Mucosal immunotherapy of tuberculosis: Is there a value in passive IgA?

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Summary Immunotherapeutic approaches, which have been considered for tuberculosis (TB), include immuno-potentiating or suppressing agents, cytokines, antibodies, DNA vaccines, non-pathogenic mycobacteria and mycobacterial extracts. While most or all of these potential agents showed at least some degree of promise in various experimental models, few progressed to clinical trials, yielding only moderately encouraging, though controversial results. Consequently, further research is required, as the need for an immunological agent, adjunct to chemotherapy, remains strongly justified. Its purpose is to shorten the currently protracted (6–9 months) drug treatment and thus increase compliance rates, which are most disappointing in areas with the highest disease prevalence. Using a mouse model of *Mycobacterium tuberculosis* (Mtb) infection, we recently reported, that an intranasally given monoclonal IgA antibody significantly reduced the bacterial load in the infected lungs, and that this protective effect of IgA could be further extended by co-inoculation with interferon gamma (IFNγ). In this review, we describe the main features of IgA and its cellular receptors, the extent and possible mechanisms of passive vaccination with an IgA monoclonal antibody against the α-crystallin antigen of Mtb and discuss the potentials of this approach in the wider context of immunotherapy of TB.
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Introduction

By causing approximately 2 million deaths annually, tuberculosis (TB) remains a major failure of global health management, despite the concerted efforts over many decades to bring this ancient disease

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under control. Three factors have mostly contributed to this failure to eradicate TB: (1) ability of the pathogen, *Mycobacterium tuberculosis* (Mtb), to undergo latency and survive in the host for long periods of time, without causing clinical disease; (2) failure of the BCG vaccine to protect against the infection; and (3) requirement for protracted drug treatment (6–9 months), leading to poor compliance in several disease endemic areas and the associated emergence of drug resistant strains.[1] It is estimated that one-third of the world's population is latently infected with Mtb. This huge reservoir of the pathogen ensures a continuous spread of the disease, yet chemoprophylactic intervention in poverty stricken endemic areas in South-East Asia and Africa is not feasible in the face of limited health care resources. In these areas, while neonatal BCG vaccine is protective against the most severe disseminated forms of TB, BCG vaccination is not effective against the most common pulmonary disease. It is, therefore, quite obvious that a more efficient vaccine will have to be developed if the TB epidemic is ever to be brought under control. So far, many subunit vaccine candidates have been explored, mainly in animal studies, but only a few have performed better than BCG[2] and entered the early stages of clinical trials. The first to do so is a BCG-boosting vaccinia virus based, Ag85 vaccine, which is currently in a phase I trial in the UK and Africa.[3] Chemotherapy of TB has an extremely high cure rate when delivered correctly, but the protracted duration of the treatment is the main factor leading to poor compliance in areas with limited resources, with serious detrimental impact on TB control. The need for the long duration of chemotherapy is due to the fact that Mtb is a slow growing organism and that following the initial killing of the majority of bacilli, it reverts to the state of latent persistency. As drugs cannot kill these non-dividing "persister" bacilli, there is a clear need to devise novel strategies that could complement and shorten the current chemotherapy regimen period. To this effect, immunotherapy of TB, applied concurrently or as an adjunct treatment to chemotherapy, appears to hold the greatest promise.

Immunotherapy of TB

Previous attempts at immunotherapy as an adjunct to chemotherapy in TB patients and in animal experimental models produced initially discouraging or only modestly beneficial results.[4–7] More recently, DNA plasmids expressing a number of mycobacterial antigens have been explored for their immunotherapeutic capacity. DNA coding for Ag85A, which is effective prophylactically, failed to protect when given after the challenge[8] and DNA coding for several antigens failed to prevent relapse when given after chemotherapy.[9] In contrast, Lowrie et al. using the DNA for hsp65 succeeded in preventing the post-chemotherapy relapse[10,11] following the intravenous infection of mice. Most recently, inoculation of DNA coding for either Ag85 or for IL-12, concurrently with chemotherapy, reduced significantly the Mtb relapse both in the lungs and spleens of aerosol infected C57BL/6 mice.[12]

Clinical studies aiming to improve chemotherapy by either immunopotentiation with levamisole[13] and *M. vaccae*[14] or by immunosuppression with corticosteroids[15] have been negative, but IFNγ administered to multidrug resistant TB patients in aerosolised form led to clinical improvement.[16] However, the overall outcome of several other IFNγ studies was only moderately satisfactory.[17–19] Disappointingly, recent results from the first large-scale randomised trial of IL-2 therapy showed that it was somewhat detrimental, rather than beneficial, in terms of bacterial clearance.[20] Notably, a new immunotherapeutic vaccine (called 'RUTI'), based on a detoxified extract of Mtb in liposome form prevented relapse to some degree in the 'Cornell model' and has been proposed for the immunoprophylactic treatment of latent tuberculous infection.[21]

The effectiveness of passive antibody treatment for various intracellular bacterial infections, including TB has been recently reviewed.[22,23] This concept is contentious partly due to the notion that antibodies cannot easily reach the intracellular pathogens. This reservation has been applied particularly to organisms, which are confined to phagosomes (i.e. Mtb), and thus further segregated from the cytoplasm. However, this view is under reconsideration in the light of increasing evidence, that antibodies interfering with some extracellular stages of the infection can influence the intracellular fate of the pathogen. Unlike the variable, and sometimes contradictory outcomes from early studies with polyclonal antisera, recent application of monoclonal antibodies (mAbs) showed consistently protective activity against a number of intracellular pathogens, such as *Cryptococcus neoformans*,[24] *Listeria monocytogenes*[25] and *Ehrlichia schaffensis*.[26] Several studies also examined the potential of passively administered mAbs for protection against Mtb infection. Thus, intratracheal infection with anti-arabinomannan IgG3 opsonised Mtb prolonged the survival of mice, but

enhanced the granulomatous infiltration of the lungs, while the bacterial load was not reduced.[27] In contrast, Hamasur et al.[28] reported a substantial decrease in the lung bacterial load, in addition to prolonged long-term survival, in mice inoculated intravenously with a lipoarabinomannan (LAM) specific IgG1 mAb. Opsonization of *M. bovis* with an IgG1 mAb against the MPB83 surface glycoprotein also prolonged the survival of mice and changed the morphology of lung granulomas.[29] In contrast with the above-mentioned studies, in which antibody opsonisation of bacilli led to decreased uptake or prolonged survival, another study utilising an antibody against heparin-binding hemagglutinin (HBHA) glycoprotein, demonstrated reduced bacterial dissemination from the lungs, due to the antibody inhibiting the interaction of HBHA with epithelial cells.[30] Very recently, antisera from BCG vaccinated individuals were shown to enhance the mediators of both innate and acquired cell-mediated immunity against mycobacterial infection in vitro.[31] In that study, anti-LAM specific IgG was shown to be an important component of the observed immuno-stimulating capacity of serum antibodies.

