Available online at www.sciencedirect.com
SCIENCE DIRECT®
Microbes and Infection xx (2006) 1–8
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

Passive serum therapy with polyclonal antibodies against *Mycobacterium tuberculosis* protects against post-chemotherapy relapse of tuberculosis infection in SCID mice☆

Evelyn Guirado, Isabel Amat, Olga Gil, Jorge Díaz, Virginia Arcos, Neus Caceres, Vicenç Ausina, Pere-Joan Cardona*

Unitat de Tuberculosi Experimental, Department of Microbiology, Fundació Institut per a la Investigació en Ciències de la Salut Germans Trias i Pujol, Universitat Autònoma de Barcelona, Crta del Canyet s/n, 08916 Badalona, Catalonia, Spain

Received 26 September 2005; accepted 1 December 2005

Abstract

We investigated the protective role of immune-sera against reactivation of *Mycobacterium tuberculosis* infection in SCID mice and found that passive immunization with sera obtained from mice treated with detoxified *M. tuberculosis* extracts (delivered in liposomes in a composition known as RUTI) exerted significant protection. Our SCID mouse model consisted of aerosol infection by *M. tuberculosis*, followed by 3 to 8 weeks of chemotherapy with isoniazid + rifampicin (INH + RIF) (25 and 10 mg/kg, respectively). After infection and antibiotic administration, two groups of mice were treated for up to 10 weeks with intraperitoneal passive immunization using hyperimmune serum (HS) obtained from mice infected with *M. tuberculosis*, treated with chemotherapy (INH + RIF) for 8 weeks and inoculated with RUTI (HS group) or with normal serum (CT group). Significant differences were found between HS and CT groups in the number of bacilli in the lungs (3.68 ± 2.02 vs. 5.72 ± 1.41 $\log_{10}$ c.f.u.), extent of pulmonary granulomatomous infiltration (10.33 ± 0.67 vs. $31.2 \pm 1.77\%$), and percentage of animals without pulmonary abscesses (16.7% vs. 45.5%). These data strongly suggest a protective role of specific antibodies against lung dissemination of *M. tuberculosis* infection.

Keywords: *Mycobacterium tuberculosis*; Murine model; SCID mouse; Serum therapy; Antibody-mediated immunity

1. Introduction

Cell-mediated immunity (CMI) by Th1-specific cells plays a central role in the control of *Mycobacterium tuberculosis* infection; interferon gamma (IFN-γ) secreted by Th1cells, in synergy with tumor necrosis factor (TNF), is crucial for the endogenous activation of antimycobacterial mechanisms, such as the production of nitric oxide and reactive nitrogen intermediates (RNI) by the inducible form of nitric oxide synthase (iNOS) [1–3]. In this process, both CD4 and CD8 T cells play a paramount role as effector cells, which also lead to cytotoxic activity of infected macrophages [3,4]. Although B cells are found in *M. tuberculosis* granuloma, especially during the chronic phase of infection [5], the suggestion that the antibody response plays a role in protection against *M. tuberculosis* remains controversial. However, results from classical studies do not support a protective role for antibody-mediated immunity against *M. tuberculosis* [6]. In fact, in human tuberculosis, the presence of specific antibodies (Abs) against LAM or against antigen-85 complex correlated with the absence of disseminated tuberculosis in a pediatric population [7] and with a positive outcome in tuberculous Mexican Indians [8], respectively. Furthermore, several studies in murine models have revealed a protective role for IgM, IgG

☆ Parts of this study were presented at the Keystone Symposian – Tuberculosis: Integrating Host and Pathogen Biology, Whistler, British Columbia, Canada, April 2–7, 2005.

* Corresponding author. Tel.: +34 93 497 8894; fax: +34 93 497 8895.
E-mail address: pcardona@ns.hugtip.scs.es (P.-J. Cardona).

1286-4579/$ - see front matter © 2006 Published by Elsevier SAS.
doi:10.1016/j.micinf.2005.12.004

and IgA monoclonal antibodies (mAbs) against different antigens of *M. tuberculous* in early lung infection [9,10].

Passive immunization with specific polyclonal antibodies (pAbs) and mAbs also has a protective effect against intracellular pathogens such as *Ehrlichia chaffeensis* in SCID mice [11,12]. However, passive immunization of pAbs did not protect against other intracellular pathogens, such as *Listeria monocytogenes* [13] or *Cryptococcus neoformans* [14], whereas passive immunization with mAbs did [15,16].

The positive immune response that we observed upon treatment with chemotherapy (8 weeks with INH + RIF) and detoxified *M. tuberculosis* extracts (delivered in liposomes in a composition known as RUTI) in a latent tuberculosis mouse model encouraged us to assess the protective action of the strong humoral response elicited [17]. The present investigations reveal that passive immunization with hyperimmune serum (HS) obtained from mice immunized with RUTI restricts dissemination of bacilli in the lungs of SCID mice infected with *M. tuberculosis*. The data from our animal model strongly suggest that the antibody response against multiple antigens of *M. tuberculosis* plays an important protective role against tuberculosis infection.

2. Material and methods

2.1. Mice

Specific pathogen-free (*spf*), 6- to 8-week-old, female, DBA/2 and CB17/Icr-SCID mice were obtained from Harlan Iberica (Sant Feliu de Codines, Catalonia, Spain). The animals were shipped in appropriate conditions with the corresponding certificate of health and origin. All the animals were kept under controlled conditions in a P3 High-Security Facility with sterile food and water ad libitum.

