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Identification of guinea pig γδ T cells and characterization during pulmonary tuberculosis

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Abstract

Guinea pigs are an alternative small animal model for many disease studies. Here we describe a pan-γδ monoclonal antibody (anti-TCRδ1) specific for the constant region of human T cell receptor delta chains that cross-reacts with a subpopulation of guinea pig (*Cavia porcellus*) lymphocytes. The phenotype and distribution of this subpopulation is consistent with the guinea pig γδ T cell subset. FACS analysis of fresh PBMC and splenocytes from naïve guinea pigs revealed the presence of a subset of cells that stained with the anti-TCRδ1 mAb. The relative percentage of anti-TCRδ1 positive cells in PBMC and tissues is similar to that described for γδ T cells in other species. Immunohistochemistry of tissues also revealed a distribution of anti-TCRδ1 positive cells consistent with γδ T cells. These data are further supported by staining of a polyclonal guinea pig T cell line that became progressively CD4 and CD8 negative in long-term culture. Analysis of PBMC from guinea pigs following aerosol infection with virulent *Mycobacterium tuberculosis* revealed no apparent changes in the steady-state percentage of blood $\gamma\delta^+$ T cells. Taken together, these data suggest that the anti-TCRδ1 antibody recognizes the γδ T cell subset in guinea pigs. This reagent may be useful for examining γδ T cells in various disease models where the guinea pig is a more desirable model for study.

Keywords: Mycobacteria; Infection; Guinea pig; Histochemistry; T cells; FACScan analysis

1. Introduction

The T cell receptor (TCR) is expressed as a heterodimeric protein on the cell-surface of all T cells and confers the exquisite specificity to foreign antigens that typifies the adaptive immune system. The T cells

Abbreviations: HMBPP, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; IPP, isopentenyl pyrophosphate; MEPP, monoethyl-pyrophosphate.

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can be broadly divided into two subsets based upon the TCR gene-family expressed: either the αβ or γδ TCR. The γδ T cell subset has been implicated in a wide-range of immunological processes including tumor immunity, microbial immunity, and autoimmune diseases (reviewed in Carding and Egan, 2002). However, the precise function of the γδ T cell subset in various immunological phenomena has remained elusive. Human αβ T cells comprise the majority of circulating T cells while γδ T cells make up about 5% under normal physiological conditions but are also found in various tissues such as the gut and skin (Carding and Egan, 2002). During certain infections in humans, a distinct subset of γδ T cells can account for up to 50% of circulating T cells (Balbi et al., 1993; Bertotto et al., 1993; Caldwell et al., 1995; Hara et al., 1992; Munk et al., 1990; Sumida et al., 1992). This suggests an important role for these cells in microbial immunity.

A number of studies support a role for γδ T cells in infectious diseases including tuberculosis (Ladel et al., 1995). Studies in the mouse model of tuberculosis suggest that γδ T cells may affect the early influx of inflammatory cells into the infectious foci of the lungs (D'Souza et al., 1997). These studies also show that γδ TCR deficient mice exhibit increased pathology following a high-dose aerosol infection. In humans, some γδ T cells express high-levels of granulysin, a microbicidal protein found to be active against a range of microbial pathogens including *Mycobacterium tuberculosis* (Spada et al., 2000; Stenger et al., 1998). Moreover, human Vδ2Vγ2 T cells can kill intracellular Mycobacteria (Dieli et al., 2000; Dieli et al., 2001). These Vδ2Vγ2 T cells mount adaptive immune responses to *Mycobacterium bovis* BCG in monkeys and expansion of Vδ2Vγ2 T cells coincided with clearance of BCG and immunity to fatal tuberculous disease (Shen et al., 2002). Taken together, these data support a role for the γδ T cells in tuberculosis.

The guinea pig has historically been an attractive alternative animal model system for tuberculosis studies. This is due to several factors, including the high susceptibility of these animals to *M. tuberculosis* infection and the similar pulmonary histopathology to humans that develops following aerosol infection (McMurray, 1994). In addition, guinea pigs have traditionally been the gold-standard for tuberculosis vaccine studies since they exhibit a high-degree of protection using traditional BCG vaccines. Therefore, there is a rational basis for continuing to develop this animal model for current and future studies. Unfortunately, a lack of reagents for the guinea pig has hindered progress in developing this species as a robust animal model system. For example, monoclonal antibodies to lymphocyte and monocyte surface markers have been particularly lacking. This precludes the detailed assessment of immune system status under experimental conditions. Here, we demonstrate that a previously characterized anti-human pan-δ chain monoclonal antibody (anti-TCRδ1) cross-reacts with the guinea pig. We used this antibody to quantify changes in the γδ T cell population during the acute phase of *M. tuberculosis* infection in guinea pigs. This antibody should prove useful in future studies on the role of γδ T cells in tuberculosis and other disease models where guinea pigs are preferable to mice.

2. Methods

2.1. Animals

Female Hartley strain guinea pigs (300 g) were obtained from Elm Hill Breeders (Chelmsford, MA). Uninfected guinea pigs were housed under specific pathogen free conditions at the Dana Farber Cancer Institute animal facility. Strain 2 guinea pigs (300–400 g) were used for *M. tuberculosis* infection studies and were bred in-house. Infected animals were housed in individual micro-isolator cages in a biosafety level 3 animal facility. All appropriate institutional animal protocols were obtained and followed.

