Virulence of a *Mycobacterium tuberculosis* clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-α/β

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To understand how virulent mycobacteria subvert host immunity and establish disease, we examined the differential response of mice to infection with various human outbreak *Mycobacterium tuberculosis* clinical isolates. One clinical isolate, HN878, was found to be hypervirulent, as demonstrated by unusually early death of infected immune-competent mice, compared with infection with other clinical isolates. The differential effect on survival required lymphocyte function because severe combined immunodeficiency (SCID) mice infected with HN878 or other clinical isolates all died at the same rate. The hypervirulence of HN878 was associated with failure to induce *M. tuberculosis*-specific proliferation and IFN-γ production by spleen and lymph node cells from infected mice. In addition, 2- to 4-fold lower levels of tumor necrosis factor-α (TNF-α), IL-6, IL-12, and IFN-γ mRNAs were observed in lungs of HN878-infected mice. IL-10, IL-4, and IL-5 mRNA levels were not significantly elevated in lungs of HN878 infected mice. In contrast, IFN-α mRNA levels were significantly higher in lungs of these mice. To further investigate the role of Type 1 IFNs, mice infected with HN878 were treated intranasally with purified IFN-α/β. The treatment resulted in increased lung bacillary loads and even further reduced survival. These results suggest that the hypervirulence of HN878 may be due to failure of this strain to stimulate Th1 type immunity. In addition, the lack of development of Th1 immunity in response to HN878 appears to be associated with increased induction of Type 1 IFNs.

Virulence of a microorganism is defined as "the relative capacity of a pathogen to overcome body defenses" (1). *Mycobacterium tuberculosis* is a virulent intracellular pathogen that has evolved sophisticated strategies to infect and persist in host macrophages (2–5). Generally, in 90% of immune-competent people, the infection is asymptomatic. Disease manifestations are not observed, and the infecting bacilli are either killed or they remain viable but latent. In those infected individuals that develop active disease, the bacilli appear to evade or subvert the protective cellular immune responses of the host. Infection is established, the bacilli continue to replicate in various host tissues, and clinical symptoms of disease are seen.

The development of active disease is likely determined by multiple factors. First, there is increasing evidence that host genetic factors are involved in determining the susceptibility and/or resistance of an individual to tuberculosis (TB). Particular polymorphisms are associated with TB or show evidence of linkage to susceptibility to TB (6–8). In addition, naturally occurring mutations in the human genes for IFN-γ receptor and IL-12 receptor have been shown to result in increased susceptibility to mycobacterial infection (9–11). Secondly, *M. tuberculosis* itself may undergo genetic changes, resulting in modified virulence (12). Third, the ability of a particular *M. tuberculosis* strain to elicit a strong or weak host immune response may be important in determining development of disease. For example, the *M. tuberculosis* clinical isolate CDC1551 induces an unusually high frequency of seroconversion in exposed persons (13). Despite the high rate of seroconversion, the rates of active disease are not unusually high. This dichotomy may be due to a vigorous host immune response after infection with this clinical isolate, as suggested by the observation that 32% of purified protein derivative (PPD) skin test-positive individuals had indurations of 20 mm or more and 12% had vesiculated skin test sites (13). Indeed, we have recently shown that CDC1551 infection of mice induced unusually high cytokine levels in the lungs. This cytokine induction was associated with long term survival of the CDC1551-infected animals. In the same study, we noted that HN878, another clinical isolate of *M. tuberculosis*, appeared to kill infected mice much earlier. This early mortality was associated with lower cytokine induction (14).

To further explore the determinants of *M. tuberculosis* virulence in mice, we compared HN878 to another clinical isolate, NHN5. Both strains belong to the *M. tuberculosis* genetic group 1, based on two polymorphisms that occur at high frequency in the genes encoding catalase-peroxidase (12). The clinical isolate HN878 caused 60 cases of TB in Houston from 1995 to 1998, and was responsible for at least three clusters of disease. The clinical isolate NHN5 caused 122 cases of TB and 6 disease outbreaks during the same period, including the largest TB outbreak in Texas prisons (12, 15).

To evaluate the virulence of the clinical isolates, we established pulmonary infection in mice and characterized the host-pathogen interaction in this system. We studied the ability of each *M. tuberculosis* strain to replicate in the lungs and spleen, to induce a granulomatous response in the lungs, and to cause mortality in infected immune-competent and immune-deficient mice. We also evaluated the capacity of each strain to induce cytokine mRNA in the lungs and to activate cells from the spleens and draining lymph nodes. The direct effect of intranasal cytokine administration (IFN-α/β or IFN-γ) on survival, as well as on bacillary load and cytokine mRNA levels in the lungs of HN878-infected mice, was also studied.