2.2. Animal health

Mice were checked every day using a protocol that evaluated weight loss, apparent good health (bristled hair and wounded skin) and behavior (signs of aggressiveness or isolation) and weighed once a week. Animals were euthanized with an overdose of halothane (Fluothane, Zeneca Farma, Pontevedra, Spain), to avoid suffering. Sentinel animals were used to check *spf* conditions in the facility. Tests for 25 known mouse pathogens were all negative. All experimental proceedings were approved and supervised by the Animal Care Committee of ''Germans Trias i Pujol'' University Hospital in agreement with the European Union Laws for protection of experimental animals.

2.3. Bacteria and infection

M. tuberculosis strains H37Rv Pasteur were grown in Proskauer Beck medium containing 0.01% Tween-80 to mid-log phase and stored at −70 °C in 2-ml aliquots. Mice were placed in the exposure chamber of an airborne infection device (Glas-col Inc., Terre Haute, IN, USA). The nebulization provided an approximate uptake of 20 viable bacilli within the lungs. The number of viable bacteria in the left lung and spleen homogenates were monitored by plating serial dilutions on nutrient Middlebrook 7H11 agar (Biomedics s.l., Madrid, Spain) and counting bacterial colony formation (colony forming units; c.f.u.) after incubation at 37 °C for 21 days. Special care was taken not to include hilar lymph nodes when removing the left lung so as to prevent an artificial increase of c.f.u. values. Lungs were immediately removed after euthanasia by means of a halothane overdose (Zeneca Farma, Pontevedra, Spain). Broncho-alveolar lavage (BAL) was obtained by gentle intratracheal injection of 1 ml phosphate-buffered saline (PBS) and lungs were removed immediately after euthanasia by means of an overdose of halothane (Zeneca Farma, Pontevedra, Spain).

2.4. Serum samples

Blood samples were collected from the retro-orbital veins of all animals after being anaesthetized with halothane. Samples were kept at 4 °C for 8 h; serum was obtained by centrifugation at 2500 × *g*, aliquoted and then kept at −20 °C until needed. A pool of previous positive samples was titrated, aliquoted and kept at −20 °C to be used as control for Western blotting.

2.5. mRNA quantification

The procedures for mRNA quantification are described elsewhere [18]. In short, total RNA from the middle right lobe was removed with a marketed phenol-chloroform method, RNAzol (Cinna/Biotecx, Friendswood, IX, USA). After DNase treatment with DNA-free kit (Ambion, Woodward Austin, USA), a denaturing agarose gel was used to assess the stability of RNA. A Superscript RT kit (Gibco BRL, Grand Island, NY, USA) was used to obtain cDNA by reverse transcription of 5 μg of RNA, following the manufacturer's recommendations. The quantitative analysis for IFN-γ, IL-12p40, RANTES, iNOS, IL-4, IL-10 and TNF was performed using a LightCycler™ System (Roche Biochemicals, Idaho Falls, ID, USA). Real-time PCR was carried out in glass capillaries to a final volume of 10 μl in the presence of 1 μl of 10× reaction buffer (Taq Polymerase, dNTPs, $MgCl_2$, SYBRGreen, Roche Biochemicals), 1 μl of cDNA (or water as negative control, which was always included), $MgCl_2$ to a final concentration of 2–5 mM and primers to a final concentration of 0.5 μM were also added. Following PCR, a single peak was present in the melting-curve analysis, corresponding to a single species of the appropriate estimated size on agarose gel electrophoresis. Expression of hypoxanthine guanine phosphoribosyl transferase (HPRT) mRNA expression was analyzed for every target sample to normalize for efficiency in cDNA synthesis and RNA loading. A ratio based on the HPRT mRNA expression was obtained for each sample.

2.6. Histology and morphometry

The procedures for histology and morphometry have been described elsewhere [20]. Briefly, two right lung lobes from

each mouse were fixed in buffered formalin and subsequently embedded in paraffin. All samples were stained with hematoxylin–eosin, trichrome of Masson and Ziehl Neelsen. For histometry, 5-μm-thick sections from each sample were stained with hematoxylin–eosin and photographed at 6× magnification using a Stereoscopic Zoom SMZ800 microscope (Nikon, Tokyo, Japan) and a Coolpix 990 digital camera (Nikon). Sections of eight lung lobes were studied for each sample. Scion Image (Scion Corporation, Frederick, MD, USA) and Photoshop 5.0 (Adobe Systems Inc., San José, CA, USA), were used to quantify the area of each single lesion and the total tissue area on photomicrographs. In all cases, sections were blindly evaluated.

2.7. Fragmented *M. tuberculosis* cells

Liposome preparations of detoxified, fragmented *M. tuberculosis* (RUTI) were kindly provided by Archivel Technologies, s.l. (Mataró, Catalonia, Spain), manufactured under strict GMP standards. The manufacturing process of RUTI has been published elsewhere [17]. Briefly, *M. tuberculosis* H37Rv are cultured for 3 weeks in Middlebrook 7H11 agar at 37 °C, and colonies are carefully removed and mechanically disrupted using silica-zirconia beads in PBS buffer with 4% Triton X-114. After centrifuging at $3000 \times g$ to remove undisrupted cells, the pellet is centrifuged at $27{,}000 \times g$, the lipidic supernatant is removed and the final product is washed, pasteurized at 65 °C for 40 min and lyophilized. Finally, liposomes are prepared after mixing the cell extracts at high speed with phosphatidylcholine and sodium cholate (20:1.4). The peptide composition of RUTI is determined using SDS–PAGE and Western blot analysis as previously described [17].