2.2. Aerosol infection with M. tuberculosis

For infection of animals, a single aliquot of *M. tuberculosis* H37Rv was thawed and diluted in 0.9% saline plus 0.05% Tween-80 (ST buffer) and added to a Lovelace nebulizer attached to a nose-only aerosol exposure chamber (Intox Products; Albuquerque, NM). Bacteria were diluted to a concentration predetermined by prior calibration experiments to deliver a dose of approximately 20 CFU into the lungs of guinea pigs. Three animals were euthanized at various time points after infection by overdose of sodium pentobarbital. The spleen and right caudal lobe of

the lung were sterilely removed and placed in separate Teflon-on-glass homogenizers containing 5 ml sterile ST buffer. Homogenized tissues were serially diluted and plated in duplicate on Middlebrook 7H11 agar plates (Remel; Lenexa, KS). The plates were incubated for 3 weeks at 37 °C and then counted to determine the total CFU in the lung lobe or spleen. All animals were infected as evidenced by the recovery of viable bacterial colonies from the lung. The minimum detectable number of bacteria in this assay is approximately 50 CFU.

2.3. Cell lines and culture

Single cell suspensions of naïve guinea pig spleen were generated by mincing tissue and passing it through a #60 wire mesh screen and then allowed to gravity settle in a 50 ml conical tube. The supernatant was spun down and resuspended in hypotonic lysis buffer to remove RBCs. The remaining leukocytes were washed three times in T cell media composed of RPMI 1640 medium (Life Technologies, Gaithersburg, MD) containing 10% fetal calf serum (FCS) (Hyclone, Logan, UT) and used for FACS analysis or in vitro culture. To generate the guinea pig T cell line, splenocytes were cultured in RPMI 1640 with 10% FCS, 100 U/ml penicillin/streptomycin (15140-122, Life Technologies), 60 μM non-essential amino acids (1140-050, Life Technologies), 0.8 mM L-glutamine (25030-081, Life Technologies), and 2-mercaptoethanol (55 nM). The cells were stimulated with 2 μg/ml phytohemagglutinin (PHA) (Difco) to promote proliferation and the media was supplemented with 2 nM human IL-2. T cells were periodically re-stimulated with PHA and IL-2 as described above. Freshly isolated allogeneic whole spleen cells irradiated at 5000 rad were also added during restimulation. Cells were cultured in a humidified 5% CO_2 incubator at 37 °C.

2.4. Lymphocyte proliferation assay

Guinea pig whole blood was obtained by cardiac puncture of ketamine anesthetized animals. PBMC were isolated by Ficoll gradient centrifugation as described previously (Pollack et al., 1981). Proliferation to PPD was determined by measuring the antigen-specific reactivity of isolated PBMC from each *M. tuberculosis* infected animal. The isolated PBMC (2×10^5/well) were cultured in triplicate in 96-well flat bottom plates (Costar, Corning, NY) with 10 μg/ml PPD (Mycos Research, Loveland, CO), phytohemagglutinin A (1 μg/ml) (Difco), or T cell media alone. Plates were incubated at 37 °C and 5% CO_2 for 5 days and then pulsed with [^{3}H]-thymidine (6.7 Ci/mol) for 6 h. Cells were then harvested using a Skatron model 11019 (Sterling, VA) and analyzed in a liquid scintillation counter beta-plate 1205 (Wallac, Boston, MA). The stimulation index was calculated by dividing the response to PPD by the media control.

2.5. Phosphoantigen stimulation of guinea pig γδ T cells

To determine the reactivity of guinea pig γδ T cells to phosphoantigens that have been shown previously to stimulate human Vγ2Vδ2 T cells, spleen and PBMC were harvested from two individual 300 g Hartley strain guinea pigs. The bacterial small phosphate antigens were prepared as described previously (Bukowski et al., 1998). Spleen single-cell suspensions and PBMC were prepared as described above and then cultured at 1×10^6 cells/ml in 1 ml in a 24-well plate with either T cell media alone, partially purified (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) from a *Mycobacterium fortuitum* supernatant (1/1000 dilution), or with the isopentenyl pyrophosphate (IPP) analog, mono-ethyl-pyrophosphate (MEPP) (1/1000 dilution of crude stock). Similar experiments were carried out with the bisphosphonate antigen Risedronate (Procter and Gamble, Cincinnati, OH) using half-log serial dilutions from 100 to 1 μg/ml. After 3 days of culture, recombinant human IL-2 was added to 2 nM final concentration. The cells were cultured at 37 °C and 5% CO_2 for up to 2 weeks. Plates were observed daily by microscopy for outgrowth and expansion of non-adherent blasts.

