Intractable mycobacterial infections associated with genetic defects in the receptor for interferon gamma: what does this tell us about immunity to mycobacteria?

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Introductory articles

Interferon-γ-receptor deficiency in an infant with fatal Bacille Calmette-Guérin infection
E Jouanguy, F Altare, S Lamhamedi, P Revy, J-F Emile, M Newport, M Levin, S Blanche, E Seboun, A Fischer, J-L Casanova

The attenuated strain of Mycobacterium bovis bacille Calmette-Guérin (BCG) is the most widely used vaccine in the world. In most children, inoculation of live BCG vaccine is harmless although it occasionally leads to a benign regional adenitis. In rare cases, however, vaccination causes disseminated BCG infection, which may be lethal. Impaired immunity of the host is generally thought to be the pathogenic mechanism. Disseminated BCG infection has been reported in children with inherited immune disorders. Most of these children had severe combined immunodeficiency, which is characterized by an absence of T cells, and some had chronic granulomatous disease, which is marked by an impairment of the phagocyte respiratory burst. Rare cases of BCG infection have also been reported in association with the acquired immunodeficiency syndrome. We examined the five genes coding for interferon-γ, interferon-γR1, IRF1, TNF-α, and TNF-αR1 in a child with fatal idiopathic disseminated BCG infection.

We found a mutation of the gene for interferon-γR1. There was no detectable interferon-γR1 on the cells from the affected child. These findings provide further evidence of the importance of interferon-γ in the response to mycobacterial infection. (N Engl J Med 1996;335:1956–61)

A mutation in the interferon-γ-receptor gene and susceptibility to mycobacterial infection
MJ Newport, CM Huxley, S Huston, CM Hawrylowicz, BA Oostra, R Williamson, M Levin

Background. Genetic differences in immune responses may affect susceptibility to mycobacterial infection, but no specific genes have been implicated in humans. We studied four children who had an unexplained genetic susceptibility to mycobacterial infection and who appeared to have inherited the same recessive mutation from a common ancestor. Methods. We used microsatellite analysis, immunofluorescence studies, and sequence analysis to study the affected patients, unaffected family members, and normal controls. Results. A genome search using microsatellite markers identified a region on chromosome 6q in which the affected children were all homozygous for eight markers. The gene for interferon-γ receptor 1 maps to this region. Immunofluorescence studies showed that the receptor was absent on leukocytes from the affected child. Sequence analysis of complementary DNA for the gene for interferon-γ receptor 1 revealed a point mutation at nucleotide 395 that introduces a stop codon and results in a truncated protein that lacks the transmembrane and cytoplasmic domains. Conclusions. Four children with severe mycobacterial infections had a mutation in the gene for interferon-γ receptor 1 that leads to the absence of receptors on cell surfaces and a functional defect in the up-regulation of tumor necrosis factor α by macrophages in response to interferon-γ. The interferon-γ pathway is important in the response to intracellular pathogens such as mycobacteria. (N Engl J Med 1996;335:1941–9)
Tuberculosis has become a global emergency. One of the most important barriers to progress in the management of tuberculosis is our lack of understanding of the immune mechanisms that control the growth of mycobacteria in man. It is generally believed that Th1 lymphocytes are important, but the evidence is circumstantial or extrapolated from rodents, and we have almost no convincing data on the final effector pathways that kill the organisms. A fruitful approach to discovering how mycobacteria are controlled in mice has been to study the course of infection in animals modified by the administration of neutralising antibodies or by gene knockout to induce temporary or permanent deficiency of a single cytokine or cytokine receptor. The introductory articles comprise two recent studies of children with intractable mycobacterial infections but no recognisable immunodeficiency syndrome, which have revealed defects resulting in failure to express receptors for interferon gamma (IFN-γ). It is now therefore certain that these receptors are fundamental to immunity to some member of the genus of bacteria in man. These are fascinating papers and represent a step forward in our understanding, but they also raise a whole series of questions about possible differences between rodent and human immune systems, and about the effector mechanisms of antitybacterial immunity.

The patients investigated

Vaccination with Bacille Calmette-Guérin (BCG), an avirulent derivative of the organism responsible for bovine tuberculosis, occasionally causes disseminated infection. In some of the affected individuals there is massive proliferation of the organisms with little evidence of a local T cell mediated reaction, suggesting a very fundamental defect in immune competence. Jouanguy and colleagues studied such a case. She was a Tunisian girl who had been given Pasteur BCG at one month of age. By 2.5 months she had fever and regional adenitis. She then developed hepatosplenomegaly, granulomatous dermatitis, pneumonia, and multiple osteolytic lesions. Biopsy specimens revealed poorly circumscribed areas of macrophages filled with acid fast bacilli subsequently identified as BCG. There were no associated epithelioid cells, giant cells, or lymphocytes. Despite appropriate chemotherapy and treatment with IFN-γ she died at 10 months. The first lesson we learn from this is that even optimal chemotherapy to which the mycobacteria are fully sensitive is ineffective without help from the immune system.