2.8. Treatments

Hyperimmune serum (HS) was obtained at week 23 post-infection from DBA/2 mice infected by aerosol with *M. tuberculosis* strain H37Rv Pasteur, treated orally with rifampicin and isoniazid (10 and 25 mg/kg, respectively) for 5 days a week from weeks 9 to 17 post infection. RUTI (245 μg) was administered subcutaneously at weeks 17, 19 and 21 post infection.

Protection studies were carried out in aerosol-infected SCID mice and treated with rifampicin and isoniazid (10 and 25 mg/kg, respectively) from week 3 until week 8. Mice were then divided into two groups and treated with HS (HS group) or non-immune serum from healthy DBA/2 mice (CT group). In both cases, sera were inoculated intraperitoneally with 100 μl of serum every 3 days (a total of four inoculations). Mice were sacrificed at week 10.

The serum obtained from infected DBA/2 mice treated with chemotherapy alone presented low levels of antibodies against antigen 38 (the $\log_{10}$ of the titer was below 2). Hence, this serum was not used in any other serum-therapy experimental group.

2.9. Humoral response

Western blot was carried out as described [17] following preparative SDS–PAGE (12% acrylamide NuPAGE BisTris Gel, Invitrogen), electrotransferred onto nitrocellulose sheets (Millipore, Bedford, MA, USA) using a semidry transfer method (Invitrogen, Carlsbad, CA, USA) and cutting into strips corresponding to 16 lanes. The strips were incubated with 1:25 to 1:51,200 diluted sera, or 1:2 to 1:256 diluted bronchoalveolar lavage (BAL) for 1 h at room temperature. Appropriate horseradish peroxidase (HRP)-labeled secondary goat anti-mouse antibody (Southern Biotechnology Associates Inc., Birmingham, AL, USA), chemiluminescent substrate Super Signal West Pico (Pierce) and X-ray films Hyperfilm ECL (Amersham Biosciences, Arlington Heights, IL, USA) were used. A molecular weight standard was run in each gel (Invitrogen). The humoral response was analyzed using a pre-established peptidic profile (6, 10, 17, 19, 27, 30, 38, 40, 47, 50, 55, 65, 70 kDa) [17].

2.10. Measurement of cytokines in serum

The concentrations of IFN-γ and TNF in serum were measured using specific ELISA kits (Endogen Inc., Cambridge, MA, USA). The detection limit of assays for IFN-γ and TNF was 15 and 5.1 pg/ml, respectively.

2.11. Statistical analysis

Sigma Stat (Jandel Scientific Software, San Rafael, CA, USA) was used to discover differences between experimental groups with the Mann–Whitney rank sum test. Differences were significant for $P < 0.05$.

3. Results

3.1. Treatment of infected mice with HS markedly decreases the number of bacilli in the lungs

Infected SCID mice had 5.98 ± 0.43 $\log_{10}$ c.f.u. in the lungs and 3.51 ± 0.29 $\log_{10}$ c.f.u. in the spleen at 3 weeks after aerosol infection. After five further weeks of chemotherapy these values were 2.98 ± 0.026 $\log_{10}$ c.f.u. and 2.12 ± 1.02 $\log_{10}$ c.f.u., respectively. Fig. 1 shows that serum therapy (HS group) inhibited reactivation after chemotherapy; this inhibition was more evident in the lungs, where mean c.f.u. values decreased by 2 $\log_{10}$ (3.68 ± 2.02 vs. 5.72 ± 1.41 $\log_{10}$ c.f.u.). The bacillary counts in the spleen also decreased, although not significantly (4.75 ± 1.84 vs. 5.79 ± 1.02 $\log_{10}$ c.f.u.). It must be highlighted that the lowest point of detection was at 2 $\log_{10}$. This explains why six mice had the same values, and indicates that some of them could have even lower counts.

3.2. Serum therapy protects against bacillary dissemination in the lungs

Macroscopic exploration of the lungs in necropsy at week 10 post-infection revealed the presence of big abscesses (diameter equal to or higher than 3 mm) in 10 out of 12 mice in the CT (control) group. In contrast, in the HS (serum therapy) group, only 6 out of 11 mice presented abscesses, which

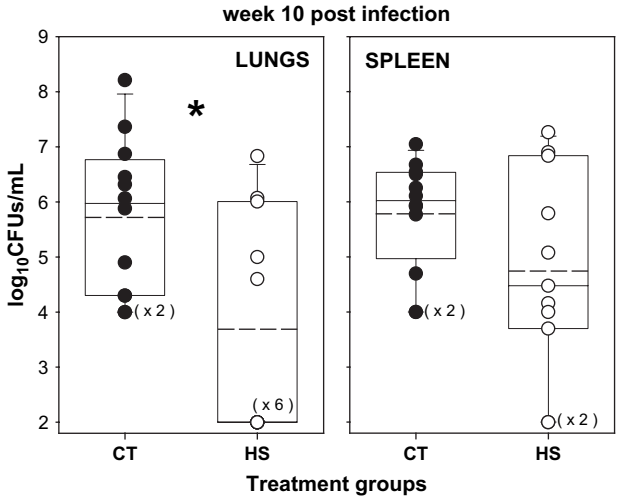

Fig. 1. Bacillary counts at week 10 post-infection in control (CT) animals and animals treated with serum therapy (HS). C.f.u. from lungs and spleens are plotted as individual values from $n = 12$ (control group) and $n = 11$ (HS group) mice and as a box representing statistical values. Continuous and dotted lines within the box indicate the median and the mean values, respectively. The boundary of the box closest to zero indicates the 25th percentile, whereas the boundary of the box farthest from zero indicates the 75th percentile. Whisker caps show the 5th and 95th percentile. Differences were significant when marked with an asterisk for $P < 0.05$.