Materials and Methods

Clinical Isolates of *M. tuberculosis*. *M. tuberculosis* clinical isolates NHN5, HN60, and HN878 were recovered from patients in

Abbreviations: cfu, colony forming unit; TB, tuberculosis; TNF-α, tumor necrosis factor-α; SCID, severe combined immunodeficiency.

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Houston, TX, and characterized as described (12). The clinical isolate CDC1551 (T. M. Shinnick, Centers for Disease Control, Atlanta, GA) (13, 14) and the laboratory strain H37Rv (Trudeau Institute, Saranac Lake, NY) were also studied. Working stocks of all *M. tuberculosis* isolates were prepared by growing the bacteria for 7 days at 37°C with daily agitation in Middlebrook 7H9 medium (Difco) supplemented with ADC (albumin-dextrose complex), and containing 0.05% Tween 80 (Sigma). *M. tuberculosis* stocks (10^8-10^9 bacilli/ml) were stored at −70°C until use. All procedures were performed in a Biosafety Level III (BSL III) laboratory.

Mice. Eight-week-old female B6D2/F_1 mice (Charles River Breeding Laboratories), male CB-17 severe combined immune deficient scid/scid (SCID) and coisogenic CB-17 mice (Taconic Farms), all free of common pathogens, were used for these experiments. Mice were housed in group cages in The Rockefeller University Laboratory Animal Research Center BSL III facility.

Aerosol Infection. For each experiment, frozen stocks of bacteria were thawed, followed by 30 s sonication in a water bath sonicator (Laboratory Supplies, Hicksville, NY; Model G112SPIT) to disperse clumps. A Lovelace nebulizer (In-Tox Products, Albuquerque, NM) was used to generate the aerosol from 1.5 to 8×10^6 bacilli/ml in 10 ml of saline containing 0.04% Tween 80 (16). For each experiment, 24 mice were exposed for 30 min to the aerosol, resulting in implantation of 50–600 organisms into the lungs of each mouse. All of the protocols were approved by the Animal Care and Use Committee of The Rockefeller University.

Intranasal Treatment. Natural purified murine IFN-α/β (specific activity 5×10^7 units/mg protein) was prepared from cultures of C243-3 cells and standardized against the international reference preparation muIFN-α/β (G-002-9004-5411) provided by the National Institutes of Health, Bethesda, MD, as previously described (17). Purified IFN-α/β was diluted in 1% BSA/PBS to a final concentration of 1×10^6 units/ml for administration to mice. Lyophilized recombinant murine IFN-γ (Valbiotech, Paris, France) was reconstituted in sterile water to a final concentration of 4×10^6 units/ml. Stocks of IFN-γ and IFN-α/β were stored at −80°C. Cytokines were kept at 4°C and administered intranasally for 5 consecutive days per week for four weeks for a total of 20 days (final dose of 10^4 units/mouse/day) (18). Ten μl applied directly to the nostrils were readily aspirated by the mouse. Control mice were untreated.

Colony-Forming Unit (cfu) Assays. The growth of *M. tuberculosis* isolates in the lungs and spleens of mice was evaluated by preparing homogenates of these organs and plating as described (14). After 10–14 days, distinct colonies were observed in HN878 cultures whereas 4–5 more days were necessary to visualize NHN5 colonies.

Reverse Transcription (RT)-PCR for Cytokine mRNA Detection. The lower lobe of the left lung of each infected mouse was removed and immediately frozen in liquid N_2; mRNA was prepared as previously described (19). PCR was performed with paired 5′ and 3′ primers; the conditions used for β-actin, tumor necrosis factor (TNF)-α, IL-12p40, IL-10, IL-6, and IFN-γ were as previously described (19). Primers and conditions for IL-4 and IL-5 were as described (20). Primers for muIFN-α types 1, 2, and 7 (cat no. 5476-3, CLONTECH) were combined and used together as follows: 1 cycle of 15′ at 95°C and 35 cycles of 1′ at 95°C, 1′ at 65°C, and 1.5′ at 72°C. Results are expressed as relative densitometry units normalized to the values for β-actin (19).

