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Short communication

Differential distribution of WC1⁺ γδ TCR⁺ T lymphocyte subsets within lymphoid tissues of the head and respiratory tract and effects of intranasal *M. bovis* BCG vaccination

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ABSTRACT

BCG vaccination of neonatal calves induces significant protection against bovine tuberculosis. The enhanced protection observed in neonatal calves may be linked to an enhanced capacity for IFNγ production by innate cells, including $WC1^+$ γδ T cells, which constitute a major population in young cattle. Intranasal BCG vaccination of mice induces high levels of IFNγ in the lungs, which may enhance protection against subsequent challenge with virulent strains of mycobacteria. We used an intranasal BCG vaccination model in calves to study the effect on the distribution of $WC1^+$ γδ T cells expressing two alternate forms of WC1: WC1.1 and WC1.2. These subsets of $WC1^+$ γδ T cells have previously been shown to have a differential capacity for IFNγ secretion. Our results indicate that there is a selective expansion/recruitment of γδ T cells expressing the IFNγ-associated WC1.1 isoform in tissues of the lungs and upper respiratory tract following intranasal BCG vaccination.

1. Introduction

T cells expressing the alternate receptor consisting of γ and δ chains (often referred to as TCR1) are found in all vertebrate species, though the relative numbers of γδ-expressing T cells varies. For example, in humans and mice, γδ T cells comprise ∼10% of the circulating T cells, while in ruminants they may constitute up to 50–60%. There are two distinct subsets of bovine γδ T cells, based on expression of workshop cluster antigen-1 (WC1). WC1 is a 220 KDa glycoprotein, encoded by a complex family of genes in cattle, sheep, goats, pigs and camelids (Mackay et al., 1986, 1989; Clevers et al., 1990; Carr et al., 1994) and also in humans and mice, although in these the antigen is not expressed and the gene family is less complex. In cattle, 13 members of the WC1 gene family have been identified, on two loci on chromosome 5 (Herzig and Baldwin, 2009). The extra-cellular domains of WC1 share homology with the scavenger receptor cysteine-rich (SRCR) family, though its function remains enigmatic. It has been postulated that WC1 may be a pattern recognition receptor or a regulator of lymphocyte receptor signalling (Herzig and Baldwin, 2009).

Distinct functional sub-populations of bovine $WC1^+$ γδ T cells have been identified, characterised by the expression of either the WC1.1 or WC1.2 isoform (Rogers et al., 2005a; Rogers et al., 2005b; Chen et al., 2004). Within the $WC1^+$ population, the $WC1.1^+$ sub-population is the principal IFNγ-secreting population, while the WC1.2-expressing cells are more responsive to mitogen stimulation. It is likely that these two sub-types of $WC1^+$ γδ T cell play discrete roles in the innate immune response, and may display distinct behaviour in response to infection or vaccination.

Potential roles for γδ T cells in innate immune responses have been investigated in cattle. Depletion of $WC1^+$ γδ from *Mycobacterium bovis* infected calves increased levels of IL-

Abbreviations: WC, workshop cluster; FCM, flow cytometry.

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4, decreased IgG2 and, importantly, decreased secretion of innate IFNγ (Kennedy et al., 2002) suggesting that these cells have a role in directing Th1 bias. WC1+ γδ T cells are among the first cells to accumulate at sites of infection and concurrently reduce in number in the peripheral blood, implying a migration from the blood to the tissues (Pollock et al., 1996). Bovine WC1+ γδ T cells may play a significant role in shaping the adaptive immune response through early interactions with dendritic cells, resulting in secretion of significant amounts of IFNγ (Price et al., 2007).

Bovine tuberculosis caused by *M. bovis* is a major economic and potential zoonotic problem in the UK. Use of the BCG vaccine in cattle has been hampered by the fact that it has not been possible to distinguish between infected and vaccinated animals by current diagnostic tests, and that BCG vaccination may have variable efficacy as seen in humans (Suazo et al., 2003). Experimentally, BCG vaccination is effective against bovine TB (Hope and Vordermeier, 2005) with BCG vaccination of neonatal calves eliciting significant protection against challenge with *M. bovis* (Hope et al., 2005; Buddle et al., 2003). Several studies in mice and cattle have indicated that enhanced protection against experimental infection with virulent mycobacteria may be achieved by delivering BCG via the intratracheal route (Buddle et al., 1995; Falero-Diaz et al., 2000). This protective effect is linked to rapid production of IFNγ production in the lungs in mice (Lyadova et al., 2001). In addition, intranasal infection of mice with *M. bovis* BCG was shown to induce increased levels of γδ T cells by 7 days post-infection, and γδ T cells isolated from the lungs of infected mice showed high levels of IFNγ production and cytotoxic activity against infected macrophages (Dieli et al., 2003). These studies indicate that effects on sub-populations of lymphocytes post-vaccination are of key importance in the development of protective immune responses.