were of small size (diameter equal to or smaller than 2 mm). Therefore, only 16.7% of the CT group mice presented no abscesses in the lung, whereas this percentage increased to 45.5% in the HS group. Fig. 2 shows low-power microphotographs of the lobes obtained for histological and histometry studies, revealing an increased number of abscesses and granulomas in the CT group when compared with mice treated with serum therapy at week 10 post-infection (HT group). Granulomas were formed essentially by a macrophage-rich core infiltrated with mononuclear cells and neutrophils (Fig. 3A) that were progressively enlarged by a strong neutrophilic infiltration (Fig. 3B) to form an abscess at the end, i.e. a localized collection of necrotic tissue surrounded almost exclusively by fragmented, and rarely, entire neutrophils.

The percentage of pulmonary granulomatous infiltration, defined as the ratio obtained after dividing the infiltrated area by the total area of the lung section, and then multiplying by 100, decreased significantly (Fig. 4), up to threefold, in the serum-therapy group compared with the control group ($10.33 \pm 0.67\%$ vs. $31.2 \pm 1.77\%$) despite the fact that the global area was higher in the latter.

3.3. Pulmonary cytokine and iNOS expression tend to be lower in the serum therapy group

As seen in Fig. 5, no significant differences were found in the lung expression of IFN-γ, RANTES, TNF, IL-12p40 and iNOS at week 10 of the two groups, although levels were consistently lower in mice treated with HS compared with mice in the CT group.

3.4. Absence of antibodies against peptide 40 to 70 in BAL of SCID mice

Serological titers ($\log_{10}$ of the titer) of IgA and IgM antibodies against the different antigens identified in RUTI, as previously described [17], were below 2. The production pattern of different IgG isotypes in the serum of SCID mice treated with serum therapy (Fig. 6) was the same as the hyperimmune serum (HS) obtained from DBA/2 immunized with RUTI, i.e. IgG2a > IgG2b > IgG1 > IgG3 predominance, although antibodies were much less abundant in SCID mice than in immunized DBA/2 mice (a mean difference of 1 $\log_{10}$; data not shown). In SCID mice, levels of antibody in BAL were much lower than in serum and, interestingly, we were not able to detect antibodies against Ag 40 to 70 in BAL.

3.5. Absence of IFN-γ and TNF in the hyperimmune sera

Concentrations of IFN-γ and TNF in the HS were below the detection limits of the commercial kits used.

4. Discussion

After a long period of oblivion, there is an increasing interest in studying antibody-based immunity to understand fully the immunological response against intracellular pathogens such as *C. neoformans*, *L. monocytogenes*, *E. chaffeensis* and *M. tuberculosis* [9,10,12,15,16]. Experimental murine models

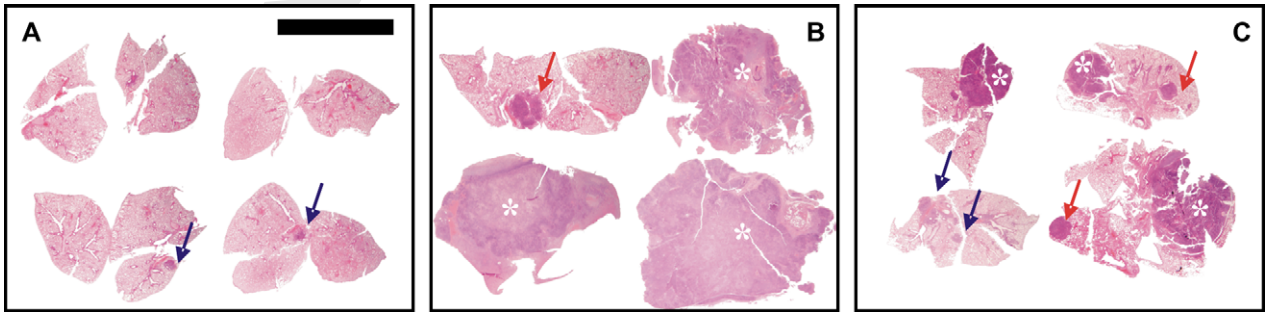

Fig. 2. Histology of representative hematoxylin–eosin stained sections from entire pulmonary lobes at week 8 (A) and week 10 post-infection, at low-power magnification (10×). (B) shows sections from the control (CT) group and (C) shows sections from the group treated with serum therapy (HS). Abscesses are marked with a white asterisk; granulomas are marked with a blue arrow and enlarged granulomas with a red arrow. The bar represents 3 mm.

