Genetics of type-1 cytokines in human intracellular infectious diseases: a spectrum of novel genetic deficiencies demonstrates the essential role of type-1 cytokines in immunity against nontuberculous mycobacteria, *M. bovis* BCG and salmonellae

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ABSTRACT

Human genetic factors play an important role in determining the outcome of infections caused by intracellular pathogens, including mycobacteria and salmonellae.[1] The genetic elements involved and the mechanisms by which these control disease susceptibility *vs* resistance, however, remain incompletely characterised. Recent studies on patients with idiopathic severe infections due to otherwise poorly pathogenic mycobacteria (nontuberculous mycobacteria or *Mycobacterium bovis* BCG) or *Salmonella* species have revealed that many of these patients are unable to produce or respond to IFN-γ. This inability results from causative, deleterious genetic mutations in any one of five different genes in the type-1 cytokine cascade, encoding IL-12p40, IL-12Rβ1, IFN-γR1, IFN-γR2 or STAT1. The mutational events can lead to complete or partial deficiency, and are mostly autosomal recessive but can be dominant negative as well. The immunological, clinical and histopathological phenotypes resulting from the 11 groups of genetic type-1 cytokine (receptor) deficiencies that have been identified thus far differ significantly. These findings are summarised, discussed and placed in a broader context in relation to genetic disease predisposition and molecular and cellular mechanisms of protective immunity to these intracellular pathogens.

OUTLINE

Protective immunity to intracellular pathogens such as mycobacteria and salmonellae depends on effective cell-mediated immunity (CMI) in which T-cell-macrophage interactions play a crucial role.[2] A major effector mechanism of CMI is the activation of infected macrophages by type-1 cytokines, particularly interferon-gamma (IFN-γ).[2] IFN-γ is produced by antigen-specific T-helper 1 (Th1) cells and natural killer (NK) cells, and binds to IFN-γR1/R2 receptor complexes at the cell surface of macrophages.

The production of IFN-γ by T cells and NK cells itself is tightly regulated by another cytokine, interleukin-12 (IL-12). IL-12 is a heterodimer, composed of a p40 and a p35 subunit, and is produced by antigen-presenting cells such as macrophages, monocytes and dendritic cells[2] following the activation of Toll-like receptors by bacterial ligands.[3] Thus, IL-12 plays a major role in promoting and linking both innate and adaptive immunity. IL-12 binds to IL-12 receptor β1/β2 complexes at the surface of Th1 and NK cells. Whereas IL-12p40 predominantly interacts with the IL-12Rβ1 chain, IL-12p35 primarily binds to IL-12Rβ2. Two additional cytokines with IL-12-like activities have recently been identified, namely IL-18 and IL-23, both of which are produced by antigen-presenting cells. IL-18 acts mainly in synergy with IL-12 and plays an accessory role in promoting optimal IFN-γ production.[4] Interestingly, IL-23 shares one subunit

with IL-12, notably p40, which is coupled to a unique second chain, p19.[5] Similarly, the receptor for IL-23 consists of an IL-12Rβ1 subunit which is complexed to an as yet unidentified p19R chain. As expected, IL-12 and IL-23 display similar functions, including the stimulation of IFN-γ production, although their functional profiles are not completely identical.[5] Other cytokines can act as additional factors in promoting Th1 development and IFN-γ production, such as the recently identified TCCR ligand,[6] interferon-α and chemokines.[7]

IFN-γ, in synergy with TNF-α, is able to activate microbicidal mechanisms with antimycobacterial activity in murine macrophages.[8] Although IFN-γ is clearly able to affect the growth of *M. bovis* BCG also in human macrophages, reports on a similar role for IFN-γ in *M. tuberculosis*-infected human macrophages have been conflicting.[8] Recent evidence now suggests that IFN-γ may require the presence of other factors, such as 1,25-dihydroxy-vitamin D3 and TNF-α, or human lymphocytes, in order to exhibit anti-*M. tuberculosis* activity in human macrophages, but this issue clearly needs further study.[9] Furthermore, IFN-γ is well known to enhance MHC class I and II expression and to modulate the expression of other molecules involved in antigen presentation, such as proteasomes and transporters-associated-with-antigen-processing (TAP), thus promoting optimal CD4 and CD8 T-cell activation.[10] An additional component of CMI is the activation of cytolytic effector T cells that are able to kill infected macrophages, thereby inhibiting bacterial proliferation through a variety of mechanisms.[11] Effective CMI typically leads to the local containment of the pathogen inside well-organised granulomatous lesions, with epitheloid and giant cell formation and relatively few detectable micro-organisms.