2.6. Monoclonal antibodies and FACS

The monoclonal antibodies used in these studies include CT5 (pan guinea pig lymphocyte), CT6, and CT7 (specific for guinea pig CD8 and CD4 respectively) (Serotec, Raleigh, NC); P3 was used as the nonspecific mouse IgG1 isotype control antibody (Kohler and Milstein, 1975). Anti-TCRδ1 (5A6.E9)

is a monoclonal antibody specific for the constant region of human TCR delta chains and has been described previously (Band et al., 1987). FACS analysis was performed as previously described (Parker et al., 1990). Briefly, primary antibodies were added to single cell suspensions at saturating concentrations for 1 h, washed with staining buffer (PBS containing 2% FCS and 0.01% azide) and then incubated for 1 h with 30 μg/ml FITC-conjugated donkey anti-mouse IgG (Jackson Immunologicals, West Grove, PA). After staining, cells were washed with staining buffer and analyzed with a FACSORT flow cytometer (Becton Dickinson, Mountain View, CA) and gated on the lymphocyte subpopulation. Dead cells were excluded using propidium iodide (Sigma Chemical Co., St. Louis, MO).

2.7. Immunohistochemistry

Thymus tissue was obtained from 3-week-old guinea pigs and spleen was obtained from adult animals. Tissue samples were mounted in OCT compound (Tissue-Tek, Torrance, CA), frozen in liquid nitrogen, and stored at −80 °C. Frozen tissue sections (5 μm thick) were fixed in acetone for 10 min, air dried, and stained by an indirect immunoperoxidase method using avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA) and 3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, MO) as the chromogen.

3. Results

3.1. Generation of a double-negative guinea pig T cell line

A non-specific T cell line was derived using guinea pig spleen cells stimulated with PHA. This line was kept in continuous long-term culture by periodic restimulation with PHA and irradiated allogeneic splenocytes. In addition, cultures were supplemented with human IL-2 which was found previously to possess cross-species activity for guinea pigs (Martensen et al., 1987 and data not shown). Periodic FACS analysis of this line demonstrated an initial outgrowth of $CD4^+$ T cells at two weeks (Fig. 1B). Subsequent time points at day 35 and day 45 revealed that the level

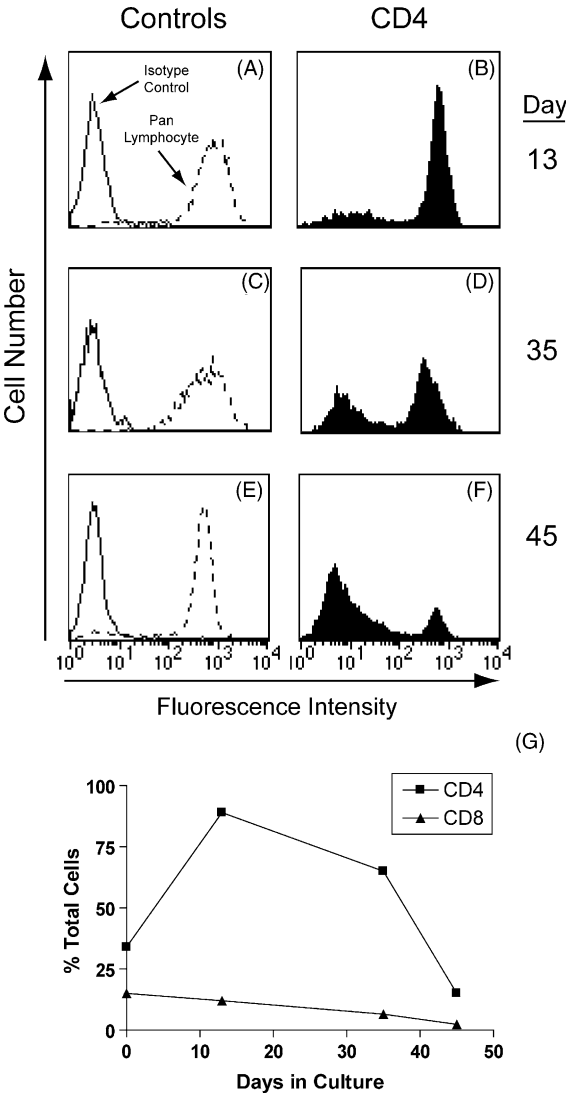

Fig. 1. A T cell line with an increasing proportion of double negative cells. A poly-clonal guinea pig T cell line was generated by repeated PHA stimulation and culture with allogeneic irradiated splenocytes feeder cells. Periodic FACS analysis was performed in order to assess the phenotype of the cell line. Long-term culture of guinea pig splenocytes resulted in gradual loss of $CD4^+$ T cells (B, D and F) but were not replaced by $CD8^+$ T cells (G). The cell line remained positive when stained with the pan-lymphocyte CT5 antibody (A, C and E). The percentage of $CD4^+$ cells from panels (B), (D), and (F) together with the $CD8^+$ cell percentage in the culture are plotted (G). The day 0 time point in panel (G) is the percentage of $CD4^+$ and $CD8^+$ cells from the original guinea pig spleen used to generate the T cell line.

of $CD4^+$ cells diminished significantly (Fig. 1D, F and G). Moreover, the line did not contain significant $CD8^+$ cells at later time points (Fig. 1G), although the line did continue to exhibit positive staining with the pan-lymphocyte antibody CT5 (Fig. 1A, C and E). We designated this polyclonal T cell line as gp1.