Passive IgA immunotherapy

IgA and its receptors

Several major reviews have been devoted to the biology of IgA and to its role in immunity to mucosal pathogens.[32–35] In summary, IgA is the most abundantly produced antibody isotype, predominant at mucosal surfaces and the second in concentration of the circulating serum immunoglobulins. The human mucosal IgA occurs predominantly in dimeric form, complexed with the poly immunoglobulin receptor (pIgR), while serum IgA is mostly monomeric, and can be of either IgA1 or IgA2 type. The difference between IgA1 and 2 is in a short peptide sequence, that determines the nature of glycosylation of the protein.[36] In contrast, mouse serum IgA is of one isotype only, resembling the human IgA1 type. The activities of the serum IgA are mediated by its monocyte/neutrophil receptor CD89 (FcαR), which binds IgA with relatively low affinity of $10^6 M^{-1}$.[37] This triggers potent inflammatory reactions, including antibody-dependent cytotoxic clearance (ADCC), endocytosis, phagocytosis, generation of super-oxide radicals, cytokines and inflamatory mediators.[35] Despite extensive efforts, no mouse CD89 counterpart has been identified. Secretory IgA

(sIgA), being prominent at mucosal surfaces, plays an important role in the early defensive mechanisms against invading pathogens in the gastrointestinal, respiratory and urogenital tracts. It is secreted into mucosal fluids by a well-described process known as transcytosis, that involves binding to the pIgR on the basolateral side of the epithelial lining, followed by detachment and trans-location to the apical side in a tight complex with the secretory component.[32,35] sIgA can bind and intercept invading pathogens during the transcytosis or in the mucosal fluids, leading to their neutralisation or 'exclusion' of the infection. An additional possible mechanism of action of sIgA, is elimination of IgA complexes from serum, by binding of complexes to pIgR and subsequent excretion by transcytosis.[38] These important and well documented functions of sIgA in immunity to mucosal pathogens, make sIgA in theory, an ideal immunotherapeutic that could be applied topically against many respiratory, gut-associated and urogenital pathogens. However, sIgA is not easy to produce in recombinant form, although advances in expression technology have been achieved.[39]

In addition to CD89 and pIgR, other IgA receptors have also been described. The asialoglycoprotein receptor (ASGPR) plays a major role in IgA catabolism by hepatocytes and its binding to IgA is carbohydrate dependent.[40] Transferrin receptor, CD71, which binds IgA1 but not IgA2, is well expressed by renal mesangial cells,[41] while mouse Peyer's patch M cells express a novel type IgA receptor, that binds mouse IgA and human IgA2 but not IgA1.[42] Recently, a new IgA/IgM receptor (Fcα/μR) on B cells and monocytes has been identified[43] and most recently, we reported that murine IgA also binds to intracellular galactoside-binding lectin of macrophages, galectin-3 (Gal-3, Mac-2), in a carbohydrate dependent fashion.[44] Although the precise role of this interaction remains to be elucidated, we hypothesise that it may be important for phagosome formation and maturation, following phagocytosis of IgA-opsonised particles or organisms. Table 1 summarises the current knowledge of IgA receptors, their tissue distribution and the various biological functions ascribed to them.

Passive IgA against viral and bacterial infections

Given the variety of IgA receptors, and also the different molecular forms of IgA, it is not surprising that this antibody isotype plays many defensive roles against mucosal infections. The possible

Table 1   IgA receptors: expression, tissue distribution, specificity of binding and suggested functions

Receptor name or symbol	H = human M = mouse	Cell type	Isotype bound	Function	Reference
FcαR, CD89	H	Myeloid	IgA1, IgA2	Phagocytosis, cell activation, ADCC	35
Poly IgR	H, M	Epithelial	Poly IgA	Transcytosis: epithelial cells	32
ASGPR (asialoglycoprotein)	H > M	Hepatocytes	IgA2	Clearance of serum IgA	40,91
Fcα/μR	H, M	B, myeloid	IgM, IgA	Phagocytosis	43
Transferrin CD71	H, M	B, mesangial	IgA1	Binding immune complexes	41
Gal-3/Mac-2	H, M	Macrophage, epithelial	IgA, IgE	Phagosome biogenesis?	44
M cell, IgA	M	M	Mouse sIgA hIgA2	Transcytosis to GALT	42
β 1,4-galactosyl transferase	H, M	Lymphocyte, myeloid, epithelial	IgA, IgM, IgG	Internalisation of immune complexes?	92
T cell IgAR	M	T	IgA	Unknown	93
Epithelial IgAR	H	Intestinal epithelial	mIgA	Unknown	94
NK cell IgAR	H	NK	SIgA	Cell killing	95

mechanisms involve the agglutination of microbes, inhibition of their motility, blocking of their attachment to the mucosal epithelium by targeting bacterial adhesins, clearance of microbial products and activation of phagocytic cells. Thus, IgA is thought to play a major role against respiratory viral pathogens, either by immune exclusion or neutralisation of viral activity. Immune exclusion is a concept that is defined by the combined activities of IgA antibodies and the mucus blanket that covers the epithelium of the respiratory tract. While mucus provides a physical barrier that restricts viral access to epithelial lining, the antibodies can cross-link and agglutinate viral particles and thus reduce their ability to penetrate mucus. Ciliary activity then moves the mucus with the trapped viral particles to the nasopharynx, from which it is cleared. Since sIgA is polymeric, its virus-agglutinating capacity is clearly superior to that of IgG, and the presence of the secretory component renders it also more resistant to proteolysis. Neutralisation, on the other hand, occurs when IgA binds to virus particles and prevents them from infecting target cells and spreading the infection. Intracellular neutralisation of viruses within epithelial cells can happen during transcytosis of IgA.[45] Various degrees of protection have been achieved using passively administered monoclonal IgA against viral pathogens.[46–50]

IgA can also act against pathogenic bacteria, as reported in a number of studies.[51–62] The mechan-

isms of IgA action against bacteria are not fully understood, though immune exclusion was reported to be the main IgA-mediated protective mechanism against *Salmonella typhimurium*[56] and *Vibrio cholerae*[58] and agglutination was shown to play a role in inhibition of *Chlamydia trachomatis* genital infection.[62] Direct neutralisation, via binding to a defined virulence antigen, was responsible for prevention of *Helicobacter felis* gastric infection,[60] while multiple mechanisms (i.e. exclusion of the infection, Fcα-dependent stimulation of phagocytic cells) were suggested for IgA-mediated protection against *Shigella flexneri*, the aetiological agent of dysenteric shigellosis.[59]

In addition to viruses and bacteria, monoclonal IgA antibodies have also been used for inhibition of fungal and protozoan infections.[24,63,64] In one study,[24] direct comparison of different antibody isotypes specific for the same antigen of *Cryptococcus neoformans* fungus, revealed that IgA was the most effective isotype, although its effectiveness was somewhat diminished by its inferior intravascular half-life, compared with IgG1.

