Tuberculosis 95 (2015) 48–53

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

IMMUNOLOGICAL ASPECTS

BALB/c mice display more enhanced BCG vaccine induced Th1 and Th17 response than C57BL/6 mice but have equivalent protection

M. Carmen Garcia-Pelayo [a], Véronique S. Bachy [a, b], Daryan A. Kaveh [a], Philip J. Hogarth [a, *]

[a] Animal & Plant Health Agency (APHA), Woodham Lane, Addlestone KT15 3NB, United Kingdom
[b] Current address: Orbio Laboratoire, 12C rue du 35eme Regiment d'Aviation, 69500 Bron, France

ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form
29 October 2014
Accepted 31 October 2014

Keywords:
Tuberculosis
BCG
Vaccine
Th1
Th17

SUMMARY

It is generally assumed that the inbred mouse strains BALB/c (*H-2*^{d}) and C57BL/6 (*H-2*^{b}) respond to mycobacterial infection with distinct polarisation of T helper responses, with C57BL/6 predisposed to Th1 and BALB/c to Th2. We investigated this in a BCG-immunisation, *Mycobacterium bovis* challenge model. Following immunisation, lung and spleen cell cytokine responses to *in vitro* re-stimulation with a cocktail of seven secreted, immunogenic, recombinant mycobacterial proteins were determined. In both lung and spleen, BALB/c cells produced at least 2-fold more IFN-γ, and up to 7-fold more IL-2 and IL-17 than C57BL/6 cells, whereas IL-10 production was reciprocally increased in C57BL/6 mice. These data suggest that, contrary to reports in the literature, specific mycobacterial antigens are able to induce strong Th1 and Th17 responses in BALB/c mice following BCG vaccination, whilst in C57BL/6 mice, the Th1 response is partly counterbalanced by IL-10. After subsequent *M. bovis* low dose challenge, protection, as measured in the lungs and dissemination to the spleen, was equivalent in BALB/c and C57BL/6 mice, indicating that BCG-induced immunity was equivalent in both strains. Thus, the differential immune responses do not appear to have a role in protection, but further, as yet unidentified, specific immune responses play a significant role.

1. Introduction

In inbred mouse strains, defense against intracellular pathogens such as mycobacteria, salmonella and leishmania involves an early innate immune response and a late adaptive cell-mediated immunity with T lymphocytes being the main effector cells. The innate response is mainly controlled by the expression of the single dominant gene *Bcg/Ity/Lsh* which affects the ability of the murine host to restrict proliferation of these pathogens within macrophages [1,2]. The strains BALB/c (*H-2*^{d}) and C57BL/6 (*H-2*^{b}) bear the same susceptible allele of the *Bcg/Ity/Lsh* gene (2), but have shown contrasting susceptibilities to certain intracellular pathogens. Thus, BALB/c mice are susceptible to *Leishmania major*, *Yersinia enterocolitica* and *Chlamydia trachomatis* infection, whereas C57BL/6 mice are resistant [3–6]. Such dichotomy was linked to the balance between T helper cell populations; leishmania infection triggers a strong Th2 response in susceptible BALB/c mice, characterized by increased production of IL-4 and IL-10, whereas resistant C57BL/6 mice preferentially activate Th1 cells producing IFN-γ and IL-12 [3,4]. A similar shift towards Th1 responses in C57BL/6 and Th2 in BALB/c was also reported in response to mycobacterial vaccination; C57BL/6 mice exhibiting an enhanced protective immunity to intravenous re-challenge with *Mycobacterium bovis* BCG (BCG) when compared to BALB/c [7]. The lack of a strong protective response in BALB/c has been associated with a reduced ability to express the Th1 cytokine IL-12, and subsequent lower levels of IFN-γ [8]. Furthermore, treatment of BALB/c mice with recombinant IL-12 enhanced host defense against intravenous *Mycobacterium tuberculosis* infection [9]. Another study suggested that the suppressed Th1 response to mycobacterial infection in BALB/c mice was not a result of an enhanced Th2 response; BALB/c mice developed a greater early innate response, but failed to develop a strong Th1 response compared to C57BL/6 mice [10].

Experimental studies have therefore shown divergent results on the immune profile and the nature of responses to mycobacterial infections in mice of different genetic backgrounds. In this study we analysed the cytokine secretion profile of lymphocytes derived from BCG immunised C57BL/6 and BALB/c mice and compared protective immunity after intranasal challenge with virulent *M. bovis*. Contrary to previous studies, we found that BCG

* Corresponding author.
E-mail address: philip.hogarth@apha.gsi.gov.uk (P.J. Hogarth).

http://dx.doi.org/10.1016/j.tube.2014.10.012

vaccinated BALB/c mice mounted a larger Th1 and Th17 response, with increased levels of IFN-γ, IL-2 and IL-17, whereas C57BL/6 mice produced more IL-10, characteristic of a Th2 response. Interestingly, BCG vaccination generated comparable protective immunity in both strains, indicating that the differential immune responses observed do not have a critical role in protection against primary infection with *M. bovis*.

2. Materials & methods

2.1. Ethics statement

All animal work was carried out in accordance with the UK Animal (Scientific Procedures) Act 1986; under appropriate personal and project licences. The study protocol was approved by the APHA Animal Use Ethics Committee (UK Home Office PCD number 70/6905).

