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A Novel Human IgA Monoclonal Antibody Protects against Tuberculosis

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Abstract

Abs have been shown to be protective in passive immunotherapy of tuberculous infection using mouse experimental models. In this study, we report on the properties of a novel human IgA1, constructed using a single-chain variable fragment clone (2E9), selected from an Ab phage library. The purified Ab monomer revealed high binding affinities for the mycobacterial α-crystallin Ag and for the human FcαRI (CD89) IgA receptor. Intranasal inoculations with 2E9IgA1 and recombinant mouse IFN-γ significantly inhibited pulmonary H37Rv infection in mice transgenic for human CD89 but not in CD89-negative littermate controls, suggesting that binding to CD89 was necessary for the IgA-imparted passive protection. 2E9IgA1 added to human whole-blood or monocyte cultures inhibited luciferase-tagged H37Rv infection although not for all tested blood donors. Inhibition by 2E9IgA1 was synergistic with human rIFN-γ in cultures of purified human monocytes but not in whole-blood cultures. The demonstration of the mandatory role of FcαRI (CD89) for human IgA-mediated protection is important for understanding of the mechanisms involved and also for translation of this approach toward development of passive immunotherapy of tuberculosis.

Immunotherapy aims to shorten the chemotherapy of tuberculosis (TB), thereby reducing treatment default rates and in turn decreasing transmission of infection and development of drug-resistant *Mycobacterium tuberculosis* strains. Passive Ab treatment is of interest for use in immunocompromised HIV-infected populations, who show faster disease progression and toxicity from overlapping HIV and TB treatments, and for the treatment of multidrug-resistant TB (1). Although the role of Abs has been controversial (2), systemic infection of mice with *M. tuberculosis* can be reduced by inoculation of mice with Ag-specific mouse mAbs (3–6) or polyspecific human serum IgG (7, 8). Moreover, mouse polyspecific antiserum can act in synergy with the chemotherapy of *M. tuberculosis*-infected mice (9).

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Ab treatment has also been reported to protect against infection by various other intracellular pathogens (10).

A mouse IgA mAb (TBA61) against the α-crystallin (Acr) Ag of *M. tuberculosis* was reported to reduce early pulmonary *M. tuberculosis* infection in BALB/c mice through intranasal (i.n.) inoculation rather than through the systemic route (11–13). The need for postinfection inoculations of the Ab to obtain protection indicated action on *M. tuberculosis* released from killed macrophages. Concurrent inoculations with IFN-γ (14) and anti–IL-4 mAb (15) prolonged protection and reduced relapse from short-term chemotherapy of *M. tuberculosis*-infected mice (16), suggesting action against chemotherapy-generated persister *M. tuberculosis* bacilli (17).

Translation of this approach to the immunotherapy of TB in humans would require development of a fully human IgA Ab, so that anti-mouse Ig responses are avoided. Importantly, there are key differences between the human and mouse IgA systems that need to be taken into account. In humans, the protective function of IgA is mediated in large part through interaction with FcαRI/CD89 (18–20). Mice, however, lack a homologue of CD89 due to a translocation event and are presumed to use an as yet undefined alternative receptor for IgA-mediated effector function. As a result of these important differences, we chose to generate a novel Acr-specific human IgA1 and use mice transgenic for human CD89 (CD89tg) to evaluate whether passive inoculation with the human IgA1 could protect against *M. tuberculosis* infection.

Materials and Methods

Screening of the human single-chain variable fragment phage library

The Tomlinson I&J libraries from Geneservice, Cambridge (21) (http://www.geneservice.co.uk/products/proteomic/scFv_tomlinsonIJ.jsp; distribution was terminated in 2008), were subjected to three rounds of panning. Immunotubes (Maxisorb; Nunc) were coated with recombinant Acr (LRP-0019.3; Lionex Diagnostics, Braunschweig, Germany) in 1 ml carbonate buffer (pH 9.6) at concentrations 20, 2.0, and 0.2 μg/ml in the first, second, and third panning rounds, respectively. The immunotubes were subsequently washed three times with PBS and blocked with 2% skimmed milk in PBS for 2 h at 20°C before further three washings with PBS. Then, ~10^{13} single-chain variable fragment (scFv) phage was added and incubated for 2 h with rotation. Unbound scFv phage were removed by 10 washes with PBS/0.1% Tween 20 and 10 washes with PBS. The bound scFv phage were eluted by incubation with 0.5 ml Tris-PBS for 10 min at room temperature. Two hundred fifty microliters of the eluate was used to infect 1.75 ml freshly prepared bacilli of the TG1 strain of *Escherichia coli*.