Mucosal IgA therapy of TB

With an interest to analyse the possible protective role of IgA antibodies for TB, we generated IgA mAbs against mycobacterial antigens.[65] With the intention of evaluating them for passive

Table 2 Summary of the data from the passive antibody immunisation studies (from[66,73] and unpublished), in BALB/c mice infected with aerosolised *M. tuberculosis*.

Inoculation	Antigen specificity/Ig isotype	Reduction factor, experimental/PBS pulmonary CFU counts at:	
		9 days	4 weeks
TBA61	Acr/IgA	8.9	1.0
TBA84	PstS1/IgA	4.5	ND
MOPC 315	Nitrophenylated proteins/IgA	1.7	ND
TB68	Acr/IgG1	3.9	ND
IFNγ	NA	4.0	1.5
TBA61+IFNγ	Acr/IgA	11.2	**18.5**

ND = not determined; NA = not applicable.

vaccination in mice, we found that the best transmission into the lungs was achieved following their intranasal (i.n.) administration. We then compared these mAbs for their protective capacity in Balb/c mice challenged with either aerosolised or intranasally (i.n.) delivered Mtb bacilli.[66] The results have been summarised in Table 2. Mice were given mAbs before and after the challenge, and the bacterial load in the lungs determined 9 days later. We found that the IgA mAb TBA61, which is specific for the α-crystallin (acr, 16 kDa) antigen, was superior to both an IgG1 of the same antigen and epitope specificity, and also to another IgA mAb, specific for the PstS1 (38 kDa) antigen, in terms of inhibition of early Mtb infection in the lungs. TBA61 inoculation of mice resulted in approximately 10-fold reduction following either aerosol or i.n. infection. Although the initial experiments were performed with ascitic fluid preparations, subsequent experiments with affinity purified mAbs confirmed the original outcome. Since the IgA preparation we used in our experiments contained both monomeric and polymeric IgA, it was important to distinguish whether there were any differences in protective efficacy between these two molecular forms. We separated monomer and polymer IgA by size-exclusion chromatography and standardised the preparations in terms of antigen binding titres and protein concentration, prior to inoculation to mice. The results showed, that both fractions exerted similar levels of inhibition of Mtb lung infection. An important feature of IgA-mediated protection in our experiments was, that both pre- and post-challenge mAb inoculations were required for optimal protection. The acr antigen specificity, IgA isotype and pre- plus post-challenge antibody inoculations regimen, were all important for the observed inhibitory effect in this experimental model.

The main concern about the IgA-mediated inhibition of the early Mtb lung infection was its apparent transience, as it could not be observed beyond the 9-days post-challenge experimental period. Therefore, we tested if the duration of this protection could be further extended by co-inoculation with interferon gamma (IFNγ). IFNγ is known to be a critical component of protective immunity against mycobacterial infections. Humans deficient in the gene for IFNγ or the IFNγ-receptor show enhanced susceptibility to TB[67] and depletion of IFNγ by antibodies leads to reactivation of latent infection in mice,[68] suggesting a critical role for IFNγ also in the control of latent infection. However, clinical trials utilising IFNγ as an immunotherapeutic for TB showed that it was of little value in the absence of antimicrobial agents.[17,69] The mechanisms of IFNγ action are pleiotropic, involving stimulation of macrophage bactericidal functions, mediated by TNFα, nitric oxide (NO) and reactive nitrogen and oxygen intermediates (RNI, ROI). The resulting bacteriostasis or killing of Mtb was found in mouse,[70,71] but apparently not in human[72] macrophages. Actions of IFNγ other than those targeted on macrophages could also play a role.

In our recent experiments, intended to prolong the passive protection, we inoculated IFNγ to mice i.n., 3 days before aerosol Mtb challenge, and then again together with IgA mAb, just before and 2 days after the challenge. Lungs were harvested either before (9 days) or 4 weeks after the infection. The results (Table 2 and Reljic et al.[73]) showed that, although IFNγ co-inoculation did not significantly enhance the IgA inhibition of the infection at the early time point (9 days), it synergistically prolonged and enhanced the CFU inhibitory effect at the later time point (28 days). These results were corroborated by reduced lung pathology in the IgA/IFNγ treated mice.

Mechanism of IgA action against TB

Based on the experimental evidence from our in vivo and in vitro studies, we can exclude some of the potential mechanisms for the observed inhibition of Mtb lung infection in mice. "Immune exclusion" could be discounted on the grounds that the 24h harvest of lungs, following IgA treatment and aerosol challenge of mice, did not result in diminished bacterial counts.[66] Moreover, IgA mAb on its own did not inhibit uptake of the bacilli by peritoneal macrophages in vitro, nor did it affect their subsequent intracellular replication.[66,73] Most importantly, the observed synergy between IgA and IFNγ renders any such mechanisms based on blocking of infection, unlikely. Agglutination of bacilli by the IgA, which could limit their invasiveness, is also unlikely, based on the following observations. Firstly, the bacteria were not pre-opsonised by the antibody, while their subsequent agglutination in the lungs is improbable, given the low number of bacilli in the inoculum (100 CFU), the mono-disperse nature of the aerosol delivery process, and the relatively large surface/volume of the lungs. Secondly, the polymeric IgA, which could be expected to be superior to monomer for inducing bacillary agglutination, was not more protective than the monomer in our studies.[66] Similarly, the outcome of the comparison between monomer and polymer also excludes the possible role of either pIgR or the so-called "reverse transcytosis", that could facilitate dissemination of bacteria to other organs. No increased spleen infection was observed in mice treated with IgA and IFNγ, 4 weeks after the challenge.[73]

The acr specificity of the IgA mAb could be significant. Although the precise role of the acr1 antigen in the pathogenesis of TB is not known, there is evidence that its expression is required for bacterial growth inside macrophages.[74,75] In that case, targeting of the acr antigen with TBA61 could possibly interfere with the intracellular fate of the bacilli. However, an IgG1 against the same acr epitope as the TBA61 IgA mAb, was less inhibitory in our mouse studies,[66] suggesting the mandatory importance of the IgA isotype.

