Therapeutic Efficacy of High-Dose Intravenous Immunoglobulin in *Mycobacterium tuberculosis* Infection in Mice

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Intravenous immunoglobulin (IVIg) is used to treat patients with primary antibody deficiencies and, at high doses, to treat a range of autoimmune and inflammatory disorders. With high-dose IVIg (hdIVIg), immuno-modulatory mechanisms act on a range of cells, including T cells, B cells, and dendritic cells. Here, we demonstrate that the treatment of *M. tuberculosis*-infected mice with a single cycle of hdIVIg resulted in substantially reduced bacterial loads in the spleen and lungs when administered at either an early or late stage of infection. Titration of the IVIg showed a clear dose-response effect. There was no reduction in bacterial load when mice were given equimolar doses of another human protein, human serum albumin, or maltose, the stabilizing agent in the IVIg preparation. HdIVIg in vitro had no inhibitory effect on the growth of *M. tuberculosis* in murine bone marrow-derived macrophages. In addition, the effect of hdIVIg on bacterial loads was not observed in nude mice, suggesting the involvement of conventional T cells. Analysis of T cells infiltrating the lungs revealed only small increases in CD8$^+$ but not CD4$^+$ T-cell numbers in hdIVIg-treated mice. The mechanism of action of hdIVIg against tuberculosis in mice remains to be determined. Nevertheless, since hdIVIg is already widely used clinically, the magnitude and long duration of the therapeutic effect seen here suggest that IVIg, or components of it, may find ready application as an adjunct to therapy of human tuberculosis.

Mycobacterium tuberculosis remains one of the most successful pathogens of mankind, infecting one-third of the world's population and causing over 2 million deaths annually (35). The incidence of tuberculosis (TB) has increased in recent years and this is attributed to a number of factors, including coinfection with human immunodeficiency virus and the emergence of multidrug-resistant strains of the *M. tuberculosis* bacterium (24). The chemotherapeutic regimens available for treatment of TB are far from ideal, requiring the ingestion of multiple anti-TB drugs in combination over prolonged periods. The side effects of current drug regimens, combined with the protracted duration of treatment, frequently result in poor patient compliance, treatment failures, and associated emergence of drug resistance with major financial implications (26). The close supervision of treatment that is needed to raise efficacy to acceptable levels, such as the World Health Organization's DOTS program, pushes the cost beyond the reach of many of the world's populations most in need. The development of novel, shorter treatments for TB is now an urgent requirement.

The possibilities of immunotherapy deserve more attention than they have received in the past, not least because immunotherapy could circumvent the problems of drug resistance. However, this must be approached with caution, because the disease is itself a consequence of the immune response and one must stimulate protective and not harmful aspects of the response. *M. tuberculosis* is a facultative intracellular pathogen, and it is cell-mediated Th1 type immunity, comprising cytokine-mediated monocyte activation and T-cell cytotoxicity toward infected macrophages, that is the major component of the protective immune response. The role of antibodies in protection is less clear but is being reevaluated in light of a number of recent publications (7, 13, 34).

Indeed, it was the concern of one of us (S.J.) that antibodies that were being administered to patients in the form of high-dose intravenous immunoglobulin (hdIVIg) might theoretically exacerbate latent or undiagnosed tuberculosis that led to the present study. IVIg is a human blood product prepared from the plasma of from 1,000 to 15,000 donors per batch. It has been widely used in the treatment of primary and secondary antibody deficiencies and in these circumstances it is administered at replacement dose (200 to 400 mg/kg of body weight at 2- to 4-week intervals) (28). In contrast, hdIVIg, given at 2 g/kg/month and used as an immunomodulatory agent, was first described for immune-mediated thrombocytopenia (18) but is now widely used in treating a range of neurological, hematological, immunological, dermatological, and rheumatological immune and inflammatory disorders (29). Recently, the use of IVIg as an anti-infectious agent in viral and bacterial infections has been reviewed (4), and it has been

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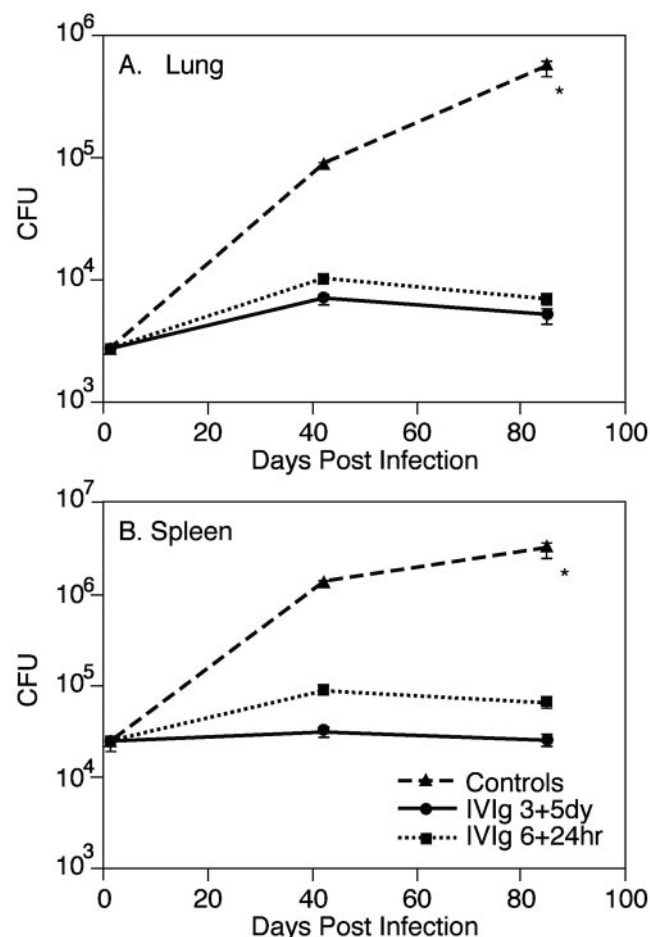† Both authors contributed equally.