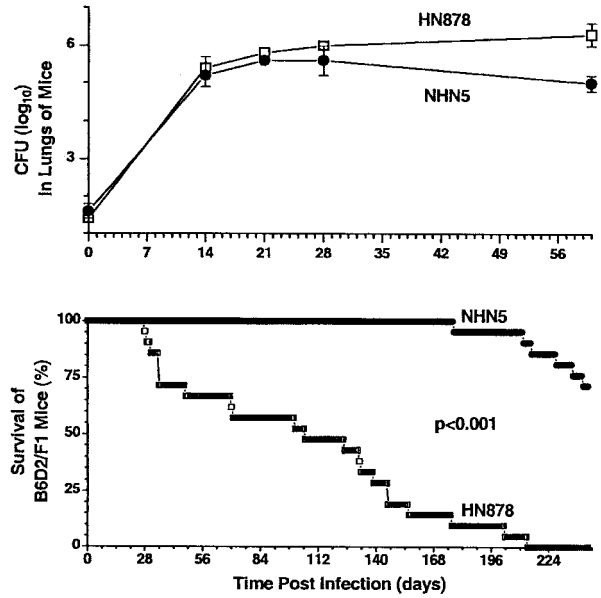

Fig. 1. Survival and bacterial load in lungs of B6D2/F_1 mice infected with *M. tuberculosis* clinical isolates. Mice were infected by aerosol with 1.6 log_{10} NHN5 (●) or with 1.4 log_{10} HN878 (□). (*Upper*) Growth of mycobacteria in lungs. Results are from one representative experiment. Each time point is the mean of 3–4 mice per group per time point; error bars indicate SD. (*Lower*) Survival of infected mice. Results are from one representative experiment with 22 mice per group.

Lymphocyte Proliferation Assay. Spleen and draining lymph nodes were removed at indicated time points and processed as described (21). Cells isolated from spleen and lymph nodes were cultured in RPMI 1640 medium supplemented with 10% FCS, penicillin, and streptomycin (50 μg/ml, final concentration; R10). Cells were cultured at 2×10^5 cell/100 μl in 96-well U-bottom plates. The cells were incubated in R10 with Con A (5 μg/ml final concentration; Sigma) or H37Ra homogenate (20 μg/ml final concentration; Difco), incubated at 37°C for 3 days or 5 days respectively, and then pulsed with [^{3}H]thymidine (1 μCi/well) for an additional 18 h. Incorporation of [^{3}H]thymidine was measured by β-scintillation counting. Values were expressed as stimulation index (S.I.) calculated as: cpm in the cultures with added antigen or mitogen/cpm in the control cultures (no stimulation).

Statistical analysis of the *in vitro* assays and cytokine mRNA data were performed by using an independent *t* test. Kaplan-Meier analysis was used to determine statistical significance of the differences in survival of mice. $P < 0.05$ were considered significant.

Results

Effect of Infection with *M. tuberculosis* NHN5 and HN878 on cfu and Survival of Mice. To compare the relative virulence of two *M. tuberculosis* clinical isolates in the murine model of tuberculosis, immune-competent B6D2/F_1 mice were infected by aerosol with about 50 bacteria/mouse and monitored for bacillary growth in the tissues and for survival. The growth of the two *M. tuberculosis* strains in lungs of infected mice was very similar during the first 21 days postinfection (Fig. 1 *Upper*). The estimated doubling time from day 1 to day 21 postinfection for HN878 and NHN5 was 26 h and 30 h, respectively. Thereafter, the cfu of NHN5 declined slightly whereas the cfu of HN878 remained the same or even increased slightly by day 28 postinfection. Subsequently, about 1 log_{10} higher cfu was seen in the lungs and spleens of HN878-infected mice, compared with infection with NHN5

Table 1. Growth of *M. tuberculosis* clinical isolates in B6D2/F_1 mice

Time postinfection, days	HN878* (lungs/spleen)	NHN5* (lungs/spleen)
0	2.2/0	2.0/0
30	7.4/4.7	6.6/4.5
49	6.8/5.1	5.8/4.4
70	7.1/5.2	5.8/4.5
96	7.6/5.4	6.3/4.6

*cfu (log_{10}) are means of four mice/group in one representative experiment.

