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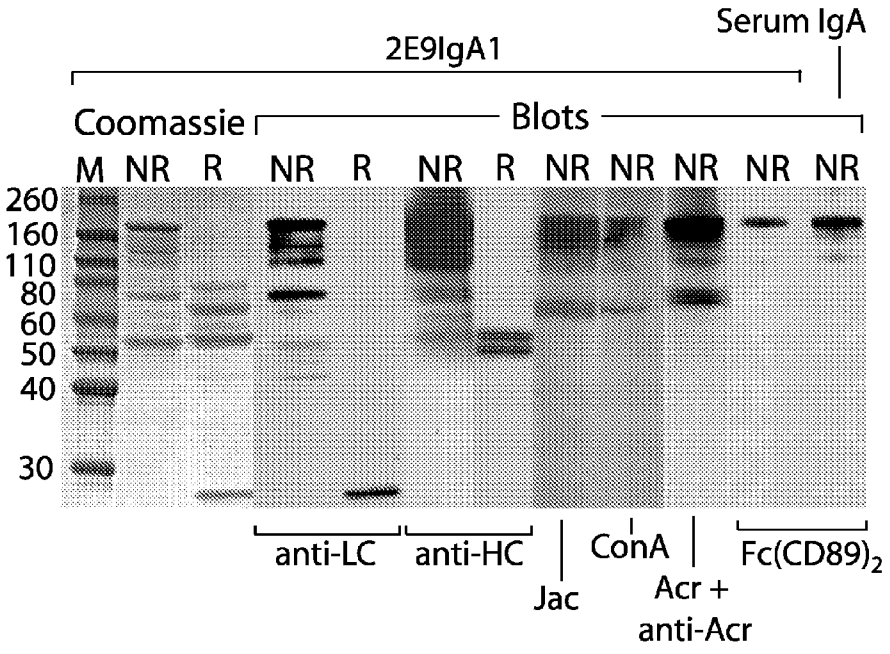


Figure 1

(57) Abstract: The present invention relates to antibodies to Mycobacterium tuberculosis, and to methods, compositions and cells associated with such antibodies. Such antibodies are useful of the treatment of tuberculosis, particularly but not exclusively through passive immunotherapy.

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Antibodies to Mycobacteria

The present invention relates to antibodies to Mycobacterium tuberculosis, and to methods, compositions and cells associated with such antibodies. Such antibodies are useful for the treatment of tuberculosis, in particular through passive immunotherapy.

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 antibody (Ab) treatment is of particular interest for use in immunocompromised HIV-infected populations, who show faster disease progression and toxicity from overlapping HIV and TB treatments, and also 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). 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). 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).

The immunological system in mice is, however, different in many ways to the human system, in particular with respect to the mode of action of IgA and the pathways of passive immunotherapy. In particular, there are significant differences between IgA in mice and IgA in humans. In fact the amino acid sequence of the constant regions of the heavy chain of mouse IgA is only 58% identical to that of human IgA1. Importantly, mouse IgA cannot bind to human CD89 (FcαRI) because of differences

in amino acid sequence and glycosylation at the Fc site known to be critical for interaction with human CD89 (20; Wines BD *et al*. 2011. J. Biol. Chem. 286(38):33118-24). Furthermore, mice lack an equivalent of human CD89 (FcαRI) which plays a major role in IgA-mediated effector mechanisms. 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 mouse IgA-mediated effector function.

In a first aspect the present invention provides an isolated human monoclonal antibody which binds to mycobacterial alpha crystallin. Preferably the antibody binds to alpha crystallin of *M. tuberculosis*.

Preferably the antibody binds with an affinity (K_D) of 9×10^{-8} M or higher, preferably 6.9×10^{-8} M or higher, optionally 5×10^{-8} M or higher. For the avoidance of doubt, a higher affinity is represented by a smaller K_D value. Methods to determine the K_D of an antibody are well known in the art, and one technique is described below.

It is preferred that the antibody binds to an epitope of alpha crystallin other than that bound by the mouse antibodies TBA61 or TBG65. This can be determined by testing whether an antibody binds competitively with TBA61 or TBG65, e.g. using techniques described below, and well known to the person skilled in the art.

In one embodiment the antibody binds to alpha crystallin competitively with 2E9IgA1. Where two antibodies bind competitively with each other it is known that they are targeted to the same epitope. A preferred antibody is one which binds to alpha crystallin both competitively and preferentially to 2E9IgA1, for example an antibody which targets the same epitope as 2E9IgA1 but with a higher affinity.

In certain embodiments the antibody can be a whole antibody, i.e. containing all the components of a natural antibody (in particular including complete Fab and Fc portions), or it can be an antibody fragment, provided said fragment retains the necessary functions to provide the desired biological effect.

A wide variety of antibody fragments have been developed including:

- Fab
- scFv
- dsFv
- (dsFv)2
- Diabody
- Minibody
- Flex minibody
- Bi-specific fragments
- Bi-specific antibodies

It is envisaged that such fragments, provided all scaffold and constant regions are human, can form embodiments of the present invention. However, it is highly preferred that any antibody fragment has the capacity to induce an immunological effect against a mycobacterium bound to by the antibody fragment. The Fc region of an antibody is the principal coordinator of such functionality, and thus it is preferable that at least a portion of the Fc region is present. An alternative is a bi-specific antibody fragment in which 1 portion binds the antigen and the other binds to, and activates, a modulator of immunological function (e.g. a leucocyte surface molecule). Overall, it is generally preferred that the antibody is a substantially whole human antibody.

It is preferred that the antibody is glycosylated, and it is generally preferred that it is glycosylated with a human glycosylation pattern appropriate to the particular class of antibody. The glycosylation pattern will be determined by both the sequence of the antibody and the cell in which the antibody is expressed. A completely human glycosylation pattern can be achieved by expressing a human antibody in a human cell. However, in alternative embodiments of the invention the antibody could be expressed in a non-human cell, and thus the glycosylation pattern may be non-human.

It is preferred that the antibody of the present invention is able to activate CD89. CD89 (FcαRI) is an important receptor in mediating passive immunotherapy in

humans, and accordingly it is preferred that the antibody is able to invoke this effect in a subject via this receptor. In order to achieve this, the antibody must contain regions which bind to and activate CD89. In the case of a whole antibody such features would be contained in the Fc region of IgA.