FIG. 1. Effect of IVIg on the growth of *M. tuberculosis* in mice. C57BL/6 mice were infected with H37Rv and then received two identical i.p. injections of IVIg at either 6 and 24 h or 3 and 5 days postinfection. Viable counts were conducted on spleen and lungs on days 0, 42, and 85 postinfection. Results are expressed as means ± the standard error of the mean (SEM) of five mice per group and are representative of at least four independent experiments. *, $P < 0.01$ as measured by Student's *t* test.

demonstrated that IVIg given in combination with ampicillin is protective against pneumococcal pneumonia (10). In this study, we investigated the capacity of hdIVIg to influence the course of infection in a murine model of TB.

MATERIALS AND METHODS

Mice. C57BL/6, BALB/c, and nude (BALB/c nu/nu) mice aged 8 to 12 weeks were obtained from breeding colonies maintained under specific pathogen-free conditions in the Division of Biological Services, National Institute for Medical Research. Experiments were carried out in the United Kingdom according to the Home Office Animals Scientific Act of 1986.

Bacterial cultures. *Mycobacterium tuberculosis* (H37Rv) was grown in 7H9 Middlebrook medium supplemented with 0.05% Tween 80 and 10% Middlebrook ADC enrichment (Difco Laboratories, Surrey, United Kingdom) at 37°C for 14 days, aliquoted, and stored at −80°C. Aliquots were thawed and diluted in phosphate-buffered saline (PBS) prior to use.

Infection of mice with *M. tuberculosis*. Mice were infected with *M. tuberculosis* (H37Rv) via the intravenous (i.v.) route, each animal receiving 0.2 ml (2×10^5 live bacteria) via the lateral tail vein. The infection was monitored at various intervals by removing the lungs and spleens of sacrificed animals and determining the number of viable bacteria present. The baseline level of infection was established by harvesting organs at 18 h postinfection and determining viable counts.

Tissues were weighed and homogenized by being shaken with 2-mm glass beads in chilled saline with a Mini Bead Beater (Biospec Products, Bartlesville, Okla.) and 10-fold dilutions (in PBS) of the suspension were plated on 7H11 Middlebrook medium (Difco Laboratories). The numbers of CFU were determined after the plates had been incubated at 37°C for 21 days.

Administration of IVIg in mice. Octagam (Octapharma, Vienna) was used for the in vivo studies, which uses pooled normal human IgG obtained from healthy donors. Mice received two doses of 0.5 ml via the intraperitoneal (i.p.) route at various time points postinfection. This dose represented a final dose of 2 g/kg of body weight, as is used in clinical practice. Human serum albumin (HSA) (BPL, Elstree, United Kingdom) or maltose (Sigma-Aldrich, Poole, United Kingdom) was also administered via the i.p. route at an equimolar dose to IVIg for HSA and at the same dosage as contained within Octagam for maltose (100 mg/ml).

Generation of bone marrow-derived macrophages. Bone marrow-derived macrophages (BMMφ) were prepared from C57BL/6 mice and cultured in Iscove's modified Dulbecco's medium (LifeTechnologies, Paisley, Scotland), supplemented with 5% fetal calf serum, 50 μM mercaptoethanol, 2 mM glutamine, and 10% L929 cell supernatant (as a source of macrophage colony-stimulating factor). These cells were plated into 12-well plates at 5×10^5 cells/well and incubated at 37°C. After 72 h (day 3), the medium and nonadherent cells were removed and replaced with fresh medium containing 10% L929 cell supernatant; this was repeated on day 5. Cells were used on day 6 as a source of macrophages.

Growth of *M. tuberculosis* in bone marrow-derived macrophages. Day 6 BMMφ were infected with *M. tuberculosis* (H37Rv) at a multiplicity of infection of 2:1 for 6 h. The medium was then removed and replaced with fresh medium containing 25 mg/ml IVIg. The IVIg preparation used for these in vitro studies was Gammanorm (Octapharma, Vienna); this was dialyzed twice in Iscove's modified Dulbecco's medium to remove stabilizing agents and then filter sterilized. Gammanorm and Octagam are both immunoglobulin products produced by Octapharma (Vienna, Austria), but Gammanorm is used clinically for subcutaneous administration and contains glycine, rather than maltose, as a stabilizing agent. This preparation was used for the in vitro studies, as it is much more concentrated than Octagam (165 mg/ml as opposed to 50 mg/ml), and thus smaller volumes were required to achieve the desired concentration. Plates were incubated at 37°C, and viable counts were performed on days 0, 3, and 6 postinfection. Wells were washed with 1 ml of PBS, and cells were lysed with 2% saponin (100 μl/ml) and incubated at 37°C for 1.5 to 2 h. Serial dilutions (10 fold) were prepared in PBS and plated on 7H11 Middlebrook medium. The number of CFU was determined after plates had been incubated at 37°C for 21 days.

