, H., S. Hamano, G. Senaldi, T. Covey, R. Faggioni, S. Mu, M. Xia, A. C. Wakeham, H. Nishina, J. Potter, C. J. Saris, and T. W. Mak. 2001. WSX-1 is required for the initiation of Th1 responses and resistance to L. major infection. *Immunity.* 15: 569-578.
132. Chen, Q., N. Ghilardi, H. Wang, T. Baker, M. H. Xie, A. Gurney, I. S. Grewal, and F. J. de Sauvage. 2000. Development of Th1-type immune responses requires the type I cytokine receptor TCCR. *Nature* 407: 916-920.
133. Takeda, A., S. Hamano, A. Yamanaka, T. Hanada, T. Ishibashi, T. W. Mak, A. Yoshimura, and H. Yoshida. 2003. Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. *J. Immunol.* 170: 4886-4890.
134. Khader, S. A., G. K. Bell, J. E. Pearl, J. J. Fountain, J. Rangel-Moreno, G. E. Cilley, F. Shen, S. M. Eaton, S. L. Gaffen, S. L. Swain, R. M. Locksley, L. Haynes, T. D. Randall, and A. M. Cooper. 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. *Nat. Immunol.* 8: 369-377.
135. Chackerian, A. A., S. J. Chen, S. J. Brodie, J. D. Mattson, T. K. McClanahan, R. A. Kastelein, and E. P. Bowman. 2006. Neutralization or absence of the interleukin-23 pathway does not compromise immunity to mycobacterial infection. *Infect. Immun.* 74: 6092-6099.

136. Villarino, A., L. Hibbert, L. Lieberman, E. Wilson, T. Mak, H. Yoshida, R. A. Kastelein, C. Saris, and C. A. Hunter. 2003. The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. *Immunity.* 19: 645-655.
137. Srinivasan, A., R. M. Salazar-Gonzalez, M. Jarcho, M. M. Sandau, L. Lefrancois, and S. J. McSorley. 2007. Innate immune activation of CD4 T cells in salmonella-infected mice is dependent on IL-18. *J. Immunol.* 178: 6342-6349.
138. Guia, S., C. Cognet, de Beaucoudrey L., M. S. Tessmer, E. Jouanguy, C. Berger, O. Filipe-Santos, J. Feinberg, Y. Camcioglu, J. Levy, S. Al Jumaah, S. Al-Hajjar, J. L. Stephan, C. Fieschi, L. Abel, L. Brossay, J. L. Casanova, and E. Vivier. 2008. A role for interleukin-12/23 in the maturation of human natural killer and CD56+ T cells in vivo. *Blood* 111: 5008-5016.
139. Presky, D. H., H. Yang, L. J. Minetti, A. O. Chua, N. Nabavi, C. Y. Wu, M. K. Gately, and U. Gubler. 1996. A functional interleukin 12 receptor complex is composed of two beta-type cytokine receptor subunits. *Proc. Natl. Acad. Sci. U. S. A* 93: 14002-14007.
140. Desai, B. B., P. M. Quinn, A. G. Wolitzky, P. K. Mongini, R. Chizzonite, and M. K. Gately. 1992. IL-12 receptor. II. Distribution and regulation of receptor expression. *J. Immunol.* 148: 3125-3132.
141. Szabo, S. J., A. S. Dighe, U. Gubler, and K. M. Murphy. 1997. Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. *J. Exp. Med.* 185: 817-824.
142. Lighvani, A. A., D. M. Frucht, D. Jankovic, H. Yamane, J. Aliberti, B. D. Hissong, B. V. Nguyen, M. Gadina, A. Sher, W. E. Paul, and J. J. O'Shea. 2001. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. *Proc. Natl. Acad. Sci. U. S. A* 98: 15137-15142.
143. Belladonna, M. L., J. C. Renauld, R. Bianchi, C. Vacca, F. Fallarino, C. Orabona, M. C. Fioretti, U. Grohmann, and P. Puccetti. 2002. IL-23 and IL-12 have overlapping, but distinct, effects on murine dendritic cells. *J. Immunol.* 168: 5448-5454.
144. Parham, C., M. Chirica, J. Timans, E. Vaisberg, M. Travis, J. Cheung, S. Pflanz, R. Zhang, K. P. Singh, F. Vega, W. To, J. Wagner, A. M. O'Farrell, T. McClanahan, S. Zurawski, C. Hannum, D. Gorman, D. M. Rennick, R. A. Kastelein, M. R. de Waal, and K. W. Moore. 2002. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* 168: 5699-5708.