2.2. Animals

Female BALB/c and C57BL/6 mice were obtained from SPF facilities at Charles River UK Ltd and used at 8 weeks of age. All animals were housed in appropriate containment facilities at APHA.

2.3. Mycobacteria

The vaccination strain used was the human vaccine *M. bovis* BCG Danish 1331, prepared as per manufacturer's instructions (SSI, Denmark).

M. bovis isolate AF2122/97 was grown to mid-log phase in Middlebrook 7H9 broth (Gibco, UK) supplemented with 4.16 g/L pyruvic acid, 10% v/v OADC and 0.05% v/v Tween 80 (all Sigma, UK), subsequently frozen at −80 °C, and used for all virulent challenges.

2.4. Mycobacterial antigens

A cocktail of 7 secreted, immunogenic recombinant mycobacterial proteins (Rv1886c, Rv0251, Rv0287, Rv0288, Rv3019c, Rv3763, and Rv3804c) was used for antigen-specific stimulation. Rv1886, Rv3019c, Rv3804c and Rv3763 were purchased from Lionex GmbH, Germany. Rv0251, Rv0287 and Rv0288 were purchased from Proteix s.r.o., Czech Republic.

2.5. Immunisation and mycobacterial challenge

Mice were immunized with a single intradermal (i.d.) injection of 2×10^5 CFU of BCG. Placebo control mice were immunized with PBS. Six weeks following immunisation, mice were either challenged intranasally (i.n.) with approx. 400 CFU of virulent *M. bovis*, as previously described [11], or euthanised and the lungs and spleens removed for cell isolation prior to immunological assays. Intranasal challenge in our laboratory results in retention of 75% of the administered dose to the lungs [11]. Four weeks after challenge, the mice were euthanized and the lungs and spleens removed and homogenised. These homogenates were serially diluted and plated out on modified Middlebrook 7H11 agar media [12]. Bacterial colonies were enumerated four weeks later following incubation at 37 °C.

2.6. Cell isolations and stimulations

Spleen, and interstitial lung lymphocytes were prepared as previously described [13]. Cells were cultured with a pool of 7 recombinant antigens as described, each antigen at a final concentration of 2 μg/ml for all assays.

2.7. IFN-γ ELISPOT

5×10^5 cells were incubated in duplicate in 96 well filter plates (MSIPS4510 Millipore, Ireland) with or without antigen for 16 h and the frequency of IFN-γ secretors detected by ELISPOT (Mabtech, Sweden), as per manufacturer's instructions.

2.8. Cytokine production

Spleen and lung interstitial lymphocytes (5×10^6/ml) were cultured in the presence of antigen cocktail for 72 h (37 °C/5% CO_2) prior to harvest of supernatant. Production of IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-17 and IL-22 was measured by a capture ELISA using the Mesoscale Discovery (MSD®, Mesoscale, USA) platform according to manufacturer's instructions.

2.9. Statistical analyses

All data were analysed by 1-way ANOVA with Tukey's post test, using GraphPad Instat 3 statistical package (GraphPad, USA). Mycobacterial counts were previously $\log_{10}$ transformed and ELISA/ELISPOT data were corrected by subtracting the unstimulated from the antigen-stimulated culture values prior to analysis.

3. Results

3.1. Frequency of IFN-γ producing cells after BCG vaccination

Given the importance of IFN-γ responses in protection against tuberculosis, we evaluated the frequency of antigen-specific IFN-γ producing cells by ELISPOT six weeks after BCG immunisation, by *in vitro* re-stimulation with a cocktail of mycobacterial antigens. In the spleen (Figure 1A), vaccination induced a significantly greater frequency of IFN-γ secretors compared to the placebo controls in both BALB/c and C57BL/6 strains, indicating functional Th1 cells. The frequency of secretors was similar between both strains with a mean value of 613 SFU/million cells in BALB/c and 619 SFU/million cells in C57BL/6. In the lungs, however (Figure 1B), whilst vaccination induced a significant increase in IFN-γ secreting cells in both strains; the frequency was significantly higher in BALB/c (924 vs. 578 SFU/million cells) indicating a stronger mucosal antigen-specific immune response.

In order to compare these data to previously published studies, we evaluated the frequency of IFN-γ producing cells using PPD-B to stimulate responses. PPD was a less potent inducer of IFN-γ producing cells (Figure 2A), even used at 5 times normal concentration at 50 μg/ml. In vaccinated mice, PPD stimulated a maximum of 186 SFU/million cells in BALB/c and 102 SFU/million cells in C57BL/6 strains, more than threefold lower than the M7 cocktail responses described in Figure 1, and not significantly higher than naïve controls. Despite this poor performance, the frequency of Th1 responders induced by BCG vaccination was greater (N.S.) in BALB/c than C57BL/6 mice when stimulated by PPD.

Although defined, M7 still represents a mixture of antigens. To investigate strain differences & potential MHC restriction in antigen recognition following vaccination, spleen cells from BALB/c and C57BL/6 were subjected to IFN-γ ELISPOT six weeks after BCG immunisation and re-stimulated *in vitro* with the individual antigens of the M7 cocktail (Figure 2B). BALB/c mice responded exclusively to Rv0288 and C57BL/6 to Rv0287, whilst both strains responded to Rv3019c & Rv3763 & M7, indicating that the M7 cocktail induces a balanced response in both strains.