In order to investigate potential roles for WC1+ γδ T cells in vaccine-induced responses in calves we studied the distribution of WC1+ γδ T cells in the head and respiratory tract following intranasal BCG vaccination. Differences in the distribution of cells expressing the alternate isoforms of WC1 were also assessed. We demonstrated functionally relevant alterations in WC1+ cells in calves 1 week following intranasal BCG vaccination.

2. Materials and methods

2.1. Intranasal BCG vaccination

Four 5–10-month-old Friesian calves were selected on the basis of their lack of response to mycobacterial antigens (purified protein derivatives from *M. avium* (PPD-A) and *M. bovis* (PPD-B) as previously described (Hope et al., 2005)). Calves were vaccinated intranasally by introduction of a 6" long catheter into the left nostril. Approximately 1.5×10^7 cfu of *Mycobacterium bovis* Bacille Calmette Guerin strain Pasteur (BCG Pasteur) suspended in 2 ml of sterile PBS was delivered into the nostril, followed by 2 ml of PBS alone. The calves showed no adverse effects of vaccination. After 7 days, the animals were killed by captive bolt. Tissues from three age-matched unvaccinated calves were used for control samples. All animal procedures used were approved by the local ethics committee, consisting of expert and lay members.

Table 1
Tissues taken post-mortem.

Tissue	Location	Abbreviation
Cardiac (medial) lung (right)	Lung	RC
Cardiac (medial) lung (left)	Lung	LC
Intermediate lung	Lung	I
Diaphragmatic lung (right)	Lung	RD
Diaphragmatic lung (left)	Lung	LD
Pallatine tonsil (right)	Distal palate	Pall R
Pallatine tonsil (left)	Distal palate	Pall L
Retropharyngeal lymph node (right)	Pharynx	RPLN R
Retropharyngeal lymph node (left)	Pharynx	RPLN L
Pharyngeal tonsil	Pharynx	Ph ton
Cervical lymph node	Draining distal trachea	CLN

2.2. Isolation of peripheral blood mononuclear cells

PBMC were isolated from blood collected at the time of slaughter, as previously described (Price et al., 2007). Cells were re-suspended at 2×10^5 cells per ml in PBS with 0.1% BSA and 0.01% sodium azide (PBS/BSA/Azide) for immuno-staining.

2.3. Isolation of mononuclear cells from tissues

Pieces of tissue approximately 2.5 cm^3 and selected peripheral lymph nodes were removed at post-mortem (Table 1) and washed thoroughly in sterile PBS. The lymph nodes were sliced open and flushed repeatedly with PBS to liberate cells from within the tissue. Other tissue samples were homogenised in a pestle and mortar containing sterile PBS. The resulting cell suspensions were passed through a 40 μm nylon cell strainer (Becton Dickinson, Oxford, UK). The mononuclear cells were then isolated by density gradient centrifugation over Histopaque 1083 (Sigma Aldrich, Gillingham, UK) as described (Price et al., 2007). Cells were re-suspended at 2×10^5 cells per ml for immuno-staining.

2.4. Flow cytometric analysis

Cells suspended at 2×10^5 per ml were incubated for 10 min with 10% normal mouse serum. Cells were then incubated with either an isotype-matched control antibody or antibody to the bovine γδ T cell receptor TCR1 (mAb GB21a; IgG2b, kindly provided by Dr W. Davis, WSU, Pullman, USA) in conjunction with either anti-pan WC1 (CC39-Alexa Fluor 647 IgG1; AbD-Serotec, Oxford, UK) anti-WC1.1 (mAb BAQ159A; IgG1, W. Davis) or anti-WC1.2 (mAb CACTB32A; IgG1, W. Davis) antibodies. Isotype-specific goat anti-mouse secondary reagents were then added (goat anti-mouse IgG2b tri-colour or IgG2b FITC; goat anti-mouse IgG1 phycoerythrin; Southern Biotechnology Associates, Birmingham, USA). Flow cytometric acquisition was carried out on a FACSCalibur flow cytometer (Becton Dickinson, Oxford, UK) and the data was analysed using FCS Express version 3 (DeNovo Software, Ontario, Canada). A minimum of 200,000 events were collected.