It is preferred that the antibody is an IgA, i.e. a human IgA. As mentioned above, IgA in humans and IgA in mice are very different entities, with different functionality. Furthermore, IgG is the standard class of antibody used to generate therapeutic antibodies in humans, and as such the development of a human therapeutic IgA antibody against a TB antigen is a particularly significant and surprising development. In light of the prejudice in the art towards using IgG, and technical difficulties and uncertainties in creating a therapeutic human IgA, it is surprising to have arrived at the present invention.

Advantages associated with IgA in the context of the present invention include:

- IgA appears to be better suited to function at mucosal sites such as in the lungs than other antibody classes, e.g. IgG.
- IgA appears to be well suited for administration by inhalation, which would deliver it to the lungs.
- IgA interaction with CD89 can result in better triggering of effector mechanisms (such as cell killing and ADCC [antibody dependent cell-mediated cytotoxicity]) than equivalent engagement of FcgammaR (the receptors specific for IgG).
- IgA does not activate the classical pathway of complement, whereas IgG (specifically human IgG1, IgG3 and to a lesser extent IgG2) does. It may be advantageous to use an antibody that is incapable of complement activation, particularly since excessive complement activation can be associated with undesirable inflammation.

In a preferred embodiment the antibody is an IgA1. Due to its long hinge region, IgA1 is more likely than IgG to be able to mediate bivalent binding (and therefore higher avidity binding) to antigens spaced distantly apart, i.e. IgA has greater reach from Fab tip to Fab tip. Higher avidity binding may allow lower dosing strategies, which might be more cost effective.

It is highly preferred that the antibody of the present invention is capable of inducing passive immunotherapy of mycobacterial tuberculosis in a subject, more preferably a human. It appears that an IgA antibody which binds to mycobacterial alpha crystallin is generally capable of achieving this result. However, it is possible that some such antibodies may not achieve this result, e.g. perhaps because they bind an epitope which is not available for binding in an in vivo context, and such antibodies are less preferred. It is possible to test for such activity using the transgenic mouse model described below. Promising initial indications of activity in mice can be confirmed through other trials, e.g. human trials.

In one embodiment of the present invention the antibody comprises CDRs comprising or consisting of the following amino acid sequences:
Heavy chain – (CDR1) GFTFSSYA, (CDR2) IQSLGIVT and (CDR3) AKRPATFDY (Sequence ID Nos 9-11)
Light chain – (CDR1) QSISSY (Sequence ID No 12), (CDR2) RAS and (CDR3) QRRSFPLT (Sequence ID No 13)

Amino acid sequences are given in the N to C direction, unless otherwise stated.

As is well known in the art, it is the CDRs which are principally responsible for the specific binding of an antibody to an epitope. Accordingly, any antibody which comprises the abovementioned CDRs can be expected to bind to alpha crystallin. These CDRs could, for example, be engineered into any recombinant human Fv; optimisation of framework regions may be required, but this is within the abilities of the skilled person.

The antibody may suitably comprise a variable region, e.g. an Fv, comprising:

a V_H portion comprising the sequence: EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTIQSL GIVTAYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRPATFDYWGQGT LVTVSS (Sequence ID No 14)

and/or a V_L portion comprising the sequence:

DIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYRASKLQSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQRRSFPLTFGQGTKLEIK
(Sequence ID No 15)

Portions in which the CDR sequences are identical to the above-mentioned sequences, and which have framework regions having 80% or higher, preferably 90% or higher, preferably 95% or higher, sequence identity to the above-mentioned sequences also form part of the present invention, provided the antibody binds alpha crystallin with an affinity of 9 x 10^{-8} M or higher, preferably 6.9 x 10^{-8} M or higher, optionally 5 x 10^{-8} M or higher. In this regard it is noted that the sequence of framework regions can vary to some extent without affecting antigen binding. In particular, conservative amino acid changes which do not significantly disrupt protein folding can be easily accommodated in the framework region without compromising antigen binding.

In a preferred embodiment the antibody comprises both V_H or V_L portions as defined above. However, it is possible that the antibody may comprise one or other of the V_H or V_L portions mentioned above, and a different V_H or V_L portion, e.g. one having different framework sequences, but the same CDRs, provided the antibody still binds alpha crystallin with an affinity of 9 x 10^{-8} M or higher, preferably 6.9 x 10^{-8} M or higher, optionally 5 x 10^{-8} M or higher.

As is known in the art, an antibody comprises a variable region comprising a heavy portion and a light portion (or chain). Each of these portions comprises three CDRs, as mentioned above, and framework or scaffold regions. The framework or scaffold regions act to support the CDRs and position them correctly for interaction with a target antigen.

In a particularly preferred embodiment the antibody comprises a Fab which comprises:

a heavy chain having the amino acid sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTIQSL
GIVTAYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRPATFDYWGQGT
LVTVSSSPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTAR

NFPPSQDASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCP (Sequence ID No 16)

and/or a light portion comprising the amino acid sequence:

DIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYRASKLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQRRSFPLTFGQGTKLEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (Sequence ID No 17)

The antibody can suitably comprise:

- a heavy chain comprising or consisting of the amino acid sequence of Sequence ID No 2, and
- a light chain comprising or consisting of the amino acid sequence of Sequence ID No 4.

In one embodiment the antibody comprises or consists of human monoclonal antibody 2E9IgA1. 2E9IgA1 is a monomer of IgA.

The antibody of the invention can either be a monomer or a dimer, e.g. a dimer of 2E9IgA1.

The antibody can be a secretory IgA. Typically this is in the form of an IgA dimer bound to a secretory component.

In a second aspect, the present invention provides a method of treating or preventing tuberculosis in a subject, said method comprising administering to said subject an antibody as set out above.

The antibody should be administered at a therapeutic dose. Suitable doses and dosage regimes can be determined by the person skilled in the art, e.g. following routine clinical trials.

Suitably the method is a method of passive immunotherapy.

In a preferred embodiment the method is for treating TB in immune-compromised subjects, e.g. those suffering from HIV/AIDS. Alternatively or additionally, the method may be for treating drug resistant TB, e.g. multi-drug resistant TB.

In a preferred embodiment the antibody is administered in combination with an adjuvant, carrier, diluent or excipient. In a preferred embodiment the antibody is administered in combination with interferon gamma and/or antagonist of interleukin-4, e.g. an antibody against interleukin 4. It has been shown that co-administration with these agents may provide a synergistic increase in efficacy. The administration of interferon gamma and/or an antibody against interleukin 4 can occur simultaneously with the administration of the antibody, or at a different time point within the same dosage regime.