145. Oukka, M., T. Korn, A. Awasthi, V. Dardalhon, G. Galileos, L. Riol Blanco, V. Kuchroo, and E. Bettelli. 2008. OR.98. IL-23 Receptor (IL-23R) GFP Reporter Mice Reveal New Populations of IL-17-producing and IL-23-responsive Cells. *Clinical Immunology* 127: S39.
146. Ivanov, I. I., B. S. McKenzie, L. Zhou, C. E. Tadokoro, A. Lepelley, J. J. Lafaille, D. J. Cua, and D. R. Littman. 2006. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 126: 1121-1133.
147. Rachitskaya, A. V., A. M. Hansen, R. Horai, Z. Li, R. Villasmil, D. Luger, R. B. Nussenblatt, and R. R. Caspi. 2008. Cutting Edge: NKT Cells Constitutively Express IL-23 Receptor and ROR{gamma}t and Rapidly Produce IL-17 upon Receptor Ligation in an IL-6-Independent Fashion. *J. Immunol.* 180: 5167-5171.
148. Annunziato, F., L. Cosmi, V. Santarlasci, L. Maggi, F. Liotta, B. Mazzinghi, E. Parente, L. Fili, S. Ferri, F. Frosali, F. Giudici, P. Romagnani, P. Parronchi, F. Tonelli, E. Maggi, and S. Romagnani. 2007. Phenotypic and functional features of human Th17 cells. *J. Exp. Med.* 204: 1849-1861.
149. Sonobe, Y., J. Liang, S. Jin, G. Zhang, H. Takeuchi, T. Mizuno, and A. Suzumura. 2008. Microglia express a functional receptor for interleukin-23. *Biochem. Biophys. Res. Commun.* 370: 129-133.
150. Gocke, A. R., P. D. Cravens, L. H. Ben, R. Z. Hussain, S. C. Northrop, M. K. Racke, and A. E. Lovett-Racke. 2007. T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity. *J. Immunol.* 178: 1341-1348.
151. Chen, Z., A. Laurence, Y. Kanno, M. Pacher-Zavisin, B. M. Zhu, C. Tato, A. Yoshimura, L. Hennighausen, and J. J. O'Shea. 2006. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. *Proc. Natl. Acad. Sci. U. S. A* 103: 8137-8142.
152. Watford, W. T., B. D. Hissong, J. H. Bream, Y. Kanno, L. Muul, and J. J. O'Shea. 2004. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol. Rev.* 202: 139-156.
153. Naeger, L. K., J. McKinney, A. Salvekar, and T. Hoey. 1999. Identification of a STAT4 binding site in the interleukin-12 receptor required for signaling. *J. Biol. Chem.* 274: 1875-1878.
154. Mathur, A. N., H. C. Chang, D. G. Zisoulis, G. L. Stritesky, Q. Yu, J. T. O'Malley, R. Kapur, D. E. Levy, G. S. Kansas, and M. H. Kaplan. 2007. Stat3 and Stat4 direct development of IL-17-secreting Th cells. *J. Immunol.* 178: 4901-4907.
155. Yang, X. O., A. D. Panopoulos, R. Nurieva, S. H. Chang, D. Wang, S. S. Watowich, and C. Dong. 2007. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J. Biol. Chem.* 282: 9358-9363.
156. Cho, M. L., J. W. Kang, Y. M. Moon, H. J. Nam, J. Y. Jhun, S. B. Heo, H. T. Jin, S. Y. Min, J. H. Ju, K. S. Park, Y. G. Cho, C. H. Yoon, S. H. Park, Y. C. Sung, and H. Y. Kim. 2006. STAT3 and NF-{kappa}B

Signal Pathway Is Required for IL-23-Mediated IL-17 Production in Spontaneous Arthritis Animal Model IL-1 Receptor Antagonist-Deficient Mice. *J. Immunol.* 176: 5652-5661.

157. Park, H., Z. Li, X. O. Yang, S. H. Chang, R. Nurieva, Y. H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6: 1133-1141.
158. Harrington, L. E., R. D. Hatton, P. R. Mangan, H. Turner, T. L. Murphy, K. M. Murphy, and C. T. Weaver. 2005. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6: 1123-1132.
159. Kaplan, M. H., Y. L. Sun, T. Hoey, and M. J. Grusby. 1996. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* 382: 174-177.