Several pieces of evidence from our studies point to an immuno-stimulatory role of IgA, which can be further enhanced by the action of IFNγ, and possibly by other factors. This is based on our finding, that mouse monoclonal IgAs, irrespective of their antigenic specificity, inhibit the proliferation of mouse macrophage cell lines.[76] This growth-inhibitory activity of IgA, but not IgG and IgM, was accompanied by stimulation of TNF-α and NO production, and subsequent FAS-independent apop-

tosis. We also demonstrated, that IgA can synergise with IFNγ to induce TNF-α and NO production of thioglycollate-elicited[76] or Mtb infected[73] peritoneal macrophages, leading also to an increased bactericidal capacity. The in vitro actions of IgA (summarised in Table 3) may play a stimulatory role for mouse macrophages also in vivo, that can be further potentiated by IFNγ. This, in turn, suggests a role for a mouse Fcα macrophage receptor, capable of a signalling function similar to that of the human Fcα/CD89 receptor. One possible candidate for such receptor could be the recently discovered B and myeloid cell Fcα/μ receptor.[43]

In our endeavours to identify the IgA-binding structure on mouse macrophages we utilised detergent lysates of macrophages.[44] These experiments revealed that IgA binds to the intracellular lectin Gal-3 (Mac-2), which has previously been shown to bind IgE and a number of other non-immunoglobulin ligands (reviewed in Liu et al.[77]). Although Gal-3 is not directly involved in phagocytosis, its interaction with IgA could still play an important role in IgA-mediated inhibition of intracellular Mtb. Thus, Gal-3 was previously shown to accumulate only in those phagosomes, which contained live Mtb through the binding to phosphatidylinositol mannosides (PIM), and appeared to influence the clearance of late infection.[78] IgA antibodies recognising mycobacterial surface constituents, as used in our studies, could thus have an

Table 3 Effects of IgA on macrophages in vitro (from[76] and unpublished)

Assay (read-out)	Cell type	
	J774 line	Fresh macrophages*
Cell growth	Inhibition	NA
[^{3}H]-Thymidine uptake	Inhibition	NA
IgA binding (flow cytometry)	Positive	Positive
Annexin-V+propidium iodide staining	Positive	NT
LDH activity	Positive	Positive
TNFα production	Increased	Increased
NO production	Increased	Increased
FAS/CD95 induced apoptosis	Negative	NT
Synergy with IFNγ	Yes	Yes
Gal-3 binding	Yes	NT

LDH, lactate-dehydrogenase; NO, nitric oxide; NA, not applicable; NT, not tested.
  *Thioglycollate induced peritoneal macrophages.

additional targeting opportunity to influence the course of intracellular infection, by virtue of interfering with the interactions between opsonised bacteria and phagosomal membrane. Such interactions could negatively influence bacterial survival, since close apposition with phagosomal membrane is thought to be important for the capacity of Mtb bacilli to inhibit phagosomal maturation and fusion with lysosome. In addition, secreted Gal-3, with its well-described chemoattractant properties for monocytes,[80] could also play a role in monocyte accumulation in vivo, in tissue sites harbouring IgA-opsonised organisms, leading to their clearance by phagocytosis. Gal-3 expression by monocytes/macrophages is cell differentiation dependent[81] and can be induced by IFNγ,[82] which may in part explain the potential of IFNγ to enhance the bactericidal effect of IgA against Mtb infected macrophages.

An intriguing possibility for IgA/Gal-3 interaction to play a role in Mtb infection, and IgA biology in general, is that IgA-opsonised microbes could target the Gal-3/FcγRII complex on the surface of macrophages, as Gal-3 has recently been shown to interact specifically with the low affinity receptor for IgG, FcγRII/CD32.[83] Since Gal-3 interacts with IgA,[44] the IgA-opsonised bacilli could indirectly target FcγRII as the phagocytic receptor. Human FcαR also associates with FcγRII and entirely depends on its signalling capacity for induction of the microbicidal function of macrophages,[35] thus indicating potentially similar modes of action of IgA in both species. Interestingly, Gal-3 can also bind to both IgE and its high affinity receptor FcϵRI on mast cells,[84,85] thus indicating a potentially important immuno-modulatory pathway. Since IgA utilisation of the FcγRII receptor in this scenario would depend on the expression of Gal-3, which in turn is monocyte differentiation dependent,[81] this may represent a regulatory mechanism by which sIgA in mucosal fluids, could target opsonised particles or microbes only to differentiated resident macrophages and not immature monocytes, thus reducing the risk of unnecessary inflammatory reactions.

Although additional work is required to understand fully the molecular mechanisms involved in the IgA-mediated inhibition of Mtb lung infection in our experiments, we propose that the key mechanism is likely to involve IgA potentiation of bactericidal functions of infected macrophages. This could involve either the induction of activation-dependent apoptosis, leading to killing of the bacilli, or the IgA/Gal-3 mediated inhibition of bacterial interaction with phagosomal membrane, which is required for the inhibition of phago-lysosome fusion.[79] A hypothetical model of these
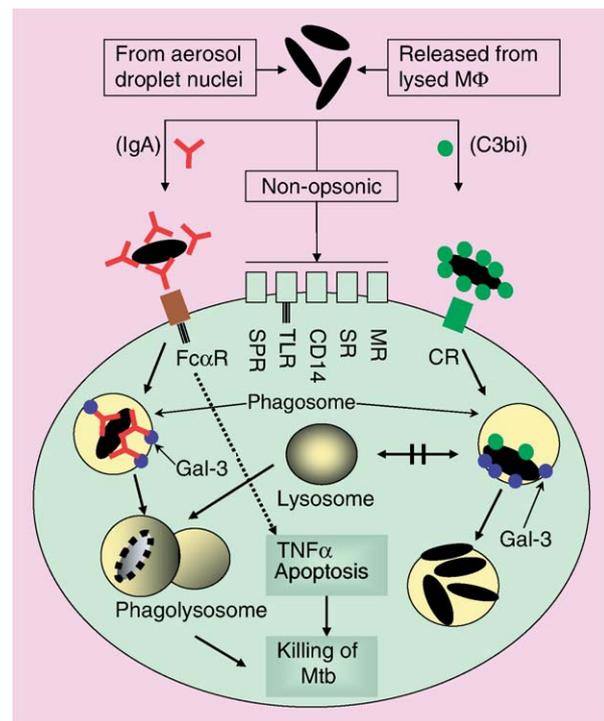

Figure 1 Schematic illustration of the hypothetical mechanisms by which passive IgA anti-acr mAbs could restrict the multiplication of *M. tuberculosis* in macrophages. C3bi-opsonised bacilli targeting CR 1, 3 and 4 on alveolar macrophages do not induce pro-inflammatory signals and phago-lysosome fusion during natural infection. This allows tubercle bacilli, sheltered in the phagosome, to multiply. In contrast, following passive anti-acr IgA therapy, FcαR-mediated uptake of IgA-opsonised bacilli results in the killing of bacilli. This could occur either by (i) induction of TNFα dependent macrophage apoptosis, or by (ii) binding of phagosomal Gal-3 with IgA preventing the interaction of the opsonized bacilli with the phagosomal membrane (left-hand side of the scheme) and thus enabling phago-lysosome fusion. Non-opsonic receptors implicated in mycobacterial infection of macrophages: SPR, surfactant protein receptor; TLR, Toll-like receptors; SR, scavanger receptor; MR, mannose receptor.

IgA bactericidal mechanisms is depicted schematically, in Fig. 1.