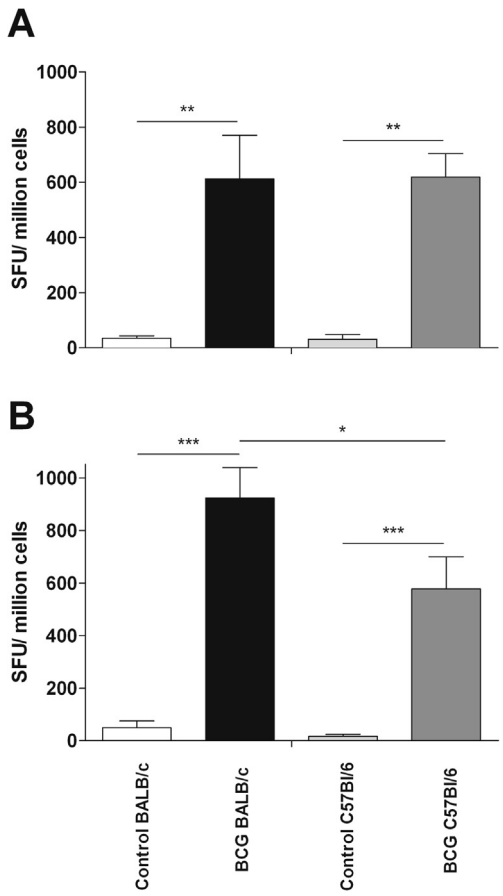

Figure 1. Frequency of IFN-γ secreting cells in spleen (A) and lung (B) from BALB/c or C57BL/6 mice immunised i.d. with 2×10^5 CFU of BCG six weeks earlier. Following a 16 h re-stimulation *in vitro* with a cocktail of 7 mycobacterial recombinant proteins, cells were enumerated for IFN-γ production by ELISPOT. Bars represent means ± SEM ($n = 6$); $^{**}p < 0.01$, $^{***}p < 0.001$, versus naive controls; $^{*}p < 0.05$, BCG BALB/c versus BCG C57BL/6. Data representative of 2 independent studies.

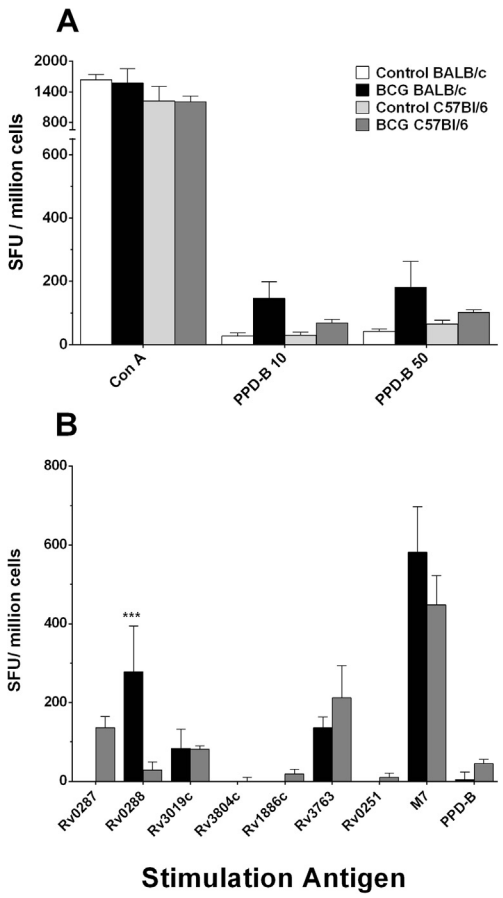

Figure 2. Frequency of IFN-γ secreting cells in spleen from BALB/c/or B6 mice immunised i.d. with 2×10^5 CFU of BCG six weeks earlier. Following a 16 h re-stimulation *in vitro* with 10 or 50 μg PPD-B (A), or the individual components of M7 cocktail (B), cells were enumerated for IFN-γ production by ELISPOT. Results are shown as means ± SEM for groups of 6 mice; $^{***}p < 0.001$, BALB/c M7 *vs* C57BL/6.

3.2. Magnitude of antigen-specific cytokine recall responses

To further characterize the antigen-specific immune response induced by BCG, we assessed the magnitude of the mucosal and systemic cytokine responses six weeks after vaccination. After *in vitro* re-stimulation of lung and spleen cell cultures with a cocktail of mycobacterial antigens, cytokine production was measured by ELISA. BCG vaccinated BALB/c splenocytes produced significantly higher levels of both the type 1 cytokines IFN-γ and IL-2; and the type 17 cytokines IL-17 and IL-22 than C57BL/6 vaccinates (Figure 3). In contrast, BCG vaccination of C57BL/6, induced significantly higher levels of the immunosuppressive cytokine IL-10, compared to BALB/c. In lung lymphocyte cultures (Figure 4), the pattern of responses was broadly similar, with significant increases in the production of IFN-γ, IL-4, IL-6, TNF-α, IL-2, IL-17 and IL-22 in BALB/c and significantly higher levels of IL-10 in C57BL/6. Interestingly, as with the IFN-γ ELISPOT, antigen-specific responses from C57BL/6 lung derived lymphocytes are significantly lower, in magnitude as well as frequency.