Recently, several patients have been reported with unusually severe and sometimes fatal infections due to usually poorly pathogenic mycobacteria, in the absence of any recognised primary or secondary immunodeficiencies. These patients mostly suffered from infections due to *M. avium*, other nontuberculous mycobacterial species or *M. bovis* BCG, but also other pathogens have been reported including salmonellae and *M. tuberculosis*. The patients described thus far often fail to form well-organised granulomata at the sites of their lesions. A common feature of these individuals was their inability to produce or respond to IFN-γ *in vitro*. This appeared to be due to deleterious mutations in any one of five different genes in the type-1 cytokine cascade, notably *IL12B* (encoding IL-12 p40), *IL12RB1* (encoding IL-12Rβ1), *IFNGR1*, *IFNGR2* (encoding IFN-γR1 and IFN-γR2 chains, respectively) or *STAT1* (encoding IFN-γR-associated STAT1). The mutational events resulted in either (i) recessive, nonfunctional null alleles, (ii) recessive, partially functional alleles, or (iii) dominant-negative alleles, causing partial functionality. Upon further analysis, these deleterious mutations appear to comprise a spectrum of genetically controlled deficiencies in which the extent of the defect correlates with the severity of the clinical, immunological, and histopathological phenotypes observed.
Recent developments and findings regarding defects in the IL-12/IL-12R and IFN-γ/IFN-γR/STAT1 systems respectively will be discussed.[12,13] These novel defects reveal essential molecular and cellular mechanisms of protective cell-mediated immunity against intracellular pathogens. The intriguing observation that these patients appear to be selectively susceptible to otherwise poorly pathogenic mycobacteria and *Salmonella* species will be discussed as well. Finally, comparison of the different clinical, immunological and cellular phenotypes in the various types of genetic defects reveals a spectrum of genetic disorders, which establishes genotype-phenotype relationships but also elucidates additional host- or environment-dependent variability in disease manifestations.

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Discussion following lecture by T.H.M. Ottenhoff

Van der Meer: Regarding the interaction between the T lymphocyte and the macrophage, you did not point to defects of IL-18. The question is, of course, is IL-18 an irrelevant molecule, didn't we look carefully enough, or could it be that the IL-23/IL-12 combination takes out enough of the redundancy to cause trouble.

Ottenhoff: We have not been able to find any defects in IL-18 production and IL-18 receptor expression in those patients we have analysed. Other laboratories did not find any mutations or defects in that system either. In my view IL-18 is mainly an amplifier of IL-12 and perhaps also IL-23. Maybe IL-18 deficiency is not sufficient to cause a dramatically enhanced disease susceptibility to mycobacteria.

Netea: You have also investigated the patients with receptor defects for IL-12 and interferon-γ and found no difference in IL-18 receptor expression?

Ottenhoff: Correct.

Netea: One of the mechanisms for the synergism between IL-12 and IL-18 could be that IL-12 actually induces expression of IL-18, and IL-18 would do a lot of the work.

Ottenhoff: If you activate the cells with phytohaemagglutinin (PHA), you see normal expression of the IL-18 receptor α component. So that certainly is not IL-12-dependent in that system, or at least not IL-12-receptor-β1-dependent.

Netea: But I don't know whether PHA is a relevant stimulus. Could it be going to the T cell directly without the need of a macrophage?

Ottenhoff: PHA is a good system to screen for major defects, but physiologically IL-18 actually could contribute.

Netea: Have you looked at the IL-18 receptor in these patients with IL-12 receptor defects?

Ottenhoff: Yes, we looked at it, but in these patients there is no defect in IL-18 receptor α expression.

Netea: That would imply that those who are saying that IL-12 is working through induction of IL-18 receptor are probably wrong?

Ottenhoff: That is true. We have published this.[1] If you activate T cells in patients with IL12R-β1 defects, there is a small residual IL-12 response. This is of course β1 STAT4-independent, and most likely works through MAP kinase. Perhaps that pathway would be sufficient to induce IL-18 receptor expression in these patients.

Van Furth: Do you have an explanation for the occurrence of tuberculosis, which only arose in three patients, and the other mycobacterial infections, while activation of macrophages is only mildly affected? In contrast, AIDS patients develop tuberculosis early on, and when the CD4 count is really low infections with other mycobacteria develop.