Advantages of passive IgA

Tuberculous infection is transmitted by the mucosal route, i.e. by inhalation of bacterial droplets into the alveoli in the lungs. Therefore, it could be expected that high affinity IgA antibodies produced by the infected host can first encounter the incipient infection within about 2–3 weeks after

infection. This time-lag is due to the need for the reactive B cells to undergo affinity maturation and isotype switch within germinal centres, and subsequent transcytosis of polymeric IgA into mucosal sites harbouring the infection. By this time, the bacillary amplification is well under way and most of the bacilli are likely to be intracellular,[86] i.e. within infected macrophages, thus evading IgA action. This may be at least in part an explanation for the lack of evidence that IgA plays any protective role during primary tuberculous infection, either in humans or experimental animals. On the other hand, IgA antibody levels have been associated with protection to leprosy.[87] BCG vaccination also induces substantial IgA responses, and it was reported that IgA deficient mice were less able to control BCG infection, despite partial compensation by increased specific IgM responses.[88] LAM appears to be the major antigenic target of IgA response, following oral BCG vaccination.[89] Whether the IgA antibodies induced by BCG play a role in protection against Mtb is not known.

There are several functional differences between the passively inoculated mAbs and those induced by the natural infection or BCG vaccination. They deserve to be emphasised, since they may help to justify the potential usefulness of the passive IgA immunotherapy:

(i) *Quantitative aspect*: By direct application of purified, concentrated mAb, higher effective antibody concentrations in the bronchoalveolar lavage can be achieved, compared to naturally produced either circulating or sIgA antibody.

(ii) *Antigen specificity*: Unlike the polyclonal response induced by live bacteria, mAbs are specific for a single antigen, thus eliminating possible interference from non-protective or deleterious antibodies of the same or different isotype.

(iii) *Timing of IgA action*: Instantaneous availability of an effective IgA mAb at the time of pathogenic invasion could be expected to have a stronger impact, particularly since the number of bacilli is low, compared to replicating bacilli at later stages. This makes passively delivered IgA suitable also for prophylaxis against tuberculous infection in individuals at high risk of infection.

(iv) *Triggering of phagocytic functions*: This is an important, albeit poorly explored aspect of passive immunity at mucosal sites. It is generally considered that sIgA is not pro-inflammatory, but acts as a first line of defence against the mucosal entry of pathogens, by an "immune exclusion" mechanism. The presence of the covalently attached secretory component prevents sIgA from direct binding to an alternative Fcα receptor that may be present on phagocytic cells, requiring instead CD11b/CD18 (Mac-1, CR3) as an accessory molecule.[90] Human myeloid cells express an Fcα receptor (CD89), and even though its counterpart has not been identified in mice, an IgA binding structure must also exist on murine cells. Unlike sIgA, passively administered IgA mAb (composed usually of both monomeric and polymeric forms) can directly bind to an Fcα receptor on phagocytes and induce more efficiently bactericidal functions in these cells. In this regard, passive IgA immunisation therefore offers a distinct advantage over naturally occurring or vaccine induced sIgA.

Further improvements and potential applications of the IgA/IFNγ therapy

The acr antigen, which has been the immunological target of the IgA mAb in our studies, is known to be over-expressed at the latent stage of tuberculous infection.[74] Therefore, the protective effect of the antibody could be even more pronounced in respect of 'latent/dormant' bacilli, which express high levels of this antigen. The fact that relatively small numbers of the organisms persist following treatment, may further favour the therapeutic efficacy of the passive IgA treatment. IFNγ is known to be critically involved in the control of latent Mtb infection, since depletion of IFNγ by antibodies leads to reactivation of latent infection in mice.[68] Consequently, the combined anti-acr IgA/IFNγ therapy could be developed as an adjunct to short-term chemotherapy. A potential shortening of the treatment period would reduce costs and increase compliance rates. An alternative application of IgA/IFNγ could be for the immunoprophylaxis in AIDS patients who are at a high risk of TB reactivation or exogenous infection. However, before progressing to such clinical applications, further improvements in the efficacy and duration of protection need to be developed in animal models. As the existing protection data have all been derived at the acute stage of infection, future studies should give priority to ascertain the effect of passive IgA treatment for preventing the post-chemotherapy relapse in the Cornell experimental model.

Concluding remarks

Passive immunotherapy with IgA mAbs may have an important potential as an adjunct therapy for TB and other respiratory lung infections. Moreover, it can be considered also for the prophylaxis of immunocompromised individuals (e.g. HIV infected) at a high risk of developing TB. The IgA isotype is particularly well suited for this purpose, given the broad range of its pro-inflammatory actions. We have shown, using an experimental mouse model of pulmonary TB, that an IgA mAb against the acr antigen of *M. tuberculosis*, can inhibit the infection by approximately 10-fold, and that this inhibition can be extended by co-inoculation with IFNγ. Synergistic stimulation of macrophage bactericidal functions by i.n. administered IFNγ and IgA, probably by targeting the bacilli to a currently unidentified mouse Fcα receptor appears to be the likely mechanism for the observed inhibition of lung infection in mice. Such potential immunotherapeutic strategies deserve further research effort as they have a significant potential to improve TB control by shortening the currently protracted drug treatment period in patients with active TB. Alternatively, an effective prophylactic application in AIDS patients and other immunocompromised individuals at high risk of contracting TB could also be of major benefit, since vaccination in these cases is contraindicated. Further research and development of the reported immunotherapeutic procedure could lead to considerable improvements in the foreseeable future, thus making its clinical application a realistic target.