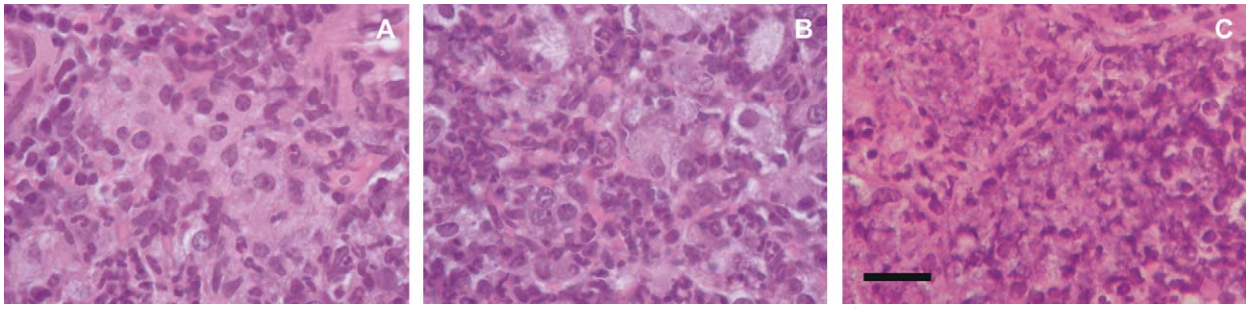

Fig. 3. Histology of the lung lesions at week 10 post-infection at high-power magnification (1000×). Granulomas are formed essentially by a macrophage-rich core infiltrated with mononuclear cells and neutrophils (A). Enlarged granulomas have a strong neutrophilic infiltration (B). Abscesses show a localized collection of necrotic tissue surrounded almost exclusively by fragmented, and rarely, entire neutrophils (C). The bar represents 100 μm.

have demonstrated that cell-mediated immunity is crucial in controlling infections of these intracellular pathogens; infections by these bacteria mostly elicit responses of specific T cells that activate infected macrophages with IFN-γ or result in direct T-cell cytotoxicity against the infective agent [3]. It is noteworthy that antibody responses were not detected in murine models of tuberculosis induced with an aerosol, or only a limited production against glycolipids [19]. A strong antibody response was only detected during the final stages of infection, when lungs were highly infiltrated by granulomas and mice were close to dying (data not published). Interestingly, in murine models of tuberculosis, the high percentage of B cells in the granulomas found during the chronic phase [5] did not correlate with production of antibodies. Thus, the role of the antibody response to tuberculosis infection is controversial. Furthermore, the comparison between murine experimental models and human tuberculosis reveals important differences. In humans, the initial strong inflammatory response in the infectious foci generates intragranulomatous necrosis (*caseum*), and liquefaction of the caseous foci is the source of a large extracellular bacillary population [20]; this phenomenon is never observed in infected mice. In mice, *M. tuberculosis* spreads both through the bloodstream and by getting out the pulmonary granulomas through foamy macrophages that occupy the alveolar spaces; it is thought that some of these infected foamy macrophages harbor a single or just a few bacilli (considered to be in a latent state) that may reactivate [18,21].

The complete predominance of cell-mediated immunity in the control of *M. tuberculosis* infection in mice encouraged us to use the model of SCID infection, as other researchers did before [12,16], to remove all specific immunity and thus explore the protective effect of antibody-mediated immunity. We investigated antibody therapy in a mouse model where the extreme virulence of *M. tuberculosis* infection kills 100% of untreated mice in just 4 weeks. Specifically, we wanted to study the protective effect of antibodies against reactivation, for which we used a mouse model treated with a short period of chemotherapy. Since immunotherapy with RUTI [17] allowed the control of reactivation and induced a bactericidal activity, we hypothesized that protection was effected not only by triggering a poly-antigenic Th1 or a cytotoxic response, but also by the strong antibody response elicited following inoculation with RUTI. Although chemotherapy by itself is a strong inducer of antibody production in humans and potentially plays some role in the control of the bacilli [22,23], this is not the case in mice. Using DBA/2 mice, the production of antibodies after chemotherapy is very limited, as reported above and previously described [17]. On the other hand, this production is quite higher in C57BL/6. Thus, future experiments will dissect the individual contributions of each of these factors to the generation of protective serum antibodies.

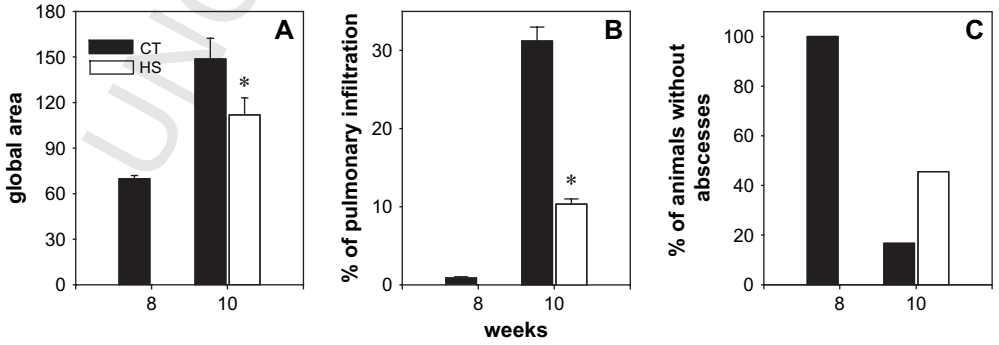

Fig. 4. Quantification of pulmonary infiltration in the lung at week 10 post-infection in control (CT) and serum-therapy (HS) groups. (A) shows the global area of the studied lobes in pre-established (arbitrary) area units. (B) shows the percentage of pulmonary infiltration obtained after dividing the infiltrated area by the total area of the lobes, and then multiplying the result by 100. Values represent the mean value and standard deviation from $n = 12$ (control group) and $n = 11$ (HS group) mice. Differences were significant when marked with an asterisk for $P < 0.05$. (C) shows the percentage of animals without abscesses.

