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Ag85A-specific CD4$^+$ T cell lines derived after boosting BCG-vaccinated cattle with Ad5-85A possess both mycobacterial growth inhibition and anti-inflammatory properties

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ABSTRACT

There is a need to improve the efficacy of the BCG vaccine against human and bovine tuberculosis. Previous data showed that boosting bacilli Calmette-Guerin (BCG)-vaccinated cattle with a recombinant attenuated human type 5 adenovirally vectored subunit vaccine (Ad5-85A) increased BCG protection and was associated with increased frequency of Ag85A-specific CD4$^+$ T cells post-boosting. Here, the capacity of Ag85A-specific CD4$^+$ T cell lines – derived before and after viral boosting – to interact with BCG-infected macrophages was evaluated. No difference before and after boosting was found in the capacity of these Ag85A-specific CD4$^+$ T cell lines to restrict mycobacterial growth, but the secretion of IL-10 *in vitro* post-boost increased significantly. Furthermore, cell lines derived post-boost had no statistically significant difference in the secretion of pro-inflammatory cytokines (IL-1β, IL-12, IFNγ or TNFα) compared to pre-boost lines. In conclusion, the protection associated with the increased number of Ag85A-specific CD4$^+$ T cells restricting mycobacterial growth may be associated with anti-inflammatory properties to limit immune-pathology.

1. Introduction

Bovine tuberculosis (bTB), caused mainly by *Mycobacterium bovis*, poses economical, animal welfare and human health problems [1]. Development of vaccines for cattle, to be used in conjunction with current regulations, is part of control and eradication strategies for England and Wales for controlling bTB [2,3].

Cattle vaccinated with the live attenuated *M. bovis* bacillus Calmette-Guerin (BCG) and boosted with adenovirus type 5 (Ad5) expressing *Mycobacterium tuberculosis* Ag85A (Ag85A) (Ad5-Ag85A) showed improved protection against pathology associated with *M. bovis* [4,5]. Recently, we adapted the use of the antigen-unbiased T cell expansion method from Geiger et al. [6] to the bovine system [7]. This approach avoids initial *in vitro* bias caused by expansion of T cell lines by specific repeated cycles of antigen stimulation. We reported that boosting BCG-vaccinated cattle with Ad5-85A increased the frequency of Ag85A-specific CD4$^+$ T cell lines, which correlated with protection, but there was no change in T-cell antigen avidity or epitope-recognition repertoire; the avidity of Ag-85A specific CD4$^+$ T cells was not modulated by viral boosting [7]. Therefore, it was of interest to further characterise the functional properties of these Ag85A-specific CD4$^+$ T cell lines derived from BCG-primed and Ad5-85A-boosted cattle.

In this study, the capacity of these Ag85A-specific CD4$^+$ T cells – generated either before or after Ad-85A boost – to control mycobacteria *in vitro* and their cytokine profile, after culture for 24 h with BCG-infected macrophages, have been evaluated. Our data suggest that boosting BCG with Ad5-85A enhances protection by increasing the number of Ag85A-specific CD4$^+$ T cells capable of controlling mycobacteria, whilst also potentially developing anti-inflammatory properties to limit immune-pathology.

2. Materials and methods

2.1. Animals

Experiments were carried out according to the UK Animal (Scientific Procedures) Act 1986 under project license PPL70/7737. The study protocol was approved by the APHA Animal

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Use Ethics Committee (UK Home Office PCD number70/6905) and has been reported previously [5]. Briefly, all animals were vaccinated with 1×10^6 Colony Forming Units (CFU) *M. bovis* BCG Danish 1331 subcutaneously at week (wk) 0; Ad5-85A boosted cattle were inoculated at wk 8 with 2×10^9 infectious units of Ad5-85A by intradermal injection on the shoulder; all animals were challenged endobronchially with 2×10^3 CFU *M. bovis* AF2122/97 strain at wk 12 [5]. Peripheral blood mononuclear cells (PBMC) were cryo-preserved pre- (wk 8) and post-boost (wk 11) and used to generate $CD4^+$ T cell lines. The present study utilised Ag85A-specific $CD4^+$ T cell lines, from three BCG-primed Ad5-85A-boosted cattle and one BCG-vaccinated control, acquired in the study described previously [7]. Thirteen pre-boost cell lines were used from two animals (three from one animal and ten from the BCG control) and thirteen post-boost cell lines were used from three animals (five from one animal and four from each of the remaining animals).

2.2. Isolation and selection of pre-/post- boost Ag85A-specific $CD4^+$ T cell lines

Polyclonal $CD4^+$ T cell libraries were generated from pre-boost (wk 8) and post-boost (wk 11) PBMC using a method adapted from Geiger et al. [6], as described previously [7]. Ag85A-specific $CD4^+$ T cells were identified by screening the different polyclonal cell cultures for their capacity to proliferate using $1 \times 10^5 - 2 \times 10^5$ $CD4^+$ T cells per culture and 5 μg/ml (initial screening) or 10 μg/ml (subsequent screening) recombinant Ag85A (Lionex GmbH, Germany) and 5×10^3 $CD14^+$ as antigen presenting cells per well of 96-well U-bottom plates. Ag85A-specific $CD4^+$ T cell lines were expanded, after each 11 day Ag85A-selective culture, using 1 μg/ml lectin from *Phaseolus vulgaris* leucoagglutinin PHA-L (PHA – Sigma-Aldrich) in the presence of 10 U/ml recombinant human interleukin 2 (Gentaur, Belgium) and $CD14^+$ feeder cells for nine days and cryopreserved. All Ag85A-specific $CD4^+$ T cell lines used in these experiments had undergone three sequential rounds of Ag85A-PHA stimulation.

