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## Role of gamma/delta T cell receptor-expressing lymphocytes in cutaneous infection caused by *Staphylococcus aureus*

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### SUMMARY

The high number of  $\gamma\delta$ -expressing T cells found in the epithelial lining layer suggests that they form a first line of defence against invading pathogens. To evaluate the role of  $\gamma\delta$  T cell-receptor (TCR)-expressing cells in cutaneous infection caused by *Staphylococcus aureus*, mice lacking  $\gamma\delta$ -expressing T cells (TCR $\delta^{-/-}$ ) were inoculated intradermally with *S. aureus*, and compared with *S. aureus*-infected congenic TCR $\delta^{-/-}$  control mice. The number of bacteria recovered from the skin of TCR $\delta^{-/-}$  mice was significantly higher ( $P = 0.0071$ ) at early time-points after inoculation compared to the number of bacteria isolated from infected TCR $\delta^{-/-}$  congenic controls. Nevertheless, inflammatory responses measured as serum IL-6 levels, were significantly lower in TCR $\delta^{-/-}$  mice than in the control group. A possible explanation for this discrepancy was the observation of significantly decreased overall numbers of infiltrating cutaneous T lymphocytes, which are important producers of IL-6. These results support the notion that the  $\gamma\delta$ -expressing T cells that reside at the epithelial lining layer of the skin is of importance for early containment of the bacteria, thereby limiting their replication and spread.

**Keywords** dermatitis gamma/ delta TCR mice *Staphylococcus aureus*

### INTRODUCTION

Recent experiments have demonstrated that gamma/delta ( $\gamma\delta$ ) T cell-receptor (TCR)-expressing cells are involved in the regulation and resolution of inflammatory processes that are associated with infectious diseases and autoimmunity. Nevertheless, the physiological role of  $\gamma\delta$  T cells is not fully understood.  $\gamma\delta$  T cells have been shown to accumulate at sites of inflammation that are associated with certain viral and parasitic infections, such as malaria and *Toxoplasma gondii* [1].  $\gamma\delta$  T lymphocytes also accumulate in certain human infectious diseases that are associated with granulomatous responses, such as the reactive lesions of leprosy and cutaneous leishmaniasis [2]. Experimental infection with intracellular bacteria, such as *Listeria monocytogenes*, has indicated that  $\gamma\delta$  T lymphocytes may be involved in the regulation of the infection by suppressing the formation of liver lesions [3]. In addition, experimental infection with *Nocardia asteroides* showed an essential role for intraepithelial  $\gamma\delta$  lymphocytes in the survival of the host [4].

The majority of T cells bear the T cell-receptor (TCR) alpha/beta complex ( $\alpha\beta$ ) that recognizes antigen peptides only in the context of self major histocompatibility complex (MHC) molecules [5]. In man,  $\gamma\delta$  TCR lymphocytes constitute on average 5% of all the T cells in normal peripheral blood [6], in organized lymphoid organs and in the skin- and gut-associated lymphoid tissues [2]. Most human  $\gamma\delta$  T cells display the V $\gamma$ 9V $\delta$ 2 rearrangement and lack the expression of both CD4 and CD8 accessory molecules. These T cells constitute a small subset both in the neonatal and adult thymus, but the population expands with age in the peripheral blood, which suggests that positive selection occurs in the periphery following sustained antigenic stimulation [7,8].

In the mouse,  $\gamma\delta$  T cell populations with distinct clonal compositions have been found in the epidermis, intestine, lung, female reproductive tract, tongue and lactating mammary gland. In the murine epidermis, the vast majority of T lymphocytes express TCR  $\gamma\delta$  rather than TCR  $\alpha\beta$ . In the mouse epidermis, the  $\gamma\delta$  T cells which are also known as Thy-1<sup>+</sup> dendritic epidermal T cells (DETCs) [9,10], expresses essentially a single type of  $\gamma\delta$  heterodimer [11], which is known as the junctionally monomorphic V $\gamma$ 5/V $\delta$ 1<sup>+</sup> TCR [12]. The  $\gamma\delta$  T cells vary in density between different skin sites and different strains [9], with values as high as 580 cells/mm<sup>2</sup> in ear epidermis, measured in whole mounts of epidermis (horizontal sections). Precursors of DETC appear primarily

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in the fetal liver and traverse the fetal thymus as a single wave of cells on their way to the epidermis. At later time-points in thymic ontogeny, cells that express other subsets of  $\gamma\delta$  receptors appear in similar waves but migrate preferentially to other tissues [13–15]. The DETCs that appear in the skin cannot be replaced in the adult animal by new precursors that express the same subset of  $\gamma\delta$  TCR receptors, because the generation of DETC precursors is restricted to specific stages of fetal development. In animals that congenitally lack these  $\gamma\delta$  DETC, the epidermis is populated by bone marrow-derived TCR  $\alpha\beta$ , CD8<sup>+</sup> DETCs [16].