160. Filipe-Santos, O., J. Bustamante, A. Chapgier, G. Vogt, L. de Beaucoudrey, J. Feinberg, E. Jouanguy, S. Boisson-Dupuis, C. Fieschi, C. Picard, and J. L. Casanova. 2006. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. *Semin. Immunol.* 18: 347-361.
161. Picard, C., C. Fieschi, F. Altare, S. Al-Jumaah, S. Al-Hajjar, J. Feinberg, S. Dupuis, C. Soudais, I. Z. Al-Mohsen, E. Genin, D. Lammas, D. S. Kumararatne, T. Leclerc, A. Rafii, H. Frayha, B. Murugasu, L. B. Wah, R. Sinniah, M. Loubser, E. Okamoto, A. Al-Ghonaium, H. Tufenkeji, L. Abel, and J. L. Casanova. 2002. Inherited interleukin-12 deficiency: IL12B genotype and clinical phenotype of 13 patients from six kindreds. *Am. J. Hum. Genet.* 70: 336-348.
162. Altare, F., D. Lammas, P. Revy, E. Jouanguy, R. Doffinger, S. Lamhamedi, P. Drysdale, D. Scheel-Toellner, J. Girdlestone, P. Darbyshire, M. Wadhwa, H. Dockrell, M. Salmon, A. Fischer, A. Durandy, J. L. Casanova, and D. S. Kumararatne. 1998. Inherited interleukin 12 deficiency in a child with bacille Calmette-Guerin and Salmonella enteritidis disseminated infection. *J. Clin. Invest* 102: 2035-2040.
163. Pulickal, A. S., S. Hambleton, M. J. Callaghan, C. E. Moore, J. Goulding, A. Goodsall, R. Baretto, D. A. Lammas, S. T. Anderson, M. Levin, and A. J. Pollard. 2008. Biliary cirrhosis in a child with inherited interleukin-12 deficiency. *J. Trop. Pediatr.* 54: 269-271.
164. Mansouri, D., P. Adimi, M. Mirsaeidi, N. Mansouri, S. Khalilzadeh, M. R. Masjedi, P. Adimi, P. Tabarsi, M. Naderi, O. Filipe-Santos, G. Vogt, L. de Beaucoudrey, J. Bustamante, A. Chapgier, J. Feinberg, A. A. Velayati, and J. L. Casanova. 2005. Inherited disorders of the IL-12-IFN-gamma axis in patients with disseminated BCG infection. *Eur. J. Pediatr.* 164: 753-757.
165. Elloumi-Zghal, H., M. R. Barbouche, J. Chemli, M. Bejaoui, A. Harbi, N. Snoussi, S. Abdelhak, and K. Dellagi. 2002. Clinical and genetic heterogeneity of inherited autosomal recessive susceptibility to disseminated Mycobacterium bovis bacille calmette-guerin infection. *J. Infect. Dis.* 185: 1468-1475.
166. Verhagen, C. E., T. de Boer, H. H. Smits, F. A. Verreck, E. A. Wierenga, M. Kurimoto, D. A. Lammas, D. S. Kumararatne, O. Sanal, F. P. Kroon, J. T. Van Dissel, F. Sinigaglia, and T. H. Ottenhoff. 2000. Residual type 1 immunity in patients genetically deficient for interleukin 12 receptor beta1 (IL-12Rbeta1): evidence for an IL-12Rbeta1-independent pathway of IL-12 responsiveness in human T cells. *J. Exp. Med.* 192: 517-528.
167. de Jong, R., F. Altare, I. A. Haagen, D. G. Elferink, T. de Boer, V. van Breda, P. J. Kabel, J. M. Draaisma, J. T. Van Dissel, F. P. Kroon, J. L. Casanova, and T. H. Ottenhoff. 1998. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. *Science* 280: 1435-1438.
168. Altare, F., A. Durandy, D. Lammas, J. F. Emile, S. Lamhamedi, F. Le Deist, P. Drysdale, E. Jouanguy, R. Doffinger, F. Bernaudin, O. Jeppsson, J. A. Gollob, E. Meinl, A. W. Segal, A. Fischer, D. Kumararatne, and J. L. Casanova. 1998. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* 280: 1432-1435.
169. Lichtenauer-Kaligis, E. G., T. de Boer, F. A. Verreck, S. van Voorden, M. A. Hoeve, E. van de Vosse, F. Ersoy, I. Tezcan, J. T. Van Dissel, O. Sanal, and T. H. Ottenhoff. 2003. Severe Mycobacterium bovis BCG infections in a large series of novel IL-12 receptor beta1 deficient patients and evidence for the existence of partial IL-12 receptor beta1 deficiency. *Eur. J. Immunol.* 33: 59-69.