Lung histology. Mice were administered a lethal injection of anesthetic (Sagatal; RMB Animal Health, Ltd., Dagenham, United Kingdom) via the i.p. route and the lungs were perfused with 1 ml of 10% neutral buffered formalin solution (Sigma-Aldrich) via the trachea in situ and with an additional 1 ml on removal of the lungs. The lungs were then placed in 10 ml of neutral buffered formalin for 3 days prior to histology. Following dehydration in a graded series of ethanol and clearing in xylene, the lungs were embedded in fibrowax (VWR International, Leicestershire, United Kingdom). Sections of 6-μm thickness were stained with hematoxylin and eosin.

Flow cytometry. Lungs were harvested, homogenized, and digested in saline containing 125 U/ml collagenase, 60 U/ml DNase, and 60 U/ml hyaluronidase (Sigma-Aldrich) at 37°C for 30 min. Lung tissue was then passed through a 70-μm cell strainer (Falcon, London, United Kingdom), and red blood cells were lysed in RBC lysis buffer (Sigma-Aldrich) for 2 min. Cells were then washed in PBS and resuspended in fluorescence-activated cell sorter (FACS) buffer (PBS plus 1% fetal calf serum plus 0.05% azide). Fc block (anti-CD16/CD32, clone 2.4G2; BD PharMingen, Oxford, United Kingdom) was added to prevent nonspecific antibody binding, and cells were incubated at 4°C for 15 min. Cells were then stained using directly conjugated antibodies (all from BD PharMingen, Oxford, United Kingdom): phycoerythrin anti-CD4 (clone H129.19), allophycocyanin anti-CD8 (clone 53-6.7), fluorescein isothiocyanate anti-CD44 (clone IM7), or fluorescein isothiocyanate anti-CD3ε (clone 145-2C11). Cells were then incubated at 4°C for 30 min. After being stained, cells were washed in PBS and fixed in 4% paraformaldehyde for 2 h. Following fixation, cells were washed in PBS and resuspended in FACS buffer, and acquisition was performed in a FACSCalibur (Becton Dickinson, Oxford, United Kingdom) using forward and side scatter characteristics to identify the lymphocyte population. Data analysis was performed using Win MDI (Scripps Research Institute, California).

RESULTS

IVIg provides protection against *M. tuberculosis* infection in vivo. To investigate the effect of hdIVIg on *M. tuberculosis*
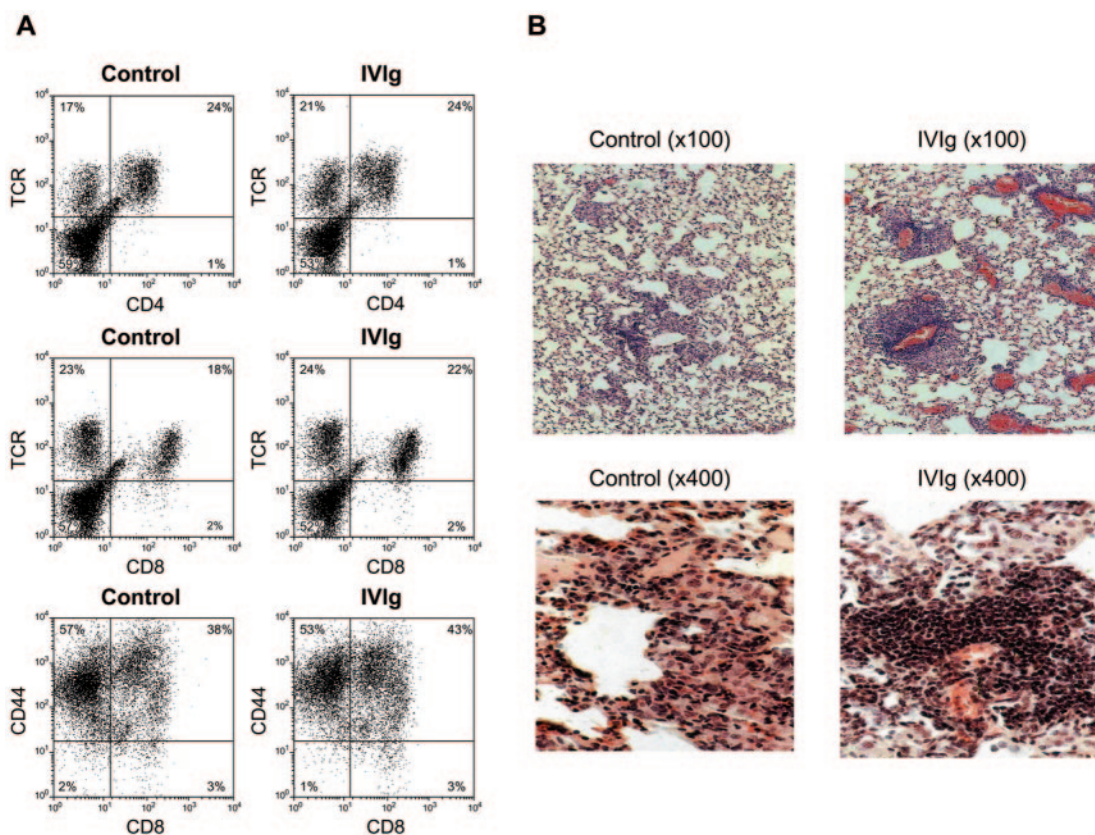
FIG. 2. Analysis of pulmonary immune response. (A) Cells extracted from the lungs of C57BL/6 mice at day 42 postinfection were stained for surface expression of CD4, CD8, CD3ε, or the activation marker CD44. Results show cells pooled from the lungs of five mice per group and are representative of three independent experiments. (B) Lung sections of IVIg-treated or control mice were stained with hematoxylin and eosin at day 42 postinfection. Shown at 100× and 400× magnifications.