Acknowledgements

References

1. WHO/IUATLD. Anti-tuberculosis drug resistance in the world. Repor 2: prevalence and trends. Geneva, 2000.
2. Rosenkrands I, Agger EM, Olsen AW, Korsholm KS, Andersen CS, Jensen KT, et al. Cationic liposomes containing mycobacterial lipids: a new powerful Th1 adjuvant system. *Infect Immun* 2005;**73**:5817–26.
3. McShane H, Pathan AA, Sander CR, Goonetilleke NP, Fletcher HA, Hill AV. Boosting BCG with MVA85A: the first candidate subunit vaccine for tuberculosis in clinical trials. *Tuberculosis (Edinburgh)* 2005;**85**:47–52.
4. Cannetti G. Host factors and chemotherapy of tuberculosis. In: Barry III CE, editor. *Chemotherapy of tuberculosis*. London: Butterworth; 1965. p. 175–91.
5. Grange JM. Immunotherapy of tuberculosis. *Tubercle* 1990;**71**:237–9.
6. Lecoeur HF, Lagrange PH, Truffot-Pernot C, Gheorghiu M, Grosset J. Relapses after stopping chemotherapy for experimental tuberculosis in genetically resistant and susceptible strains of mice. *Clin Exp Immunol* 1989;**76**:458–62.
7. Venkataprasad N, Ledger P, Ivanyi J. The effect of glucosaminylmuramyl dipeptide injection to mice on the course of tuberculous infection and in vitro superoxide anion production. *Int Arch Allergy Immunol* 1997;**114**:23–9.
8. Turner J, Rhoades ER, Keen M, Belisle JT, Frank AA, Orme IM. Effective preexposure tuberculosis vaccines fail to protect when they are given in an immunotherapeutic mode. *Infect Immun* 2000;**68**:1706–9.
9. Repique CJ, Li A, Collins FM, Morris SL. DNA immunization in a mouse model of latent tuberculosis: effect of DNA vaccination on reactivation of disease and on reinfection with a secondary challenge. *Infect Immun* 2002;**70**:3318–23.
10. Lowrie DB, Tascon RE, Bonato VL, Lima VM, Faccioli LH, Stavropoulos E, et al. Therapy of tuberculosis in mice by DNA vaccination. *Nature* 1999;**400**:269–71.
11. Lowrie DB, Silva CL. Enhancement of immunocompetence in tuberculosis by DNA vaccination. *Vaccine* 2000;**18**:1712–6.
12. Ha SJ, Jeon BY, Kim SC, Kim DJ, Song MK, Sung YC, et al. Therapeutic effect of DNA vaccines combined with chemotherapy in a latent infection model after aerosol infection of mice with *Mycobacterium tuberculosis*. *Gene Ther* 2003;**10**:1592–9.
13. Gatner EM, Anderson R. Immune responses and immunostimulation in tuberculosis therapy. *S Afr Med J* 1982;**61**:707–10.
14. Mwinga A, Nunn A, Ngwira B, Chintu C, Warndorff D, Fine P, et al. *Mycobacterium vaccae* (SRL172) immunotherapy as an adjunct to standard antituberculosis treatment in HIV-infected adults with pulmonary tuberculosis: a randomised placebo-controlled trial. *Lancet* 2002;**360**:1050–5.
15. Madras TR. Study of chemotherapy regimens of 5 and 7 months' duration and the role of corticosteroids in the treatment of sputum-positive patients with pulmonary tuberculosis in South India. *Tubercle* 1983;**64**:73–91.
16. Condos R, Raju B, Canova A, Zhao BY, Weiden M, Rom WN, et al. Recombinant gamma interferon stimulates signal transduction and gene expression in alveolar macrophages in vitro and in tuberculosis patients. *Infect Immun* 2003;**71**:2058–64.
17. Raad I, Hachem R, Leeds N, Sawaya R, Salem Z, Atweh S. Use of adjunctive treatment with interferon-gamma in an immunocompromised patient who had refractory multidrug-resistant tuberculosis of the brain. *Clin Infect Dis* 1996;**22**:572–4.
18. Lauw FN, van Der Meer JT, de Metz J, Danner SA, van Der Poll T. No beneficial effect of interferon-gamma treatment in 2 human immunodeficiency virus-infected patients with *Mycobacterium avium* complex infection. *Clin Infect Dis* 2001;**32**:e81–2.
19. Ulrichs T, Fieschi C, Nevicka E, Hahn H, Brezina M, Kaufmann SH, et al. Variable outcome of experimental interferon-gamma therapy of disseminated bacillus Calmette–Guerin infection in two unrelated interleukin-12Rbeta1-deficient Slovakian children. *Eur J Pediatr* 2005;**164**:166–72.
20. Johnson JL, Ssekasanvu E, Okwera A, Mayanja H, Hirsch CS, Nakibali JG, et al. Randomized trial of adjunctive interleukin-2

in adults with pulmonary tuberculosis. *Am J Respir Crit Care Med* 2003;**168**:185–91.

21. Cardona PJ, Amat I, Gordillo S, Arcos V, Guirado E, Diaz J, et al. Immunotherapy with fragmented *Mycobacterium tuberculosis* cells increases the effectiveness of chemotherapy against a chronical infection in a murine model of tuberculosis. *Vaccine* 2005;**23**:1393–8.

22. Glatman-Freedman A. Advances in antibody-mediated immunity against *Mycobacterium tuberculosis*: implications for a novel vaccine strategy. *FEMS Immunol Med Microbiol* 2003;**39**:9–16.

23. Casadevall A. Antibody-mediated immunity against intracellular pathogens: two-dimensional thinking comes full circle. *Infect Immun* 2003;**71**:4225–8.

24. Mukherjee J, Scharff MD, Casadevall A. Protective murine monoclonal antibodies to *Cryptococcus neoformans*. *Infect Immun* 1992;**60**:4534–41.

25. Edelson BT, Unanue ER. Intracellular antibody neutralizes *Listeria* growth. *Immunity* 2001;**14**:503–12.

26. Li JS, Yager E, Reilly M, Freeman C, Reddy GR, Reilly AA, et al. Outer membrane protein-specific monoclonal antibodies protect SCID mice from fatal infection by the obligate intracellular bacterial pathogen *Ehrlichia chaffeensis*. *J Immunol* 2001;**166**:1855–62.

27. Teitelbaum R, Glatman-Freedman A, Chen B, Robbins JB, Unanue E, Casadevall A, et al. mAb recognizing a surface antigen of *Mycobacterium tuberculosis* enhances host survival. *Proc Natl Acad Sci USA* 1998;**95**:15688–93.

28. Hamasur B, Haile M, Pawlowski A, Schroder U, Kallenius G, Svenson SB. A mycobacterial lipoarabinomannan specific monoclonal antibody and its F(ab′) fragment prolong survival of mice infected with *Mycobacterium tuberculosis. Clin Exp Immunol* 2004;**138**:30–8.

29. Chambers MA, Gavier-Widen D, Hewinson RG. Antibody bound to the surface antigen MPB83 of *Mycobacterium bovis* enhances survival against high dose and low dose challenge. *FEMS Immunol Med Microbiol* 2004;**41**:93–100.

30. Pethe K, Alonso S, Biet F, Delogu G, Brennan MJ, Locht C, et al. The heparin-binding haemagglutinin of *M. tuberculosis* is required for extrapulmonary dissemination. *Nature* 2001;**412**:190–4.

31. de Valliere S, Abate G, Blazevic A, Heuertz RM, Hoft DF. Enhancement of innate and cell-mediated immunity by antimycobacterial antibodies. *Infect Immun* 2005;**73**:6711–20.

32. Mestecky J, Lue C, Russell MW. Selective transport of IgA. Cellular and molecular aspects. *Gastroenterol Clin North Am* 1991;**20**:441–71.

33. Lamm ME. Interaction of antigens and antibodies at mucosal surfaces. *Annu Rev Microbiol* 1997;**51**:311–40.

34. van Egmond M, Damen CA, van Spriel AB, Vidarsson G, van Garderen E, van de Winkel JG. IgA and the IgA Fc receptor. *Trends Immunol* 2001;**22**:205–11.

35. Monteiro RC, van de Winkel JG. IgA Fc receptors. *Annu Rev Immunol* 2003;**21**:177–204.

36. Kerr MA. The structure and function of human IgA. *Biochem J* 1990;**271**:285–96.

37. Wines BD, Hulett MD, Jamieson GP, Trist HM, Spratt JM, Hogarth PM. Identification of residues in the first domain of human Fc alpha receptor essential for interaction with IgA. *J Immunol* 1999;**162**:2146–53.

38. Robinson JK, Blanchard TG, Levine AD, Emancipator SN, Lamm ME. A mucosal IgA-mediated excretory immune system in vivo. *J Immunol* 2001;**166**:3688–92.