170. Sakai, T., M. Matsuoka, M. Aoki, K. Nosaka, and H. Mitsuya. 2001. Missense mutation of the interleukin-12 receptor beta1 chain-encoding gene is associated with impaired immunity against Mycobacterium avium complex infection. *Blood* 97: 2688-2694.
171. Aksu, G., C. Tirpan, C. Cavusoglu, S. Soydan, F. Altare, J. L. Casanova, and N. Kutukculer. 2001. Mycobacterium fortuitum-chelonae complex infection in a child with complete interleukin-12 receptor beta 1 deficiency. *Pediatr. Infect. Dis. J.* 20: 551-553.
172. Fieschi, C., S. Dupuis, E. Catherinot, J. Feinberg, J. Bustamante, A. Breiman, F. Altare, R. Baretto, F. Le Deist, S. Kayal, H. Koch, D. Richter, M. Brezina, G. Aksu, P. Wood, S. Al-Jumaah, M. Raspall, A. J. Da Silva Duarte, D. Tuerlinckx, J. L. Virelizier, A. Fischer, A. Enright, J. Bernhoft, A. M. Cleary, C. Vermylen, C. Rodriguez-Gallego, G. Davies, R. Blutters-Sawatzki, C. A. Siegrist, M. S. Ehlayel, V. Novelli, W. H. Haas, J. Levy, J. Freihorst, S. Al-Hajjar, D. Nadal, V. De Moraes, O. Jeppsson, N. Kutukculer, K. Frecerova, I. Caragol, D. Lammas, D. S. Kumararatne, L. Abel, and J. L. Casanova.

2003. Low penetrance, broad resistance, and favorable outcome of interleukin 12 receptor beta1 deficiency: medical and immunological implications. *J. Exp. Med.* 197: 527-535.
173. Caragol, I., M. Raspall, C. Fieschi, J. Feinberg, M. N. Larrosa, M. Hernandez, C. Figueras, J. M. Bertran, J. L. Casanova, and T. Espanol. 2003. Clinical tuberculosis in 2 of 3 siblings with interleukin-12 receptor beta1 deficiency. *Clin. Infect. Dis.* 37: 302-306.
174. Staretz-Haham, O., R. Melamed, M. Lifshitz, N. Porat, C. Fieschi, J. L. Casanova, and J. Levy. 2003. Interleukin-12 receptor beta1 deficiency presenting as recurrent Salmonella infection. *Clin. Infect. Dis.* 37: 137-140.
175. Cleary, A. M., W. Tu, A. Enright, T. Giffon, R. Waal-Malefyt, K. Gutierrez, and D. B. Lewis. 2003. Impaired accumulation and function of memory CD4 T cells in human IL-12 receptor beta 1 deficiency. *J. Immunol.* 170: 597-603.
176. Haerynck, F., S. M. Holland, S. D. Rosenzweig, J. L. Casanova, P. Schelstraete, and F. De Baets. 2008. Disseminated Mycobacterium avium infection in a patient with a novel mutation in the interleukin-12 receptor-beta1 chain. *J. Pediatr.* 153: 721-722.
177. Ehlayel, M., B. L. de, F. Fike, S. A. Nahas, J. Feinberg, J. L. Casanova, and R. A. Gatti. 2008. Simultaneous presentation of 2 rare hereditary immunodeficiencies: IL-12 receptor beta1 deficiency and ataxia-telangiectasia. *J. Allergy Clin. Immunol.* 122: 1217-1219.
178. Altare, F., A. Ensser, A. Breiman, J. Reichenbach, J. E. Baghdadi, A. Fischer, J. F. Emile, J. L. Gaillard, E. Meinl, and J. L. Casanova. 2001. Interleukin-12 receptor beta1 deficiency in a patient with abdominal tuberculosis. *J. Infect. Dis.* 184: 231-236.
179. Moraes-Vasconcelos, D., A. S. Grumach, A. Yamaguti, M. E. Andrade, C. Fieschi, L. de Beaucoudrey, J. L. Casanova, and A. J. Duarte. 2005. Paracoccidioides brasiliensis disseminated disease in a patient with inherited deficiency in the beta1 subunit of the interleukin (IL)-12/IL-23 receptor. *Clin. Infect. Dis.* 41: e31-e37.