infection, C57BL/6 mice were infected with virulent *M. tuberculosis* (H37Rv) via the i.v. route, and IVIg was administered i.p. at a dose of 2 g/kg of body weight. This dose was split into two identical injections, given at 6 h and 24 h postinfection or on day 3 and day 5 postinfection. As can be seen from Fig. 1, the number of viable bacteria recovered from both the spleen and the lungs showed a significant reduction (approximately 100 fold) in IVIg-treated animals compared to the control group. This effect increased over time and was consistently slightly greater in mice treated with IVIg at the later time points (days 3 and 5 postinfection) than in those treated at 6 and 24 h postinfection; however, this difference was not statistically significant. The effect was durable up to at least 133 days postinfection, well beyond the half-life of IVIg (11 to 21 days in humans), which is likely to be considerably shorter in the mouse, due to the development of a mouse antihuman response beginning at 7 to 10 days.

Consistent with these results, histological analysis of the pulmonary infiltrate in hdIVIg and control *M. tuberculosis*-infected mice revealed that both groups contained well formed granulomas. Some of the granulomas in the hdIVIg-treated lungs showed more lymphocyte infiltration (Fig. 2). In addition, when lungs from control and IVIg-treated mice were analyzed by flow cytometry at day 42 postinfection, the results revealed a small (3 to 5%) but significant increase in the

numbers of infiltrating CD8⁺ T cells. There was no significant difference in the numbers of infiltrating CD4⁺ T cells (Fig. 2).

IVIg protects against *M. tuberculosis* infection in a dose-dependent manner. IVIg is used at replacement dose in humans for the treatment of antibody deficiency; however, it is used at high dose (2 g/kg) as an immunomodulator. The treatment administered in our experiments reflects this high dose regimen utilized in humans. We therefore conducted a dose-response study to determine the dose-dependency of the protection against *M. tuberculosis* infection. As can be seen from Fig. 3, the best protection was achieved at a dose of 2 g/kg; however, the protection was dose dependent, showing a greater reduction in bacterial burden with increasing IVIg dose.

IVIg treatment provides protection against *M. tuberculosis* infection in an established late infection. To study the effects of hdIVIg in a well-established infection, mice were infected and IVIg was administered on day 108 postinfection. As can be observed in Fig. 4, the administration of IVIg at this time point reduced the bacterial burden in the spleen and the lungs to a level similar to that observed with the group that had received IVIg on days 3 and 5 postinfection. Indeed, there was no significant difference at day 133 postinfection between the groups that had received early and late treatment.

Human serum albumin or maltose provide no protection against *M. tuberculosis* infection in vivo. The administration of
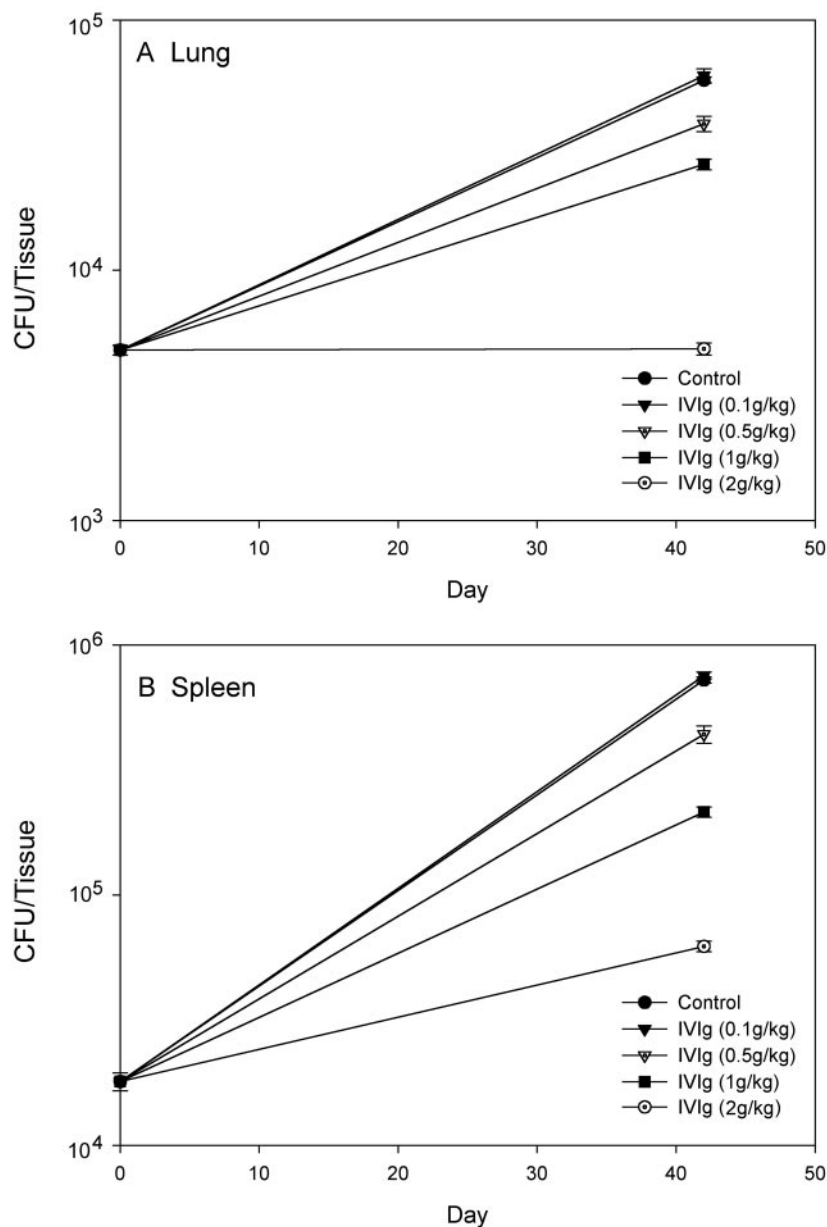
FIG. 3. Dose response of IVIg on *M. tuberculosis* in mice. BALB/c mice were infected i.v. with H37Rv and treated i.p. on day 16 postinfection with saline (control) or with 0.1 g/kg, 0.5 g/kg, 1 g/kg, or normal dose (2 g/kg) IVIg. Viable counts were conducted on day 42 postinfection. Results are expressed as means ± SEM of five mice per group.