2.3. Bovine monocyte/macrophage cell culture

Autologous bovine $CD14^+$ (monocytes) or granulocyte-macrophage colony stimulating factor (GM-CSF)-matured $CD14^+$ cells (macrophages [Mφ]) were cultured at 37 °C (5% CO_2) in complete medium consisting of RPMI 1640 containing 2 mM GlutaMax, 25 mM HEPES, 0.1 mM non-essential amino acids, 5×10^{-5} M β-mercaptoethanol, 50 μg/ml Gentamicin (all from Life Technologies, UK), and 10% foetal calf serum (FCS) (Sigma-Aldrich, UK) (complete medium). For Mφ differentiation, monocytes were cultured at a density of 1×10^6/ml in medium containing recombinant bovine GM-CSF diluted 1/100 (Bio-rad, UK) for six days in Corning Ultra-low adhesion flasks (Sigma-Aldrich); cells were fed GM-CSF on day three. After six days, Mφ were harvested by incubating flasks

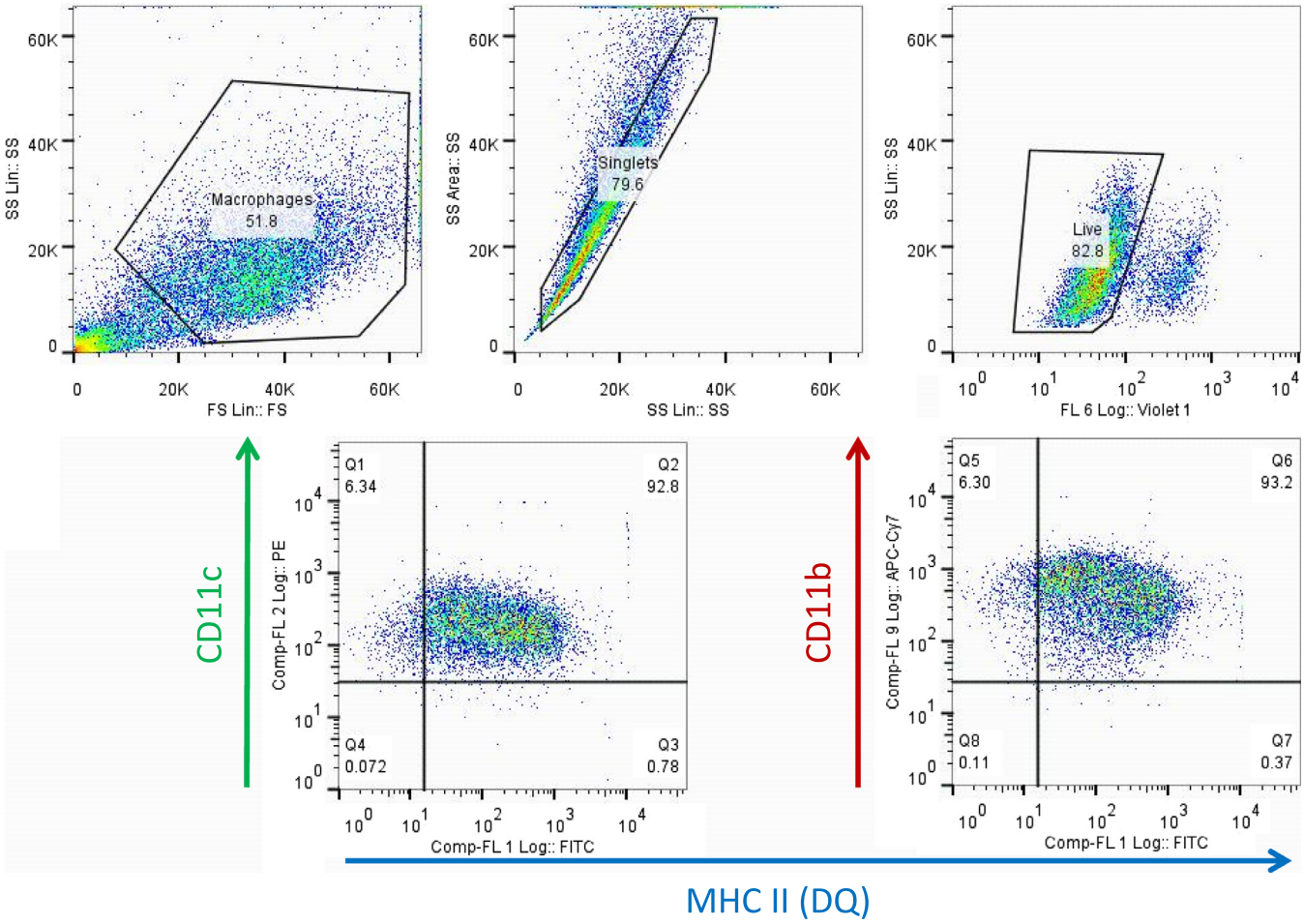

Fig. 1. Characterisation of $CD14^+$cell-derived mature macrophage phenotype. Representative plots of $CD14^+$ cells matured into macrophages (Mφ) by culturing in medium supplemented with GM-CSF for six days as described in materials and methods. Harvested Mφ were selected based on their large FSC/SSC phenotype and live Mφ were analysed for CD11c, CD11b and MHC class II (DQ) as described. Plots shown were derived from GM-CSF-matured $CD14^+$ obtained from a cow infected naturally with *M. bovis* that was used to develop the methodology.

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