The second study involved four children from the same small town in Malta who presented with disseminated mycobacterial infections. The mycobacterial species isolated were M. fortuitum, M avium (two strains) and M chelonae. One child also had prolonged boilomellosis. Three of the children had died at the time of publication.

Elucidation of the genetic defect

The strategy used to investigate these children was based on the hypothesis that the deficiency was due to homozygosity for an abnormal recessive gene. The parents of the Tunisian girl were first cousins, and three of the M altase children were known to be related while clues as to the mechanism of the susceptibility, but this confirmed the presence of an abnormality. The group studying the Tunisian child had been using a candidate gene approach, analysing only those genes considered likely to be critical for mycobacterial immunity. Discussions between the two groups led to the focusing of attention on the IFN-γ receptor because there were striking clinical similarities between the M altase children and this particular case of disseminated BCG infection. The gene turned out to be abnormal in the Tunisian child as well, though interestingly, at the molecular level, the defect in the Tunisian child was different from that found in the M altase families.

The gene for IFN-γ receptor 1 (IFN-γR1) from the Tunisian child had a single nucleotide deletion that resulted in the creation of a premature stop codon near the N-terminus, in a region that encodes the extracellular domain of the receptor, so the receptor would probably be non-functional. In fact, we do not know if this abnormal receptor would have retained any functionality. There was very little mRNA derived from the gene, suggesting that the abnormal transcript was rapidly degraded. No expression of IFN-γR1 could be detected with monoclonal antibodies on the EBV-transformed cell line from this child, and 125I-IFN-γ failed to bind.

In contrast, the M altase children had a single nucleotide substitution (A for C) rather than a deletion. It allowed normal levels of expression of the mRNA, but introduced a premature stop codon so that the resultant protein was truncated with no membrane or intracellular domain. The cells of these patients may therefore conceivably release truncated extracellular domains but, as expected, flow cytometric analysis with monoclonal antibodies to IFN-γR1 failed to show any membrane-associated receptor, while the parents' cells (homozygous) had intermediate levels of expression. Expression remained undetectable after incubation of the patients' cells with dexamethasone, which increases still further the membrane expression of IFN-γR1 on normal cells.

Is the role of IFN-γ in man different from its role in mice?

The pattern of disease susceptibility resulting from loss of competent receptors for IFN-γ might provide some clues as to the mechanism of the susceptibility, but this susceptibility may be different in the two species. In mice, knocking out the gene for IFN-γ receptor 1 for its receptor leads to susceptibility to a wide range of
What it all means for IFNc

What can we deduce from these findings? IFNc formation of granulomas. Even if we assume that the logical abnormalities detected are due to the functional failure of some un- Appendix 1: Other cytokines - TNF (see text)

- Endotoxin-activated
- Other mechanisms (not specified)

Figure 1  Interferon gamma receptors are ubiquitously expressed. Even if we consider only the macrophage, IFNγ regulates multiple functions at different stages of the cell-mediated immune response. The affected children initiate Th1 responses, but there are no granulomas and the mycobacteria are not killed. The problem could be anywhere within stages 2 and 3.

Antigen presentation, initiation of the immune response

First, macrophages are important antigen-presenting cells involved in the initiation of the response. IFNγ upregulates expression of the major histocompatibility complex on macrophage membranes, and these glyco-proteins present antigen-derived peptides to T lympho-cytes. However, there does not appear to be a defect in response initiation in these children. The tuberculin-driven T cell proliferation was normal in the Tunisian girl and, still more striking, she had a positive tuberculin reaction (16 mm induration) to 10 IU of purified protein derivative (PPD). The absence of IFNγ-R1 therefore clearly does not block initiation of a Th1 response. By themselves, these findings do not eliminate the possibility that regulation of MHC expression is the problem in these patients. It is possible that the response is initiated by other antigen-presenting cells - for example, dendritic cells - but that the Th1 lymphocytes generated cannot "see" the infected macrophages. In fact, mice that lack IFNγ-R1 show decreased expression of MHC class II molecules on macrophages following systemic infection with BCG.