3.2. Cross-reactivity of a pan-Cδ TCR monoclonal antibody with guinea pig T cells

Possible explanations for the loss of CD4 and CD8 positive T cells in the gp1 cell line included outgrowth of B cells, $CD4^-CD8^-\alpha\beta$ T cells, NK cells, or γδ T cells. We investigated this last possibility by screening the gp1 cell line with a panel of monoclonal antibodies specific for various human γ or δ chains (Table 1). This approach has previously proven successful in identifying cross-reactive antibodies to the guinea pig CD1 cell-surface proteins (Dascher et al., 1999; Hiromatsu et al., 2002). One mAb from this panel designated anti-TCRδ1 reacted with the majority of the cells (77%) in the gp1 cell line (Fig. 2A–D). This antibody is specific for the constant region of all human TCR delta chains and thus is a pan-γδ mAb in humans (Band et al., 1987). Concurrent staining of the gp1 line with antibodies to guinea pig CD4 and CD8 surface proteins revealed that the proportion of cells expressing these

Table 1
Anti-γδ TCR specific antibodies tested

mAb designation	Specificity	Isotype	Source or reference
Anti-TCRδ1 (5A6.E9)	Cδ	IgG1	Band et al. (1987)
TCR γ/δ-1 (11F2)	Cγ	IgG1	Borst et al. (1988)
Anti-CγM	Cγ	nd	Porcelli et al. (1991)
510 (52)	Cδ	IgG1	Davodeau et al. (1993)
B1	γδ	IgG1	Pharmingen
F11	γδ?	nd	Ferrini et al. (1989)
515	γδ non-Vδ1, Jδ2	IgG1	Davodeau et al. (1993)
A13	Vδ1	IgG1	Ferrini et al. (1989)
δTCS1	Vδ1/Jδ1, Jδ2	IgG1	Wu et al. (1988)
LL112	Vδ1	nd	Porcelli et al. (1991)
LL113	Vδ1	nd	Porcelli et al. (1991)
TS8-2	Vδ1	nd	Porcelli et al. (1991)
TS9-3C10	Vδ1	nd	Porcelli et al. (1991)
3/62	Vδ1	nd	Porcelli et al. (1991)
R9.12.6.2	Vδ1	nd	Porcelli et al. (1991)
BB3	Vδ2	IgG1	Ciccone et al. (1988)
4G6	Vδ2	IgG1	Deusch et al. (1991)
7A8	Vδ2/Jδ1	IgG1	Deusch et al. (1991)
389	Vδ2	IgG1	Davodeau et al. (1993)
15D (C448.15D)	Vδ2	IgG1	Li et al. (1996)
Anti-TiVδ2	Vδ2	nd	Miossec et al. (1990)
6.2	Vδ2	IgM	Bender et al. (1993)
P11.5.B	Vδ3	IgG1	Romagne et al. (1996)
23D12	Vγ1.2,1.3,1.4	IgG1	Kabelitz et al. (1994)
18C2	Vγ1.2,1.3,1.4	nd	Kabelitz et al. (1994)
4A11	Vγ1.4	IgG1	De Libero et al. (1991)
94	Vγ1.4	nd	Romagne et al. (1996)
B10.11.17	Vγ1.8	nd	Romagne et al. (1996)
TiγA	Vγ2	IgG2a	Jitsukawa et al. (1987)
7A5	Vγ2	IgG1	Janssen et al. (1991)
360	Vγ2	IgG1	Davodeau et al. (1993)
292	Vγ2	IgG1	Davodeau et al. (1993)
4D7	Vγ2	IgG1	Deusch et al. (1991)
B3	Vγ2	IgG1	Pharmingen

nd: no data available.