(Table 1). Mice infected with HN878 showed accelerated mortality compared with mice infected with NHN5 (Fig. 1). Median survival times were 106 days for HN878 vs. 236 days for NHN5 ($P < 0.001$). To confirm these results, the experiment was repeated with a higher infecting inoculum (≈600 bacteria/mouse). Again, the growth of both strains in the lungs during the first 28 days was similar. Thereafter, about a 1 log_{10} higher cfu was observed in HN878-infected lungs. Median survival times were 126 days for mice infected with HN878 vs. 215 days for mice infected with NHN5 ($P < 0.001$). Taken together, the experiments show that even a 1 log difference in the number of bacilli in the lungs can result in a difference in survival between mice infected with different strains.

Survival and cfu in Infected SCID and Wild-Type Mice. To examine the role of acquired immunity on survival of mice infected with the two clinical isolates, we infected SCID mice and their immune-competent CB-17 controls with about 300 bacteria/mouse, and monitored the cfu in lungs and survival. Early growth of the two *M. tuberculosis* clinical isolates was similar (Fig. 2 *Upper*). SCID mice infected with either strain succumbed within a similar time period (Fig. 2 *Lower*). The 50% survival time for HN878 was 28 days and for NHN5 was 27 days. Further comparisons among

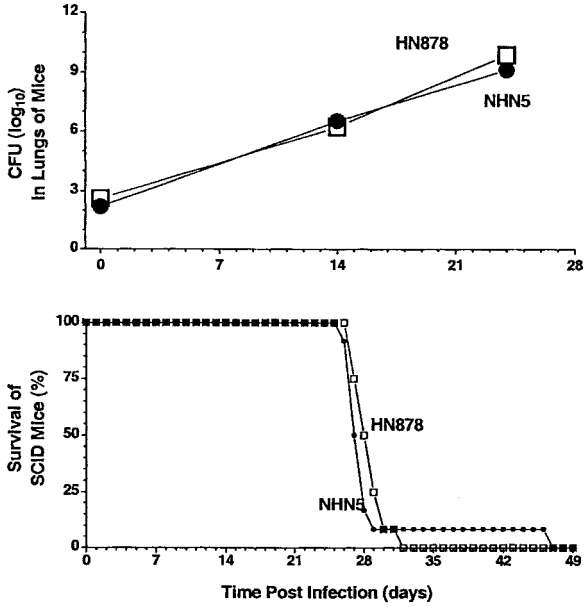

Fig. 2. Survival and bacterial load in lungs of SCID mice infected with *M. tuberculosis* clinical isolates. Mice were infected by aerosol with 2.2 log_{10} NHN5 (●) or with 2.6 log_{10} HN878 (□). (*Upper*) Growth of mycobacteria in lungs. Results are from one representative experiment. Each time point is the mean of 4–5 mice per group per time point. (*Lower*) Survival of infected mice. Results are from one representative experiment with 10 mice per group.

Table 2. Survival of mice infected with *M. tuberculosis* strains

Mouse strain*	*M. tb*	Lung cfu†	Median survival‡
SCID CB-17	HN878	2.6§	28§
	NHN5	2.5§	27§
	HN60	2.9	27
	CDC1551	2.1	32
	H37Rv	2.5	32
Wild-type CB-17	HN878	2.9§	30§
	NHN5	2.5§	260§
	HN60	2.5	252
	CDC1551	2.3	228
	H37Rv	2.6	197

*Ten to twelve mice/group.
†log_{10} bacilli implanted.
‡Days.
§Means of two experiments.

HN878, NHN5, and the clinical isolates HN60, CDC1551, and the laboratory strain H37Rv, showed that infection of SCID mice with any of these strains led to very similar median survival times (Table 2). In the control CB-17 mice infected with NHN5 and HN878, early growth of both strains in the lungs (days 1–24) was similar, with generation times of 25.9 hr and 26.5 hr, respectively. In this immune-competent mouse, HN878 was again more virulent, with a median survival time of 30 days (Table 2). H37Rv, CDC1551, HN60, and NHN5 were all less virulent, and the mice infected with these strains survived much longer. Thus, in the two immune-competent mouse strains tested (B6D2/F_1 and CB-17), HN878 inhibited or failed to induce an acquired immune response, and the mice succumbed early to the infection.

Cell Proliferation and IFN-γ Production *in Vitro*. To further characterize the role of acquired immunity in the response to different strains of *M. tuberculosis*, we investigated the state of T cell activation in immune-competent mice (B6D2/F_1) infected by aerosol with 100 bacteria/mouse.