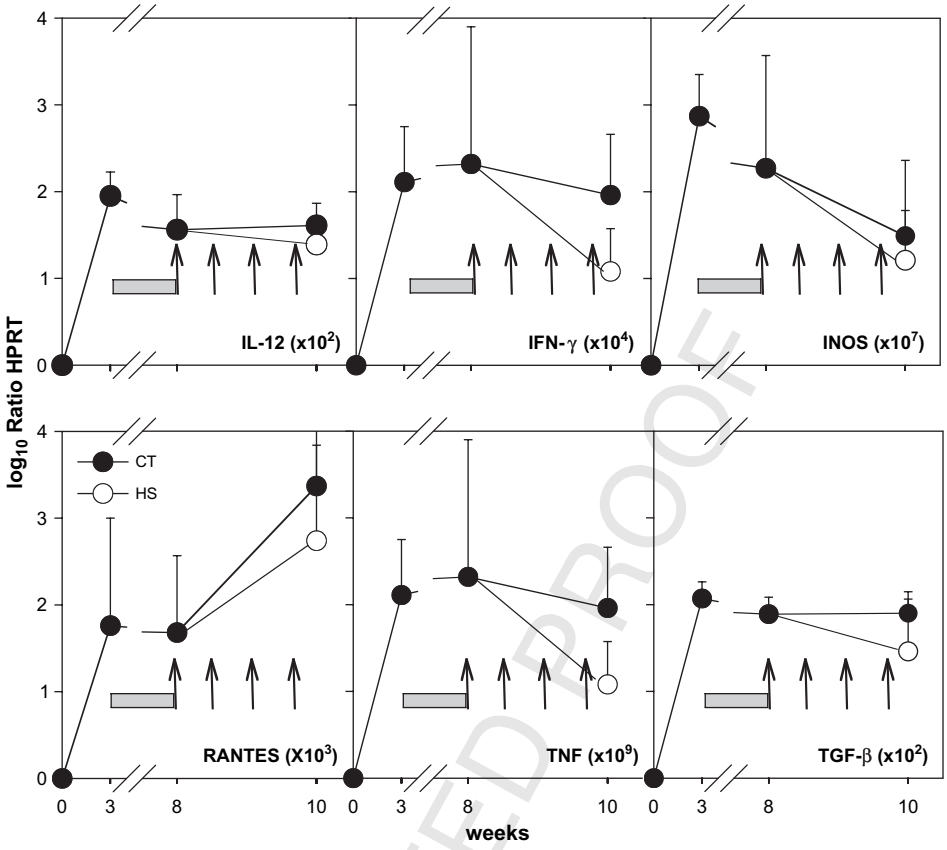

Fig. 5. Expression of cytokines, RANTES and iNOS in the lungs. These are expressed as the $\log_{10}$ of the ratio obtained after dividing every value by the expression of HPRT in each sample and multiplying it by a factor (ranging from 10^2 to 10^9). No significant differences between CT and HS groups were detected. Arrows show every HS inoculation. The gray bar shows the chemotherapy period.

In fact, the result obtained after inoculation with HS from previously immunized mice overwhelmed all expectations. This was already evident at a macroscopic level, when no large abscesses were found in passively-immunized mice; bacillary load was 100-fold lower in treated mice compared with untreated, with up to threefold differences in pulmonary infiltration between the HS and CT groups. Besides, the bacillary reduction was correlated with a decrease in the inflammatory response in the HS group. Whether such a reduction is proportional to the bacillary load or on the contrary, remains relatively higher and helps the control of bacillary growth, is not known, and should be clarified in future studies.

Globally, these data suggest that the protective effect is localized in the intra-alveolar space, because it is known that the control of bacterial reactivation is associated to the control of pulmonary dissemination [21]. Furthermore, HS therapy appears to have little effect on the spleen, consistent with a limited systemic effect of HS therapy. Concerning the mechanisms involved in the control of infection, our data only indicate that it is independent of the presence of a Th1 response, as suggested by other authors [24,25]. The suggestion that protection of serum therapy in our experiments is independent of a Th1 response is supported by the type of hosts used (SCID mice), by the finding that the expression of Th1-associated cytokines did not increase upon treatment, and also because the response was not mediated by IgA antibodies. The levels of IgA and IgM were negligible in the hyperimmune serum and, therefore, the protective effect appears to be mediated only by IgG antibodies. This data confirms the protective effect of IgG recently found by Hamasur et al. [26], who have demonstrated the protective value of IgG mAbs against LAM.

A protective role of IgG mAbs in experimental models of murine tuberculosis is also suggested in the literature in experiments that used *M. tuberculosis* or BCG cells pre-coated with mAbs before inoculation in mice. Specifically, *M. tuberculosis* cells coated with IgG3 against arabinomannan (AM) enhanced granulomatous formation and survival of intratracheally inoculated mice [27]; *M. bovis* BCG cells coated with IgG2a and IgG3 anti heparin-binding hemagglutinin adhesin (HBHA) mAbs before intranasal inoculation limited extrapulmonary dissemination and reduced the number of bacilli in the spleen [28], whereas *M. bovis* cells coated with IgG2b anti-MPB83 mAbs before intravenous inoculation increased long-term survival but did not reduce the bacterial load [29]. In our study, we obtained a strong humoral response against a wide antigenic range with an IgG2a - IgG2b > IgG1 > IgG3 predominance. Additional studies are required to identify with greater precision the serum fraction(s) that is (are) responsible for the protective effect described in this work (immunoglobulin fraction, fractionation by antibody isotype, etc).