Ottenhoff: One explanation for this paradox is that there is no exposure of these patients, who are mainly from Western countries, to *M. tuberculosis*. Another explanation is that in endemic areas these patients die prematurely and a genetic diagnosis is never made. A third option is that once a patient presents with TB, a genetic defect is not considered. In the three TB cases that have been reported so far this was more or less discovered by coincidence. These defects may be more common in tuberculosis. A fourth, less likely and more academic option is that *M. tuberculosis* inhibits, at least in part, signalling through the interferon-γ receptor STAT1 pathway in macrophages. Because *M. tuberculosis* does this, the macrophage may be less sensitive to interferon-γ, and therefore in TB such defects would have less impact than for instance in BCG or *M. avium*.

De Jongh: Sarcoidosis has many similarities with tuberculosis. Are there defects in patients with sarcoidosis?

Ottenhoff: We have tested about ten sarcoidosis patients, but it is too early to say.

Kuijpers: Interferon-γ receptor-deficient patients may have an increased susceptibility to viral diseases.[2] It is a very weak association, but isn't it the biggest surprise that it is so monogenic?

Ottenhoff: I agree. That is why I concluded that quantitative ligation of the interferon-γ receptor is important. It indicates how important full-blown activation through the interferon-γ receptor pathway is for protection. I think in the case of viral infections or toxoplasmosis, there may be sufficient backup pathways to compensate for these defects, but in the case of mycobacterial and salmonella infection these are not sufficient.

McAdam: What about the ages of the patients with the defects? You suggested the poor life expectancy in the tropics. Certainly we have screened for type-1 pathway defects in

our adult TB patients and have not found any, but that probably means that the others died of viral infections or something else much younger.

Ottenhoff: Patients with complete interferon-α receptor 1 and 2 defects usually present during early life and quite often die before the age of ten. So that is really a severe, often fatal syndrome. IL-12 receptor deficiencies are a little less severe. Those patients can present at an early age but even at 30 years and older. They often survive quite long.

De Groot: Do you have data on the genetics of parents, grandparents and other family members, and have you gone back some generations to see if there were comparable diseases?

Ottenhoff: Yes, all the families we have analysed from Turkey are consanguineous. They can be traced back to one precedent generation. The parents are always heterozygous for the mutations. We performed extensive family analyses and showed that these really segregated recessive traits, as you would expect. At the moment we are investigating whether there are founder effects in Turkey, if certain alleles are more common in the population than other alleles, and if these alleles are maintained in the population and sometimes cause major disease in these families.

De Groot: How would this fit in with the theories on HLA and genetic susceptibility at population level? By changing the HLA make-up over generations apparently disease becomes modified? How does this relate to these cellular defects?

Ottenhoff: That is a difficult question. The defects I described work at the level of innate immunity as well as of the adaptive immunity: first there will be a major deficiency in innate immunity, and this will later on also lead to a major defect in acquired immunity. At that stage there is interaction with HLA leading to enhancement or a decreased susceptibility. These genes I have discussed have a major impact, especially on the early, innate phase, at that stage of course apart from HLA.

De Vries: Also, such a rare mutant, which results in a rather severe phenotype, works out in a recessive way. The genetic situation is completely different when you are talking about HLA alleles which are polymorphic at relatively high frequencies, and which are working in a dominant or co-dominant way. This situation is open to selection.

Netea: Regarding IL-12 p40, could it be, for example, that there are TB patients who produce too much p40 and in that way would be blocking the normal p40/p35 heterodimer action?

Ottenhoff: There is great variation in p40 production between different individuals, that I know, but I am not aware that this is related to tuberculosis.

Appelmelk: I am curious, how are you planning to go on?

Ottenhoff: It is difficult to decide. Should we opt for a candidate gene approach? That is something we are doing. One of these is the interleukin-10 gene. If you want a totally unbiased approach you should go for a genome scan and do linkage analysis and then try to positionally clone the genes involved. That is not easy. It would be a good idea to start using micro-arrays and compare genetic expression profiles of tuberculosis patients with exposed but protected individuals, and try to find which pathways light up in susceptible *vs* protected individuals and to define the different cascades.

Van Agtmael: Has this pathway been evaluated in the patients with *Legionella*, for instance during the recent outbreak in Bovenkarspel in the Netherlands?

Speelman: I can answer that: with Professor van Dissel we are investigating this. So far the results have not been very exciting.

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