2.5. Statistical analyses

Statistical analyses were carried out with Minitab release 15.0 (Minitab Inc, Coventry, UK) using a Mann Whitney non-parametric test and p values of <0.05 were considered significant.

3. Results and discussion

In order to establish a potential role for γδ TCR+ T lymphocytes in BCG vaccination induced immune responses

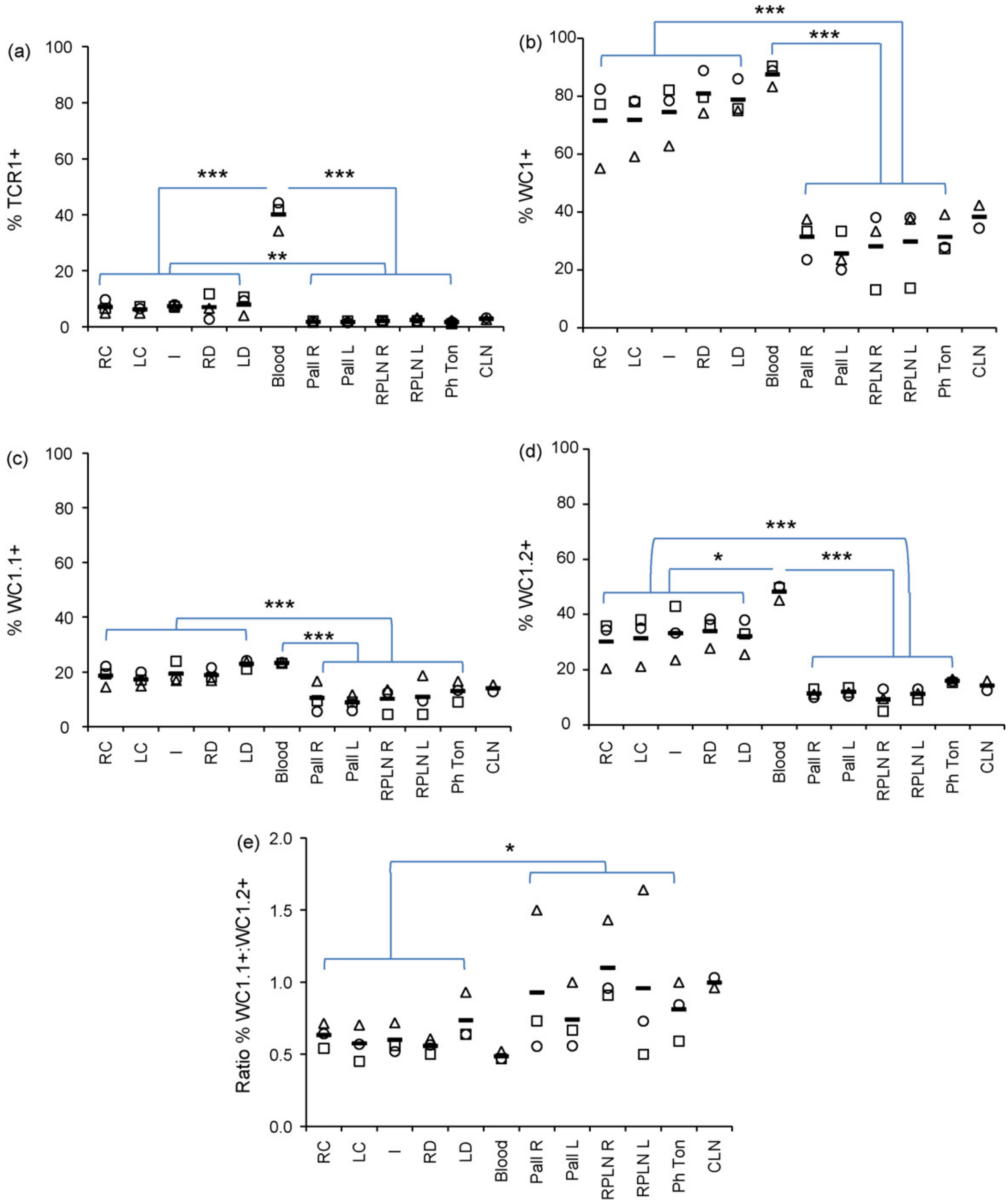

Fig. 1. Percentages of γδ T cell subsets in control, non-vaccinated, cattle. The percentage of cells expressing the γδ TCR (TCR1; a) and percentages of TCR1+ T cells expressing WC1 (b), WC1.1 (c) or WC1.2 (d) were assessed in tissues of the respiratory tract and head (see Table 1 for abbreviations) by FCM. The ratio of WC1.1:WC1.2 cells is shown in (e). Individual animal data is shown with means (solid bars). Statistically significant differences were assessed using Mann Whitney non-parametric analysis. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

we measured the percentage of T cells expressing the γδ TCR, and subsets of γδ T cells expressing isoforms of the WC1 receptor, in lymphoid tissues of the head and respiratory tract of cattle. Control, non-vaccinated calves were compared with calves that had been vaccinated intranasally with BCG 7 days prior to analysis.