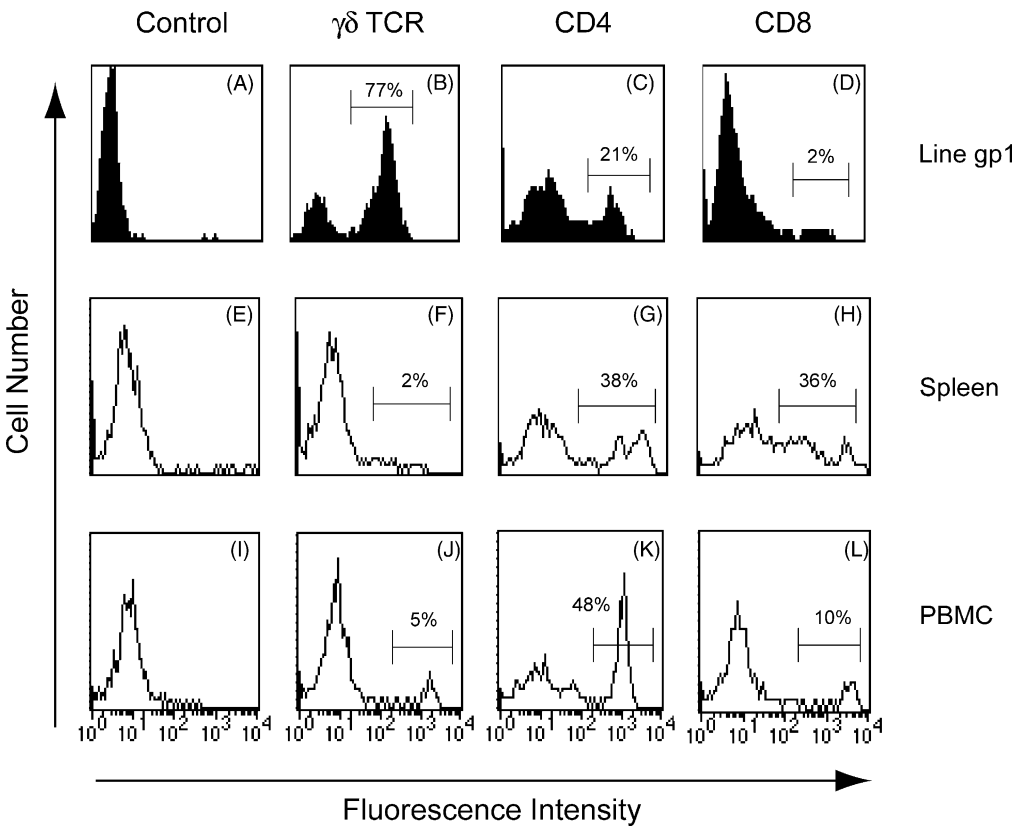

Fig. 2. Reactivity of a pan-δ TCR antibody with the gp1 T cell line. FACS analysis of the T cell line gp1 after 45 days in continuous culture (A–D), fresh naïve guinea pig splenocytes (E–H), and PBMC (I–L). Cells were stained as described in the methods with isotype control mAb P3 (A, E and I), mAb anti-TCRδ1 specific for γδ T cells (B, F and J), mAb CT7 specific for CD4 (C, G and K), and mAb CT6 specific for CD8 (D, H and L). The percentage of lymphocytes staining with the various antibodies is displayed above the gate marker. Note that the total $CD4^+$ plus $CD8^+$ population (C and D) is equal to 23% which is the percentage of anti-TCRδ1 negative cells in panel (B).

markers was 21 and 2%, respectively. Thus the total contaminating $CD4^+$ and $CD8^+$ cells in the gp1 line is about 23%, a value precisely equal to the proportion of anti-TCRδ1 negative cells in the gp1 T cell line. Thus, the gp1 line appears to be a polyclonal T cell line that is predominantly (77%) composed of γδ T cells with a small contamination (23%) of $CD4^+$ and $CD8^+$ αβ T cells.

3.3. Expression of γδ T cells in normal guinea pig PBMC and tissues

We used the anti-TCRδ1 antibody to further characterize the γδ T lymphocyte subpopulation in guinea pigs and compare it with that of other species. Freshly isolated splenocytes from naïve guinea pigs were stained with the anti-TCRδ1 mAb. Approximately 2% of the total lymphocytes in the spleen were detected with anti-TCRδ1 (Fig. 2E–H). This value is similar to those found in mouse spleen (Haas et al., 1993). Approximately 5% of PBMC were also positive with the anti-TCRδ1 mAb for one individual tested (Fig. 2I–L). The FACS analysis in Fig. 2 is representative of three individual naïve female guinea pigs tested. The mean percentage of γδ T cells in guinea pig PBMC from three individual outbred animals was 8.6% with a standard deviation of 3.35% and standard error of 1.93%. This is slightly higher than the published value of 0.5–2% for the mouse (Haas et al., 1993; Itohara et al., 1989). Other experiments with both Hartley and strain 2 guinea pigs yielded similar results (data not shown). Thus, the percentage of γδ T cells in guinea pig blood and tissue is similar to that found in human and rodents but lower than ruminants.

We next used the anti-TCRδ1 antibody to stain tissue sections in order to examine the distribution of cells within lymphoid organs. Frozen sections of guinea pig thymus and spleen were stained with the anti-TCRδ1 mAb and detected with an immunoperoxidase secondary reagent. Staining of medullary thymocytes is consistent with the presence of mature γδ T cells in this region of the thymus (Fig. 3A and B). Staining of the spleen with the anti-TCRδ1 mAb showed a scattered distribution of γδ T cells that was not specifically associated with the periarteriolar lymphoid sheaths but appeared evenly distributed throughout the red pulp. This tissue distribution and frequency is consistent with the FACS analysis presented above (Fig. 2F) and is similar to that shown for γδ T cells in rodents and humans (Bucy et al., 1989; Falini et al., 1989).