Suitably the antibody is administered via the airways, e.g. intranasally or via oral or nasal inhalation. It appears that IgA targeting alpha crystallin is particularly well adapted to have therapeutic effect when administered via the airways. Methods for formulating antibodies for nasal administration are known in the art, e.g. for administration as an aerosol via a nasal spray or nebulisation.

The antibodies may be administered in the form of droplets containing the pharmaceutical formulation, the droplets having a diameter of from 100 to 5000 µm, preferably 500 to 4000 µm, more preferably 1000 to 3000 µm. In terms of volume, the droplets may be in the range of 0.001 to 100 µl, preferably 0.1 to 50 µl, more preferably 1.0 to 25 µl. Smaller particles may be desired if an aerosol is to be formed, in which case particles suitably have diameters in the range of 0.1 to 50 µm, preferably 1 to 25 µm, more preferably 1-5 µm. To obtain such droplets the formulation may be administered as an atomised nasal spray or via nebulisation, as is known in the art.

Alternatively the antibodies may be in the form of an aerosol of a powder, in which case the same particle sizes as mentioned above are suitable.

The size of particles (droplets or powder) has an effect on the ability of a formulation to travel into the lungs; smaller particles generally being able to travel further into the

lungs. Particle size can thus be selected to determine to some extent the area to be treated by the formulation. It is typically desirable to select a particle size which allows delivery of the antibody to the bronchi, bronchioles, and alveoli. However, a more restrictive delivery may be desired in some instances e.g. to limit or exclude delivery to the alveoli to avoid alveoli associated problems such as inflammation and fibrotic scarring of the lungs.

The term "preventing" includes reducing the severity/intensity of, or initiation of, a mycobacterial infection.

The term "treating" includes post-infection therapy and amelioration of a mycobacterial infection.

In a third aspect the present invention provides a genetically modified cell or cell line expressing or adapted to express an antibody as described above. To this end, the cell or cell line will contain nucleic acid sequences suitable to be expressed in said cell to produce the antibody.

Suitably the cell or cell line is mammalian, e.g. a CHO or human cell line. The cell line may suitably be a hybridoma cell line. One exemplary embodiment is the cell line 2E9IgA1 transfectant CHO-K1 cells described below.

Suitably the cell or cell line comprises a nucleic acid comprising Sequence ID No 1 and a nucleic acid comprising Sequence ID No 3, each of said nucleic acid sequences being adapted for expression in said cell or cell line.

In a fourth aspect the present invention provides an expression vector comprising an expressible region encoding an antibody as described above.

The expression vector suitably comprises the nucleic acid sequence of Sequence ID No 1 and/or Sequence ID No 3.

The expression vector suitably comprises the nucleic acid sequence of Sequence ID No 1 and/or Sequence ID No 3 associated with suitable sequences to drive expression in a cell, e.g. mammalian cell.

An exemplary expression vector is the mammalian expression vector VHexpress, but other suitable vectors are well known and readily available in the art.

In a fifth aspect the present invention provides a pharmaceutical formulation comprising an antibody as described above. Such a formulation may comprise the antibody and a pharmaceutically acceptable carrier, diluent, adjuvant or excipient.

The pharmaceutical formulation may suitably be formulated for passive immunotherapy.

The pharmaceutical formulation may suitably be formulated for delivery via the airways, e.g. via intranasal delivery.

Such formulations may be adapted to form droplets containing the pharmaceutical formulation, e.g. as an aerosol.

Alternatively the formulation may be adapted to form an aerosol of a powder.

The pharmaceutical formulation may suitably comprise interferon gamma and/or an antibody against interleukin 4.

In a sixth aspect the present invention provides an antibody as set out above for use in the prevention or treatment of mycobacterial infection, e.g. tuberculosis.

In a seventh aspect the present invention provides the use of an antibody as defined above in the manufacture of a medicament for the treatment of mycobacterial infection, e.g. tuberculosis.

In an eighth aspect the present invention provides a pharmaceutical formulation of said antibody in association with an apparatus adapted to form an aerosol of the

pharmaceutical formulation. For example a nasal spray or nebuliser containing said pharmaceutical formulation.

The invention will now be described, by way of example only, with reference to the accompanying figures.

Brief Description of the Figures

- Figure 1 - Characterisation 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 pre-inoculated 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 post-infection. Organs were harvested 4 wk post-infection. *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 post-infection. Organs were harvested 4 wk post-infection. 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).

- Figure 5 shows the DNA sequence encoding the heavy chain of 2E9IgA1 (5' to 3')(arranged by exon; the hinge is encoded at the start of the CH2 exon. Leader sequence is not included) – Sequence ID No 1.
- Figure 6 shows the amino acid sequence of the heavy chain of 2E9IgA1 (N terminus to C terminus)(arranged VH, CH1, hinge, CH2, CH3; CDRs are highlighted in VH) – Sequence ID No 2.
- Figure 7 shows the DNA sequence encoding the light chain of 2E9IgA1 (5' to 3') (arranged by exon; leader sequence is not included). – Sequence ID No 3.
- Figure 8 shows the amino acid sequence of the light chain of 2E9IgA1 ((N terminus to C terminus) (arranged into VL and Ck domains. CDRs are highlighted) – Sequence ID No 4.
- Figure 9 shows the results of a Western blot analysis of antibody binding to wild type and truncated Acr. Truncation involved removal (by genetic means) of the 20-mer peptide sequence SEFAYGSFVRTVSLPVGADE (Sequence ID No 18) to form AcrΔ; the sequences correspond to a dominant overlapping CD4/CD8 T cell epitope of Acr.

The present inventors have developed a novel antibody which offers the potential to treat TB in humans. They generated a novel Acr-specific human IgA1 and used 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-D-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′) (Sequence ID No 5) and Link Seq New (5′-CGACCCGCCACCGCCGCTG-3′) (Sequence ID No 6) for the VH and DPK9 (5′-CATCTGTAGGAGACAGAGTC-3′) (Sequence ID No 7) and PHEN (5′-CTATGCGGCCCCATTCA-3′) (Sequence ID No 8) 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 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 non-reducing 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 non-covalently 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; thus TBA61 and TBG65 did not bind to Acr variant missing the peptide SEFAYGSFVRTVSLPVGADE (Sequence ID No 18) (ACRΔ), indicating that their epitope is fully or partially contained within this sequence; in contrast, 2E9 did bind to the same truncated Acr

variant, indicating that its epitope is not contained within this sequence (See Fig 9). Neither antibody bound
linear peptides of Acr (28) (data not shown). We conclude that 2E9IgA1 and TBA61 recognize different (yet structurally undefined) epitopes on Acr.