To investigate the role of  $\gamma\delta$  TCR-expressing lymphocytes in cutaneous staphylococcal infection, we compared bacterial clearance in  $\gamma\delta$  TCR knock-out (TCR $\delta^{-/-}$ ) mice and heterozygous littermate control mice (TCR $\delta^{+/-}$ ) using a recently described model of cutaneous *S. aureus* infection [17,18]. Our results indicate that resident cutaneous  $\gamma\delta$  T cells act as an early antibacterial defence.

## MATERIALS AND METHODS

### Mice

Male mice of mixed (129  $\times$  C57BL/6) background, which lacked  $\gamma\delta$  T cells as a result of targeted germline mutation in the TCRD gene [19], were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The mutated TCRD locus was back-crossed onto the DBA/1 (originally from the Jackson Laboratory) background for six generations. By subsequent intercrossing of mice that were either heterozygous or homozygous for the mutated TCRD, we obtained both homozygous ( $\gamma\delta$  T cell-deficient) or heterozygous (normal T cell phenotype) offspring littermates. The different T cell phenotypes were determined by flow cytometry analysis of blood cells, using a monoclonal antibody (GL3) that was specific for TCR $\gamma\delta$ .

Female, 12–26-week-old DBA/1 mice, were housed in the animal facility of the Department of Rheumatology and Inflammation Research, University of Göteborg, under standard conditions of light and temperature and fed standard laboratory chow and water *ad libitum*.

### Bacterial strain

The DBA/1 mice were inoculated intracutaneously with *S. aureus* LS-1 strain, which is harboured naturally on the skin of many mouse strains [20]. LS-1 strain has been shown to produce large amounts of toxic shock syndrome toxin 1 (TSST-1) and to be coagulase and catalase positive. The bacteria were kept frozen at  $-20^{\circ}\text{C}$  in 5% bovine serum albumin and 10% dimethylsulphoxide (C<sub>2</sub>H<sub>6</sub>OS) in phosphate buffered saline (PBS) (pH 7.4) until use. Just prior to use, the bacteria were thawed and washed in PBS. Viable counts were used to check the number of live bacteria in each bacterial solution.

### Experimental protocol

Two experiments were performed using DBA/1 mice. The mice were inoculated with bacteria intracutaneously on the shaved back during neurolept analgesia (Dormicum<sup>®</sup>, Hoffmann-La Roche Ltd., Basel, Switzerland; Hypnorm<sup>®</sup>, Janssen Pharmaceuticals, Beerse, Belgium). In experiment I, 31  $\gamma\delta$  T cell receptor knock-out mice (TCR $\delta^{-/-}$ ) and 31 congenic control mice (TCR $\delta^{+/-}$ ) were inoculated at two sites with 0.1 ml of saline that contained either  $2 \times 10^8$  or  $2 \times 10^7$  colony-forming units (CFU) of *S. aureus* (a total of  $4 \times 10^8$  or  $4 \times 10^7$  CFU per mouse). In experiment II, 19  $\gamma\delta$  T cell receptor knock-out mice (TCR $\delta^{-/-}$ ) and 19 control mice

(TCR $\delta^{+/-}$ ) were inoculated at a single site with  $1 \times 10^8$  CFU of *S. aureus* (a total of  $1 \times 10^8$  CFU per mouse). The mice were monitored individually and sacrificed by cervical dislocation 2 days or 7 days after bacterial inoculation. The mice were evaluated clinically for local inflammatory reactions and weight development. In experiment I, skin samples corresponding to single injection sites on each mouse were dissected for histopathological and immunohistochemical evaluation at the time intervals indicated. Skin samples of the second injection site were resected for bacterial analysis. Blood samples were taken for bacterial counts, granulocyte counts, total white blood cell counts, interleukin-6 (IL-6) levels, immunoglobulins (IgG1, IgG2a, IgG3 and IgM), and for specific antibodies to staphylococcal cell walls and TSST-1 (see below).

In experiment II, skin samples from the injection site as well as blood samples were obtained for bacterial analysis at the time intervals indicated. Blood samples were also analysed for granulocyte counts and total white blood cell counts.