The eluate (after taking a sample for phage titration) was plated onto large 2TY agar dishes (245 × 245 mm) with carbenicillin (50 μg/ml). After incubation at 37°C for 18 h, 10 ml 2TY broth (15% glycerol) was added and the colonies scraped. This suspension was mixed with the same volume of 50% glycerol and incubated with rotation at room temperature for 10 min and then with shaking at 37°C until reaching an OD of 0.5–0.8 at 600 nm. After adding ~5×10^9 PFU helper-phage KM13, the suspension was incubated at 37°C for 30 min without shaking and then 30 min with shaking. Cells centrifuged at 3500 rpm for 10 min were resuspended in 250 ml 2TY broth (0.1% glucose, 50 μg/ml carbenicillin and kanamycin) and grown with rapid shaking for 18 h at 30°C. Phage were prepared by polyethylene glycol/NaCl precipitation and used for two separate rounds of panning as described above.

After incubation for 18 h at 30°C, the samples were replicated, grown for 5–6 h at 37°C, then superinfected with 5×10^{10} KM13 and grown for 1 h at 37°C (all incubations with shaking). After centrifugation and removal of supernatants, 2TY broth (50 µg/ml carbenicillin and kanamycin) was added to the sediments, and scFv production was induced by 1 mM isopropyl β-ᴅ-thiogalactoside during 18 h at 30°C. One hundred microliters of phage or soluble scFv were tested for binding to Acr-coated Maxisorp immunoplates (Nunc) by ELISA using anti-M13/HRP mAb (GE Healthcare) and tetramethylbenzidine (Sigma) as substrate.

Generation of recombinant 2E9IgA1

The Acr-binding scFvs were initially cloned into the pGEMT-Easy TA cloning vector. The VH and Vκ gene regions of the selected scFv 2E9 were then amplified by PCR using the primers LMB3 (5′-CAGGAAACAGCTATGAC-3′) and Link Seq New (5′-CGACCCGCCACCGCCGCTG-3′) for the VH and DPK9 (5′-CATCTGTAGGAGACAGAGTC-3′) and PHEN (5′-CTATGCGGCCCCATTCA-3′) for the Vκ. PCRs were performed in 50-µl volumes containing 1 µl diluted (1:10) cDNA, primers (4 pmol/µl), dNTPs (10 mM), and 5× Green GoTaq reaction buffer (Promega). PCR amplification involved an initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 45 s, 55°C for 1 min, and 72°C for 2 min, and a final extension at 72°C for 10 min. The VH region was inserted upstream of the human IgA1 α-chain C region sequence previously inserted (P.K. Dehal, D.T. Bowen, P.R. Crocker, and J.M. Woof, manuscript in preparation) into the mammalian expression vector VHexpress (22). Similarly, the Vκ region was inserted upstream of the Cκ sequence in the expression vector VKExpress (22). After sequence confirmation, the 2E9IgA1 VH and Vκ constructs were stably co-transfected into CHO-K1 cells, with hygromycin and gpt serving as selectable markers. Transfectants were screened by ELISA for Acr binding, and suitable clones were expanded.

Purification and characterization of 2E9IgA1

The 2E9IgA1 transfectant cells were grown as monolayers in tissue culture flasks of 10–1000 ml (Greiner) using DMEM medium (Invitrogen) with 10% FBS (Invitrogen). After reaching confluence, the harvested supernatant from 500- to 750-ml cultures was filter-sterilized and immediately subjected to affinity chromatography on anti-human IgA agarose (Sigma) or Acr Affigel-15 columns (8). Supernatants containing 0.1% Na-azide were passed at a 0.4 ml/min flow rate; after washing with PBS, the bound IgA was eluted with 0.1 M glycine pH 2.5, collecting 2-ml fractions into 0.2 ml 1 M Tris buffer. Protein-positive fractions were concentrated in Amicon Ultra concentrators (Millipore) and dialyzed against PBS. The purified Ab was quantified using the wide-range BioChemika protein quantification kit (Sigma). 2E9IgA1 was subjected to gel filtration on a Superose 6 column, washed with 20 volumes of PBS/0.1% Na-azide, and connected to an ÄKTA FPLC system (Amersham Biosciences, Chalfont St. Giles, Bucks, UK).

Purified 2E9IgA1 was separated in 12% Bis-tris gels and reduced by DTT (Invitrogen). Gels were stained with Coomassie blue or immunoblotted and developed with HRP-labeled Abs: goat anti-human κ L chain (Sigma), goat (Kirkegaard and Perry Laboratories) or mouse anti-human IgA (constant α-chain) mAb (AbD Serotec). Acr (10 µg/ml) binding was followed by anti-Acr IgG mAb (TBG65) and anti-mouse IgG–HRP (Sigma). O-linked sugars were detected by jacalin–biotin (Sigma) and N-linked sugars by Con A–biotin (Sigma), followed by development with streptavidin–HRP.