Table I

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

			Rate Constants[a]			
Sensor Chip Ligand	**Soluble Analyte**	**Reaction Stage**	**K_a (M^{-1} s^{-1})**	**K_d (s^{-1})**	**Affinity KD (M)[a]**	**Analysis Model**
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.

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 post-infection (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 post-infection. Lung CFU counts 4 wk post-infection 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 analysed 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 (7×10^{-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 actions 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 infecting M. tuberculosis organisms could engage CD89-positive alveolar macrophages and/or neutrophils, resulting in their enhanced phagocytic activity. Uptake of this complex via CD89 receptor as opposed to spontaneous (non-receptor mediated) phagocytic uptake 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 endeavour to increase further the efficacy of immunotherapy, multimerisation or site-directed mutagenesis of the 2E9IgA1 mAb could be explored.

Studies in a different CD89 transgenic 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) pro-inflammatory, 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 pro-inflammatory 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 inter-donor 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. The clinical potential of the present invention 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 post-chemotherapy 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.

Abbreviations

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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Claims

1. An isolated human monoclonal antibody which binds to mycobacterial alpha crystallin.

2. The antibody of claim 1 which binds with an affinity (K_D) of 9 x 10^{-8} M or higher.

3. The antibody of claim 1or 2 which binds to an epitope of alpha crystallin other than that bound by the mouse antibodies TBA61 or TBG65.

4. The antibody of any preceding claim which binds to alpha crystallin competitively with 2E9IgA1.

5. The antibody of any preceding claim which is able to activate CD89.

6. The antibody of any preceding claim which is an IgA.

7. The antibody of any preceding claim which is an IgA1.

8. The antibody of any preceding claim which comprises CDRs comprising the following amino acid sequences:
   - Heavy chain – GFTFSSYA, IQSLGIVT and AKRPATFDY (Sequence ID Nos 9-11)
   - Light chain – QSISSY (Sequence ID No 12), RAS and QRRSFPLT (Sequence ID No 13)

9. The antibody of any preceding claim which comprises a variable region comprising:
   - a V_H portion comprising the sequence: EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEW VSTIQSLGIVTAYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA KRPATFDYWGQGTLVTVSS (Sequence ID No 14)

- and/or a V_L portion comprising the sequence: DIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYR ASKLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQRRSFPLTFG QGTKLEIK (Sequence ID No 15).

10. The antibody of any preceding claim which comprises a Fab which comprises:
    - a heavy chain having the amino acid sequence: EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVS TIQSLGIVTAYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRPA TFDYWGQGTLVTVSSSPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPL SVTWSESGQGVTARNFPPSQDASGDLYTTSSQLTLPATQCLAGKSVTCHV KHYTNPSQDVTVPCP (Sequence ID No 16)
    - and/or a light portion comprising the amino acid sequence: DIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYRASK LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQRRSFPLTFGQGTKL EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC (Sequence ID No 17)

11. The antibody of any preceding claim which comprises:
    - a heavy chain comprising or consisting of the amino acid sequence of Sequence ID No 2, and
    - a light chain comprising or consisting of the amino acid sequence of Sequence ID No 4.

12. The antibody of any preceding claim which comprises human monoclonal antibody 2E9IgA1.

13. A method of treating or preventing tuberculosis in a subject, said method comprising administering to said subject an antibody as set out in any preceding claim.

14. The method of claim 13 which is a method of passive immunotherapy.

15. The method of claim 13 or 14 which is for treating TB in an immunocompromised subject or for treating drug resistant TB.

16. The method of any one of claims 13 to 15 wherein the antibody is administered in combination with an adjuvant, carrier, diluent or excipient.

17. The method of any one of claims 13 to 16 wherein the antibody is administered in combination with interferon gamma and/or antagonist of interleukin 4.

18. The method of any one of claims 13 to 17 wherein the antibody is administered via the airways.

19. A genetically modified cell or cell line expressing or adapted to express an antibody as described above.

20. The cell or cell line of claim 19 is mammalian, e.g. a CHO or human cell line.

21. The cell or cell line of claim 19 or 20 which comprises a nucleic acid comprising Sequence ID No 1 and a nucleic acid comprising Sequence ID No 3, each of said nucleic acid sequences being adapted for expression in said cell or cell line.

22. An expression vector comprising an expressible region encoding an antibody as claimed in any one of claims 1 to 12.

23. The expression vector of claim 22 comprising the nucleic acid sequence of Sequence ID No 1 and/or Sequence ID No 3.

24. The expression vector of claim 23 comprising the nucleic acid sequence of Sequence ID No 1 and/or Sequence ID No 3 associated with suitable sequences to drive expression in a mammalian cell.

25. A pharmaceutical formulation comprising an antibody as claimed in any one of claims 1 to 12.

26. The pharmaceutical formulation of claim 25 formulated for passive immunotherapy.

27. The pharmaceutical formulation of claim 25 or 26 formulated for delivery via the airways.

28. The pharmaceutical formulation of claim 28 adapted to from an aerosol.

29. The pharmaceutical formulation of any one of claims 25 to 28 comprising interferon gamma and/or antagonist of interleukin 4.

30. An antibody as set out in any one of claims 1 to 12 for use in the prevention or treatment of mycobacterial infection.

31. The use of an antibody as set out in any one of claims 1 to 12 in the manufacture of a medicament for the prevention or treatment of mycobacterial infection.

32. A pharmaceutical formulation as claimed in any one of claims 25 to 29 in association with an apparatus adapted to form an aerosol of the pharmaceutical formulation.