### Histopathological examination

Skin samples from 62 mice (experiment I) were examined histopathologically after routine fixation and staining with haematoxylin–eosin. Blinded microscopical evaluation was carried out to characterize the size and density of the inflammatory infiltrates.

### Immunohistochemical examination

Skin samples from the mice in experiment I were also analysed regarding the occurrence of CD11b<sup>+</sup> cells (i.e. macrophages) and CD3<sup>+</sup>-expressing T cells. Briefly, skin samples were frozen in isopentane prechilled with liquid nitrogen, and kept at  $-70^{\circ}\text{C}$  until cryosectioned. All the sections were fixed in cold acetone for 5 min and washed in PBS. The sections were incubated overnight in a humid atmosphere at  $+4^{\circ}\text{C}$  with unlabelled rat anti-CD11b (Mac-1; M1/70) [21] or hamster anti-CD3 (clone 145–2C11, PharMingen, San Diego, CA, USA) monoclonal antibodies [22], which were diluted in PBS containing 1% bovine serum albumin (BSA). After several washes, endogenous peroxidase was depleted by treatment with 0.3% H<sub>2</sub>O<sub>2</sub> for 5 min. Biotin-labelled rabbit antirat Ig (Vector Laboratories, Burlingame, CA, USA) and mouse antihamster IgG (were a cocktail of clones G70-204 and G94-56; PharMingen) diluted in PBS–BSA were used as secondary antibodies. The binding of biotin-labelled secondary antibodies was detected by stepwise incubation with streptavidin–biotin-complex/HRP (Dako, Denmark) and 3-amino-9-ethyl-carbazole containing H<sub>2</sub>O<sub>2</sub>. All sections were counterstained with Meyer's haematoxylin.

### Bacterial culture

Skin and blood samples were obtained for bacterial analysis. After surface disinfection with 70% alcohol, skin samples that corresponded to the injection site were deposited in sterile plastic bags, homogenized and suspended in 10 ml PBS. Skin suspensions were plated in appropriate dilutions on agar plates containing 5% horse blood and incubated at  $37^{\circ}\text{C}$  for 24 h. The number of CFUs per skin sample and per 100  $\mu\text{l}$  of blood were counted and the bacterial colonies were tested for coagulase and catalase activity.

### Serological analyses

**IL-6 assay.** The cell line B13-29, which is dependent on interleukin-6 (IL-6) for growth, has been described previously [23–25]. For IL-6 determinations, the more sensitive subclone B9 was used. B9 cells were harvested from tissue culture flasks, seeded

into microtiter plates (Nunc, Roskilde, Denmark) at a concentration of 5000 cells per well, and cultured in Iscove's medium supplemented with  $5 \times 10^{-5}$  M 2-mercaptoethanol, 10% FCS (Seralab, Sussex, UK), gentamycin (50 µg/ml) and L-glutamine. The serum samples were added for 68 h and [ $^3$ H]thymidine was then added for 4 h prior to harvesting. Each sample was tested for IL-6 in a series of twofold dilutions and compared with a recombinant IL-6 standard. B9 cells have been shown previously not to react with recombinant cytokines, such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-5, granulocyte-macrophage colony-stimulating factor, tumour necrosis factor- $\alpha$  and gamma interferon and had only weak reactivity with IL-4 [25].

**Immunoglobulins.** Total serum levels of immunoglobulin (Ig) G1, IgG2a, IgG3, and IgM were measured by the radial immunodiffusion technique [26]. Antisera and immunoglobulin standards specific for IgG1, IgG2a, IgG3 and IgM were purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA).

**Anti-TSST-1 antibodies.** Serum levels of IgM and IgG antibodies to TSST-1 were estimated by ELISA using 0.5 µg/ml of highly purified TSST-1 (Toxin Technology, Sarasota, FL, USA) as a solid phase coating. All sera were diluted serially in 0.5% PBS-BSA and incubated in the wells. To measure the level and class specificity of anti-cell-wall antibodies that were bound to the solid phase, affinity-purified and biotinylated F(ab')<sub>2</sub> fragments of goat antimouse IgG and IgM (Jackson Laboratories), which were diluted 1 : 3000 in PBS-Tween 20, were added to wells. This was followed stepwise by the addition of 0.5 µg/ml extravidin-horse-radish peroxidase (Sigma) and 2.5 mg/ml of the enzyme substrate 2,2-azino-bis-(3-ethylbenzothiazoline sulphonate) (Sigma) in citrate buffer (pH 4.2) containing 0.0075% H<sub>2</sub>O<sub>2</sub>. The absorbance at 414 nm (A<sub>414</sub>) was measured in a SpectraMax Plus spectrophotometer (Molecular Devices). All optical density values were converted to antigen-specific arbitrary units using calibration curves that were based on the optical density values obtained from serial dilutions of a pool of reference sera. The calibration curves were constructed with a computer program that employed weighted logit-log models [27,28].