In control, non-vaccinated calves, significant differences in the percentages of γδ T cell subsets were noted between tissues (Fig. 1). There were significantly higher numbers of T lymphocytes expressing the γδ TCR (TCR1) in the lung lobes compared to the lymphoid tissues of the head (Fig. 1a; $p < 0.01$), but the number of γδ TCR$^+$ T cells was lower in all tissues than in the peripheral blood ($p < 0.001$). Within the γδ TCR-expressing T lymphocyte population there were also tissue specific differences noted in the percentage of cells expressing the WC1 receptor (Fig. 1b). Notably, there was a significantly higher number of γδ T cells expressing WC1 in the lung lobes and blood, when compared to lymphoid tissues of the head ($p < 0.001$). The percentage of γδ T cells expressing WC1.1 (Fig. 1c) or WC1.2 (Fig. 1d) also differed between tissues. Significantly greater percentages of WC1.1 and WC1.2 expressing cells were present in the lung lobes and peripheral blood compared to head lymphoid tissues ($p < 0.001$). Assessment of the relative ratio of WC1.1:WC1.2 expressing γδ T cells within tissues (Fig. 1e) revealed a greater ratio of WC1.1:WC1.2 within the lymphoid tissues of the head ($p < 0.05$). The ratio in the lung and blood was less than 1 indicating a predominance of WC1.2