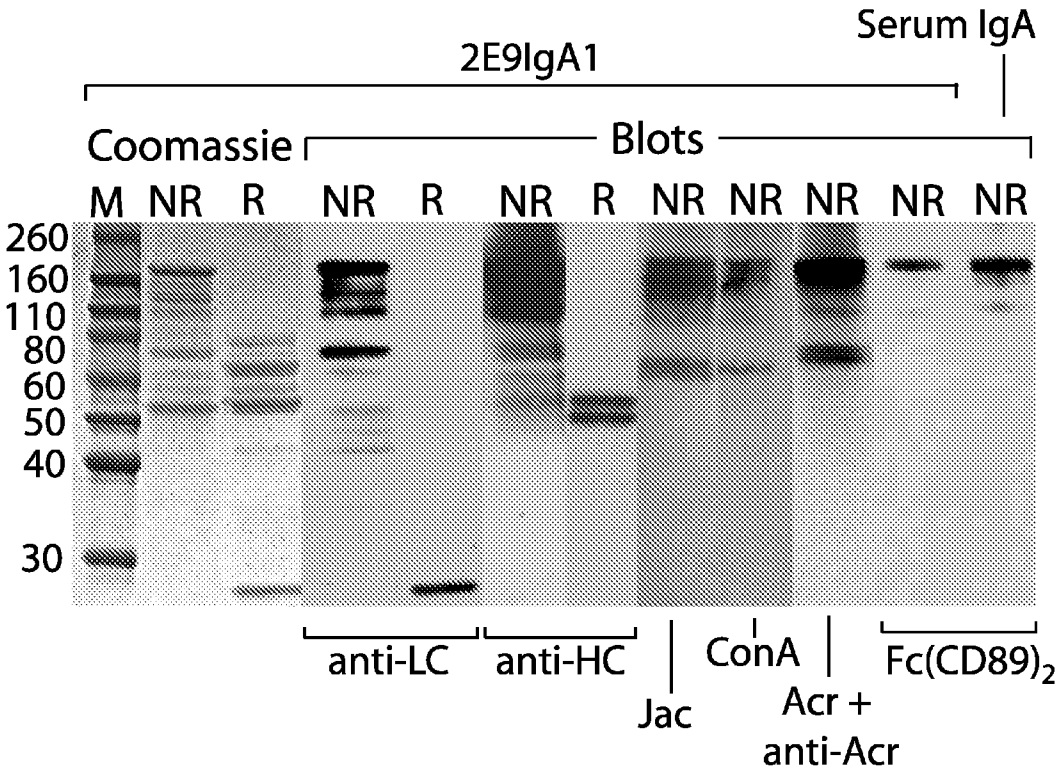
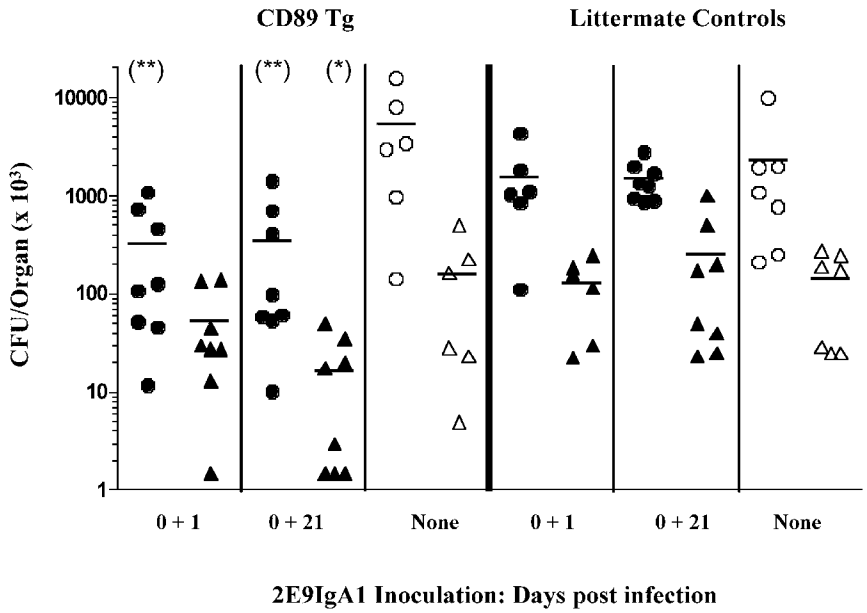
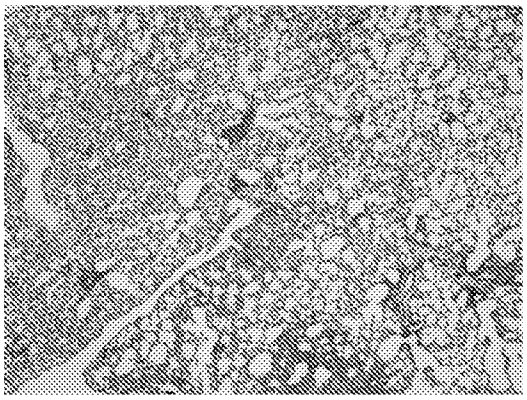

Serum IgA
2E9IgA1
Coomassie
Blots
M NR R NR R NR R NR NR NR NR NR
260
160
110
80
60
50
40
30
anti-LC
anti-HC
Jac
ConA
Acr +
anti-Acr
Fc(CD89)2

Figure 1

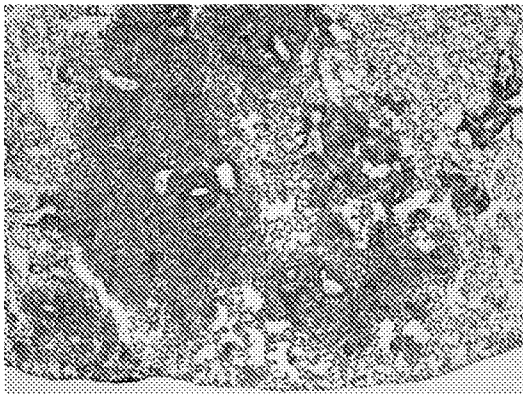
A



B



2E9IgA



PBS

Figure 2

C

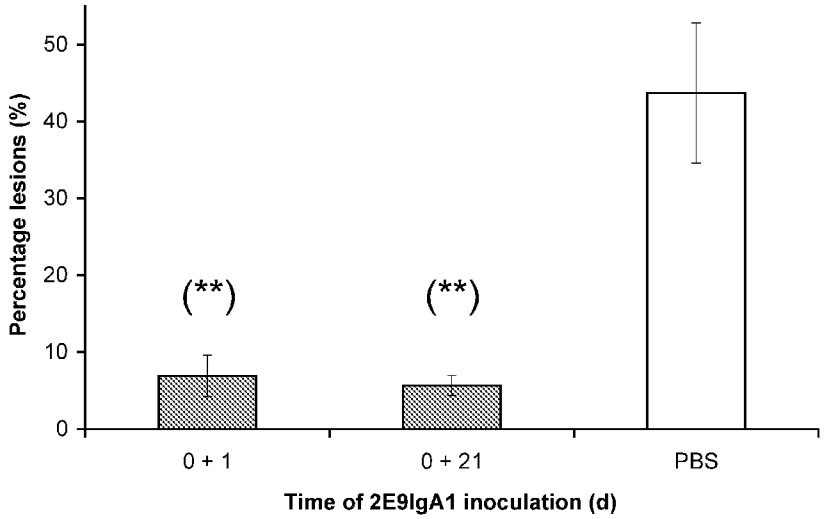


Figure 2 (continued)