However, disseminated BCG infection was not re-port in children with a complete deficiency of MHC class II molecules so this hypothesis is therefore unlikely.

Granuloma formation and enhanced release of TNFα

The next phase in the development of immunity to mycobacteria is the formation of granulomas. It seems that in mice TNFα is needed for this to occur. Here again we can blame the lack of macrophage activation by IFNγ, because exposure to IFNγ strikingly increases the ability of appropriately stimulated macrophages to release TNFα. Indeed, the failure of IFNγ to enhance endotoxin-triggered TNFα release from the macro-phages of these patients was one of the first immunological abnormalities detected. Unfortunately we do not know whether TNFα is required for granuloma formation in man, but it may be relevant that, in spite

Intractable mycobacterial infections

intracellular (including M avium) and extracellular bacteria, as well as to viruses and parasites. In man the expected effect is susceptibility to otherwise avirulent mycobacteria and, perhaps, increased susceptibility to salmonella. This difference between man and mouse may be an artefact due to the fact that in murine models these susceptibilities have been demon-strated by laboratory workers with syringes, often administering doses that would not normally be encountered, in order to obtain high rates of infection. It is not possible to predict what would happen if such an experiment were performed on IFNγ-R-deficient chil-dren. It would be rash to assume that they would not become infected with a similarly wide range of organisms. It is noteworthy that the infections seen in these five children were all caused by "atypical" mycobacteria that are ubiquitous in the environment. Would they have been abnormally susceptible to M tuberculosis if they had encountered it? It is tempting to assume that they would, but in fact treatment of M avium infection with IFNγ has been successful in man11 whereas this cytokine is not helpful in human tuberculosis. We do not know whether this is because IFNγ is not the limiting factor in the immune response of patients with tuberculosis.

Although we cannot know whether these children had been exposed to M tuberculosis, it is inconceivable that they had not been exposed to organisms such as herpes viruses that are thought to require Th1-mediated immunity. It is therefore tempting to hypothesise that there may be a real difference between mice and men in the role of IFNγ, which may be more "mycobacterium-specific" in man. An extreme view (likely to be wrong because it is so extreme) would be that mycobacteria have posed such a great threat during human evolution (and perhaps continue to kill 10 million of us each year) that the selection pressure induced by mycobacteria has caused human IFNγ to become a specialised anti-mycobacterial cytokine.

Interpretation of the relevance of the mouse data to man is further complicated by the fact that mechanisms required for the clearance of acute infections may differ from those required for clearance of established ones. For instance, mice with disrupted IFNγ genes can mount an effective cytotoxic T cell response against an acute challenge with lymphocytic choriomeningitis virus and actually clear the virus. On the other hand, IFNγ is essential for the clearance of chronic persistent infection which more closely mimics the situation in these chil-

What it all means for IFNγ function

What can we deduce from these findings? IFNγ-R1 is ubiquitously expressed on cells other than erythrocytes. The extraordinary susceptibility to infection with mycobacteria of low virulence could, strictly speaking, therefore be due to the functional failure of some un-identified accessory cell required, for instance, for the formation of granulomas. Even if we assume that the defect exerts its effect within the macrophage, there are still numerous possibilities. Macrophages play a crucial role at several different points within the pathway of cell-mediated (Th1-mediated) immunity, and IFNγ regulates all of these roles (fig 1).

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of the presence of a Th1 response, granuloma formation was not seen in the lesions of these children.12 This hypothesis therefore remains a possibility.

**MICROBACTERIAL MECHANISMS WITHIN MACROPHAGES**

Finally, IFNγ may be involved in activating the pathways in macrophages that actually kill the bacteria. How are mycobacteria killed? There is evidence that, in the mouse, IFNγ activates macrophages so that they express enhanced capacity for production of oxygen reduction products such as superoxide anion and hydrogen peroxide, and also increased expression of the inducible form of nitric oxide synthase (iNOS; also known as type II NOS). Tnfα may then act as a trigger for NO production. This molecule may have some intrinsic anticytobacterial activity,23 or it may interact with oxygen reduction products to yield peroxynitrites that may be the final effector molecules.24