**Antibodies to cell walls of *S. aureus*.** Serum levels of IgM antibodies that were specific for *S. aureus* cell-wall constituents were estimated by an ELISA in which poly L-lysine (25 µg/ml) was used to precoat the wells, and 100 µl of whole, formalin-treated (4% for 20 min) *S. aureus* LS-1 cells were used (10<sup>8</sup>/ml) to coat the wells. The subsequent steps in the assay were similar to those described above.

#### Haematological analysis

Total white blood cell counts (WBC) were determined using a haemocytometer (Toa Medical Electronics, Kobe, Japan). Blood smears were prepared and stained by the May-Grünwald-Giemsa method for differential counts.

#### Statistical evaluation

The differences between mean values were tested for significance using the non-parametric Mann-Whitney *U*-test. *P*-values  $\leq 0.05$  were considered to be statistically significant.

## RESULTS

#### Bacterial cultures

Skin samples from  $\gamma\delta$  T cell-deficient mice that received  $2 \times 10^8$  CFU of bacteria had significantly higher numbers of bacteria

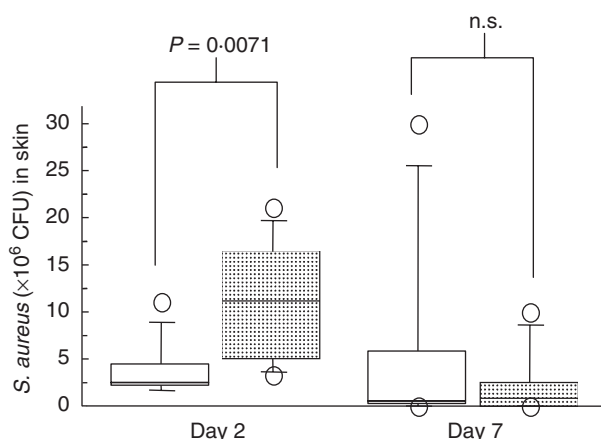
than littermate controls 2 days after inoculation ( $P = 0.0071$ ) (Fig. 1). There were no significant differences in bacterial numbers between mice that received  $2 \times 10^8$  CFU of *S. aureus* 7 days after bacterial inoculation, and no significant differences between groups of mice that were inoculated with  $2 \times 10^7$  CFU of *S. aureus* (data not shown). Bacterial cultures from blood samples 2 days after bacterial inoculation were positive in six of eight mice (75%) in the  $\gamma\delta$  T cell-deficient group that received a total of  $4 \times 10^8$  CFU, and in eight of nine mice (89%) in the control group (n.s.) that received the same dose of bacteria. Seven days after staphylococcal inoculation, 2/7 mice (29%) in both groups showed bacteraemia. Bacteraemia was absent in mice that received a total of  $4 \times 10^7$  CFU of *S. aureus*.

#### Microscopic evaluation

Microscopic evaluation of infected skin specimens revealed a dense infiltrate of granulocytes and macrophages at days 2 and 7 in mice that received  $2 \times 10^8$  CFU of *S. aureus*. The bacteria were clearly visible in some skin specimens. In contrast, mice that received  $2 \times 10^7$  CFU of *S. aureus* showed only mild inflammatory infiltrates and the bacteria were not easily detectable. This result is in accordance with previously reported findings [17]. There were no clear-cut differences between the KO and control mice with respect to inflammatory infiltrate size.