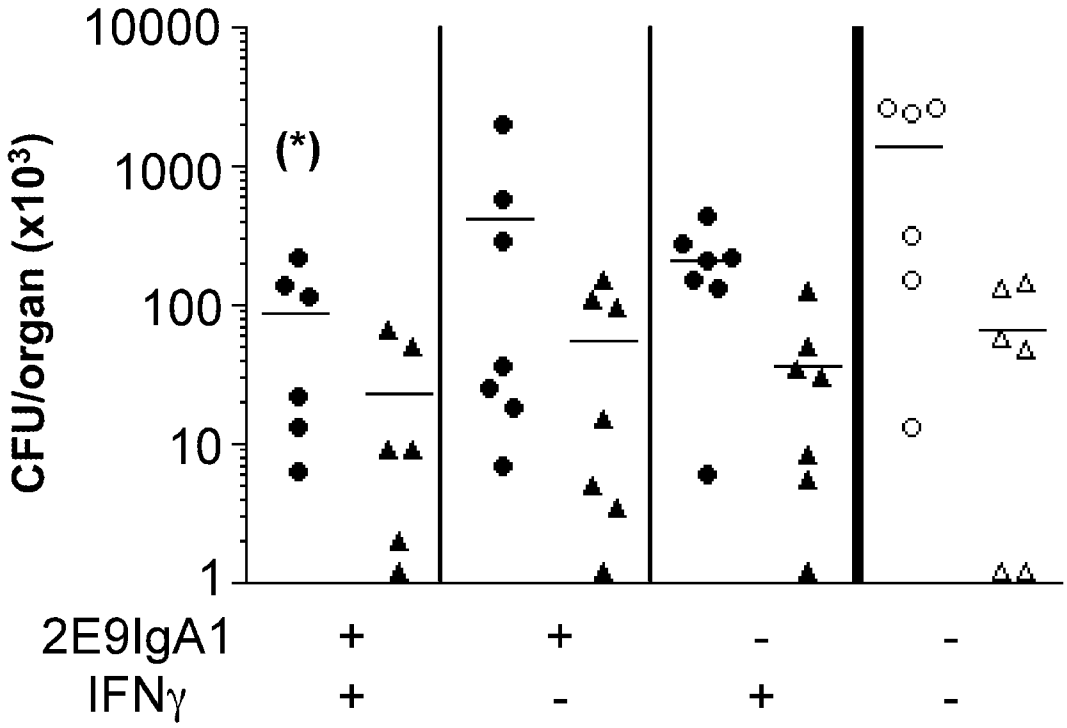


Figure 3

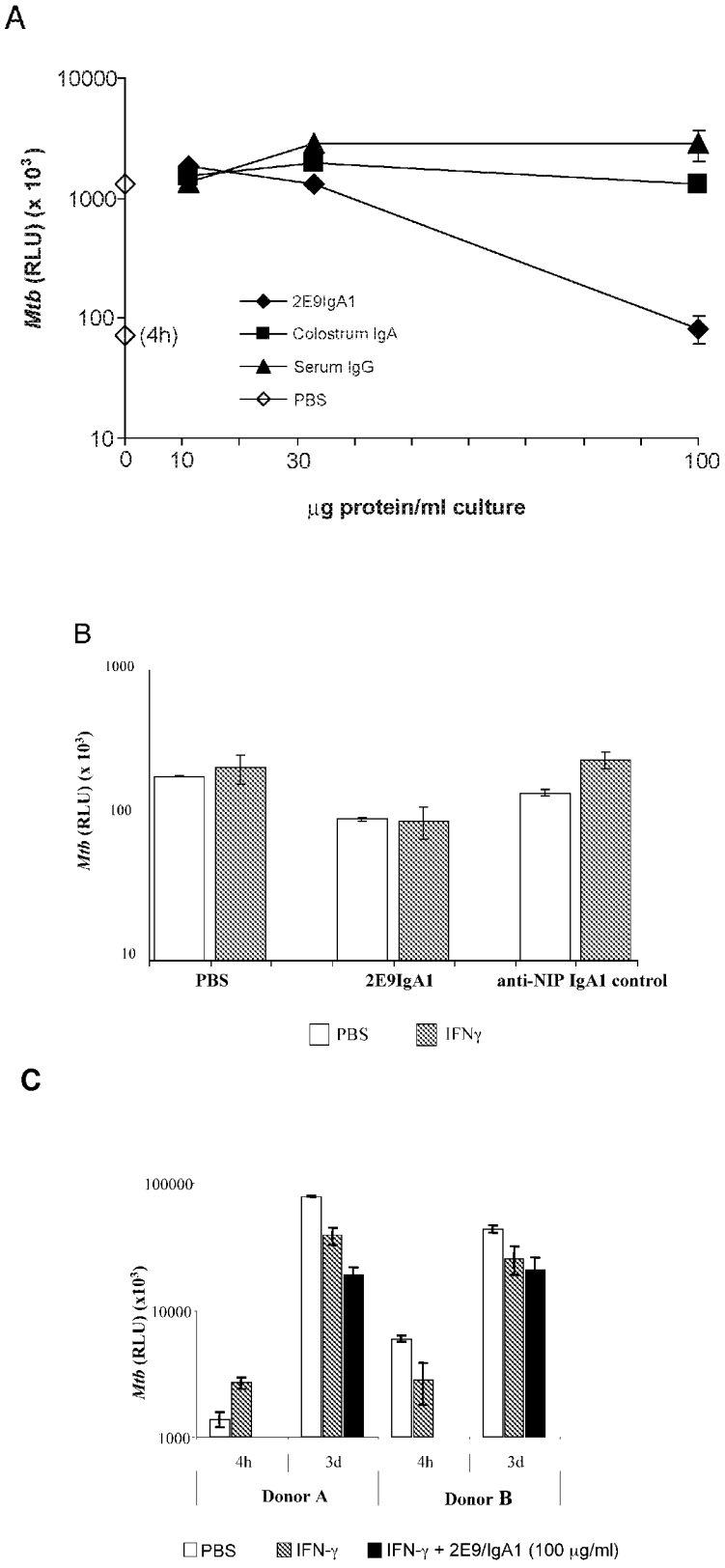
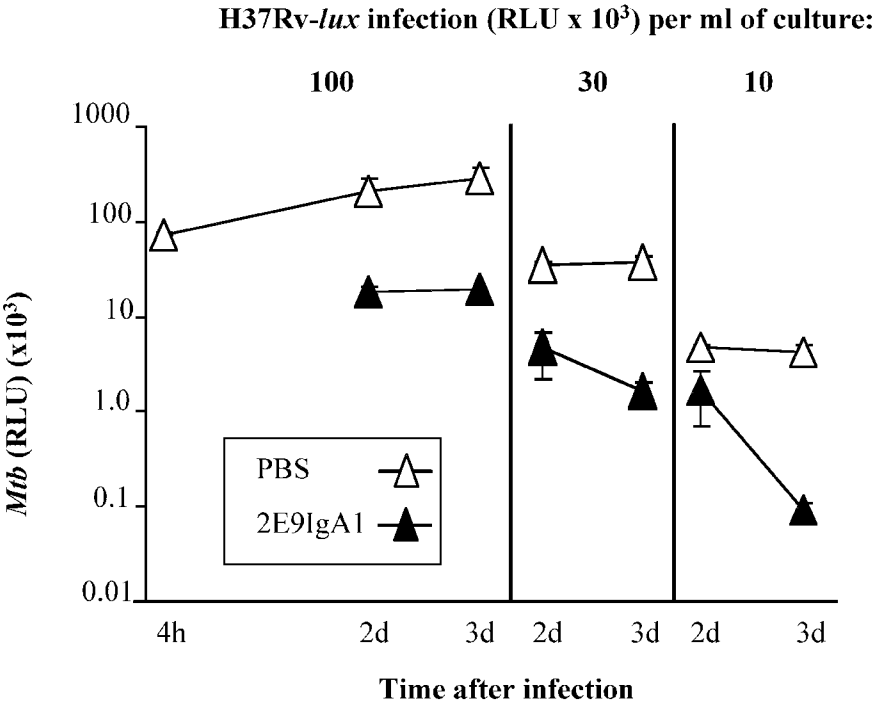