**DO HUMAN MACROPHAGES MAKE HIGH OUTPUT NITRIC OXIDE AND, IF SO, UNDER WHAT CIRCUMSTANCES?**

The regulation of high output NO production by human cells is poorly understood, and the promoter regions of the mouse and human iNOS genes are rather different. Simple exposure to IFNγ does not enable high output NO production by human monocytes. Interestingly, IFNγ used by itself also fails to cause inhibition of growth of M tuberculosis in human macrophages whereas it is very effective on murine macrophages.25 26 For this reason, in the possibility that man and mouse suffer different consequences from losing IFNγ function, an abnormality of the NO pathway must be another possible explanation for the specific mycobacterial susceptibility in these children. It was at first thought that human macrophages were unable to express iNOS, and that even if they did they would not be able to make high levels of NO due to the absence of adequate levels of tetrahydrobiopterin, an essential cofactor for iNOS function. In fact, the situation is much more complex and obscure. It now appears that human macrophages can express iNOS, detectable by antibody or reverse transcriptase polymerase chain reaction (RT-PCR), after stimulation with IFNγ and endotoxin.27 28 Thus, IFNγ could also play a part in this pathway in man. However, NO production remained trivial even when intracellular bipterin levels were raised by incubation with a membrane permeable derivative (sepiapterin). Stimuli tried included LPS, ConA, PHA, PMA, A23187, Tnfα, Gm-csf, IL-1, IL-2, IL-4, IL-7, IL-6, 1,25(OH)2-D3, and combinations of these.29 30 Neutralising transforming growth factor (TGF-β) was also ineffective, as was exposure to M avium or M tuberculosis. (Some reports of NO production by human macrophages infected with M tuberculosis have been guilty of an embarrassing error. This organism expresses a potent nitrate reductase, so it generates nitrite from nitrate. Nitrite is often quantified as a surrogate for NO release since NO rapidly decays to this anion. Experiments involving M tuberculosis that use the nitrite method therefore mean nothing if there is free nitrate in the medium.)

However, it is claimed that high output NO production is a property of alveolar macrophages from patients with tuberculous lung disease.22 23 What these authors have actually shown is rather indirect. The alveolar macrophages certainly express iNOS (that, at least, is no longer in doubt), but they also contain diaphorase activity. These authors consider diaphorase activity to be a reliable correlate of functional high output enzyme.23 31 If human macrophages really are capable of high output NO production, what signals do they require? As already outlined, many signals, including those that are effective in the mouse, have been shown not to work on human cells, even when they lead to expression of the enzyme.23 However, there is evidence that stimulation of human macrophages with IL-4, followed by further stimulation via the low affinity IgE receptor (CD13; FcεRII) in the absence of IL-4, can induce high output NO.32 33 Originally this pathway was thought to require IFNγ as well, but it now appears that it works without, though both IFNγ and TNFα enhance it. If this function of IL-4 and CD23 is confirmed we will be forced to ask whether, in human tuberculosis, immunity requires both a Th1 response (to provide IFNγ, TNFα, and granuloma formation) and a Th2 response (to provide the IL-4, CD23, and NO). Actually there is clear evidence for IgE antibody and for enhanced IL-4 expression in tuberculous individuals.24 If correct, this is totally different from the situation in mice. In the latter, even a small Th2 component greatly increases susceptibility to the disease.35

The apparent difference in disease susceptibility between IFNγ-R1-deficient mice and humans (if real) could therefore lie in the different regulation of high output NO release. Perhaps it all depends on whether the infecting organism triggers an alternative non-IFNγ-dependent pathway in the host species. In the mouse there is some evidence that other cytokines such as IFNγ and TNFα can occasionally provide such an alternative to IFNγ, even if they are less efficacious than IFNγ itself.14 Another example is the apparent ability of TNFα and IL-12 to bypass a total lack of IFNγ due to gene knockout in a murine model of Leishmania donovani infection and cause NO-dependent control of infection.14 However, mice without IFNγ-R1 may become susceptible to a wide range of infections because few of these organisms trigger sufficiently high levels of the cytokines that can bypass the defect in NO release. In contrast, the IFNγ-R1 defect may be more readily
Children with inherited defects of the interferon gamma receptor (IFN-γR) have greatly increased susceptibility to infection with mycobacteria of low virulence.

Children without IFN-γR cannot be cured of disseminated BCG infection, even when given optimal chemotherapy to which the organism is fully sensitive.

In man this defect may lead to specific susceptibility to this group of organisms; genetically modified mice that lack IFN-γ or IFN-γR are susceptible to a wide range of bacteria, viruses and parasites.

IFN-γR is expressed on most (perhaps all) nucleated cells so the critical defect is difficult to identify.

Affected children still develop Th1 responses and have positive lymphocyte proliferation and skin test responses to tuberculin, but granulomas do not form.

Likely candidate sites for the crucial defect include granuloma formation and high output nitric oxide secretion, the control of which differs markedly between man and mouse.


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Thorax 1997 52: 41
doi: 10.1136/thx.52.2008.S41

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