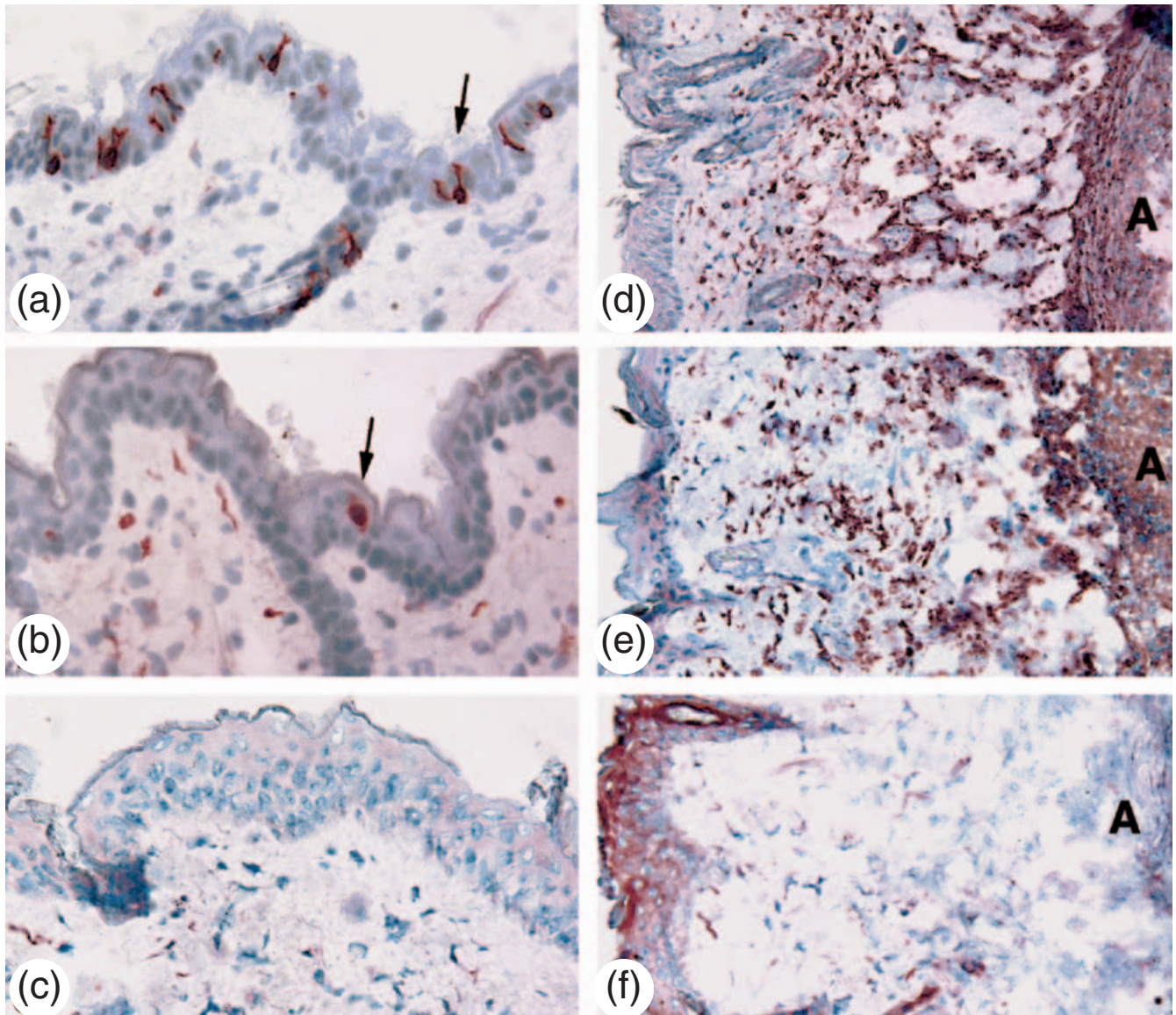
#### Immunohistochemical analysis

Immunohistochemical analysis was performed to determine the occurrence of CD11b<sup>+</sup> cells (phagocytic cells) and CD3<sup>+</sup> cells (T lymphocytes). The number of CD3<sup>+</sup> T cells in the epidermis was significantly higher in the control mice compared to the  $\gamma\delta$  T cell-deficient animals (Fig. 2), both in mice that received  $2 \times 10^8$  CFU of *S. aureus* and in mice that were inoculated with  $2 \times 10^7$  CFU of *S. aureus* at days 2 and 7 (Table 1). No significant differences were noted between the groups regarding the number of CD3<sup>+</sup> cells in the dermis, where a number of approximately 10 CD3<sup>+</sup> T cells/mm<sup>2</sup> were noted. The number of CD11b<sup>+</sup> (Mac-1) cells was high ( $>50/\text{mm}^2$ ) in all the mouse groups, with no significant differences noted at days 2 or 7 (Fig. 2).



**Fig. 1.** Numbers of *S. aureus* ( $\pm$  s.d.) in skin samples ( $n = 7-9$  for each time period and group) recovered at defined time intervals after initial intracutaneous inoculation with  $2 \times 10^8$  CFU of *S. aureus*. □,  $\gamma\delta$  TCR<sup>+/+</sup>; ▨,  $\gamma\delta$  TCR<sup>-/-</sup>.