3.3. Protective efficacy of BCG against M. bovis infection

We next compared the vaccine induced protection between BALB/c and C57BL/6 mice after low dose i.n. challenge with *M. bovis*. Mice were challenged six weeks after vaccination, and bacterial load (CFU) assessed in lungs and spleen four weeks after challenge. As shown in Figure 5, both in spleen (A) and lungs (B) BALB/c and C57BL/6 mice showed a significant reduction in the bacterial burden when compared to the placebo controls, thus indicating that BCG vaccination induces protection in both strains. Furthermore, BCG induced protection was similar in both strains with a bacterial reduction in the spleen of approximately 1.4 Log_{10} CFU in BALB/c and 1.3 Log_{10} CFU in C57BL/6. Similar results were found in the lungs with a reduction of 1.3 Log_{10} CFU and 1.2 Log_{10} CFU in BALB/c and C57BL/6, respectively.

4. Discussion

The aim of this study was to assess the nature of the immune responses and subsequent protection against *M. bovis* challenge elicited by BCG vaccination in two murine hosts with different genetic backgrounds; BALB/c ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$). We report that, despite displaying equivalent levels of protection, there are distinct differences in the immune response to vaccination, some of which challenge the commonly accepted dogma regarding these two commonly utilized strains.

BALB/c mice are capable of developing a fully functional adaptive Th1 immune response after a single BCG immunisation, as indicated by the induction of a significant frequency of both systemic and mucosal IFN-γ secreting cells coupled to the significant production of other Th1 cytokines. Overall this Th1 response was

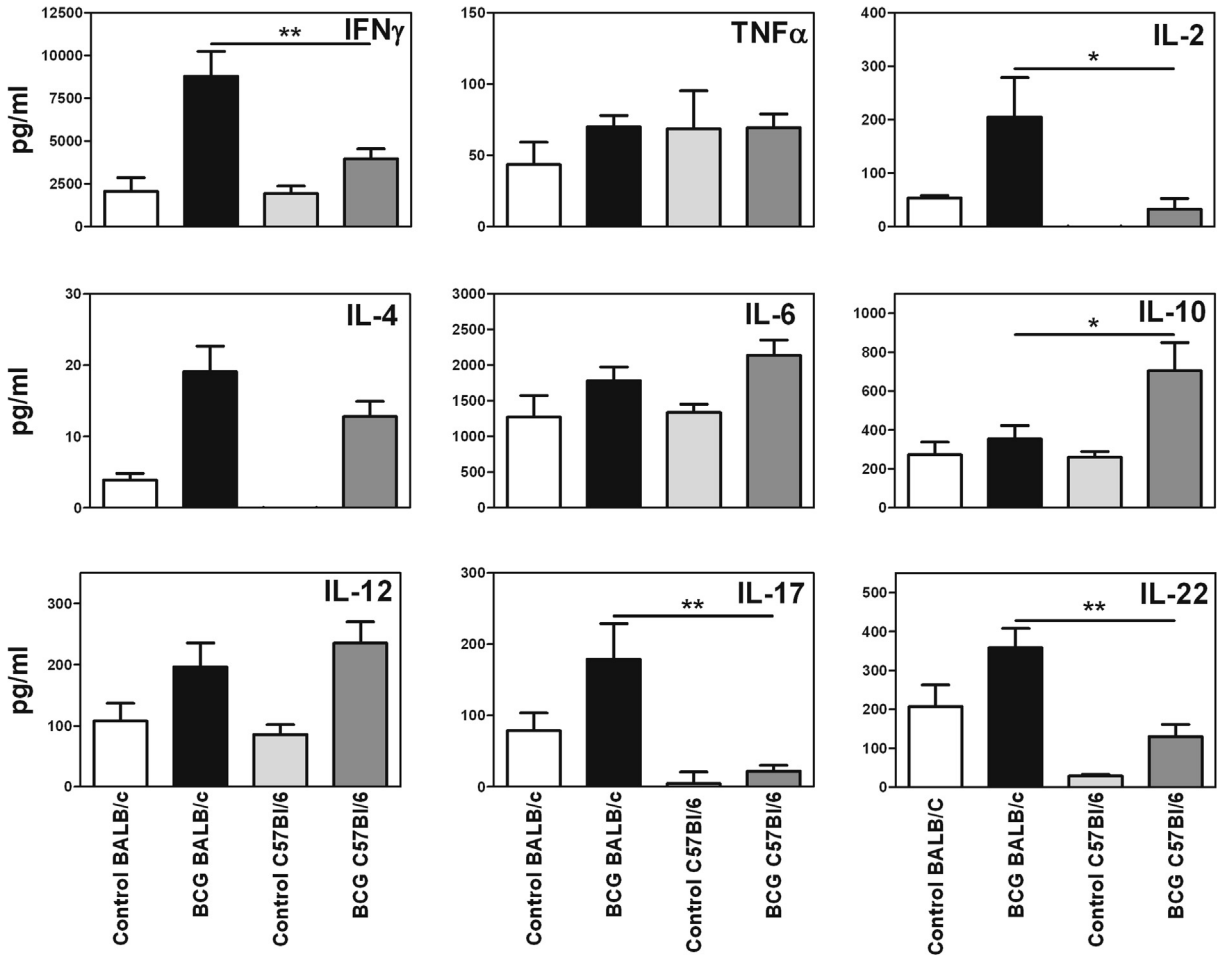

Figure 3. Cytokine production in spleen cell cultures from BALB/c or C57BL/6 mice immunised i.d. with 2×10^5 CFU of BCG six weeks earlier. The cells were stimulated *in vitro* for 72 h with a cocktail of 7 mycobacterial recombinant proteins. Bars represent means ± SEM ($n = 6$); $^*p < 0.05$, $^{**}p < 0.01$, BCG BALB/c versus BCG C57BL/6. Data representative of 2 independent studies.