39. Crottet P, Cottet S, Corthesy B. Expression, purification and biochemical characterization of recombinant murine secre-

tory component: a novel tool in mucosal immunology. *Biochem J* 1999;**341**(Part 2):299–306.

40. Stockert RJ, Kressner MS, Collins JC, Sternlieb I, Morell AG. IgA interaction with the asialoglycoprotein receptor. *Proc Natl Acad Sci USA* 1982;**79**:6229–31.

41. Moura IC, Centelles MN, Arcos-Fajardo M, Malheiros DM, Collawn JF, Cooper MD, et al. Identification of the transferrin receptor as a novel immunoglobulin (Ig)A1 receptor and its enhanced expression on mesangial cells in IgA nephropathy. *J Exp Med* 2001;**194**:417–25.

42. Mantis NJ, Cheung MC, Chintalacharuvu KR, Rey J, Corthesy B. Neutra MR Selective adherence of IgA to murine Peyer's patch M cells: evidence for a novel IgA receptor. *J Immunol* 2002;**169**:1844–51.

43. Shibuya A, Sakamoto N, Shimizu Y, Shibuya K, Osawa M, Hiroyama T, et al. Fc alpha/mu receptor mediates endocytosis of IgM-coated microbes. *Nat Immunol* 2000;**1**:441–6.

44. Reljic R, Crawford C, Challacombe S, Ivanyi J. Mouse monoclonal IgA binds to the galectin-3/Mac-2 lectin from mouse macrophage cell lines. *Immunol Lett* 2004;**93**:51–6.

45. Mazanec MB, Kaetzel CS, Lamm ME, Fletcher D, Nedrud JG. Intracellular neutralization of virus by immunoglobulin A antibodies. *Proc Natl Acad Sci USA* 1992;**89**:6901–5.

46. Outlaw MC, Dimmock NJ. Mechanisms of neutralization of influenza virus on mouse tracheal epithelial cells by mouse monoclonal polymeric IgA and polyclonal IgM directed against the viral haemagglutinin. *J Gen Virol* 1990;**71**(Part 1):69–76.

47. Taylor HP, Dimmock NJ. Mechanism of neutralization of influenza virus by secretory IgA is different from that of monomeric IgA or IgG. *J Exp Med* 1985;**161**:198–209.

48. Mazanec MB, Nedrud JG, Lamm ME. Immunoglobulin A monoclonal antibodies protect against Sendai virus. *J Virol* 1987;**61**:2624–6.

49. Weltzin R, Hsu SA, Mittler ES, Georgakopoulos K, Monath TP. Intranasal monoclonal immunoglobulin A against respiratory syncytial virus protects against upper and lower respiratory tract infections in mice. *Antimicrob Agents Chemother* 1994;**38**:2785–91.

50. Burns JW, Siadat-Pajouh M, Krishnaney AA, Greenberg HB. Protective effect of rotavirus VP6-specific IgA monoclonal antibodies that lack neutralizing activity. *Science* 1996;**272**:104–7.

51. Fubara ES, Freter R. Protection against enteric bacterial infection by secretory IgA antibodies. *J Immunol* 1973;**111**:395–403.

52. Hajishengallis G, Nikolova E, Russell MW. Inhibition of Streptococcus mutans adherence to saliva-coated hydroxyapatite by human secretory immunoglobulin A (S-IgA) antibodies to cell surface protein antigen I/II: reversal by IgA1 protease cleavage. *Infect Immun* 1992;**60**:5057–64.

53. Svanborg-Eden C, Svennerholm AM. Secretory immunoglobulin A and G antibodies prevent adhesion of *Escherichia coli* to human urinary tract epithelial cells. *Infect Immun* 1978;**22**:790–7.

54. Williams RC, Gibbons RJ. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science* 1972;**177**:697–9.

55. Wold AE, Mestecky J, Tomana M, Kobata A, Ohbayashi H, Endo T, et al. Secretory immunoglobulin A carries oligosaccharide receptors for *Escherichia coli* type 1 fimbrial lectin. *Infect Immun* 1990;**58**:3073–7.

56. Michetti P, Mahan MJ, Slauch JM, Mekalanos JJ, Neutra MR. Monoclonal secretory immunoglobulin A protects mice against oral challenge with the invasive pathogen *Salmonella typhimurium*. *Infect Immun* 1992;**60**:1786–92.

57. Czinn SJ, Cai A, Nedrud JG. Protection of germ-free mice from infection by *Helicobacter felis* after active oral or passive IgA immunization. *Vaccine* 1993;**11**:637–42.

58. Apter FM, Lencer WI, Finkelstein RA, Mekalanos JJ, Neutra MR. Monoclonal immunoglobulin A antibodies directed against cholera toxin prevent the toxin-induced chloride secretory response and block toxin binding to intestinal epithelial cells in vitro. *Infect Immun* 1993;**61**:5271–8.

59. Phalipon A, Kaufmann M, Michetti P, Cavaillon JM, Huerre M, Sansonetti P, et al. Monoclonal immunoglobulin A antibody directed against serotype-specific epitope of *Shigella flexneri* lipopolysaccharide protects against murine experimental shigellosis. *J Exp Med* 1995;**182**:769–78.

60. Blanchard TG, Czinn SJ, Maurer R, Thomas WD, Soman G, Nedrud JG. Urease-specific monoclonal antibodies prevent *Helicobacter felis* infection in mice. *Infect Immun* 1995;**63**:1394–9.

61. Iankov ID, Petrov DP, Mladenov IV, Haralambieva IH, Kalev OK, Balabanova MS, et al. Protective efficacy of IgA monoclonal antibodies to O and H antigens in a mouse model of intranasal challenge with *Salmonella enterica* serotype Enteritidis. *Microbes Infect* 2004;**6**:901–10.

62. Pal S, Theodor I, Peterson EM, de la Maza LM. Monoclonal immunoglobulin A antibody to the major outer membrane protein of the *Chlamydia trachomatis* mouse pneumonitis biovar protects mice against a chlamydial genital challenge. *Vaccine* 1997;**15**:575–82.

63. Leher H, Zaragoza F, Taherzadeh S, Alizadeh H, Niederkorn JY. Monoclonal IgA antibodies protect against *Acanthamoeba keratitis*. *Exp Eye Res* 1999;**69**:75–84.

64. Enriquez FJ, Riggs MW. Role of immunoglobulin A monoclonal antibodies against P23 in controlling murine *Cryptosporidium parvum* infection. *Infect Immun* 1998;**66**:4469–73.

65. Falero-Diaz G, Challacombe S, Rahman D, Mistry M, Douce G, Dougan G, et al. Transmission of IgA and IgG monoclonal antibodies to mucosal fluids following intranasal or parenteral delivery. *Int Arch Allergy Immunol* 2000;**122**:143–50.