3.4. Response of guinea pig cells to phosphate and amine γδ T cell antigens

A number of small phosphate containing compounds have been shown to specifically stimulate the expansion of Vγ2Vδ2 T cells. To determine if

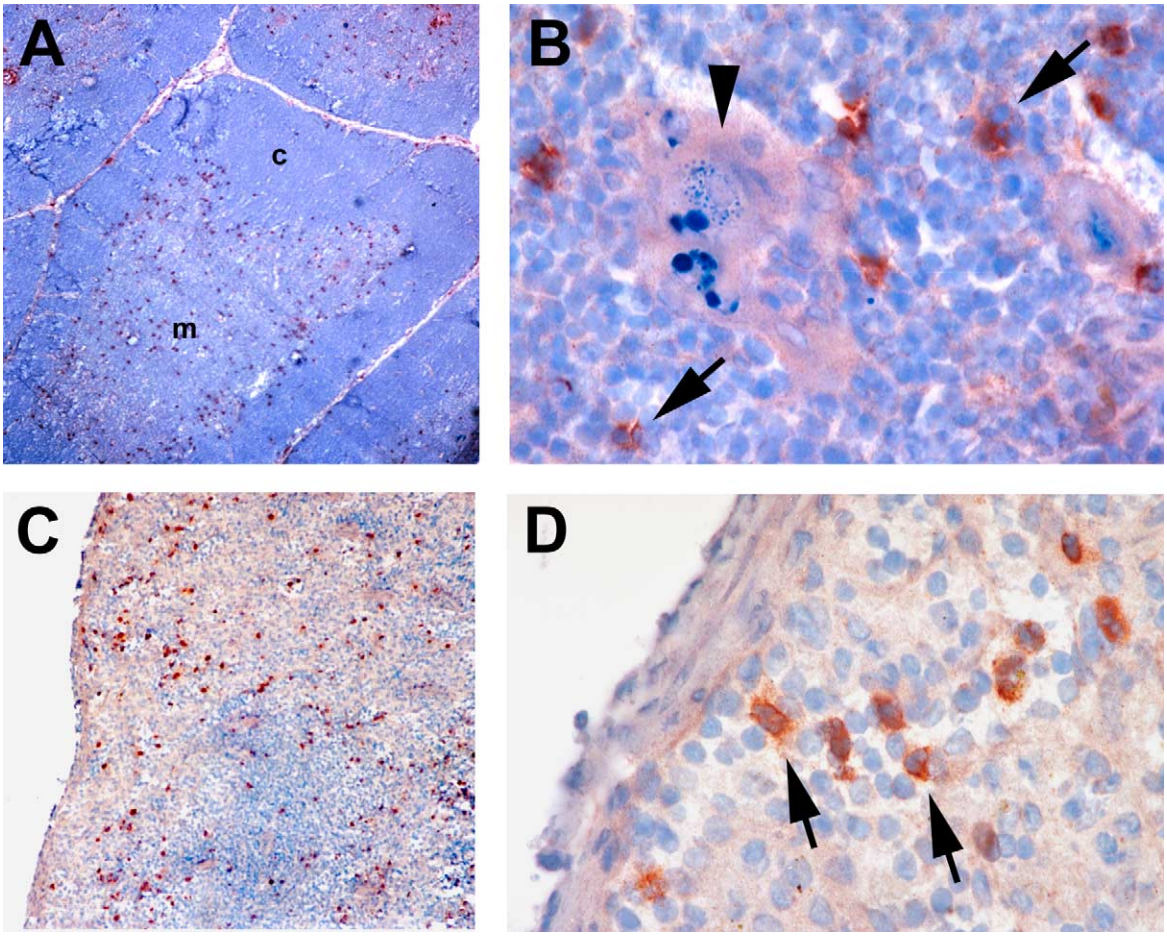

Fig. 3. Expression of γδ cells in guinea pig tissues. Frozen sections of thymus (A and B) and spleen (C and D) were stained with the mAb anti-TCRδ1. Stained sections were visualized with an anti-mouse avidin–biotin–peroxidase complex. Positive staining appears red in these sections. Panel (A) shows staining of cells in the medulla (m) but not in the cortex (c) of a thymic lobule from a 3-week-old guinea pig (100×). Panel (B) shows a higher power magnification (600×) with positive staining cells indicated by arrows. Note the apoptotic bodies being engulfed by a large mononuclear cell (arrow head). This is most likely an early Hassal's corpuscle. Panel (C) shows positive cells staining throughout the spleen tissue (100×). Panel (D) shows a higher power magnification (600×) with positive staining spleen cells indicated by arrows.