Surface plasmon resonance analysis

Analysis was performed with a BIAcore X instrument (BIAcore AB, Uppsala, Sweden) using a CM5 sensor chip coupled with either Acr or soluble rFc-(CD89)$_2$ protein (23),

giving 1093 and 1200 response units, respectively. The flow rate was set at 5 µl/min, except for kinetic analysis during which flow rate was adjusted to 20 µl/min. The 2E9IgA1 analyte was diluted in buffer containing 0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, and 0.005% (v/v) surfactant P20. The injected 35-µl aliquot was allowed to dissociate for 4 min, and subsequently the surfaces were regenerated with 2 M potassium thiocyanate. Data collected for each experiment were analyzed for association (M^{-1} s^{-1}) and dissociation rates (s^{-1}), using either the 1:1 binding model of Langmuir or the two-state reaction (conformation change) model. The best fits from 100 to 1000 nM IgA ligand concentrations were obtained using BIAevaluation software version 4.1.

Immunotherapy of infection of CD89tg mice

Groups of CD89tg mice (24) and non-transgenic littermate control mice were infected i.n. with 0.5 million H37Rv CFU (11) (= day 0). Immunotherapy was delivered i.n. as follows: 1 µg mouse IFN-γ (10,000 U/µg; Sigma) alone was administered on day −3 before infection. IFN-γ mixed with 5 µg purified 2E9IgA1 (titer 16,500) or with PBS in control groups was administered 2 h before infection and again either on day 1 or day 21 postinfection (in the experiment shown in Fig. 2) or on both these days (in the experiment shown in Fig. 3). Lungs and spleens harvested 4 wk later were homogenized using the Stomacher 80 Biomaster (Seward Ltd), and the diluted homogenate was plated on duplicate 7H11 agar plates for the CFU assay. For histology, lung fragments were placed in 5 ml 10% buffered formaldehyde, embedded in paraffin blocks, and sections were stained with H&E. The proportion of infiltrated granulomatous areas was determined by ImageJ software-based morphometry of digitized images of lung sections. All animal experiments were performed adhering to rules specified by the UK Home Office Project and personal licenses.

Modulation of human blood infection

Heparinized human blood was obtained from bacillus Calmette-Guérin–vaccinated donors (with informed consent and approval by a local ethical committee) or from a blood transfusion center. Duplicate 1 ml whole-blood cultures (25) were preincubated with 10 ng/ml human IFN-γ and 100 µg/ml 2E9IgA1 for 24 h prior to infection with 1×10^4 to 10×10^4 relative light units (RLU) luciferase-tagged H37Rv (H37Rv-*lux*) bacilli (26) for 2 h. Extracellular bacilli were killed by 4-h incubation with amikacin (200 µg/ml). After incubation in 5% CO_2 at 37°C for 1–5 d, samples were split to half volumes, centrifuged, and erythrocytes in the sediment were lysed with dH_2O. After centrifugation, pellets were resuspended in 1 ml PBS, and luminescence was measured for 20 s with a Berthold Junior luminometer using 1% *n*-decyl aldehyde (Sigma). One viable organism corresponded with 15 RLU luminescence.

Monocytes were separated from 5-ml human buffy coats using Lymphoprep and EasySep (19058; Stem Cell Technologies) without CD16 depletion. Triplicate cultures of 10^5 monocytes in 0.5 ml RPMI medium/5% human serum were incubated in the presence of 10 ng/ml IFN-γ and 20 µg/ml 2E9IgA1 for 1 d before infection with H37Rv-*lux* at 1:5 multiplicity of infection for 2 h, followed by 2-h treatment with amikacin. At harvest, adherent cells were washed gently with PBS, then lysed in 10% Triton X-100 (Sigma) for 10 min at 37°C and tested for luminescence as described earlier.

Statistical analysis

Differences in geometric mean values of CFU counts between different groups of infected mice were evaluated using the two-sample *t* test with equal variance and two-tailed distribution or using the ANOVA test with multiple comparisons of means. The correlation coefficient was calculated for CFU counts and the area of granulomatous infiltration of

lungs in individual infected mice. Differences in mean RLU values, reflecting infection of human blood cells in vitro, were evaluated by the two-sample t test.