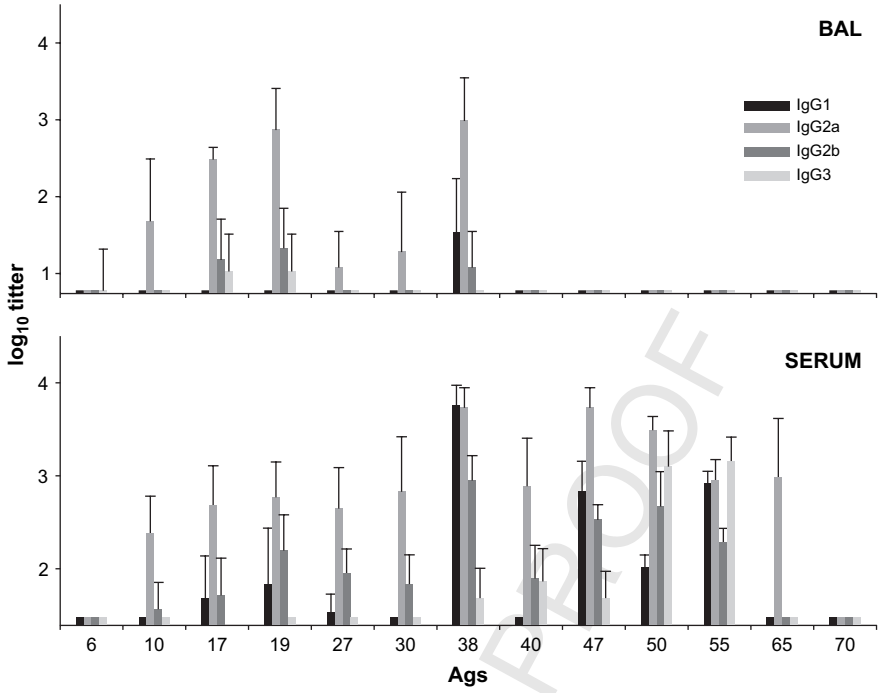

Fig. 6. IgG antibody levels in serum and BAL at week 10. Western blots were carried out using 1:25 to 1:51,200 diluted sera, or 1:2 to 1:256 diluted BAL. The results are the mean values and the standard deviations of the sera obtained from the 11 mice of the serum-therapy (HS) group for each time point.

In conclusion, passive immunization using antibodies generated against fragmented *M. tuberculosis* cells protects against reactivation of tuberculosis in SCID mice treated for a short period of time with chemotherapy, thus supporting the hypothesis that antibody-mediated immunity plays a central role in controlling *M. tuberculosis* infection.

Acknowledgements

This work was supported in part with grants from SEPAR and Fondo de Investigaciones Sanitarias (FIS 01/3104, 02/545 and 03/0757) and Archivel Technologies s.l. E.G. received a research grant from the Spanish Society for Microbiology and Infectious Diseases (SEIMC). We thank Ms. Marga Ortiz from the Center for Animal Experimentation, and the technicians of the Pathology Department, Hospital Universitari Germans Trias i Pujol, for their technical support, and Deborah Cullell Young for her excellent English style revision.

References

[1] I.E. Flesch, S.H. Kaufmann, Activation of tuberculostatic macrophage functions by gamma interferon, interleukin-4, and tumor necrosis factor, Infect. Immun. 58 (1990) 2675–2677.

[2] A.M. Cooper, D.K. Dalton, T.A. Stewart, J.P. Griffin, D.G. Russell, I.M. Orme, Disseminated tuberculosis in interferon gamma gene-disrupted mice, J. Exp. Med. 178 (1993) 2243–2247.

[3] J.L. Flynn, J. Chan, Immunology of tuberculosis, Annu. Rev. Immunol. 19 (2001) 93–129.

[4] J.E. Grotzke, D.M. Lewinsohn, Role of CD8(+) T lymphocytes in control of *Mycobacterium tuberculosis* infection, Microbes Infect. 7 (2005) 776–788.

[5] M. Gonzalez-Juarrero, O.C. Turner, J. Turner, P. Marietta, J.V. Brooks, I.M. Orme, Temporal and spatial arrangement of lymphocytes within lung granulomas induced by aerosol infection with *Mycobacterium tuberculosis*, Infect. Immun. 69 (2001) 1722–1728.

[6] A. Glatman-Freedman, A. Casadevall, Serum therapy for tuberculosis revisited: reappraisal of the role of antibody-mediated immunity against *Mycobacterium tuberculosis*, Clin. Microbiol. Rev. 11 (1998) 514–532.

[7] A.M. Costello, A. Kumar, V. Narayan, M.S. Akbar, S. Ahmed, C. Abou-Zeid, G.A. Rook, J. Stanford, C. Moreno, Does antibody to mycobacterial antigens, including lipoarabinomannan, limit dissemination in childhood tuberculosis? Trans. R. Soc. Trop. Med. Hyg. 86 (1992) 686–692.

[8] C. Sanchez-Rodriguez, C. Estrada-Chavez, J. Garcia-Vigil, F. Laredo-Sanchez, J. Halabe-Cherem, A. Pereira-Suarez, R. Mancilla, An IgG antibody response to the antigen 85 complex is associated with good outcome in Mexican Totonaca Indians with pulmonary tuberculosis, Int. J. Tuberc. Lung Dis. 6 (2002) 706–712.

[9] A. Williams, R. Reljic, I. Naylor, S.O. Clark, G. Falero-Diaz, M. Singh, S. Challacombe, P.D. Marsh, J. Ivanyi, Passive protection with immunoglobulin A antibodies against tuberculous early infection of the lungs, Immunology 111 (2004) 328–333.

[10] A. Glatman-Freedman, Advances in antibody-mediated immunity against *Mycobacterium tuberculosis*: implications for a novel vaccine strategy, FEMS Immunol. Med. Microbiol. 39 (2003) 9–16.