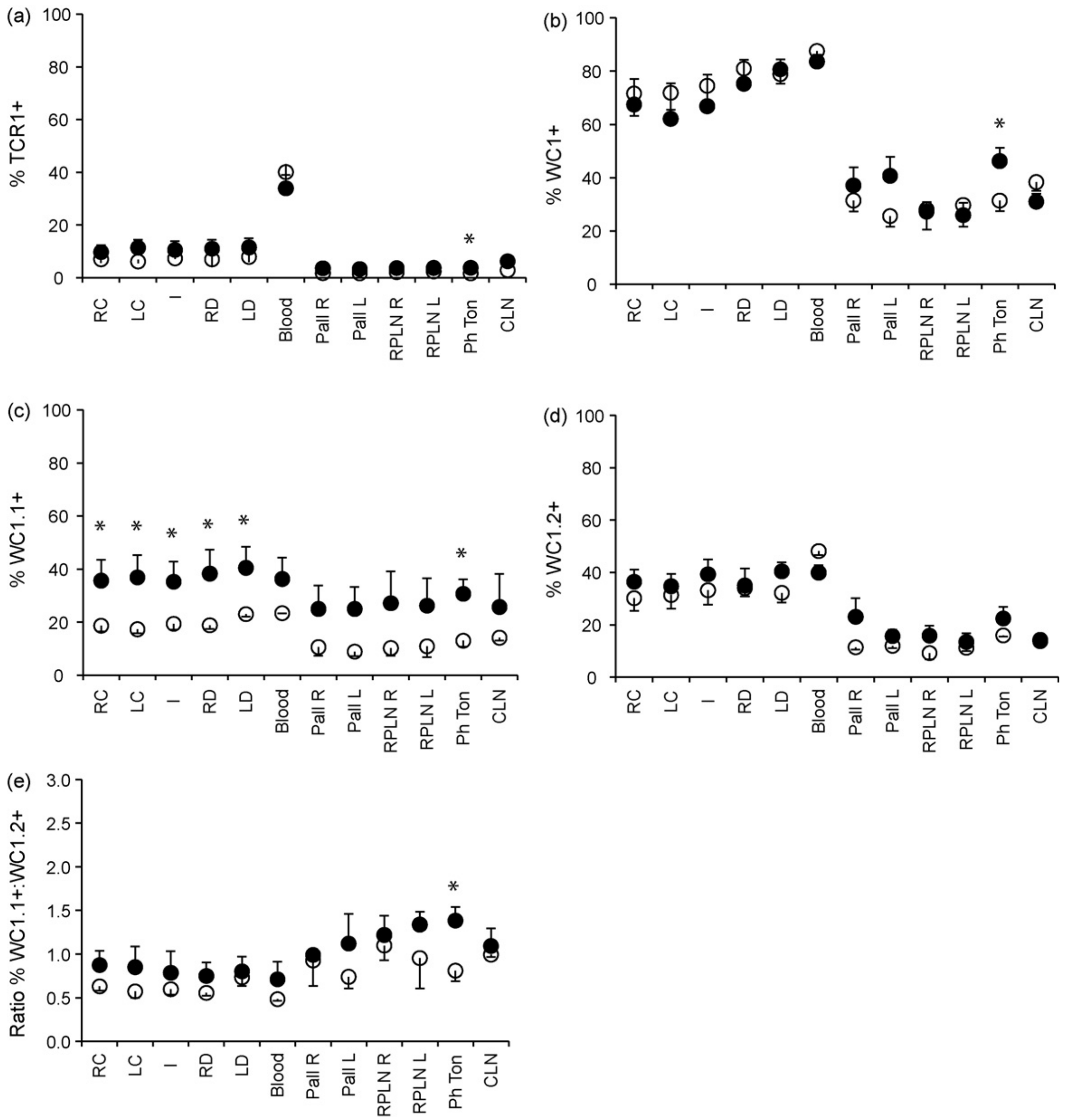

Fig. 2. Comparison of γδ T cell subsets in control and BCG vaccinated cattle. The percentage of cells expressing the γδ TCR (TCR1; a) and percentages of TCR1$^+$ T cells expressing WC1 (b), WC1.1 (c) or WC1.2 (d), WC1.1 or WC1.2 were assessed as for Fig. 1. Mean ±SE is illustrated. Differences between control (open symbols) and vaccinated (closed symbols) calves were assessed by Mann Whitney non-parametric statistical analysis. *$p < 0.05$.

expressing γδ T cells. By comparison, within the lymphoid tissues of the head the WC1.1:WC1.2 ratio was approximately 1 suggesting a more balanced population of cells expressing either of the two isoforms of WC1. Given the suggested functional differences associated with expression of either WC1.1 or WC1.2, this may have implications for immune responses being induced in different tissues (Rogers et al., 2005a, b).

The percentage of γδ TCR-expressing cells and the relative proportions which expressed WC1 and the isoforms WC1.1 and WC1.2 was subsequently assessed in calves that had been intranasally vaccinated with BCG (Fig. 2). A significant increase in the percentage of γδ TCR-expressing T cells (Fig. 2a) and of $WC1^+$ γδ TCR^+ T cells (Fig. 2b) was observed in the pharyngeal tonsil ($p < 0.05$) of BCG vaccinated calves (Fig. 2; closed symbols). Significant differences in the percentage of γδ TCR^+ T cells expressing WC1.1 (Fig. 2c), but not WC1.2 (Fig. 2d) were observed in each of the lung regions assessed and in the pharyngeal tonsil ($p < 0.05$). Increased percentages of WC1.1 expressing cells were observed in each of the tissues assessed although, due to large animal to animal variation, this did not reach statistical significance for the head lymph nodes, or blood. The ratio of WC1.1:WC1.2 was significantly increased within the draining pharyngeal tonsil (Fig. 2e; $p < 0.05$).

These data indicate that intranasal BCG vaccination induces a predominance of $WC1.1^+$ cells in the tissues of the respiratory tract. Selective recruitment and/or expansion of $WC1.1^+$ γδ T cells, which have been shown to have capacity for high level IFNγ secretion, may play an important role in the induction of the protective immune response induced by BCG vaccination in cattle.

Conflict of interest

None of the authors have any conflict of interest.

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