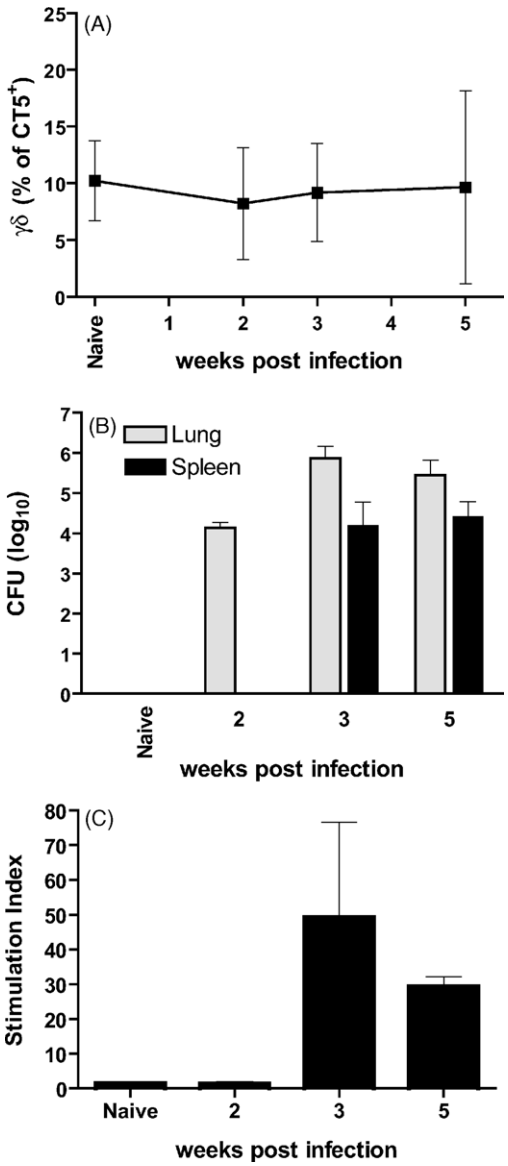

Fig. 4. Characterization of γδ T cells in PBMC following *M. tuberculosis* infection. Naïve strain 2 guinea pigs were infected by low-dose aerosol with virulent *M. tuberculosis* H37Rv strain. Groups of three animals were sacrificed at various time points after infection. Panel (A) shows the results of FACS analysis carried out on isolated PBMC from the animals to determine the percentages of circulating γδ T cells. The percentage of CT5 positive cells (pan-lymphocyte) was used to normalize each sample to account for individual variations in PBMC preparations. Each point is the mean of three animals. A two-tailed unpaired *t*-test was used to compare the means between groups. No statistically significant differences were noted between time point groups relative to the uninfected control group (day 0). Panel (B) shows the bacterial counts from the lung and spleen of the same groups of animals as in panel (A) indicating the growth of bacteria first in the lungs followed by the spleen. Panel (C) are proliferative responses of the PBMC to 10 μg/ml PPD from infected animals. Data are represented as the stimulation index which is the CPM response to PPD divided by the media control. Each bar is the mean of the stimulation index for three animals with error bars representing standard deviation. Note that the response to PPD is only observed at 3 weeks and after. This is the point at which CFU are detected in the spleen following dissemination of bacteria.

guinea pig γδ T cells could recognize phospho-antigens, we cultured spleen cells or PBMC from two guinea pigs with either the major bacterial antigen, HMBPP from *M. fortuitum* or with MEPP, an analog of IPP and HMBPP together with IL-2. Similar experiments were carried out with Risedronate, a bisphosphonate antigen with a similar specificity for the Vγ2Vδ2 T cells. No expansion of cells (other than fibroblasts) was observed during the culture period (data not shown). Identical experiments using human PBMC with these antigens typically results in a 30–200-fold expansion of the γδ T cells to 50–95% of $CD3^+$ cells in the culture by day 9 with the total expansion of the Vδ2Vγ2 T cell subset ranging from 30- to 200-fold (Bukowski et al., 1998, 1999; Das et al., 2001). Thus, it appears that guinea pigs do not respond to the known Vγ2Vδ2 T cell antigens using culture conditions known to stimulate human Vγ2Vδ2 T cells. These data are consistent with the finding that these TCR genes are unique to primates and have not been found in rodents or other mammals.

3.5. Effect of *M. tuberculosis* infection on γδ T cells in the blood

To examine the effect of acute infection on the percentage of γδ T cells, guinea pigs were infected with the virulent *M. tuberculosis* H37Rv strain. Naïve strain 2 guinea pigs were infected with a low-dose of *M. tuberculosis* using a nose-only aerosol chamber system (Dascher et al., 2003). Animals were euthanized at various time points after infection and the percentage of γδ T cells was determined by FACS (Fig. 4A). The percentage of γδ T cells from each animal was normalized against the percentage of $CT5^+$ cells (pan-lymphocyte). No statistically significant changes in the percentage of γδ T cells in the PBMC were detected over the five week period examined. We

confirmed the presence of infection and the dissemination of bacteria by quantifying the bacteria in the lung and spleen following infection (Fig. 4B). All animals exposed to aerosol were indeed infected with *M. tuberculosis* as evidenced by the presence of cultivatable bacteria from the lungs. Bacteria did not disseminate to the spleen until 3 weeks post-infection (Fig. 4B). These results are similar to previous studies using this model (Dascher et al., 2003). Histopathology of the lung also confirmed the presence of active infection with inflammatory infiltrate and granulomatous responses observed in all infected animals (data not shown). We used some of the PBMC isolated from these infected animals to check for proliferative responses to PPD (Fig. 4C). At three weeks post-infection, all animals responded to PPD indicative of a robust antigen-specific T cell immune response. These data demonstrate that a productive infection of the animals was achieved but that the infection did not result net change in the percentage of circulating γδ T cells over the time period examined.