human protein might have had a nonspecific effect on the bacterial counts. Furthermore, as the IVIg preparation used in our experiments contained maltose as a stabilizing agent, it was also important to exclude any antibacterial effect caused by this sugar. Experiments were therefore repeated including an equimolar concentration of HSA or identical amounts of maltose as contained within the IVIg preparation. Figure 5 shows that an equimolar concentration of either HSA or maltose did not reduce the number of viable bacteria in the organs of infected mice compared to control mice given normal saline. In addition, this experiment confirmed that the protective effects of IVIg observed in C57BL/6 mice were also reproduced in this model of experimental TB in BALB/c mice.

Effect of IVIg on growth of *M. tuberculosis* within macrophages in vitro. To determine whether the protective effect of hdIVIg in vivo was attributable to a direct effect on the replication of the bacterium within macrophages, *M. tuberculosis*-infected BMMφ were treated in vitro with IVIg or saline. As shown in Fig. 6, there was no significant difference in the bacterial numbers between control and IVIg-treated macrophages, suggesting that the protective effect observed in vivo is not due to a direct inhibition of infection or replication within macrophages.

IVIg provides no protection against *M. tuberculosis* infection of athymic mice in vivo. Because IVIg had no effect on the replication of the bacterium within macrophages, it was hy-
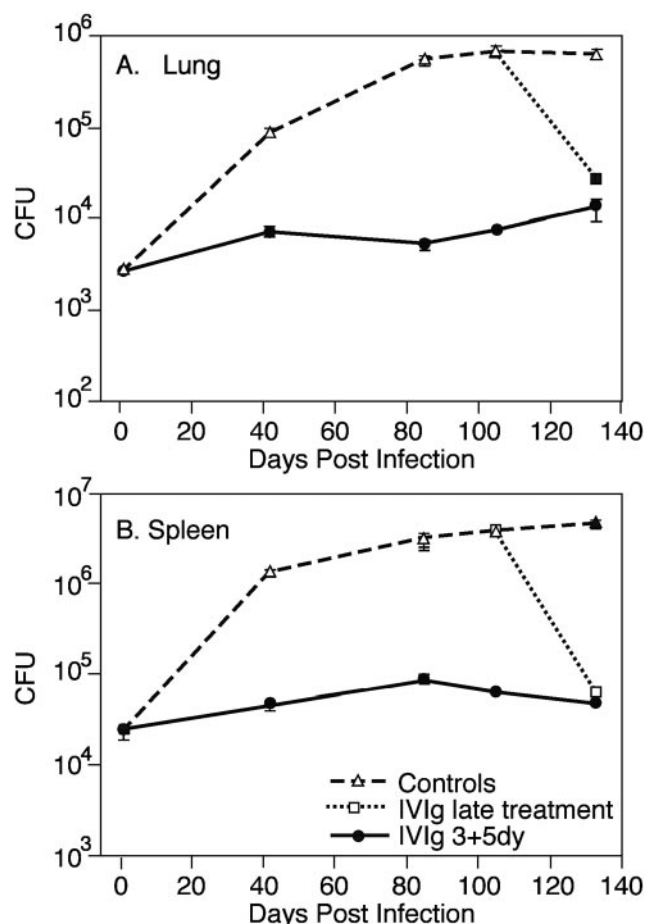
FIG. 4. Effect of late treatment with IVIg on the growth of *M. tuberculosis* in mice. C57BL/6 mice were infected i.v. with H37Rv. Viable counts were conducted on lungs and spleens on days 0, 42, and 85 postinfection to monitor the course of the infection. Late treatment with IVIg was administered i.p. on day 108 postinfection, and viable counts were conducted on day 133. Results are expressed as means ± SEM of five mice per group and are representative of two independent experiments. Similar results were observed when this experiment was repeated with IVIg given on day 60 postinfection.

pothesized that the IVIg exerts the observed protective effect via a T cell-mediated immune response. Therefore, the protection experiments were repeated using BALB/c nu/nu mice, which lack conventional CD4$^+$ and CD8$^+$ T cells. Figure 7 shows that there was no difference in the viable counts obtained from control or IVIg-treated athymic mice, suggesting that the mechanism of IVIg-induced protection may involve a conventional T-cell response.