Results

Generation and characterization of a human IgA1 mAb against Acr

Through Ag panning of a human Ab phage library, we isolated Ag-specific scFvs that bound Acr at high titer and avidity. The 2E9 clone, selected for detailed study, appeared to represent a "dominant" VH/Vκ combination, as it had identical VH and Vκ sequences to four other Acr-binding clones. After subcloning of VH and Vκ regions into α H chain and κ L chain plasmids and expression in CHO-K1 cells, a transfectant yielding Acr-specific 2E9IgA1 was generated.

On SDS-PAGE gels (Fig. 1), under nonreducing conditions, 2E9IgA1 gave a major band of ~170 kDa representing intact IgA (H_2L_2). Minor bands of lower m.w. most likely represent half molecules (HL). After reduction, bands representing H chain glycoforms were seen at around 60 kDa and an L chain band at 25 kDa, assignments confirmed by Western blotting. HPLC analysis (data not shown) showed a major peak of 170 kDa, suggesting that the half molecules seen on SDS gels associate noncovalently in solution. Lectin reactivity confirmed the anticipated N- and O-linked glycosylation. Probing of blots with Acr and Fc-$(CD89)_2$ demonstrated Ag and CD89 binding capabilities, respectively.

Purified 2E9IgA1 gave Acr-binding ELISA titers (dilution giving 30% of plateau OD) of 10–40 per microgram of protein. It bound Acr with a K_D of 6.99×10^{-8} M (Table I). A soluble form of human CD89, Fc-$(CD89)_2$, bound with similar affinity to 2E9IgA1 (1.17×10^{-7} M) and serum IgA (1.94×10^{-7} M), with K_D values in keeping with earlier reports for the IgA–CD89 interaction (27). Competition ELISA showed that whereas an IgG anti-Acr mAb (TBG65) inhibited binding of TBA61 to Acr-coated plates, 2E9IgA1 did not (data not shown). Moreover, 2E9IgA1 and TBA61 bound to different, partly overlapping, truncated Acr recombinants, and neither bound linear peptides of Acr (28) (data not shown). We conclude that 2E9IgA1 and TBA61 recognize different (yet structurally undefined) epitopes on Acr.

Immunotherapy of H37Rv infection in CD89tg mice

The influence of 2E9IgA1 inoculations on the course of infection by the H37Rv strain of *M. tuberculosis* was assessed in CD89tg mice. 2E9IgA1 was administered i.n. 2 h before infection (day 0) and on either day 1 or day 21 postinfection (Fig. 2). Both 2E9IgA1-inoculated and PBS-inoculated groups were also given 1 μg IFN-γ, shown previously to prolong TBA61 mouse IgA-mediated inhibition of infection (14). CFU values in the lungs at 4 wk postinfection (Fig. 2*A*) were significantly reduced in both 0+1 and 0+21 d 2E9IgA1-inoculated CD89tg mice (3.44×10^5 and 3.22×10^5 geometric mean CFU, respectively; t test $p = 0.006$ for both groups) compared with that of PBS-injected controls (5.15×10^6 CFU). Inoculation with 2E9IgA1 also significantly reduced splenic CFU but only in the 0+21 d group (16.1×10^3 CFU; t test $p = 0.026$) compared with that of PBS controls (1.57×10^5 CFU).

The representative H&E-stained sections of lungs of infected mice (Fig. 2*B*) demonstrate extensive granulomatous infiltration, which was substantially reduced in both 2E9IgA1 plus INF-γ treated groups of CD89tg mice. Morphometric quantitative evaluation (Fig. 2*C*) showed that both the 0+1 d and the 0+21 d inoculation schedules reduced the infiltrated granulomatous area highly significantly (t test: $p = 0.0009$ and $p = 0.0004$). The size of the lung infiltration area directly correlated with CFU counts (correlation coefficient $R^2 = 0.38$). In contrast with these differences in CD89tg mice, the CD89-negative littermate control

groups showed no significant differences either in lung and spleen CFU counts (Fig. 2*A*) or in lung granuloma areas (data not shown). Analysis of cellular composition of the lungs using formaldehyde-treated and Giemsa-stained cell suspensions showed that group mean values of macrophages (11–15%), neutrophils (8–10%), lymphocytes (14–17%), and epithelial cells (61–64%) did not differ significantly between the tested groups of mice (data not shown).