**Fig. 2.** Left row (a, b and c): CD3<sup>+</sup> epidermal T lymphocytes 2 days after bacterial inoculation with  $2 \times 10^8$  CFU of *S. aureus*. Arrows indicate single CD3<sup>+</sup> T lymphocytes in (a) control group, and (b)  $\gamma\delta$ T cell-deficient mice. (c) Tissue section stained with appropriate hamster IgG control antibodies. Right row (d, e and f): CD11b<sup>+</sup> cells 2 days after bacterial inoculation with  $2 \times 10^8$  CFU of *S. aureus* in (d) control group, and (e)  $\gamma\delta$ T cell-deficient mice. (f) Tissue section stained with appropriate rat IgG control antibodies. The letter A indicates abscess formation.

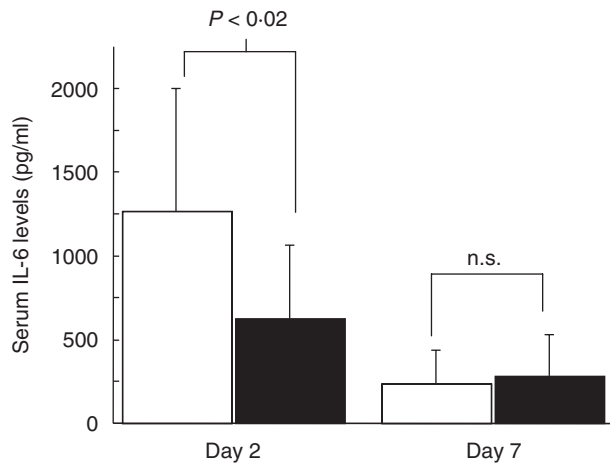
#### Serological analyses

**IL-6.** The serum levels of IL-6 in mice that received a total of  $4 \times 10^8$  CFU *S. aureus* were significantly lower in  $\gamma\delta$  knock-out mice than in control mice ( $P < 0.02$ ), with peak values around 600 pg/ml in  $\gamma\delta$ T cell-deficient mice compared to 1200 pg/ml in the controls (Fig. 3). No significant differences were noted at day 7 in groups of mice that received a total of  $4 \times 10^8$  CFU or  $4 \times 10^7$  CFU of *S. aureus*.

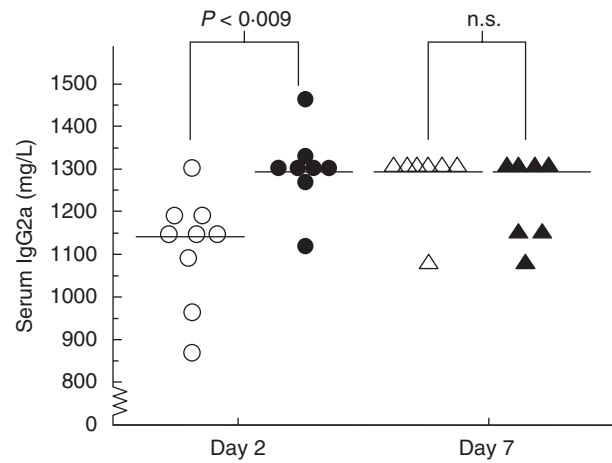
**B lymphocyte responses.** Because IL-6 is an efficient B lymphocyte-stimulating factor, we decided to analyse the serum levels of immunoglobulins and specific antibodies to staphylococcal components. Serum levels of IgG1 and IgG3 were not increased significantly 7 days after bacterial inoculation in any mouse group, and there were no significant differences between the  $\gamma\delta$ T cell-

**Table 1.** Number of epidermal CD3<sup>+</sup> T cells (mean  $\pm$  s.e.m./skin section) is significantly decreased in TCR  $\delta^{-/-}$  mice. Two to five sections were counted per mouse

No. of <i>S. aureus</i> inoculated	TCR status ( $n = 5-8$ )	Day 2	Day 7
$10^8$	TCR $\delta^{-/-}$	$48 \pm 11$	$40 \pm 10$
$P$		$<0.002$	$<0.004$
$10^8$	TCR $\delta^{-/-}$	$3 \pm 1$	$3 \pm 1$
$10^7$	TCR $\delta^{+/+}$	$40 \pm 7$	$49 \pm 9$
$P$		$<0.004$	$<0.001$
$10^7$	TCR $\delta^{-/-}$	$4 \pm 1$	$4 \pm 1$



**Fig. 3.** Serum IL-6 levels (pg/ml)  $\pm$  s.d. at specified time intervals following intracutaneous inoculation with  $2 \times 10^8$  CFU of *S. aureus* ( $n = 7-9$ ).  $\square$ ,  $\gamma\delta$  TCR $^{+/+}$ ;  $\blacksquare$ ,  $\gamma\delta$  TCR $^{-/-}$ .