DISCUSSION

The beneficial effect of hdIVIg against tuberculosis infection was as dramatic as it was unexpected. Administration soon after infection almost completely prevented increases in bacterial numbers, as judged from counts from 40 to 130 days after infection. The reduction in bacterial numbers was reproducibly slightly greater with mice treated on days 3 and 5 postinfection than with those treated at 6 and 24 h postinfection. Later

administration, in the stable plateau phase of infection, resulted in a sharp decline in bacterial numbers. No adverse effect was observed, the animals remained healthy, and histological examination did not reveal any enhancement of lesions. In consequence, far from supporting any fear that its use might exacerbate undiagnosed tuberculosis, evaluation as an adjunct to chemotherapy is indicated by the results. The high dose mirrors that which is used therapeutically in a range of inflammatory and autoimmune disorders in humans and appears to be necessary, since the beneficial effect titrated out with decreasing doses. One immediate concern was that the effect might be an indirect consequence of an immune reaction against the foreign (human) protein. This was discounted by the lack of effect of human serum albumin. Although it is conceivable that mice respond differently to human immunoglobulin than to albumin, this experiment controls for an immune reaction against a xenogeneic protein. Similarly, although sugars at high concentration can impair the growth of *M. tuberculosis*, maltose at the concentration provided by its use as a stabilizing agent in IVIg did not replicate the effect. IVIg is a highly purified product; hence, the activity is likely to be within the biological properties of the immunoglobulin itself.

Taken together, the results show a significant durable reduction in bacterial numbers in the lungs and spleens of two strains of mice following hdIVIg treatment. This effect was also seen in established infection and appears immunological in nature, in that it requires conventional T cells and persists for many months beyond the half-life of the antibody. It was suggested that this effect could be due to antibody-mediated *M. tuberculosis* neutralization in the circulation. However, this is unlikely, as the organism would be in an intracellular location within hours of inoculation, while the day 3 and 5 time points were, if anything, slightly more effective than 6 and 24 h postinfection. The durability of the effect given the half-life of antibody and the efficacy of late treatment, as well as the lack of effect in nude mice and in *M. tuberculosis*-infected BMMϕ, also argue against a direct neutralization or macrophage-dependent effect. However, this does not exclude a direct effect on macrophage ingestion of the bacteria. The lack of effect of HSA on bacterial counts makes it less likely that nonspecific inflammation caused by giving human IVIg to mice played a determinant role in the antibacterial effect. Indeed, human IVIg has been used previously in a number of autoimmune and inflammatory animal models of disease to reduce inflammation (12, 19, 25).

The protective immune response against *M. tuberculosis* is generally accepted to be cell mediated, and antibody is thought to play a much less significant role. There are a number of reasons why this is believed to be the case. (i) *M. tuberculosis* is an obligate intracellular pathogen, and it has been argued that antibody cannot gain access to the intracellular organism. (ii) Patients with primary antibody deficiencies do not appear to be at a significantly increased risk of TB and suffer infections with a range of other bacterial pathogens (9). (iii) The immunological effector response to intracellular pathogens is generally cell mediated. However, the role of antibodies in TB is being reexamined (13). It is likely that antibody may have access to *M. tuberculosis* in several situations: at the time of initial infection, within the lung macrophage during early granulomas
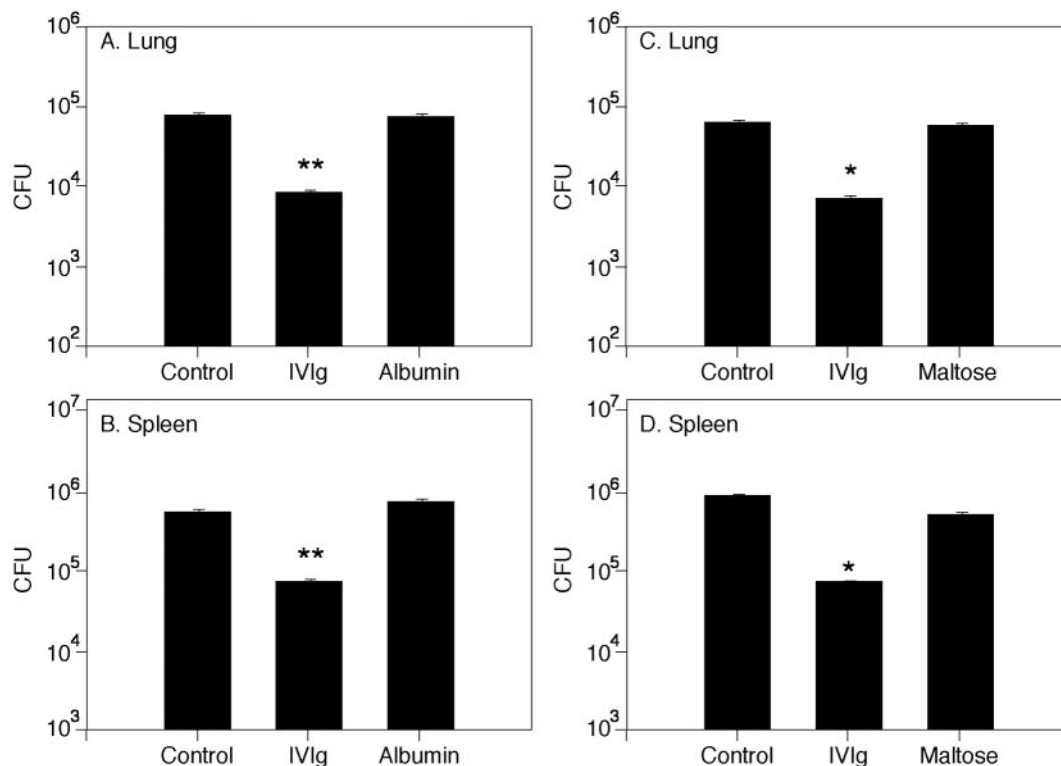
FIG. 5. Effect of IVIg or human serum albumin or maltose on growth of *M. tuberculosis* in mice. BALB/c mice were infected with H37Rv and received two identical i.p. injections of saline (controls), IVIg, human serum albumin, or maltose on days 3 and 5 postinfection. Viable counts were conducted on spleen and lungs at days 83 (panels A and B show the results of experiments with human serum albumin) or 70 (panels C and D show the results of experiments with maltose). Results are expressed as means ± SEM of five mice per group and are representative of two independent experiments. **, $P < 0.01$; *, $P < 0.05$ as measured by Student's t test.