In the following experiment, the influence of IFN-γ plus 2E9IgA1 treatment was then compared with treatments with IFN-γ alone, or 2E9IgA1 alone, or PBS (Fig. 3). All groups of CD89tg mice were infected i.n. with H37Rv and were inoculated 3 d before infection with IFN-γ alone. 2E9IgA1 and IFN-γ either as single or as combined treatment was given 2 h before and on day 1 and on day 21 postinfection. Lung CFU counts 4 wk postinfection were best inhibited by combined treatment with IFN-γ plus 2E9IgA1 (4.33×10^4 geometric mean CFU; t test: $p = 0.0427$) compared with that of PBS controls (4.67×10^5 CFU). ANOVA evaluation showed no significant inhibition by IFN-γ alone ($p = 0.227$), but inhibition by 2E9IgA1 alone was borderline significant ($p = 0.062$). Although the combined IFN-γ plus 2E9IgA1 inoculation resulted in the lowest CFU counts, the difference from the group inoculated with 2E9IgA1 alone was not significant ($p = 0.69$). Lung CFU counts in the IFN-γ–only treated group were slightly, though not significantly reduced, perhaps due to the fact that IFN-γ was inoculated four times rather than three times, as done in the experiment shown in Fig. 2. Unlike the lung CFU counts, differences in splenic CFU counts between the groups were not significant.

Modulation of H37Rv-lux infection of human whole blood and purified monocytes

Modulation of infection with H37Rv-*lux* was evaluated in cultures of whole blood from six healthy volunteer donors. The results were evaluated only from experiments where the infection increased ~10-fold during the 3-d incubation period. To assess in vitro protective capacity, 2E9IgA1 with or without 10 ng/ml IFN-γ was added to cultures. In a representative experiment (Fig. 4*A*), significant ($p = 0.009$; ~90%) decrease of RLU values of H37Rv-*lux* luminescence was imparted by 100 µg/ml 2E9IgA1 but not by colostrum IgA or IgG. In a separate experiment (Fig. 4*B*), addition of IFN-γ did not increase the significant inhibitory effect of 2E9IgA1 ($p = 0.0008$), whereas the isotype control (anti-NIP) Ab was not inhibitory. In another experiment, testing blood samples from two separate donors (Fig. 4*C*), RLU values of donor A were diminished only marginally by the addition of IFN-γ alone ($p = 0.023$) but were inhibited significantly ($p = 0.0047$) by the presence of both 2E9IgA1 and IFN-γ ($p = 0.005$). However, the inhibition in donor B was not significant ($p = 0.053$). Variation similar to that between donors A and B was observed also in other experiments with different donors, but its nature could not be analyzed in further detail with the relatively small sample of tested donors. To increase the sensitivity of inhibition, we reduced the H37Rv-*lux* infection dose from 100×10^3 RLU (used above) to 30×10^3 or 10×10^3 RLU (Fig. 4*D*). However, the degree of inhibition after 3 d of incubation improved only marginally ($p = 0.007$, $p = 0.004$, $p = 0.002$), and the lung RLU values approached the detection threshold. Incubation of purified monocytes (Fig. 4*E*) with 2E9IgA1 plus IFN-γ reduced RLU values at 72 h ($p = 0.008$) and 108 h ($p = 0.009$) of incubation, but not at 18 h. However, there was no significant inhibition by 2E9IgA1 in the absence of human IFN-γ.

Discussion

The main constituent purified from the 2E9IgA1 expressed in CHO-K1 stable transfectant cells was 170 kDa, corresponding to monomeric IgA and containing both O-linked and N-linked sugars, which are important for avoiding the formation of pathogenic immune complexes (29). Being monomeric, this IgA1 would not interact with the pIgR and hence would not form secretory IgA molecules. The binding affinity of 2E9IgA to the Acr Ag ($7 \times$

10^{-8} M) was found to be much higher than that of the mouse IgA mAb TBA61 (2.94×10^{-6} M), which was previously found to be protective in BALB/c mice.

Intranasal inoculations of 2E9IgA1 and IFN-γ reduced the pulmonary infection in CD89tg mice but not in littermate controls, demonstrating that protection depends on interaction of 2E9IgA1 with CD89. Although infection was most reduced by the combined inoculation of 2E9IgA1 with IFN-γ, statistical evaluation of the group differences by the ANOVA test failed to show significant synergy between the action of these two agents. Inhibition of infection of purified human monocytes after 72–108 h of culture was significant only when both 2E9IgA1 and human rIFN-γ were added to the culture. In contrast, there was no consistent synergy between the actions of 2E9IgA1 and IFN-γ in cultures of human whole blood. This could have been due to the action of IFN-γ, secreted by neutrophils or by other cells present in the whole blood. Our previous demonstration of longer persistence of passive protection by co-inoculation of mouse IgA anti-Acr with mouse IFN-γ could have been due to greater IFN-γ dependency of the mouse IgA receptor expression (14).