At 50 days postinfection, T cell proliferation and IFN-γ production in response to mycobacterial antigens were significantly lower in spleen cells from mice infected with HN878 than in spleen cells from mice infected with NHN5 (Fig. 3 *Top* and *Middle*). By 96 days postinfection, the proliferative responses were very low (mean S.I. 1.2 ± 0.2 for HN878; 4.3 ± 1.1 for NHN5) but still statistically significantly different ($P = 0.013$). The difference in levels of IFN-γ released by stimulated spleen cells from 96-day, HN878-infected mice compared with NHN5-infected mice was smaller (30,828 ± 11,807 pg/ml for HN878 vs. 46,928 ± 10,865 pg/ml for NHN5) but also significant ($P = 0.006$). Proliferation and IFN-γ production by cells isolated at either time point from mice infected with either strain in response to Con A were similar (not shown).

We further analyzed the response of cells isolated from draining lymph nodes at day 96 postinfection. Lymph node cells of NHN5-infected mice proliferated significantly more than cells from the lymph nodes of mice infected with HN878, which were essentially inactive (Fig. 3 *Bottom*). Proliferative responses to Con A were similar (not shown). Therefore, the increased mortality in mice infected with HN878 (Fig. 1) appeared to be associated with a decreased ability of their spleen and draining lymph node cells to proliferate and produce IFN-γ in response to *M. tuberculosis* antigens.

Leukocyte Activation in the Lungs of Infected Mice. We examined the lungs of B6D2/F_1 mice infected with HN878 or NHN5 to determine whether there were any gross microscopic differences between the infected organs. Similar granulomas with lympho-

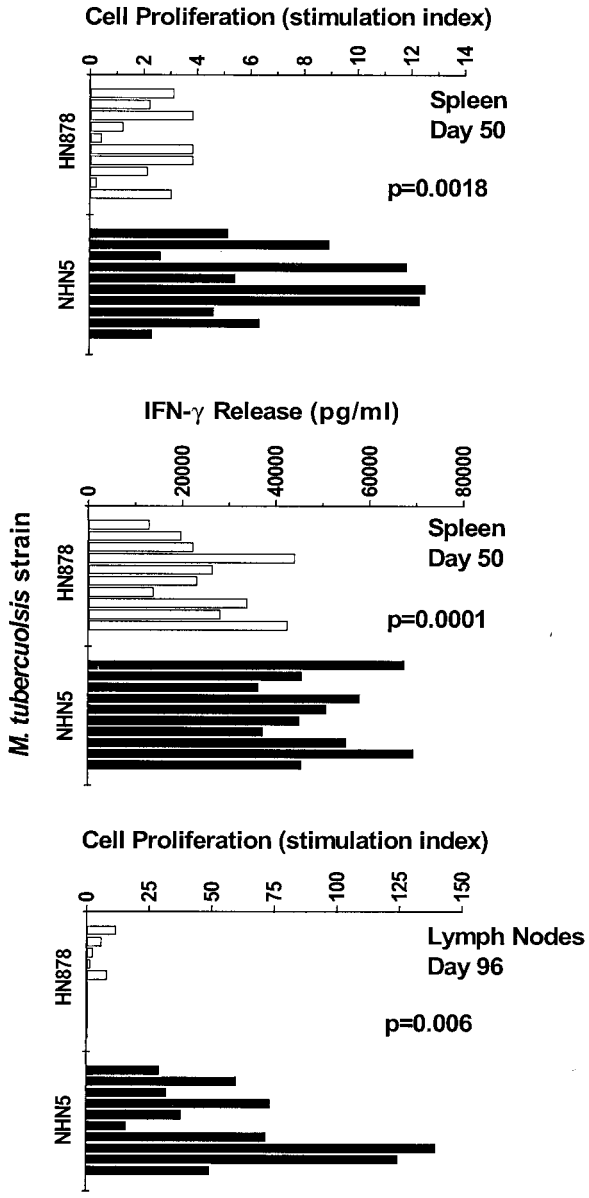

Fig. 3. Cell proliferation and IFN-γ production *in vitro*. Mice were infected by aerosol with either *M. tuberculosis* NHN5 (filled bar) or HN878 (open bar). Proliferation in response to H37Ra sonicate was measured by [^{3}H]thymidine incorporation (*Top* and *Bottom*). IFN-γ release by spleen cells was measured by ELISA (*Middle*). All results are from one representative experiment with 10 mice per group per time point.