180. Ozen, M., M. Ceyhan, O. Sanal, M. Bayraktar, and L. Mesci. 2006. Recurrent Salmonella bacteremia in interleukin-12 receptor beta1 deficiency. *J. Trop. Pediatr.* 52: 296-298.
181. Ozbek, N., C. Fieschi, B. T. Yilmaz, L. de Beaucoudrey, B. Demirhan, J. Feinberg, Y. E. Bikmaz, and J. L. Casanova. 2005. Interleukin-12 receptor beta 1 chain deficiency in a child with disseminated tuberculosis. *Clin. Infect. Dis.* 40: e55-e58.
182. Tanir, G., F. Dogu, N. Tuygun, A. Ikinciogullari, C. Aytekin, C. Aydemir, M. Yuksek, E. C. Boduroglu, L. de Beaucoudrey, C. Fieschi, J. Feinberg, J. L. Casanova, and E. Babacan. 2006. Complete deficiency of the IL-12 receptor beta1 chain: three unrelated Turkish children with unusual clinical features. *Eur. J. Pediatr.* 165: 415-417.
183. Fieschi, C., M. Bosticardo, L. de Beaucoudrey, S. Boisson-Dupuis, J. Feinberg, O. F. Santos, J. Bustamante, J. Levy, F. Candotti, and J. L. Casanova. 2004. A novel form of complete IL-12/IL-23 receptor beta1 deficiency with cell surface-expressed nonfunctional receptors. *Blood* 104: 2095-2101.
184. Scheuerman, O., L. de Beaucoudrey, V. Hoffer, J. Feinberg, J. L. Casanova, and B. Z. Garty. 2007. Mycobacterial disease in a child with surface-expressed non-functional interleukin-12Rbeta1 chains. *Isr. Med. Assoc. J.* 9: 560-561.
185. van de Vosse, E., R. A. de Paus, J. T. Van Dissel, and T. H. Ottenhoff. 2005. Molecular complementation of IL-12Rbeta1 deficiency reveals functional differences between IL-12Rbeta1 alleles including partial IL-12Rbeta1 deficiency. *Hum. Mol. Genet.* 14: 3847-3855.
186. Fieschi, C., and J. L. Casanova. 2003. The role of interleukin-12 in human infectious diseases: only a faint signature. *Eur. J. Immunol.* 33: 1461-1464.
187. Sanal, O., T. Turul, T. de Boer, E. van de Vosse, I. Yalcin, I. Tezcan, C. Sun, L. Memis, T. H. Ottenhoff, and F. Ersoy. 2006. Presentation of interleukin-12/-23 receptor beta1 deficiency with various clinical symptoms of Salmonella infections. *J. Clin. Immunol.* 26: 1-6.
188. Minegishi, Y., M. Saito, T. Morio, K. Watanabe, K. Agematsu, S. Tsuchiya, H. Takada, T. Hara, N. Kawamura, T. Ariga, H. Kaneko, N. Kondo, I. Tsuge, A. Yachie, Y. Sakiyama, T. Iwata, F. Bessho, T. Ohishi, K. Joh, K. Imai, K. Kogawa, M. Shinohara, M. Fujieda, H. Wakiguchi, S. Pasic, M. Abinun, H. D. Ochs, E. D. Renner, A. Jansson, B. H. Belohradsky, A. Metin, N. Shimizu, S. Mizutani, T. Miyawaki, S. Nonoyama, and H. Karasuyama. 2006. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity.* 25: 745-755.
189. Dorman, S. E., C. Picard, D. Lammas, K. Heyne, J. T. Van Dissel, R. Baretto, S. D. Rosenzweig, M. Newport, M. Levin, J. Roesler, D. Kumararatne, J. L. Casanova, and S. M. Holland. 2004. Clinical features of dominant and recessive interferon gamma receptor 1 deficiencies. *Lancet* 364: 2113-2121.
190. Pierre-Audigier, C., E. Jouanguy, S. Lamhamedi, F. Altare, J. Rauzier, V. Vincent, D. Canioni, J. F. Emile, A. Fischer, S. Blanche, J. L. Gaillard, and J. L. Casanova. 1997. Fatal disseminated Mycobacterium smegmatis infection in a child with inherited interferon gamma receptor deficiency. *Clin. Infect. Dis.* 24: 982-984.