[11] J.S. Li, E. Yager, M. Reilly, C. Freeman, G.R. Reddy, A.A. Reilly, F.K. Chu, G.M. Winslow, Outer membrane protein-specific monoclonal antibodies protect SCID mice from fatal infection by the obligate intracellular bacterial pathogen Ehrlichia chaffeensis, J. Immunol. 166 (2001) 1855–1862.

[12] G.M. Winslow, E. Yager, K. Shilo, E. Volk, A. Reilly, F.K. Chu, Antibody-mediated elimination of the obligate intracellular bacterial pathogen Ehrlichia chaffeensis during active infection, Infect. Immun. 68 (2000) 2187–2195.

[13] G.B. Mackaness, Cellular resistance to infection, J. Exp. Med. 116 (1962) 381–406.

[14] A. Casadevall, Antibody immunity and invasive fungal infections, Infect. Immun. 63 (1995) 4211–4218.

[15] F. Dromer, J. Charreire, A. Contrepois, C. Carbon, P. Yeni, Protection of mice against experimental cryptococcosis by anti-Cryptococcus neoformans monoclonal antibody, Infect. Immun. 55 (1987) 749–752.
[16] B.T. Edelson, P. Cossart, E.R. Unanue, Cutting edge: paradigm revisited: antibody provides resistance to Listeria infection, J. Immunol. 163 (1999) 4087–4090.
[17] P.J. Cardona, I. Amat, S. Gordillo, V. Arcos, E. Guirado, J. Diaz, C. Vilaplana, G. Tapia, V. Ausina, Immunotherapy with fragmented *Mycobacterium tuberculosis* cells increases the effectiveness of chemotherapy against a chronic infection in a murine model of tuberculosis, Vaccine 23 (2005) 1393–1398.
[18] P.J. Cardona, S. Gordillo, J. Diaz, G. Tapia, I. Amat, A. Pallares, C. Vilaplana, A. Ariza, V. Ausina, Widespread bronchogenic dissemination makes DBA/2 mice more susceptible than C57BL/6 mice to experimental aerosol infection with *Mycobacterium tuberculosis*, Infect. Immun. 71 (2003) 5845–5854.
[19] P.J. Cardona, E. Juliñan, X. Vallès, S. Gordillo, M. Muñoz, M. Luquin, V. Ausina, Production of antibodies against glycolipids from the *Mycobacterium tuberculosis* cell wall in aerosol murine models of tuberculosis, Scand. J. Immunol. 55 (2002) 639–645.
[20] S.B. Lucas, in: P.D.O. Davies (Ed.), Histopathology, Clinical Tuberculosis, vol. 8, Chapman and Hall, London, 1998, pp. 113–127.
[21] P.J. Cardona, R. Llatjos, S. Gordillo, J. Diaz, I. Ojanguren, A. Ariza, V. Ausina, Evolution of granulomas in lungs of mice infected aerogenically with *Mycobacterium tuberculosis*, Scand. J. Immunol. 52 (2000) 156–163.
[22] L.M. Pereira Arias-Bouda, S. Kuijper, A. Van Der Werf, L.N. Nguyen, H.M. Jansen, A.H.J. Kolk, Changes in avidity and level of immunoglobulin G antibodies to *Mycobacterium tuberculosis* in sera of patients undergoing treatment for pulmonary tuberculosis, Clin. Diagn. Lab. Immunol. 10 (2003) 702–709.
[23] G.H. Bothamley, Epitope-specific antibody levels demonstrate recognition of new epitopes and changes in titer but not affinity during treatment of tuberculosis, Clin. Diagn. Lab. Immunol. 11 (2004) 942–951.
[24] Y. Zhang, S. Pacheco, C.L. Acuna, K.C. Switzer, Y. Wang, X. Gilmore, G.R. Harriman, I.N. Mbawuike, Immunoglobulin A-deficient mice exhibit altered T helper 1-type immune responses but retain mucosal immunity to influenza virus, Immunology 105 (2002) 286–294.
[25] T. Moore, C.O. Ekworomadu, F.O. Eko, L. MacMillan, K. Ramey, G.A. Ananaba, J.W. Patrickson, P.R. Nagappan, D. Lyn, C.M. Black, J.U. Igietseme, Fc receptor-mediated antibody regulation of T cell immunity against intracellular pathogens, J. Infect. Dis. 188 (2003) 617–624.
[26] B. Hamasur, M. Haile, A. Pawlowski, U. Schröder, G. Källenius, S.B. Svenson, A mycobacterial lipoarabinomannan specific monoclonal antibody and its $F(ab')_2$ fragment prolong survival of mice infected with *Mycobacterium tuberculosis*, Clin. Exp. Immunol. 138 (2004) 30–38.
[27] R. Teitelbaum, A. Glatman-Freedman, B. Chen, J.B. Robbins, E. Unanue, A. Casadevall, B.R. Bloom, A mAb recognizing a surface antigen of *Mycobacterium tuberculosis* enhances host survival, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 15688–15693.
[28] K. Pethe, S. Alonso, F. Biet, G. Delogu, M.J. Brennan, C. Locht, F.D. Menozzi, The heparin-binding haemagglutinin of *M. tuberculosis* is required for extrapulmonary dissemination, Nature 412 (2001) 190–194.
[29] M.A. Chambers, D. Gavier-Widen, R.G. Hewinson, Antibody bound to the surface antigen MPB83 of Mycobacterium bovis enhances survival against high dose and low dose challenge, FEMS Immunol. Med. Microbiol. 41 (2004) 93–100.