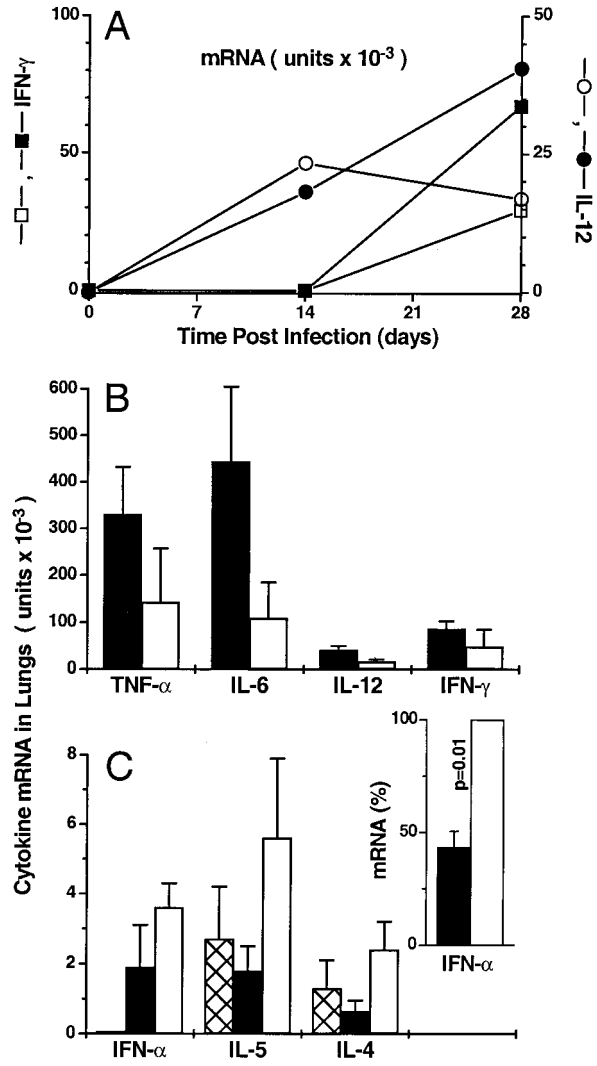

Fig. 4. Cytokine mRNA levels (normalized to β-actin mRNA levels) in lungs of mice infected with *M. tuberculosis*. (*A*) Cytokine mRNA at 14 and 28 days postinfection with NHN5 (filled symbols) and HN878 (open symbols): IFN-γ mRNA (□, ■) and IL-12 (○, ●). (*B*) mRNA at 28 days postinfection with NHN5 (filled bar) or HN878 (open bar). Uninfected mouse lungs had very low or undetectable levels of cytokine mRNA (not shown). (*C*) mRNA at 28 days postinfection with NHN5 (filled bar) or HN878 (open bar) or uninfected (cross-hatched bar). (*C Inset*) Relative levels of IFN-α mRNA in lungs of mice infected for 14 days with NHN5 (filled bar) or HN878 (open bar). Results are means of three independent experiments expressed as percentage activity relative to HN878-infected lungs. *A*, *B*, and *C* are single representative experiments.

cyte cuffs were observed at 50 days postinfection in lungs of both groups of mice (see Fig. 6, which is published as supplemental data on the PNAS web site, www.pnas.org). Thus, the histologic appearance during the first 2 months of infection did not explain the striking differences in survival seen between mice infected with the two clinical isolates.

We therefore evaluated the state of leukocyte activation after *M. tuberculosis* infection. Cytokine mRNA expression in the lungs of infected mice at 14 and 28 days postinfection was determined by using a semiquantitative assay of specific mRNA levels (RT-PCR). In two independent experiments, IL-12 and IFN-γ mRNA showed increased levels over the 28 days postinfection, with higher expression levels in response to NHN5 infection (Fig. 4*A*). At day 28, the expression levels of the mRNAs for Th1-type cytokines IFN-γ, TNF-α, IL-6, and IL-12 were all lower in the lungs of HN878-infected mice (Fig. 4*B*). In addition, IL-10 mRNA levels were also lower in HN878-infected mice (299 ± 133 units x 10^3 for NHN5 vs. 32 ± 10 for HN878). The lower cytokine levels detected in the lungs of mice infected with HN878 indicate less Th1 immune activation in mice infected with this strain. IL-4 and IL-5 mRNA levels in the lungs were not induced above background levels at 14 days in response to infection with both strains (not shown). At day 28 postinfection, mRNA levels for these cytokines had only doubled in the lungs of HN878-infected mice, compared with uninfected controls and were somewhat reduced in the lungs of NHN5-infected mice (Fig. 4*C*). In contrast, IFN-α mRNA levels were significantly induced above uninfected controls and were higher in the lungs of mice infected with HN878 compared with mice infected with