**Fig. 4.** Serum IgG2a levels (mg/L). Horizontal bars represent the median values at specified time intervals following intracutaneous inoculation with  $2 \times 10^8$  CFU of *S. aureus* ( $n = 7-9$ ).  $\circ$ ,  $\gamma\delta$  TCR $^{+/+}$ ;  $\bullet$ ,  $\gamma\delta$  TCR $^{-/-}$ ;  $\triangle$ ,  $\gamma\delta$  TCR $^{+/+}$ ;  $\blacktriangle$ ,  $\gamma\delta$  TVR $^{-/-}$ .

**Table 2.** *In vivo* antibody responses to *S. aureus* components following intradermal inoculation with TSST-1 secreting *S. aureus* in congenic mice with respect to  $\gamma\delta$ TCR expression

Size of inoculum (CFU)	Mice	Staphylococcal cell wall antibody*		TSST-1 antibody*	
		Day 2	Day 7	Day 2	Day 7
$4 \times 10^8$	$\gamma\delta^{-/-}$	$30\,300 \pm 7513$	$59\,090 \pm 29\,102$	$805 \pm 473$	$1226 \pm 634$
		n.s.	n.s.	n.s.	n.s.
	$\gamma\delta^{+/+}$	$33\,458 \pm 11\,137$	$74\,812 \pm 17\,013$	$634 \pm 64$	$1331 \pm 331$
$4 \times 10^7$	$\gamma\delta^{-/-}$	$25\,437 \pm 4905$	$36\,600 \pm 12\,567$	$446 \pm 38$	$1107 \pm 270$
		n.s.	n.s.	n.s.	$P = 0.05$
	$\gamma\delta^{+/+}$	$33\,500 \pm 14\,285$	$34\,650 \pm 16\,570$	$529 \pm 124$	$642 \pm 221$

\*IgM, arbitrary units  $\pm$  s.d.

deficient and control mice. In contrast, the serum levels of IgG2a were significantly higher at day 2 in  $\gamma\delta$ T cell-deficient mice compared to controls ( $P < 0.009$ ) (Fig. 4). Serum IgM levels were increased at day 7 in mice that received a total of  $4 \times 10^8$  CFU of *S. aureus*, but no significant differences were noted between the groups. The serum levels of *S. aureus* cell-wall-specific antibodies of IgM class increased significantly ( $P = 0.01$ ) between day 2 and day 7 in control mice (Table 2), with a twofold increase also seen in  $\gamma\delta$ T cell-deficient mice (n.s.) that were inoculated with a total of  $4 \times 10^8$  CFU of *S. aureus*. However, there were no significant differences between the experimental and control groups of mice that received  $4 \times 10^8$  or  $4 \times 10^7$  CFU of bacteria. The serum levels of TSST-1-specific antibodies of IgM class were also significantly increased ( $P = 0.006$ ) between day 2 and day 7 in control mice that received  $4 \times 10^8$  CFU of bacteria, while the serum levels of TSST-1 specific IgM antibodies in  $\gamma\delta$ T cell-deficient mice showed only a slight increase (n.s.). No increase in serum levels of TSST-1 specific antibodies of IgG class were detected (data not shown).

#### Clinical and haematological analysis

No significant changes in weight development were noted after inoculation with *S. aureus* between  $\gamma\delta$ T cell-deficient mice and

their congenic controls. Analysis of blood smears showed an increase in the percentage and number of granulocytes after bacterial inoculation, but no significant differences were noted between the groups (Table 3).