191. Rosenzweig, S., S. E. Dorman, J. Roesler, J. Palacios, M. Zelazko, and S. M. Holland. 2002. 561del4 defines a novel small deletion hotspot in the interferon-gamma receptor 1 chain. *Clin. Immunol.* 102: 25-27.
192. Newport, M. J., C. M. Huxley, S. Huston, C. M. Hawrylowicz, B. A. Oostra, R. Williamson, and M. Levin. 1996. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. *N. Engl. J. Med.* 335: 1941-1949.
193. Jouanguy, E., F. Altare, S. Lamhamedi, P. Revy, J. F. Emile, M. Newport, M. Levin, S. Blanche, E. Seboun, A. Fischer, and J. L. Casanova. 1996. Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. *N. Engl. J. Med.* 335: 1956-1961.
194. Roesler, J., B. Kofink, J. Wendisch, S. Heyden, D. Paul, W. Friedrich, J. L. Casanova, W. Leupold, M. Gahr, and A. Rosen-Wolff. 1999. Listeria monocytogenes and recurrent mycobacterial infections in a child with complete interferon-gamma-receptor (IFNgammaR1) deficiency: mutational analysis and evaluation of therapeutic options. *Exp. Hematol.* 27: 1368-1374.
195. Jouanguy, E., S. Dupuis, A. Pallier, R. Doffinger, M. C. Fondaneche, C. Fieschi, S. Lamhamedi-Cherradi, F. Altare, J. F. Emile, P. Lutz, P. Bordigoni, H. Cokugras, N. Akcakaya, J. Landman-Parker, J. Donnadieu, Y. Camcioglu, and J. L. Casanova. 2000. In a novel form of IFN-gamma receptor 1 deficiency, cell surface receptors fail to bind IFN-gamma. *J. Clin. Invest* 105: 1429-1436.
196. Jouanguy, E., S. Lamhamedi-Cherradi, D. Lammas, S. E. Dorman, M. C. Fondaneche, S. Dupuis, R. Doffinger, F. Altare, J. Girdlestone, J. F. Emile, H. Ducoulombier, D. Edgar, J. Clarke, V. A. Oxelius, M. Brai, V. Novelli, K. Heyne, A. Fischer, S. M. Holland, D. S. Kumararatne, R. D. Schreiber, and J. L. Casanova. 1999. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. *Nat. Genet.* 21: 370-378.
197. Glosli, H., A. Stray-Pedersen, A. C. Brun, L. W. Holtmon, T. Tonjum, A. Chapgier, J. L. Casanova, and T. G. Abrahamsen. 2008. Infections due to various atypical mycobacteria in a Norwegian multiplex family with dominant interferon-gamma receptor deficiency. *Clin. Infect. Dis.* 46: e23-e27.
198. Han, J. Y., S. D. Rosenzweig, J. A. Church, S. M. Holland, and L. A. Ross. 2004. Variable presentation of disseminated nontuberculous mycobacterial infections in a family with an interferon-gamma receptor mutation. *Clin. Infect. Dis.* 39: 868-870.
199. Villella, A., C. Picard, E. Jouanguy, S. Dupuis, S. Popko, N. Abughali, H. Meyerson, J. L. Casanova, and R. W. Hostoffer. 2001. Recurrent Mycobacterium avium osteomyelitis associated with a novel dominant interferon gamma receptor mutation. *Pediatrics* 107: E47.
200. Okada, S., N. Ishikawa, K. Shirao, H. Kawaguchi, M. Tsumura, Y. Ohno, S. Yasunaga, M. Ohtsubo, Y. Takihara, and M. Kobayashi. 2007. The novel IFNGR1 mutation 774del4 produces a truncated form of interferon-gamma receptor 1 and has a dominant-negative effect on interferon-gamma signal transduction. *J. Med. Genet.* 44: 485-491.
201. Storgaard, M., K. Varming, T. Herlin, and N. Obel. 2006. Novel mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infections. *Scand. J. Immunol.* 64: 137-139.
202. Jouanguy, E., S. Lamhamedi-Cherradi, F. Altare, M. C. Fondaneche, D. Tuerlinckx, S. Blanche, J. F. Emile, J. L. Gaillard, R. Schreiber, M. Levin, A. Fischer, C. Hivroz, and J. L. Casanova. 1997. Partial interferon-gamma receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guerin infection and a sibling with clinical tuberculosis. *J Clin. Invest* 100: 2658-2664.