A
10000
1000
100
10
(4h)
Mtb (RLU) (x 10³)
2E9IgA1
Colostrum IgA
Serum IgG
PBS
0
10
30
100
µg protein/ml culture
B
1000
100
10
Mtb (RLU) (x 10³)
PBS
2E9IgA1
anti-NIP IgA1 control
PBS
IFNγ
C
100000
10000
1000
Mtb (RLU) (x10³)
4h
3d
4h
3d
Donor A
Donor B
PBS
IFN-γ
IFN-γ + 2E9/IgA1 (100 µg/ml)

Figure 4

D



E

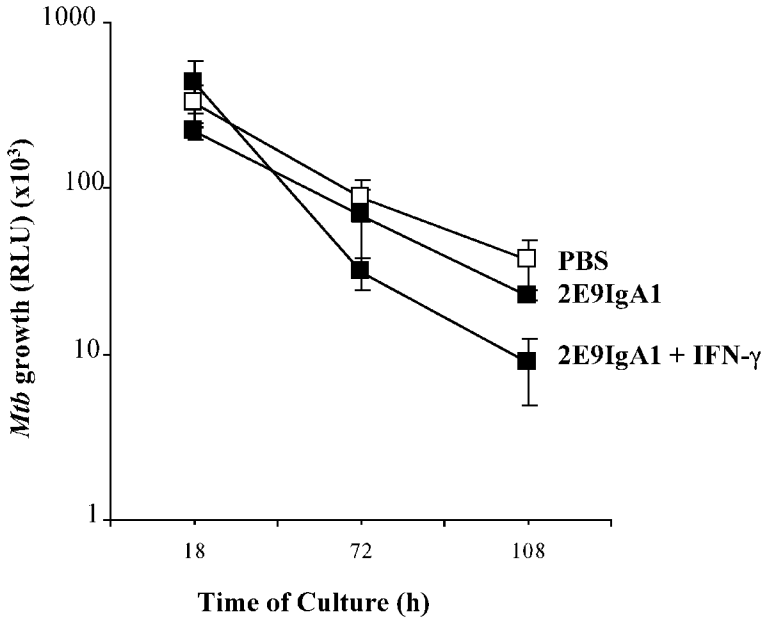


Figure 4 (continued)

Figure 5 (Sequence ID No 1)

gaggtt cagctgttgg agtctggggg aggcttggta cagcctgggg
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gccatgagct gggtccgcca ggctccaggg aaggggctgg agtgggtctc
aacgattcag tcgcttggta ttgttacagc ttacgcagac tccgtgaagg
gcaggttcac catctccaga gacaattcca agaacacgct gtatctgcaa
atgaacagcc tgagagccga ggacacggcc gtatattact gtgcgaaacg
gcctgcgacg tttgactact ggggccaggg aaccctggtc accgtctcct
cag

catccccga ccagccccaa ggtcttcccg ctgagcctct gcagcaccca
gccagatggg aacgtggtca tcgcctgcct ggtccagggc ttcttccccc
aggagccact cagtgtgacc tggagcgaaa gcggacaggg cgtgaccgcc
agaaacttcc cacccagcca ggatgcctcc ggggacctgt acaccacgag
cagccagctg accctgccgg ccacacagtg cctagccggc aagtccgtga
catgccacgt gaagcactac acgaatccca gccaggatgt gactgtgccc
tgcccag

ttccctcaa ctccacctac cccatctccc tcaactccac ctaccccatc
tccctcatgc tgccaccccc gactgtcact gcaccgaccg gccctcgagg
acctgctctt aggttcagaa gcgaacctca cgtgcacact gaccggcctg
agagatgcct caggtgtcac cttcacctgg acgccctcaa gtgggaagag
cgctgttcaa ggaccacctg agcgtgacct ctgtggctgc tacagcgtgt
ccagtgtcct gccgggctgt gccgagccat ggaaccatgg gaagaccttc
acttgcactg ctgcctaccc cgagtccaag accccgctaa ccgccaccct
ctcaaaatcc g

gaaacac attccggccc gaggtccacc tgctgccgcc gccgtcggag
gagctggccc tgaacgagct ggtgacgctg acgtgcctgg cacgcggctt
cagccccaag gacgtgctgg ttcgctggct gcaggggtca caggagctgc
cccgcgagaa gtacctgact tgggcatccc ggcaggagcc cagccagggc
accaccacct tcgctgtgac cagcatactg cgcgtggcag ccgaggactg
gaagaagggg gacaccttct cctgcatggt gggccacgag gccctgccgc
tggccttcac acagaagacc atcgaccgct tggcgggtaa acccacccat
gtcaatgtgt ctgttgtcat ggcggaggtg gacggcacct gctactga