66. Williams A, Reljic R, Naylor I, Clark SO, Falero-Diaz G, Singh M, et al. Passive protection with immunoglobulin A antibodies against tuberculous early infection of the lungs. *Immunology* 2004;**111**:328–33.

67. Ottenhoff TH, Kumararatne D, Casanova JL. Novel human immunodeficiencies reveal the essential role of type-I cytokines in immunity to intracellular bacteria. *Immunol Today* 1998;**19**:491–4.

68. Scanga CA, Mohan VP, Joseph H, Yu K, Chan J, Flynn JL. Reactivation of latent tuberculosis: variations on the Cornell murine model. *Infect Immun* 1999;**67**:4531–8.

69. Condos R, Rom WN, Schluger NW. Treatment of multidrug-resistant pulmonary tuberculosis with interferon-gamma via aerosol. *Lancet* 1997;**349**:1513–5.

70. Black CM, Catterall JR, Remington JS. In vivo and in vitro activation of alveolar macrophages by recombinant interferon-gamma. *J Immunol* 1987;**138**:491–5.

71. Sato K, Akaki T, Tomioka H. Differential potentiation of anti-mycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal macrophages activated by interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha). *Clin Exp Immunol* 1998;**112**:63–8.

72. Rook GA, Steele J, Ainsworth M, Champion BR. Activation of macrophages to inhibit proliferation of *Mycobacterium tuberculosis*: comparison of the effects of recombinant gamma-interferon on human monocytes and murine peritoneal macrophages. *Immunology* 1986;**59**:333–8.

73. Reljic R, Clark SO, Williams A, Falero-Diaz G, Singh M, Challacombe S, et al. Intranasal interferon gamma extends passive IgA antibody protection of mice against *Mycobacterium tuberculosis* lung infection. *Clin Exp Immunol* 2006;**143**:467–73.

74. Yuan Y, Crane DD, Simpson RM, Zhu YQ, Hickey MJ, Sherman DR, et al. The 16-kDa alpha-crystallin (Acr) protein of *Mycobacterium tuberculosis* is required for growth in macrophages. *Proc Natl Acad Sci USA* 1998;**95**:9578–83.

75. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, et al. Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment. *J Exp Med* 2003;**198**:693–704.

76. Reljic R, Crawford C, Challacombe S, Ivanyi J. Mouse IgA inhibits cell growth by stimulating tumor necrosis factor-alpha production and apoptosis of macrophage cell lines. *Int Immunol* 2004;**16**:607–14.

77. Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. *Biochim Biophys Acta* 2002;**1572**:263–73.

78. Beatty WL, Rhoades ER, Hsu DK, Liu FT, Russell DG. Association of a macrophage galactoside-binding protein with Mycobacterium-containing phagosomes. *Cell Microbiol* 2002;**4**:167–76.

79. de Chastellier C, Thilo L. Phagosome maturation and fusion with lysosomes in relation to surface property and size of the phagocytic particle. *Eur J Cell Biol* 1997;**74**:49–62.

80. Sano H, Hsu DK, Yu L, Apgar JR, Kuwabara I, Yamanaka T, et al. Human galectin-3 is a novel chemoattractant for monocytes and macrophages. *J Immunol* 2000;**165**:2156–64.

81. Sato S, Hughes RC. Control of Mac-2 surface expression on murine macrophage cell lines. *Eur J Immunol* 1994;**24**:216–21.

82. Liu FT, Hsu DK, Zuberi RI, Kuwabara I, Chi EY, Henderson Jr WR. Expression and function of galectin-3, a beta-galactoside-binding lectin, in human monocytes and macrophages. *Am J Pathol* 1995;**147**:1016–28.

83. Cortegano I, Pozo V, Cardaba B, Arrieta I, Gallardo S, Rojo M, et al. Interaction between galectin-3 and FcgammaRII induces down-regulation of IL-5 gene: implication of the promoter sequence IL-5REIII. *Glycobiology* 2000;**10**:237–42.

84. Wollenberg A, de la Salle H, Hanau D, Liu FT, Bieber T. Human keratinocytes release the endogenous beta-galactoside-binding soluble lectin immunoglobulin E (IgE-binding protein) which binds to Langerhans cells where it modulates their binding capacity for IgE glycoforms. *J Exp Med* 1993;**178**:777–85.

85. Frigeri LG, Zuberi RI, Liu FT. Epsilon BP, a beta-galactoside-binding animal lectin, recognizes IgE receptor (Fc epsilon RI) and activates mast cells. *Biochemistry* 1993;**32**:7644–9.

86. Wiegeshaus E, Balasubramanian V, Smith DW. Immunity to tuberculosis from the perspective of pathogenesis. *Infect Immun* 1989;**57**:3671–6.

87. Cree IA, Sharpe S, Sturrock NDC, Cochrance IH, Dawlako EC, Smith WCS, et al. Mucosal immunity to mycobacteria in leprosy patients and their contacts. *Leprosy Rev* 1988;**59**:309–16.

88. Rodriguez A, Tjarnlund A, Ivanyi J, Singh M, Garcia I, Williams A, et al. Role of IgA in the defense against respiratory infections IgA deficient mice exhibited increased susceptibility to intranasal infection with *Mycobacterium bovis* BCG. *Vaccine* 2005;**23**:2565–72.

89. Brown RM, Cruz O, Brennan M, Gennaro ML, Schlesinger L, Skeiky YA, et al. Lipoarabinomannan-reactive human secretory immunoglobulin A responses induced by mucosal bacille Calmette–Guerin vaccination. *J Infect Dis* 2003;**187**:513–7.

90. Van Spriel AB, Leusen JH, Vile H, Van De Winkel JG. Mac-1 (CD11b/CD18) as accessory molecule for Fc alpha R (CD89) binding of IgA. *J Immunol* 2002;**169**:3831–6.

91. Tomana M, Kulhavy R, Mestecky J. Receptor-mediated binding and uptake of immunoglobulin A by human liver. *Gastroenterology* 1988;**94**:762–70.

92. Tomana M, Zikan J, Kulhavy R, Bennett JC, Mestecky J. Interactions of galactosyltransferase with serum and secretory immunoglobulins and their component chains. *Mol Immunol* 1993;**30**:277–86.

93. Aicher WK, McGhee ML, McGhee JR, Eldridge JH, Beagley KW, Meyer TF, et al. Mouse IgA Fc receptor on CD3+ T cells.

Molecular forms of IgA that bind to a 38-kDa Fc alpha R protein and development of an anti-Fc alpha R antisera. *J Immunol* 1990;**145**:1745–53.

94. Kitamura T, Garofalo RP, Kamijo A, Hammond DK, Oka JA, Caflisch CR, et al. Human intestinal epithelial cells express a novel receptor for IgA. *J Immunol* 2000;**164**: 5029–34.

95. Mota G, Manciulea M, Cosma E, Popescu I, Hirt M, Jensen-Jarolim E, et al. Human NK cells express Fc receptors for IgA which mediate signal transduction and target cell killing. *Eur J Immunol* 2003;**33**:2197–205.

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