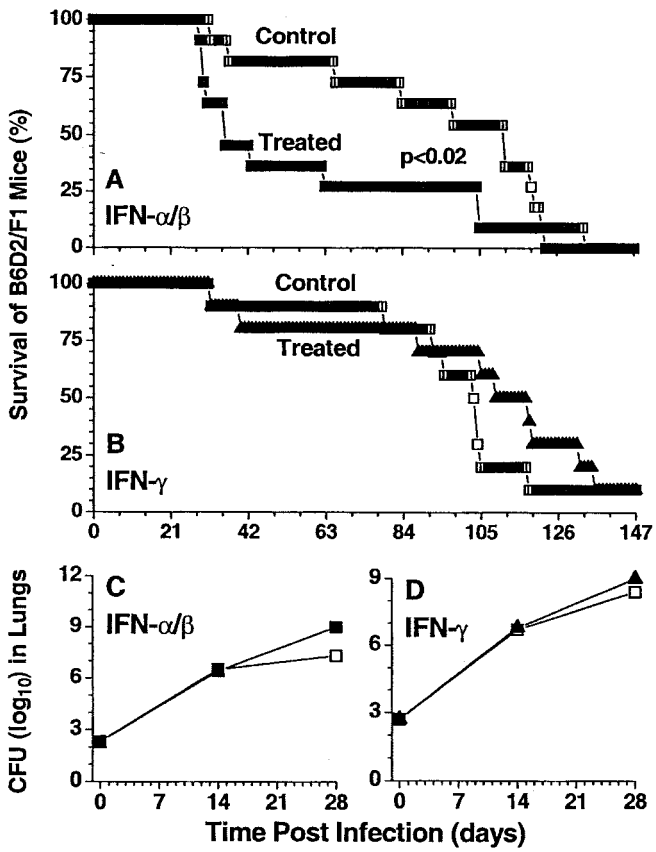

Fig. 5. Survival and bacterial load in lungs of B6D2/F_1 mice infected with *M. tuberculosis*. Mice were infected by aerosol with 2.3 $\log_{10}$ HN878 and treated intranasally with IFN-α/β (■; *A* and *C*) or IFN-γ (▲; *B* and *D*), or untreated (□). (*A* and *B*) Survival of mice (11 mice per experimental group). (*C* and *D*) Growth of HN878 in lungs of cytokine-treated (filled symbols) or untreated (open symbols) aerosol-infected mice over 28 days. Results are means of three to four mice per group per time point.

NHN5 (Fig. 4*C*). In three additional independent experiments, IFN-α mRNA levels detected in the lungs of the infected mice at day 14 postinfection were significantly higher in response to infection with HN878 compared with infection with NHN5 (Fig. 4*C*).

Effect of Treatment with IFN-α/β on the Course of HN878 Infection. To evaluate the role of Type 1 IFNs in the survival of HN878-infected mice, immune competent B6D2/F_1 mice were infected by aerosol with about 300 bacilli/mouse, followed by nasal administration of IFN-α/β (Fig. 5*A*) or of IFN-γ (Fig. 5*B*). Control mice were infected but not treated with cytokines. During the first 14 days postinfection (Fig. 5*C*), both IFN-α/β-treated and untreated mice had similar bacillary loads in the lungs. However, by day 28 postinfection, there was a 2 $\log_{10}$ higher number of bacilli in the lungs of HN878-infected mice treated with IFN-α/β compared with untreated mice. IL-12 mRNA levels in the lungs of mice infected with HN878 were further reduced by treatment with IFN-α/β by 30% (day 14) and 57% (day 28) compared with untreated HN878-infected mice. Median survival times were 35 days for mice treated with IFN-α/β vs. 112 days for the untreated HN878-infected mice ($P = 0.02$; Fig. 5*A*). When HN878-infected mice were treated with intranasal IFN-γ, no differences in survival and only small differences in the number of bacteria recovered from the lungs were noted compared with the untreated infected mice (Figs. 5 *B* and *D*). Cytokine mRNA levels in the lungs were also similar (not shown). Taken together, these results strongly indicate a role for type 1 IFNs in the regulation of expression of the protective immune response to *M. tuberculosis* infection.