larger in BALB/c than C57BL/6, apart from IL-12 which was equivalent in C57BL/6 derived cells. Previous studies, however, have reported a significantly lower Th1 response in BALB/c after systemic or pulmonary BCG infection when compared to C57BL/6 mice [7,9,10]. This discrepancy with our studies may be due to several reasons:

i) The difference in the recall antigens used for *in vitro* re-stimulation: PPD vs. a cocktail of mycobacterial proteins in our experiments. In support of this hypothesis, we found Th1 responses to be threefold lower, and not significantly above controls in when PPD-B was used for re-stimulation either at 10 or 50 μg/ml (Figure 2A). Despite this poor response to PPD, the trend toward a higher response observed in BALB/c further support our observations. These low PPD-specific responses are a consistent feature in our model and have been repeated in BALB/c mice a number of times.
ii) Previous studies utilised different strains of BCG and different immunisation routes. Our experiments used a human vaccine preparation (BCG Danish 1331) without *in vitro* growth and employed the intradermal immunisation route to closely match human vaccination protocols. In contrast, for example, Huygen et al. [7], used intravenous administration of pellicle grown BCG Pasteur GL2; whilst Wakeham et al. [10], used intra-tracheal admin of broth grown BCG Connaught.

With such variation between experimental conditions, it is therefore perhaps not so surprising that discrepancies in reported results occur.

Assessing the magnitude and frequency of key cytokines, such as IFN-γ has remained the standard measurement of vaccine induced immune responses for many years. It has become clear recently, however, that these relatively simple *in vitro* assays cannot entirely describe the complexity of the immune responses to vaccination [14]. These assays must therefore be interpreted with care as it is well documented that ESAT-6 and PPD stimulated IFN-γ correlates with bacterial load [15–17] whilst TB10.3 (Rv3019c) and TB10.4 (Rv0288) induced responses strongly correlate with protection [18,19]. Thus, in the current study we have used a defined cocktail of secreted, strongly antigenic proteins to circumvent this bias towards a single antigen or ill defined crude preparation potentially skewing the results.

We found that, following BCG vaccination, the frequency and magnitude of IFN-γ secretion are positively correlated in the mucosal response, but not at the systemic level; the frequency of secretors in the spleen is equal in both strains, but the total levels of IFN-γ are higher in BALB/c, suggesting a lower production of IFN-γ on a per *cell* basis in C57BL/6.

Another important observation of this study was that BALB/c mice exhibited a stronger Th17 response after BCG vaccination, as measured by IL-17 and IL-22. Increasing evidence suggests the