The human CD89 receptor, targeted by the 2E9IgA1 treatment, is known to be expressed strongly on neutrophils in CD89 transgenic mice (24), whereas macrophages in these mice express it when induced by GM-CSF (24) or other cytokines, which may be elevated in *M. tuberculosis*-infected mice. Dendritic cells can also express CD89 (30), and therefore their infection with *M. tuberculosis* (31) may also have been targeted by 2E9IgA1. We postulate that passive monomeric IgA bound to the surface of CD89-positive alveolar macrophages and/or neutrophils could bind the infecting *M. tuberculosis* organisms. Uptake of this complex could then activate bactericidal activity of the infected cells. It is conceivable that the efficacy of TB immunotherapy could further be enhanced by inoculation of immunomodulators that increase the expression of CD89 on target cells. It would also be of interest to ascertain whether the reported profound synergy of anti–IL-4 treatment with mouse IgA action (15, 16) could also apply with respect to the human IgA–CD89 interaction. In an endeavor to increase further the efficacy of immunotherapy, multimerization or site-directed mutagenesis of the 2E9IgA1 mAb could be explored.

Studies in a different CD89tg strain, in which CD89 expression is driven by the CD11b promoter (19) and that strongly express and shed CD89 (27), suggested that CD89 can mediate dual signals, controlled by the ITAM activation motif: a) proinflammatory, induced by IgA immune complexes leading to multimeric receptor aggregation; or b) inhibitory, induced by low-affinity binding by serum IgA, which can prevent the development of autoimmunity and inflammation. We postulate that the i.n. inoculation of 2E9IgA1 induced proinflammatory cellular responses, which could have imparted protection by promoting apoptosis of *M. tuberculosis*-infected macrophages (12).

Human and mouse IgA1 constant regions share only 58% identical amino acids and contain different O-linked and N-linked glycosylation patterns, leading to distinct biological functions. The cellular mechanisms of protection by the mouse IgA mAb (TBA61) could not have been addressed in previously reported experiments, in view of the lack of a CD89 homologue in the mouse. Therefore, availability of the novel human IgA mAb was the first opportunity for ascertaining the role of FcαRI/CD89. Consequently, our finding of its mandatory role in mediating protection is of significant novelty and importance.

The in vitro experiments indicated that 2E9IgA1 is able to reduce *M. tuberculosis* infection, consistent with our in vivo findings. Our finding of interdonor variability is not too surprising given previous results in the mouse model, where IgA-mediated inhibition of macrophage infection was less pronounced than protection in vivo (14). This outcome was attributed to the involvement of other cells, cytokines, or endogenous factors in the lungs.

There may also be contributions from polymorphic variation in FcαRI functional activity between human donors (32), akin to the recognized association of certain Fc receptors with differential disease outcomes (33). However, detailed insights into this variation in protection need larger-scale testing of human samples.

In conclusion, the described protection against *M. tuberculosis* infection by i.n. treatment with human IgA mAb and rIFN-γ represents an important step toward the possibility of passive immunotherapy in TB patients. TB research has been exploring different avenues such as diagnosis/biomarkers, drug discovery, and vaccination, all facing obstacles (34), which leaves scope for taking up new approaches. With this view, continued interest in passive immunotherapy of TB seems justified by the presented results obtained with the new human IgA mAb. The clinical potential is particularly toward HIV-associated TB, where immune compromise precludes active vaccination. Because a large proportion of AIDS patients are at risk for TB reactivation, and because simultaneous HIV/TB treatment is complicated by drug–drug interactions, the potential clinical benefits from the immunotherapy of TB could be significant. In addition, the prospect of shortening the chemotherapy regimen would have even wider impact because the currently used protracted regimens are blighted by defaulting. In this regard, the capacity of passive immunotherapy to reduce postchemotherapy relapse in mouse models (9, 16) suggests that combined immunotherapy can be effective even when started after the host infection. Finally, tackling the multidrug-resistant TB could be another challenging opportunity.

Acknowledgments

Abbreviations used in this article

Acr	α-crystallin
CD89tg	mice transgenic for human CD89
i.n.	intranasal
RLU	relative light unit
scFv	single-chain variable fragment
TB	tuberculosis