Discussion

We show here that the *M. tuberculosis* clinical isolate HN878 is hypervirulent (causes early death of infected mice) most probably because it fails to elicit the Th1-type response crucial for the control of mycobacterial infections. The Th1 response is characterized by early IL-12 production, which stimulates increased IFN-γ production and, subsequently, macrophage activation (22). IL-12 is produced by antigen presenting cells (dendritic cells and macrophages) in response to exposure to mycobacteria or their products (23, 24). Activated $CD4^+$ T cells are thought to be the major source of IFN-γ in *M. tuberculosis* infection because any reduction in the number of $CD4^+$ T cells is associated with higher susceptibility to *M. tuberculosis* both in mice (25) and in humans (26). However, $CD8^+$ T cells and other lymphoid cells [natural killer (NK) and lymphokine-activated killer (LAK)] can also produce significant amounts of IFN-γ, as demonstrated by the partial control of *M. tuberculosis* infection in CD4-deficient mice (21, 27, 28). In the present study, both lymphocyte proliferation and IFN-γ production *in vitro* were significantly lower in spleen and lymph node cells from HN878-infected mice, compared with those from mice infected with NHN5 (Fig. 3). Also, the level of induction of IL-12 and IFN-γ, as measured by the amount of mRNA in the lungs, was lower in response to infection with HN878 (Fig. 4). Thus, in mice infected with HN878, the critical mediators required for the Th1-type response were not fully induced, and the Th1-type response was therefore deficient. Interestingly, the cytokines that drive the Th2 response—IL-10, IL-4, and IL-5—were not significantly elevated in lungs of HN878-infected mice.

Our results suggest an association between the failure to induce an IL-12-mediated Th1-type response and the induction of Type 1 IFNs. In HN878-infected mouse lungs, we observed *decreased* induction of IL-12 and IFN-γ mRNA and *increased* early production of IFN-α mRNA (Fig. 4). In addition, when purified IFN-α/β was administered to the HN878-infected mice, cfu in the infected lungs were nearly 100-fold higher, and the mice died even faster (Fig. 5). This treatment was associated with a further reduction in IL-12 mRNA levels in the lungs, suggesting that Type 1 IFNs had an immunomodulatory effect on the Th1 response. Immune modulation by Type 1 IFNs has also been seen in other experimental systems. Cousens *et al.* (29) reported that IFN-α/β inhibits IL-12 and IFN-γ production by murine spleen cells stimulated *in vitro* with *Staphylococcus aureus* Cowan strain (SAC). In addition, the authors demonstrated a role for type 1 IFNs in regulating IL-12 and IFN-γ production *in vivo*: neutralization of endogenous IFN-α/β in lymphocytic choriomeningitis virus (LCMV)-infected mice resulted in increased levels of serum IL-12p40. When IFN-α/β-receptor gene-disrupted mice were infected with LCMV, serum IL-12p40 and IL-12p70 were significantly higher compared with wild-type mice. More recently, Biron and colleagues (30, 31) have identified an IFN-α/β-mediated immunoregulatory pathway in which Type 1 IFNs down-regulate IFN-γ expression in NK and T cells, thus inducing an unresponsive or refractory state after stimulation by IL-12. In another system, exposure of Leishmania-infected murine macrophages to high amounts of IFN-α/β or preincubation of macrophages with Type 1 IFNs was shown to decrease degradation of NF-κB inhibitor (I-κB) and reduce nuclear translocation of NF-κB, ultimately leading to diminished inducible nitric oxide synthase (iNOS) production (32). Taken together with these studies, our results suggest that those *M. tuberculosis* strains that induce higher levels of Type 1 IFNs may be more virulent because the development of the IL-12-dependent Th1 response is reduced.

Several mycobacterial components can stimulate macrophages to produce cytokines such as IL-12, TNF-α, and IL-6. In a previous study, we observed that, when human monocytes were exposed to the lipid fraction of the *M. tuberculosis* CDC1551 strain, higher levels of TNF-α and IL-12 were induced than after exposure to equivalent lipid fractions of the laboratory strain H37Rv (14). Lipoarabinomannan (LAM) isolated from *M. tuberculosis* has been shown to induce less TNF-α, IL-6, and IL-10 than LAM isolated from nonvirulent mycobacteria (33). It will be worthwhile to investigate whether particular clinical isolates of *M. tuberculosis* have different lipid compositions that in turn differentially induce Type 1 IFNs, as well as whether in these systems the level of Type 1 IFNs produced affects IL-12 production and thereby regulates the Th1 response.

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