## DISCUSSION

The presence of large numbers of  $\gamma\delta$  TCR-expressing lymphocytes at epithelial surfaces suggests that they have a role in the first line of defence against invading pathogens. Nevertheless, the timing of influx and regulatory responses of  $\gamma\delta$ -expressing T cells is debated and controversial. Some investigators have shown that  $\gamma\delta$ T cells are more prominent during subacute infections, or in the recovery phase following certain viral infections. The predominance of early or late  $\gamma\delta$ T cell responses following infection may be related to the type and dose of bacteria as well as to the site of infection and sampling time-points [1]. Indeed, our study suggests that the cutaneous deposition of  $2 \times 10^8$  CFU of *S. aureus* but not of lower bacterial doses leads to differential immune responses in mice that have intact T cell repertoires and those being knocked out for the  $\gamma\delta$ T cell receptor. The reasons for this threshold effect

**Table 3.** Number of leucocytes in peripheral blood 7 days after intracutaneous inoculation with *S. aureus* LS-1. The values shown represent mean  $\pm$  s.d.,  $n$  = number of mice

Mice	(n)	Size of total inoculum (CFU)	WBC ( $10^6$ /ml)	Total granulocytes ( $10^6$ /ml)	Granulocytes (%)
$\gamma\delta^{-/-}$	9	$4 \times 10^7$	$4.8 \pm 3.3$	$0.9 \pm 0.7$	$18.8 \pm 6.0$
$\gamma\delta^{+/-}$	8	$4 \times 10^7$	$4.1 \pm 2.2$	$0.8 \pm 0.5$	$18.8 \pm 4.5$
$\gamma\delta^{-/-}$	10	$1 \times 10^8$	$7.6 \pm 2.1$	$1.7 \pm 0.5$	$22.9 \pm 3.3$
$\gamma\delta^{+/-}$	10	$1 \times 10^8$	$5.8 \pm 2.0$	$1.5 \pm 0.6$	$24.8 \pm 3.8$
$\gamma\delta^{-/-}$	7	$4 \times 10^8$	$9.0 \pm 2.7$	$2.8 \pm 0.9$	$28.1 \pm 3.1$
$\gamma\delta^{+/-}$	7	$4 \times 10^8$	$9.8 \pm 2.9$	$2.7 \pm 1.2$	$29.0 \pm 6.1$

are presently unknown. In the present study, significantly higher numbers of *S. aureus* were recovered from skin samples 2 days after intracutaneous inoculation of bacteria in TCR $\delta^{-/-}$  mice compared to heterozygous TCR $\delta^{+/-}$  littermate control mice. Similar findings have been reported in experimental *Listeria monocytogenes* infection, in which mice that were depleted of  $\gamma\delta$ T cells succumbed to infection induced by high doses of this intracellular bacterium [1]. In that report, the authors proposed that one function of  $\gamma\delta^+$  T cells was to down-regulate the responses of infection-activated macrophages by inducing their apoptosis, thereby restoring macrophage homeostasis and preventing the development of chronic inflammation. The mechanisms by which  $\gamma\delta$ T cells kill activated macrophages have not been determined, but examples of cytotoxicity induced by  $\gamma\delta^+$  T cells have been reported, which include the perforin pathway and Fas ligation on target cells [29,30].

Although it has been demonstrated clearly that  $\gamma\delta$ TCRs do not recognize processed peptide Ags that are complexed to self-MHC molecules [31], there is evidence that they can recognize highly conserved non-protein Ags. In this respect, peptidoglycans and/or teichoic acid, both of which are constituents of the staphylococcal cell wall may provide activating signals to  $\gamma\delta$ T cells that, directly or indirectly, enhance bacterial elimination. In addition, it has been recently shown that  $\gamma\delta^+$  T cells may be activated directly by staphylococcal TSST-1 [32], a superantigen that is produced by the staphylococcal strain used in the present study. In the present study, skin infection with TSST-1-producing staphylococci induced predominantly IgG2a production in mice that lacked  $\gamma\delta$  TCR-expressing cells. Furthermore, decreased levels of IL-6, which is a Th2 cytokine, were found in the same mice. This finding indicate either decreased ability to produce IL-6 or decreased inflammatory stimulus (i.e. number of bacteria). Since the  $\gamma\delta$  knockout mice displayed higher rather than lower bacterial load, the first hypothesis is more valid. Interestingly, it has been suggested previously that IL-6 stimulates antibacterial macrophage activities [33], thereby decreasing the risk of infection.

Our observations of early and transient deficiencies in bacterial elimination in  $\gamma\delta$ T cell-deficient mice need further clarification. One possibility, put forward recently by Ottonen *et al.* [34], is that activated  $\gamma\delta$ T cells act directly as antibacterial effector cells in the early stages of infection. A paucity of  $\gamma\delta$ T cells would thus hamper the efficient early elimination of staphylococci, whereas in the later stages of infection compensatory mechanisms would have emerged. Altogether, our results strongly support the notion that cutaneous  $\gamma\delta$ -expressing T cells act as an early barrier to prevent staphylococcal infections of the skin.

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