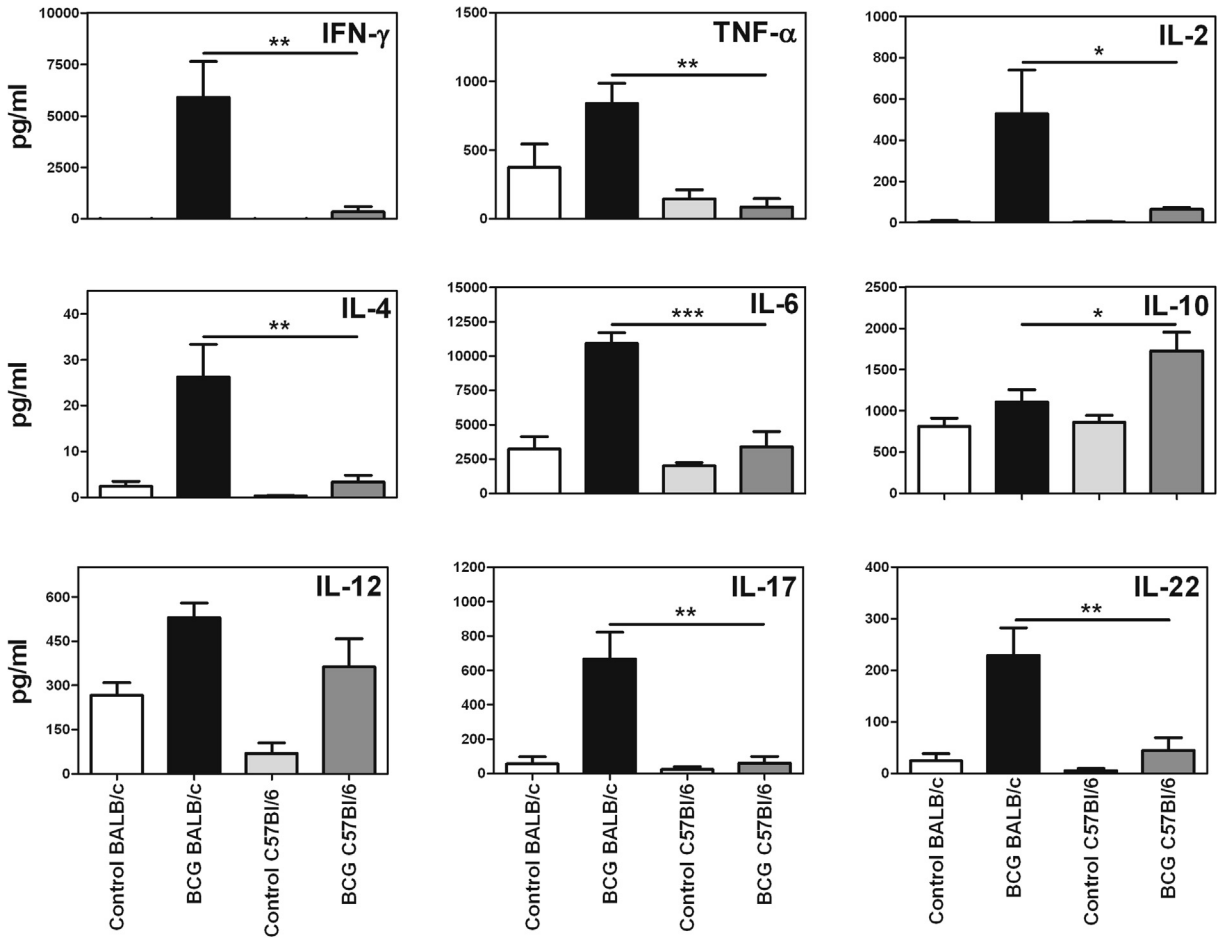

Figure 4. Cytokine production in lung cell cultures from BALB/c or C57BL/6 mice immunised i.d. with 2×10^5 CFU of BCG six weeks earlier. The cells were stimulated *in vitro* for 72 h with a cocktail of 7 mycobacterial recombinant proteins. Bars represent means ± SEM ($n = 6$); $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, BCG BALB/c versus BCG C57BL/6. Data representative of 2 independent studies.

involvement of the IL-17 pathway in defense against intracellular pathogens in murine models (reviewed in [20]) and cattle [21,22]. IL-17 induces direct bacterial killing by macrophages [23,24], and DC derived IL-12 to differentiate Th1 cells [23]. Recent data suggest IL-17 drives BCG-specific Th1 cells responses by overcoming IL-10 inhibition [25]. Consistent with these findings, our results showed that BALB/c mice secreted a higher amount of IL-17 coupled with a high secretion of IFN-γ and a decreased level of IL-10, both at the systemic and the lung mucosal level. Reciprocally, C57BL/6 mice displayed lower IL-17 and IFN-γ and increased IL-10.

Genetic differences between the two strains could account for this higher level of adaptive Th17 responses observed in BALB/c mice, although mechanisms remain to be understood at this point. Earlier studies have demonstrated genes within the MHC influence

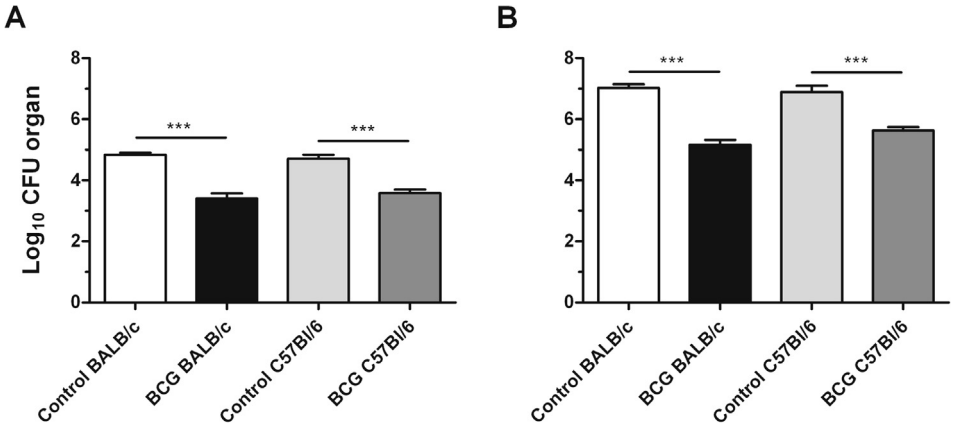

Figure 5. Protective efficacy in the BCG vaccinated and *M. bovis* challenged BALB/c or C57BL/6 mice. Six weeks after BCG vaccination, animals were intranasally challenged with 400 CFU of *M. bovis*. Four weeks post–challenge the mice were euthanised and the bacterial burden (CFU) was measured in the spleens (A) or lungs (B). Bars represent means ± SEM ($n = 8$); $^{***}p < 0.001$ versus naïve controls.

immune responses to *M. tuberculosis* [26–31]; with the H-2 locus, triggering significant differences in the production of IFN-γ after stimulation with mycobacterial antigens [29].