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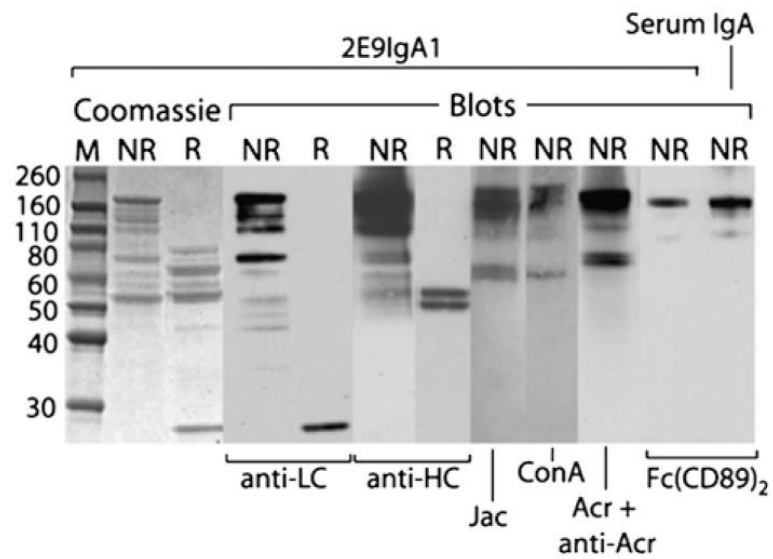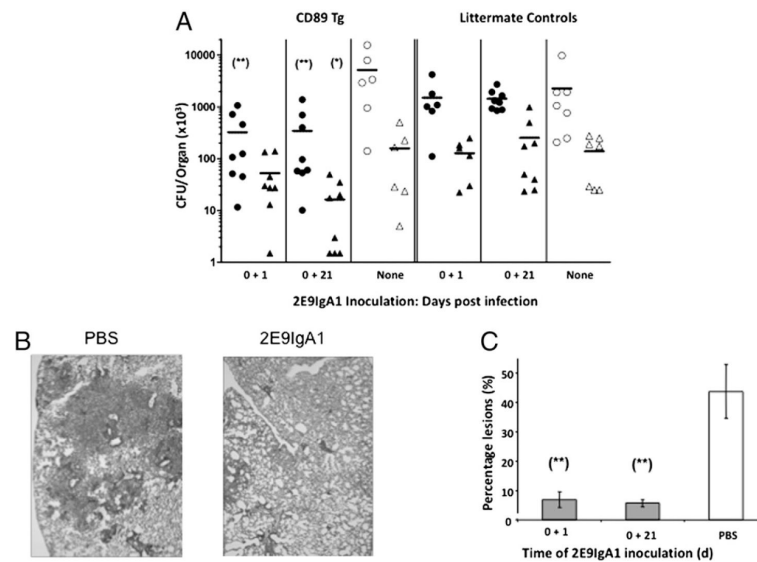
FIGURE 1.
Characterization of purified 2E9IgA1. SDS-PAGE (Coomassie) and Western blot analysis probed with anti-L chain, anti-IgA, Con A, jacalin (Jac), Acr followed by anti-Acr, and Fc-(CD89)$_2$. Serum IgA served as a control for Fc-(CD89)$_2$ binding. M, m.w. markers; NR, non-reduced samples; R, reduced samples.