4. Discussion

The γδ T cell receptor gene family is highly conserved with homologs being found in virtually all jawed vertebrates (Rast et al., 1997). Therefore, it should not be a surprise that guinea pigs, a member of the rodent order that includes mice and rats, also possess this T cell subset. In addition, the specificity of the anti-TCRδ1 antibody is well established and its capacity to recognize the γδ TCR from non-human species has been described previously. For example, the anti-TCRδ1 has been shown to cross-react with the γδ TCR of armadillos, one of the most primitive extant mammalian species (Lathrop et al., 1997). Our data strongly supports the cross-reactivity of this antibody with the γδ T cell subset in guinea pigs. This includes FACS staining by anti-TCRδ1 that identifies a subpopulation of lymphocytes in PBMC and spleen that is consistent with the expected distribution of γδ T cells. In addition, the generation of a double-negative guinea pig T cell line that stains with anti-TCRδ1 is also consistent with a phenotypic identification of γδ T cells. For a definitive identification of the cross-reacting epitope recognized by TCRδ1, it would be necessary to clone and sequence the γ and δ TCR genes from the guinea pig and to then transfect these into a TCR-negative T cell line. However, the cumulative evidence presented here is sufficient to provide a high degree of confidence for the identification the anti-TCRδ1 reactive cells as guinea pig γδ T cells.

The proportion of γδ and αβ T cells varies widely between different animal species. Primates and rodents generally have low proportions of peripheral blood γδ T cells (<10%) whereas artiodactyls, an order of mammal that includes ruminants such as sheep, cattle, and pigs, have high proportions of peripheral blood γδ T cells (20–60%) with diverse γδ T cell receptors (Hein et al., 1990; Hein and Mackay, 1991; Mackay and Hein, 1989). Chickens also have a high proportion of circulating γδ T cells (Sowder et al., 1988). We have found that guinea pigs have low proportions of γδ T cells (5–10%) in peripheral blood similar to that found in primates and other rodents.

Many γδ T cells are self-reactive although the antigens that are recognized remain elusive (Carding and Egan, 2002). One exception are the Vγ2Vδ2 γδ T cells that recognize small phosphate and amine containing compounds produced by bacteria or edible plants (Bukowski et al., 1999). However, this subset of γδ T cells has only been found in primates. We have previously noted chromosomal differences between guinea pigs and mice with respect to the CD1 gene-family, a known antigen presenting molecule for some γδ T cells (Dascher et al., 1999). Guinea pigs retain most of the CD1 genes found in primates while mice have deleted major portions of the CD1 locus retaining only the CD1d isoform (Dascher and Brenner, 2003). Thus, guinea pigs and mice have significant differences in their respective genomes that may affect their immune responses to pathogens. However, there is no evidence that this phenomena extends to the TCR gene families. To be certain, we tested guinea pigs for the presence of a functional response to either pyrophosphate or bisphosphonate antigens in vitro. Both antigens are known to specifically stimulate the Vγ2Vδ2 γδ T cell subset (Das et al., 2001). However, no expansion of cells was observed under the culture conditions used in these studies. Similar experiments performed on human PBMC typically result in a robust expansion of Vγ2Vδ2 γδ T cells. In addition, guinea pigs immunized with Risedronate and then tested by in vitro restimulation of T cells with the

same antigen showed no proliferative response in either PBMC or spleen cells (J. Bukowski, unpublished data). These data support earlier observations that the existence of the Vγ2Vδ2 γδ TCR subset is probably restricted to primates and is not present in guinea pigs and other rodents.

Previous studies have shown that γδ T cells may have some protective role in the host response to tuberculosis (Ladel et al., 1995). Moreover, murine γδ T cells have been shown to accumulate in draining lymph nodes following mycobacterial infection (Griffin et al., 1991). We wanted to examine the γδ T cell subset in guinea pigs to look for changes in this cell population following a more natural aerosol infection with *M. tuberculosis*. However, no overt changes in the percentage of γδ T cells was observed over the time period examined following infection. It is important to note that these results do not preclude the possibility of changes in V-gene specific γδ T cell subpopulations over the same time period, resulting in little or no net change in the γδ T cell percentages. In many human infectious diseases including tuberculosis, most of the increases in γδ T cells occur as a result of specific expansion of the Vγ2Vδ2 γδ subset (Balbi et al., 1993; Bertotto et al., 1993; Caldwell et al., 1995; Hara et al., 1992; Munk et al., 1990; Sumida et al., 1992). We were unable to find evidence to support the presence of a Vγ2Vδ2 T cell subset in guinea pigs which may explain the absence of an overall γδ T cell expansion during infection. However, we only examined the blood for changes in γδ T cell percentages and thus we can not rule out the possibility that there was an accumulation of γδ T cells in the tissues of these infected animals, particularly in the lungs. Unfortunately, the epitope recognized by the anti-TCRδ1 antibody is sensitive to formalin fixation and we were unable to perform immunohistology on unfixed *M. tuberculosis* infected frozen specimens as in Fig. 3 due to biohazard considerations. To address this problem, we attempted to perform antigen recovery and Tyramide amplification on the fixed infected tissue sections but were unable to detect a signal. Thus, we cannot rule out γδ T cell accumulation in granulomatous tissues.

In summary, the guinea pig provides an attractive alternative animal model for some infectious disease studies. These include a number of emerging infectious diseases and important biodefense related pathogens such as Ebola virus, anthrax, and Chlamydia infections in which mice are typically more resistant to infection (Fowler et al., 1999; Rank et al., 2000; Xu et al., 1998). We believe that the utility of the guinea pig animal model for infectious disease studies will depend on the availability of more sophisticated reagents. Therefore, this antibody puts another reagent into the toolbox of immunologists who wish to make use of the guinea pig as a model for human diseases.

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