(a stage at which the bacilli may be found extracellularly within the tissues), and during the death of the infected cell. The lack of increased severity or frequency of TB in the antibody-deficient setting does not undermine approaches designed to augment humoral responses against TB, in particular using hdIVIg. More importantly, there is increasing evidence, particularly for hdIVIg, that antibody can modify the responses of cells critical to the response against TB, such as dendritic cells and T cells (3, 6, 11, 20–22, 27, 30, 31, 33).

Commercial preparations of IVIg consist of intact IgG, with a normal IgG subclass distribution and only trace amounts of IgA and IgM. Because of the large number of donors per IVIg batch, IVIg contains a very diverse repertoire of immunoglobulins, sometimes referred to as a species repertoire, within which is a wide spectrum of pathogen specificities. The properties of IVIg are summarized in Table 1 (modified from reference 29).

Many studies into the role of antibody in TB have utilized monoclonal antibodies directed against mycobacterial antigens, particularly carbohydrate surface antigens such as lipoarabinomannan (LAM). Several studies have shown a correlation between protection and the presence of serum antibodies to mycobacterial carbohydrates; there have been suggestions that low levels of anti-LAM IgG in serum increase the risk of dissemination in childhood TB (8) and that serum antibodies to LAM are involved in classical complement activation by mycobacteria (16). A monoclonal antibody specific

for LAM has been shown to prolong survival in *M. tuberculosis*-infected mice; this antibody showed neither bactericidal nor inhibitory activity against infection or bacterial replication; however, it greatly altered the nature of the lung granulomas, suggesting it acted via enhancement of the cell-mediated re-
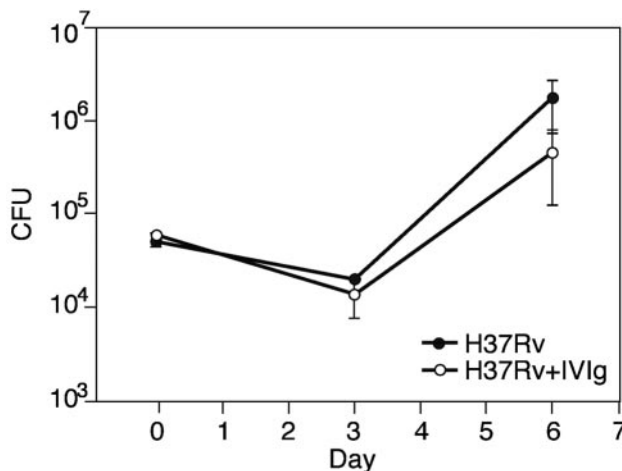

FIG. 6. Effect of IVIg on growth of *M. tuberculosis* in macrophages. Murine C57BL/6 BMMφ were infected with H37Rv at a multiplicity of infection of 2:1 for 6 h and maintained in either control medium or medium containing 25 mg/ml IVIg. Viable counts were conducted at days 0, 3, and 6 postinfection. Results are expressed as means ± SEM of triplicate wells and represent three independent experiments.
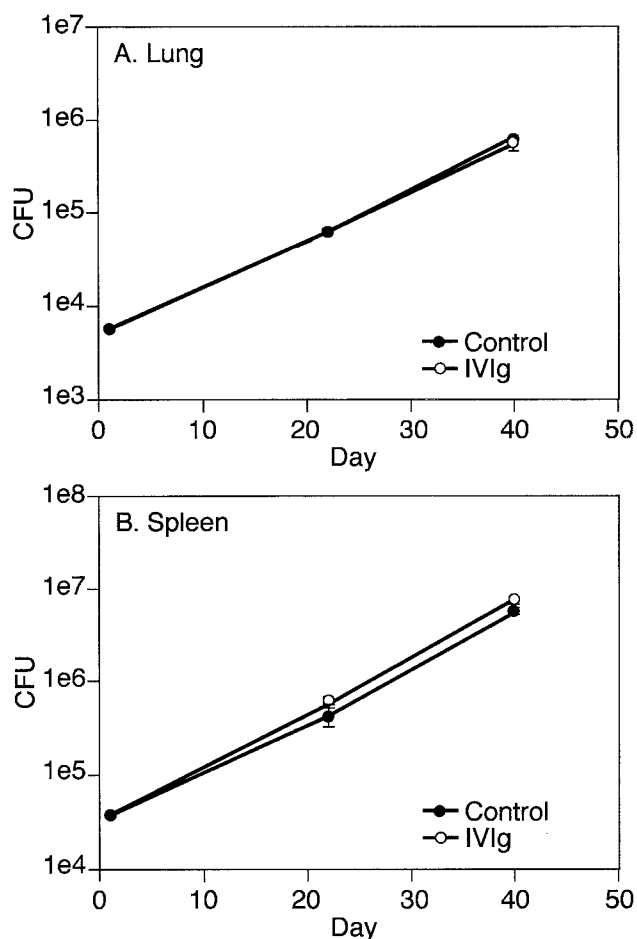
FIG. 7. Effect of IVIg on the growth of *M. tuberculosis* in athymic mice. Athymic mice (BALB/c nu⁻/nu⁻ littermates) were infected IV with H37Rv and then treated i.p. with IVIg. Controls received 0.2 ml saline i.p. Viable counts were conducted on days 0, 22, and 40 postinfection. Results are expressed as means ± SEM of five mice per group.