Figure 6 (Sequence ID No 2)

EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSYA**MSWVRQAPGKGLEWVST**IQSLGIVT**AYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC**AKRPATFDY**WGQGTLVTVSS

SPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPSQDASGDL
YTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCP

VPSTPPTPSPSTPPTPSPS

CCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGPPERDLCGCY
SVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS

GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQE
PSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMA
EVDGTCY

Figure 7 (Sequence ID No 3)

gatatcgagc tcacccagtc tccatcctcc ctgtctgcat ctgtaggaga
cagagtcacc atcacttgcc gggcaagtca gagcattagc agctatttaa
attggtatca gcagaaacca gggaaagccc ctaagctcct gatctatcgt
gcatccaagt tgcaaagtgg ggtcccatca aggttcagtg gcagtggatc
tgggacagat ttcactctca ccatcagcag tctgcaacct gaagattttg
caacttacta ctgtcaacag aggcgttcgt ttcctctgac gttcggccaa
gggaccaagc tggaaatcaa ac

gaactgt ggctgcacca tctgtcttca tcttcccgcc atctgatgag
cagttgaaat ctggaactgc ctctgttgtg tgcctgctga ataacttcta
tcccagagag gccaaagtac agtggaaggt ggataacgcc ctccaatcgg
gtaactccca ggagagtgtc acagagcagg acagcaagga cagcacctac
agcctcagca gcaccctgac gctgagcaaa gcagactacg agaaacacaa
agtctacgcc tgcgaagtca cccatcaggg cctgagctcg cccgtcacaa
agagcttcaa caggggagag tgttag

Figure 8 (Sequence ID No 4)

DIELTQSPSSLSASVGDRVTITCRAS**QSISSY**LNWYQQKPGKAPKLLIY**RAS**KLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQ**QRRSFPLT**FGQGTKLEIK

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

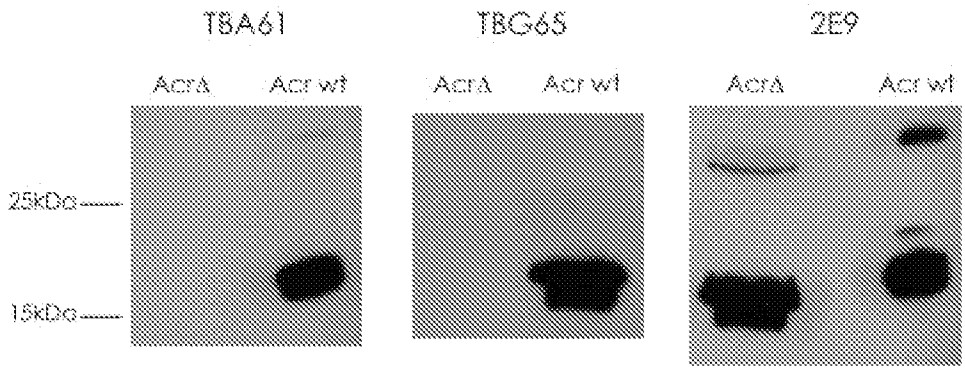


Fig 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2011/052404

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/12 A61P31/06 A61K39/40
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BUCCHERI S ET AL: "Prevention of the post-chemotherapy relapse of tuberculous infection by combined immunotherapy", TUBERCULOSIS, ELSEVIER, GB, vol. 89, no. 1, 1 January 2009 (2009-01-01), pages 91-94, XP025865050, ISSN: 1472-9792, DOI: 10.1016/J.TUBE.2008.09.001 [retrieved on 2008-11-05] abstract page 92, column 1, paragraph 3 ----- -/--	1-32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2012	02/04/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Montrone, Marco

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	S. BALU ET AL: "A Novel Human IgA Monoclonal Antibody Protects against Tuberculosis", THE JOURNAL OF IMMUNOLOGY, vol. 186, no. 5, 1 March 2011 (2011-03-01) , pages 3113-3119, XP55021377, ISSN: 0022-1767, DOI: 10.4049/jimmunol.1003189 abstract	1-32
Y	R. RELJIC ET AL: "Intranasal IFNgamma extends passive IgA antibody protection of mice against Mycobacterium tuberculosis lung infection", CLINICAL & EXPERIMENTAL IMMUNOLOGY, vol. 143, no. 3, 1 March 2006 (2006-03-01) , pages 467-473, XP55021379, ISSN: 0009-9104, DOI: 10.1111/j.1365-2249.2006.03012.x abstract	1-32
Y	RELJIC ET AL: "Mucosal immunotherapy of tuberculosis: Is there a value in passive IgA?", TUBERCULOSIS, ELSEVIER, GB, vol. 86, no. 3-4, 1 May 2006 (2006-05-01), pages 179-190, XP005416877, ISSN: 1472-9792, DOI: 10.1016/J.TUBE.2006.01.011 abstract page 180, column 2, paragraph 3 - page 181, column 2, paragraph 3 page 183; table 2 page 184, column 1, paragraphs 1,2 page 186, column 2, paragraph 2 - page 187, column 1, paragraph 1	1-32
Y	WOLBANK SUSANNE ET AL: "Characterization of human class-switched polymeric (Immunoglobulin M (IgM) and IgA) anti-human immunodeficiency virus type 1 antibodies 2F5 and 2G12", JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 77, no. 7, 1 April 2003 (2003-04-01), pages 4095-4103, XP002472382, ISSN: 0022-538X, DOI: 10.1128/JVI.77.7.4095-4103.2003 abstract	1-32

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SUN L K ET AL: "HUMAN IGA MONOCLONAL ANTIBODIES SPECIFIC FOR A MAJOR RAGWEED POLLEN ANTIGEN", BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, vol. 13, 1 August 1995 (1995-08-01), pages 779-786, XP002951000, ISSN: 0733-222X, DOI: 10.1038/NBT0895-779 abstract -----	1-32