203. Allende, L. M., A. Lopez-Goyanes, E. Paz-Artal, A. Corell, M. A. Garcia-Perez, P. Varela, A. Scarpellini, S. Negreira, E. Palenque, and A. Arnaiz-Villena. 2001. A point mutation in a domain of gamma interferon receptor 1 provokes severe immunodeficiency. *Clin. Diagn. Lab Immunol.* 8: 133-137.
204. Dorman, S. E., and S. M. Holland. 1998. Mutation in the signal-transducing chain of the interferon-gamma receptor and susceptibility to mycobacterial infection. *J. Clin. Invest* 101: 2364-2369.
205. Vogt, G., A. Chapgier, K. Yang, N. Chuzhanova, J. Feinberg, C. Fieschi, S. Boisson-Dupuis, A. Alcais, O. Filipe-Santos, J. Bustamante, B. L. de, I. Al-Mohsen, S. Al-Hajjar, A. Al-Ghonaium, P. Adimi, M. Mirsaeidi, S. Khalilzadeh, S. Rosenzweig, M. O. de la Calle, T. R. Bauer, J. M. Puck, H. D. Ochs, D. Furthner, C. Engelhorn, B. Belohradsky, D. Mansouri, S. M. Holland, R. D. Schreiber, L. Abel, D. N. Cooper, C. Soudais, and J. L. Casanova. 2005. Gains of glycosylation comprise an unexpectedly large group of pathogenic mutations. *Nat. Genet.* 37: 692-700.
206. Vogt, G., J. Bustamante, A. Chapgier, J. Feinberg, D. S. Boisson, C. Picard, N. Mahlaoui, L. Gineau, A. Alcais, C. Lamaze, J. M. Puck, B. G. de Saint, C. D. Khayat, R. Mikhael, and J. L. Casanova. 2008. Complementation of a pathogenic IFNGR2 misfolding mutation with modifiers of N-glycosylation. *J. Exp. Med.* 205: 1729-1737.
207. Rosenzweig, S. D., S. E. Dorman, G. Uzel, S. Shaw, A. Scurlock, M. R. Brown, R. H. Buckley, and S. M. Holland. 2004. A novel mutation in IFN-gamma receptor 2 with dominant negative activity: biological consequences of homozygous and heterozygous states. *J. Immunol.* 173: 4000-4008.
208. Doffinger, R., E. Jouanguy, S. Dupuis, M. C. Fondaneche, J. L. Stephan, J. F. Emile, S. Lamhamedi-Cherradi, F. Altare, A. Pallier, G. Barcenas-Morales, E. Meinl, C. Krause, S. Pestka, R. D. Schreiber,

F. Novelli, and J. L. Casanova. 2000. Partial interferon-gamma receptor signaling chain deficiency in a patient with bacille Calmette-Guerin and Mycobacterium abscessus infection. *J. Infect. Dis.* 181: 379-384.
209. Dupuis, S., C. Dargemont, C. Fieschi, N. Thomassin, S. Rosenzweig, J. Harris, S. M. Holland, R. D. Schreiber, and J. L. Casanova. 2001. Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. *Science* 293: 300-303.
210. Chapgier, A., X. F. Kong, S. Boisson-Dupuis, E. Jouanguy, D. Averbuch, J. Feinberg, S. Y. Zhang, J. Bustamante, G. Vogt, J. Lejeune, E. Mayola, B. L. de, L. Abel, D. Engelhard, and J. L. Casanova. 2009. A partial form of recessive STAT1 deficiency in humans. *J. Clin. Invest* 119: 1502-1514.
211. Chapgier, A., S. Boisson-Dupuis, E. Jouanguy, G. Vogt, J. Feinberg, A. Prochnicka-Chalufour, A. Casrouge, K. Yang, C. Soudais, C. Fieschi, O. F. Santos, J. Bustamante, C. Picard, B. L. de, J. F. Emile, P. D. Arkwright, R. D. Schreiber, C. Rolinck-Werninghaus, A. Rosen-Wolff, K. Magdorf, J. Roesler, and J. L. Casanova. 2006. Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. *PLoS. Genet.* 2: e131.
212. Dupuis, S., E. Jouanguy, S. Al-Hajjar, C. Fieschi, I. Z. Al-Mohsen, S. Al-Jumaah, K. Yang, A. Chapgier, C. Eidenschenk, P. Eid, A. Al Ghonaium, H. Tufenkeji, H. Frayha, S. Al-Gazlan, H. Al-Rayes, R. D. Schreiber, I. Gresser, and J. L. Casanova. 2003. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. *Nat. Genet.* 33: 388-391.