TABLE 1. Immunomodulatory actions of IVIg[a]

Immunomodulatory category	Effect(s)
Effects due to F(ab')₂	Antiproliferative effects
	Modulation of apoptosis and cell cycle
	Activation of specific cells
	Effects on cell adhesion
	Antibodies to pathogens and superantigens
	Anti-idiotypes
	Antibodies to immunoregulatory molecules (cytokines, TCR, CD4, and CD5)
	Effects on cytokine levels
	Effects on matrix metalloproteinases, matrix turnover
	Effects of natural antibodies on DC maturation
	Natural antibodies
Effects due to Fc receptor binding	Inhibition of phagocytosis
	Inhibition of ADCC
	Effects of antibody production, recycling through FcRn
	Effects on glucocorticoid receptor binding affinity
	Inhibition of DC maturation
	Monomeric IgG blocks access of aggregated IgG to Fc receptors
Effects due to complement-Fc binding	Inhibition of deposition of activated complement
Effects due to substances other than antibody within IVIg	IVIg contains cytokines, cytokine receptors, CD4, MHC class II and stabilizing agents (e.g., maltose, sucrose, and D-sorbitol)

[a] Intravenous immunoglobulin (IVIg) effects may be thought of as four separate components: (i) actions mediated by the variable regions F(ab'₂), (ii) actions of Fc on a range of Fc receptors (FcR), (iii) actions mediated by complement binding within the Fc fragment, and (iv) immunomodulatory substances other than antibody in the IVIg preparations. Not all of the potential mechanisms of action fit perfectly into the groupings, and several mechanisms may act concurrently (27). TCR, T-cell receptor; ADCC, antibody-dependent cellular cytotoxicity; DC, dendritic cell; FcRn, neonatal Fc receptor; MHC, major histocompatibility complex.

sponse (32). Protective effects have also been demonstrated using an IgA antibody specific for the α-crystallin antigen, delivered via the intranasal route (34). Further evidence of antibody involvement in response to *M. tuberculosis* has been provided by studies in mice and guinea pigs, where protection conferred by vaccination with mycobacterial arabinomannan protein conjugates was associated with IgG antibody, which interfered with the harmful effects of LAM (15). It has also been suggested that antibody may act to modify cytokine expression and thus influence cell-mediated responses to infection. For example, IgG1 specific for purified protein derivative has been shown to up-regulate expression of the proinflammatory cytokine tumor necrosis factor alpha in purified protein derivative-stimulated monocytes (17). Taken together, these studies show that mycobacterium-specific antibody-mediated responses may indeed alter the course of *M. tuberculosis* infection.

In the case of polyclonal human IVIg, several reports have recently emphasized its potential use as an anti-infectious agent in several diseases (4), such as infection with *Clostridium difficile* (23) and *Salmonella enterica* serovar Typhimurium

(14). More recently, IVIg has been shown to protect mice against pneumococcal pneumonia but failed to prevent bacteremia; however, IVIg-administered in combination with ampicillin was found to be fully protective (10).

Many of the known mechanisms of hdIVIg (Table 1) might be expected to lead to exacerbation of tuberculosis, in contrast to the observed findings. We do not discount a possible role for antibodies that react against mycobacterial antigens, since IVIg is produced from pooled blood from normal human donors and contains antibodies with such reactivity. The similar reduction in bacterial burden by the late treatment suggests that it is unlikely to be explained by *M. tuberculosis* being opsonized and killed or neutralized within the circulation, as the bacterium becomes intracellular within a few hours of infection. The IVIg preparation used in our studies showed

several bands on Western blotting to a lysate of *M. tuberculosis* (results not shown), indicating the presence of mycobacterium-reactive antibodies. This leaves the question unresolved as to whether the effect was mediated by repertoire [F(ab′)$_2$] or by the constant Fc region, or both. There are a number of ways in which this could be addressed in ongoing studies. (i) Fc could be administered alone, having been digested with papain, or mice could be pretreated with antimurine Fc to block any subsequent Fc interactions. (ii) Monoclonal human IgG specific for an irrelevant antigen could be used; this would have only a single specificity and thus eliminate the effect of repertoire (although it would not mimic the normal IgG subclass distribution found in polyclonal IVIg). The mechanistic possibilities which take into account the long duration of the effect and the lack of effect in nude mice and *M. tuberculosis*-infected BMMϕ include enhanced cross-priming (1, 2, 5), an effect on regulatory T cells, and an effect on T cells in terms of effector function and cytokine production.

T-cell numbers in infected tissues were not markedly influenced by IVIg treatment; consequently, it seems likely that T-cell function or specificities, rather than overall numbers, may have been significantly altered. There are numerous possible ways in which this might be brought about (Table 1). There may be more than one mechanism at play in the effects observed, since administration of IVIg early after infection resulted in bacteriostasis, whereas treatment in the plateau phase after bacteriostatic immunity has developed normally appeared to result in bacterial killing. It is nevertheless possible that one trigger in IVIg is responsible for both effects.

The long duration of the altered resistance to infection was remarkable, considering that the treatment was given only once in a split dose. This suggests that the effect of IVIg may be essentially to switch the balance of regulatory elements controlling protective immunity into a new steady state that is more effective against the bacteria. Prolonged or repeated administration of hdIVIg cannot be tested in mice but is used in many clinical contexts and might further enhance any efficacy against tuberculosis in humans. IVIg may offer a novel means by which to enhance the protective response to *M. tuberculosis*, using an agent already licensed for human use, which would function independently of pathogen resistance.

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