An effective Th1 immune response is essential for vaccine-induced protection against infection with virulent mycobacteria [32]. Therefore, we tested whether reduced BCG-induced Th17 and Th1 responses affected protection in the *M. bovis*–challenged C57BL/6 mice, but found that both mouse strains displayed similar levels of protection. Thus, it is clear that measurement of more IFN-γ production does not necessarily correlate with better protection against *M. bovis* infection. These findings are in agreement with previous studies showing that the ability to generate a large number of Th1 cells does not determine the ability to resist *M. tuberculosis* infection in BALB/c and C57BL/6 mice [33]. This has clear implications for vaccine development since it is generally assumed that vaccines able to stimulate the production of a larger number of Th1 cells would be more protective. If the resistance to *M. bovis* is not solely based in the Th1 cell component of immunity, as we understand it at present, then providing a host with the ability to generate more Th1 cells may have little or no advantageous consequences.

Acknowledgements

The authors thank the APHA Animal Sciences Unit for the excellent services and support provided. The authors thank Martin Vordermeier, APHA, for critical reading and discussions regarding the manuscript.

Funding: This work was funded by Defra under contracts SE3224 & SE3266.

Competing interests: None Declared.

Ethical approval: All animal work was carried out in accordance with the UK Animal (Scientific Procedures) Act 1986; under appropriate personal and project licenses. The study protocol was approved by the AHVLA Animal Use Ethics Committee (UK Home Office PCD number70/6905).