FIGURE 2.
Modulation of *M. tuberculosis* infection in FcαRI/CD89 transgenic mice. Mice in all three groups were preinoculated i.n. with 1 μg mouse IFN-γ 3 d before i.n. infection with 0.5 million H37Rv. Five micrograms purified 2E9IgA1 (titer 16,500) mixed with either IFN-γ (closed symbols) or PBS (open symbols) was given i.n. 2 h before infection and again either 1 d or 21 d postinfection. Organs were harvested 4 wk postinfection. *A*, Group geometric means (horizontal bars) of CFU counts in the lungs (circles) and spleens (triangles) of individual mice. **p < 0.001, *p < 0.05 (significant difference between 2E9IgA1-inoculated and PBS-inoculated groups; *t* test). *B*, H&E-stained lung sections. Original magnification ×20. *C*, Means ± SE and *t* test values of granulomatous infiltration of the lungs from CD89tg mice. **p < 0.001.
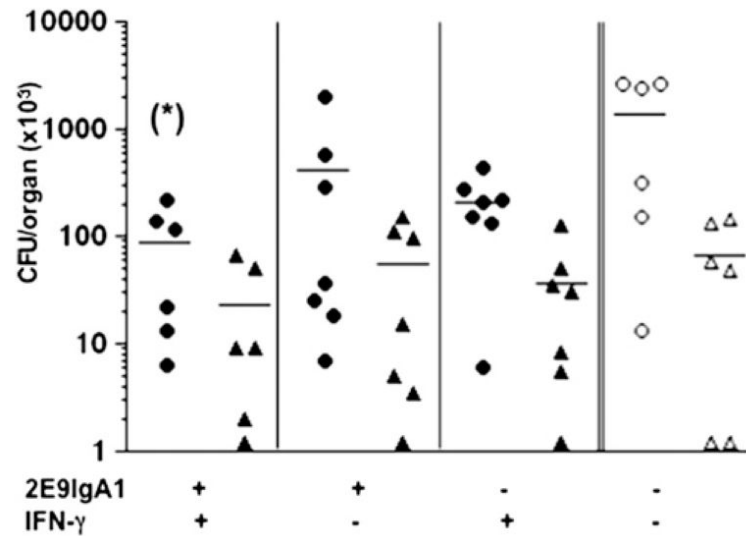
FIGURE 3.
Influence of combined and single i.n. inoculations of 2E9IgA1 and IFN-γ on *M. tuberculosis* infection. One microgram mouse IFN-γ was given 3 d before infection with 0.5 million H37Rv. Five micrograms purified 2E9IgA1 mixed with either IFN-γ or PBS was given 2 h before infection and again 1 d and 21 d postinfection. Organs were harvested 4 wk postinfection. CFU count data in the lungs (circles) and spleens (triangles) of individual mice and group geometric means (horizontal bars) are shown. *$p < 0.05$ (significant difference compared with the PBS-inoculated control group; *t* test).
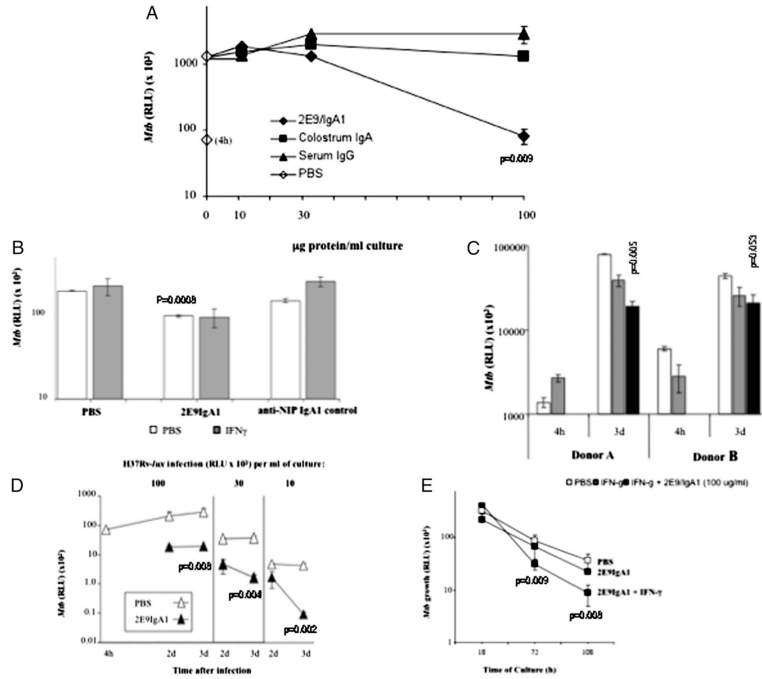
FIGURE 4.
Modulation of H37Rv-*lux* infection in vitro. Whole human blood cultures were incubated with 10 ng/ml human IFN-γ and 2E9IgA1 for 24 h prior to infection with 10^6 RLU H37Rv-*lux* for 2 h, followed by amikacin (200 μg/ml) treatment for 4 h. Chemiluminescence was determined after incubation at 37°C for 3 d. *A*, Influence of 2E9IgA, colostrum IgA, and serum IgG dose. *B* and *C*, Influence of human IFN-γ in different donors. *D*, Influence of different dosage of H37Rv-*lux* infection using 2E9IgA1 (closed symbols) or PBS (open symbols). *E*, Modulation of infection of purified human monocytes with 20 μg/ml 2E9IgA1 with or without IFN-γ before infection with H37Rv-*lux*. The *p* values represent the significance of the difference in comparison with the PBS controls (*t* test).

Table I

Binding affinity of 2E9IgA1 for Acr and Fc-(CD89)$_2$

Sensor Chip Ligand	Soluble Analyte	Reaction Stage	Rate Constants[a]		Affinity KD (M)[a]	Analysis Model
			K_a (M^{-1} s^{-1})	K_d (s^{-1})		
Acr	2E9IgA1	Early	8.9×10^4	1.5×10^{-2}	6.90×10^{-8}	Two-step linked reaction
		Late	3.0×10^{-3}	1.8×10^{-3}		
Fc-(CD89)$_2$	2E9IgA1	n.a.	5.1×10^4	5.9×10^{-3}	1.17×10^{-7}	Langmuir (1:1)
	Serum IgA	n.a.	1.3×10^4	2.7×10^{-3}	1.94×10^{-7}	

[a] Mean values obtained from analysis by the model that produced the best χ^2 (<1) value after replicate testing of three or two concentrations (50–1000 nM) of analyte.

n.a., not applicable.