213. Chapgier, A., R. F. Wynn, E. Jouanguy, O. Filipe-Santos, S. Zhang, J. Feinberg, K. Hawkins, J. L. Casanova, and P. D. Arkwright. 2006. Human complete Stat-1 deficiency is associated with defective type I and II IFN responses in vitro but immunity to some low virulence viruses in vivo. *J Immunol* 176: 5078-5083.
214. Hoflich, C., R. Sabat, S. Rosseau, B. Temmesfeld, H. Slevogt, W. D. Docke, G. Grutz, C. Meisel, E. Halle, U. B. Gobel, H. D. Volk, and N. Suttorp. 2004. Naturally occurring anti-IFN-gamma autoantibody and severe infections with Mycobacterium cheloneae and Burkholderia cocovenenans. *Blood* 103: 673-675.
215. Doffinger, R., M. R. Helbert, G. Barcenas-Morales, K. Yang, S. Dupuis, L. Ceron-Gutierrez, C. Espitia-Pinzon, N. Barnes, G. Bothamley, J. L. Casanova, H. J. Longhurst, and D. S. Kumararatne. 2004. Autoantibodies to interferon-gamma in a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity. *Clin. Infect. Dis.* 38: e10-e14.
216. Kampmann, B., C. Hemingway, A. Stephens, R. Davidson, A. Goodsall, S. Anderson, M. Nicol, E. Scholvinck, D. Relman, S. Waddell, P. Langford, B. Sheehan, L. Semple, K. A. Wilkinson, R. J. Wilkinson, S. Ress, M. Hibberd, and M. Levin. 2005. Acquired predisposition to mycobacterial disease due to autoantibodies to IFN-gamma. *J. Clin. Invest* 115: 2480-2488.
217. Patel, S. Y., L. Ding, M. R. Brown, L. Lantz, T. Gay, S. Cohen, L. A. Martyak, B. Kubak, and S. M. Holland. 2005. Anti-IFN-gamma autoantibodies in disseminated nontuberculous mycobacterial infections. *J. Immunol.* 175: 4769-4776.
218. Ulrichs, T., C. Fieschi, E. Nevicka, H. Hahn, M. Brezina, S. H. Kaufmann, J. L. Casanova, and K. Frecerova. 2005. Variable outcome of experimental interferon-gamma therapy of disseminated Bacillus Calmette-Guerin infection in two unrelated interleukin-12Rbeta1-deficient Slovakian children. *Eur. J. Pediatr.* 164: 166-172.
219. Rosenzweig, S. D., J. Yancoski, A. Bernasconi, S. Krasovec, B. E. Marciano, L. Casimir, G. Berberian, N. Simboli, M. Rousseau, and G. Calle. 2006. Thirteen years of culture-positive M. bovis-BCG infection in an IL-12Rbeta1 deficient patient: treatment and outcome. *J. Infect.* 52: e69-e72.
220. Holland, S. M. 2000. Treatment of infections in the patient with Mendelian susceptibility to mycobacterial infection. *Microbes. Infect.* 2: 1579-1590.
221. Roesler, J., M. E. Horwitz, C. Picard, P. Bordigoni, G. Davies, E. Koscielniak, M. Levin, P. Veys, U. Reuter, A. Schulz, C. Thiede, T. Klingebiel, A. Fischer, S. M. Holland, J. L. Casanova, and W. Friedrich. 2004. Hematopoietic stem cell transplantation for complete IFN-gamma receptor 1 deficiency: a multi-institutional survey. *J. Pediatr.* 145: 806-812.
222. Casanova, J. L., and L. Abel. 2002. Genetic dissection of immunity to mycobacteria: the human model. *Annu. Rev. Immunol.* 20: 581-620.
223. Ward, C. M., H. Jyonouchi, S. V. Kotenko, S. V. Smirnov, R. Patel, H. Aguila, G. McSherry, B. Dashefsky, and S. M. Holland. 2007. Adjunctive treatment of disseminated Mycobacterium avium complex infection with interferon alpha-2b in a patient with complete interferon-gamma receptorR1 deficiency. *Eur. J. Pediatr.* 166: 981-985.
224. Rapkiewicz, A. V., S. Y. Patel, S. M. Holland, and D. E. Kleiner. 2007. Hepatoportal venopathy due to disseminated Mycobacterium avium complex infection in a child with IFN-gamma receptor 2 deficiency. *Virchows Arch.* 451: 95-100.