References

[1] Skamene E, Gros P, Forget A, Kongshavn PA, St Charles C, Taylor BA. Genetic regulation of resistance to intracellular pathogens. Nature 1982;297:506–9.
[2] Ramarathinam L, Niesel DW, Klimpel GR. Ity influences the production of IFN-gamma by murine splenocytes stimulated in vitro with salmonella typhimurium. J Immunol 1993;150:3965–72.
[3] Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. J Exp Med 1989;169:59–72.
[4] Miralles GD, Stoeckle MY, McDermott DF, Finkelman FD, Murray HW. Th1 and Th2 cell-associated cytokines in experimental visceral leishmaniasis. Infect Immun 1994;62:1058–63.
[5] Bohn E, Heesemann J, Ehlers S, Autenrieth IB. Early gamma interferon mRNA expression is associated with resistance of mice against yersinia enterocolitica. Infect Immun 1994;62:3027–32.
[6] Yang X, HayGlass KT, Brunham RC. Genetically determined differences in IL-10 and IFN-gamma responses correlate with clearance of Chlamydia trachomatis mouse pneumonitis infection. J Immunol 1996;156:4338–44.
[7] Huygen K, Abramowicz D, Vandenbussche P, Jacobs F, De Bruyn J, Kentos A, Drowart A, Van Vooren JP, Goldman M. Spleen cell cytokine secretion in Mycobacterium bovis BCG-infected mice. Infect Immun 1992;60:2880–6.
[8] Yoshida A, Koide Y, Uchijima M, Yoshida TO. Dissection of strain difference in acquired protective immunity against Mycobacterium bovis Calmette-Guerin bacillus (BCG). Macrophages regulate the susceptibility through cytokine network and the induction of nitric oxide synthase. J Immunol 1995;155:2057–66.
[9] Flynn JL, Goldstein MM, Triebold KJ, Sypek J, Wolf S, Bloom BR. IL-12 increases resistance of BALB/c mice to Mycobacterium tuberculosis infection. J Immunol 1995;155:2515–24.
[10] Wakeham J, Wang J, Xing Z. Genetically determined disparate innate and adaptive cell-mediated immune responses to pulmonary Mycobacterium bovis BCG infection in C57BL/6 and BALB/c mice. Infect Immun 2000;68:6946–53.
[11] Logan KE, Gavier-Widen D, Hewinson RG, Hogarth PJ. Development of a Mycobacterium bovis intranasal challenge model in mice. Tuberculosis 2008;88:437–43. http://dx.doi.org/10.1016/j.tube.2008.05.005.
[12] Gallagher J, Horwill DM. A selective oleic acid albumin agar medium for the cultivation of Mycobacterium bovis. J Hyg Lond 1977;79:155–60.
[13] Kaveh DA, Bachy VS, Hewinson RG, Hogarth PJ. Systemic BCG immunization induces persistent lung mucosal multifunctional CD4 TEM cells which expand following virulent mycobacterial Challenge. PLoS One 2011;6. http://dx.doi.org/10.1371/journal.pone.0021566.
[14] Cooper AM. Cell-mediated immune responses in tuberculosis. Annu Rev Immunol 2009;27:393–422. http://dx.doi.org/10.1146/annurev.immunol.021908.132703.
[15] Goldsack L, Kirman JR. Half-truths and selective memory: interferon gamma, CD4(+) T cells and protective memory against tuberculosis. Tuberc Edinb 2007;87:465–73. http://dx.doi.org/10.1016/j.tube.2007.07.001. S1472-9792(07)00069-8.
[16] Vordermeier HM, Chambers MA, Cockle PJ, Whelan AO, Simmons J, Hewinson RG. Correlation of ESAT-6-specific gamma interferon production with pathology in cattle following Mycobacterium bovis BCG vaccination against experimental bovine tuberculosis. Infect Immun 2002;70:3026–32.
[17] Elias D, Akuffo H, Britton S. PPD induced in vitro interferon gamma production is not a reliable correlate of protection against Mycobacterium tuberculosis. Trans R Soc Trop Med Hyg 2005;99:363–8. http://dx.doi.org/10.1016/j.trstmh.2004.08.006. S0035-9203(04)00276-7.
[18] Logan KE, Chambers MA, Hewinson RG, Hogarth PJ. Frequency of IFN-gamma producing cells correlates with adjuvant enhancement of bacille Calmette-Guerin induced protection against Mycobacterium bovis. Vaccine 2005;23:5526–32. http://dx.doi.org/10.1016/j.vaccine.2005.07.037.
[19] Hervas-Stubbs S, Majlessi L, Simsova M, Morova J, Rojas MJ, Nouze C, Brodin P, Sebo P, Leclerc C. High frequency of CD4+ T cells specific for the TB10.4 protein correlates with protection against Mycobacterium tuberculosis infection. Infect Immun 2006;74:3396–407. http://dx.doi.org/10.1128/IAI.02086-05. 74/6/3396.
[20] Khader SA, Gopal R. IL-17 in protective immunity to intracellular pathogens. Virulence 2010;1:423–7. http://dx.doi.org/10.4161/viru.1.5.12862.
[21] Aranday-Cortes E, Bull NC, Villarreal-Ramos B, Gough J, Hicks D, Ortiz-Pelaez A, Vordermeier HM, Salguero FJ. Upregulation of IL-17A, CXCL9 and CXCL10 in early-stage granulomas induced by Mycobacterium bovis in cattle. Transbound Emerg Dis 2012. http://dx.doi.org/10.1111/j.1865–1682.2012.01370.x.
[22] Aranday-Cortes E, Hogarth PJ, Kaveh DA, Whelan AO, Villarreal-Ramos B, Lalvani A, Vordermeier HM. Transcriptional profiling of disease-induced host responses in bovine tuberculosis and the identification of potential diagnostic biomarkers. PloS One 2012;7:e30626. http://dx.doi.org/10.1371/journal.pone.0030626.
[23] Lin Y, Ritchea S, Logar A, Slight S, Messmer M, Rangel-Moreno J, Guglani L, Alcorn JF, Strawbridge H, Park SM, Onishi R, Nyugen N, Walter MJ, Pociask D, Randall TD, Gaffen SL, Iwakura Y, Kolls JK, Khader SA. Interleukin-17 is required for T helper 1 cell immunity and host resistance to the intracellular pathogen Francisella tularensis. Immunity 2009;31:799–810. http://dx.doi.org/10.1016/j.immuni.2009.08.025.
[24] Higgins SC, Jarnicki AG, Lavelle EC, Mills KH. TLR4 mediates vaccine-induced protective cellular immunity to Bordetella pertussis: role of IL-17-producing T cells. J Immunol 2006;177:7980–9.
[25] Gopal R, Lin Y, Obermajer N, Slight S, Nuthalapati N, Ahmed M, Kalinski P, Khader SA. IL-23-dependent IL-17 drives Th1-cell responses following Mycobacterium bovis BCG vaccination. Eur J Immunol 2012;42:364–73. http://dx.doi.org/10.1002/eji.201141569.
[26] Brett S, Orrell JM, Swanson Beck J, Ivanyi J. Influence of H-2 genes on growth of Mycobacterium tuberculosis in the lungs of chronically infected mice. Immunology 1992;76:129–32.
[27] Apt AS, Avdienko VG, Nikonenko BV, Kramnik IB, Moroz AM, Skamene E. Distinct H-2 complex control of mortality, and immune responses to tuberculosis infection in virgin and BCG-vaccinated mice. Clin Exp Immunol 1993;94:322–9.
[28] Pichugin AV, Khaidukov SV, Moroz AM, Apt AS. Capacity of murine T cells to retain long-term responsiveness to mycobacterial antigens is controlled by the H-2 complex. Clin Exp Immunol 1998;111:316–24.
[29] Kamath AB, Alt J, Debbabi H, Taylor C, Behar SM. The major histocompatibility complex haplotype affects T-cell recognition of mycobacterial antigens but not resistance to Mycobacterium tuberculosis in C_3H mice. Infect Immun 2004;72:6790–8. http://dx.doi.org/10.1128/IAI.72.12.6790-6798.2004.
[30] Mischenko VV, Kapina MA, Eruslanov EB, Kondratieva EV, Lyadova IV, Young DB, Apt AS. Mycobacterial dissemination and cellular responses after 1-lobe restricted tuberculosis infection of genetically susceptible and resistant mice. J Infect Dis 2004;190:2137–45. http://dx.doi.org/10.1086/425909.
[31] Pichugin AV, Petrovskaya SN, Apt AS. H_2 complex controls CD4/CD8 ratio, recurrent responsiveness to repeated stimulations, and resistance to activation-induced apoptosis during T cell response to mycobacterial antigens. J Leukoc Biol 2006;79:739–46. http://dx.doi.org/10.1189/jlb.0705392.
[32] Philips JA, Ernst JD. Tuberculosis pathogenesis and immunity. Annu Rev pathology 2012;7:353–84. http://dx.doi.org/10.1146/annurev-pathol-011811-132458.
[33] Jung YJ, Ryan L, LaCourse R, North RJ. Differences in the ability to generate type 1 T helper cells need not determine differences in the ability to resist Mycobacterium tuberculosis infection among mouse strains. J Infect Dis 2009;199:1790–6. http://dx.doi.org/10.1086/599092.