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# The role of cytokines in a *Porphyromonas gingivalis*-induced murine abscess model

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**Introduction:** *Porphyromonas gingivalis* is an important periodontopathic bacterium that is strongly associated with periodontal disease and is part of human dental plaque. Periodontal disease results from the interaction of the host with bacterial products, and T-cell-derived cytokines remain critical in the immunoregulation of periodontal disease.
**Methods:** The aim of this study was to examine the role of T helper type 1 [interleukin-12p40 (IL-12p40), interferon-γ, tumour necrosis factor (TNF)] and type 2 (IL-4, IL-10) cytokines in the immune response to a subcutaneous challenge with *P. gingivalis* using a well-established murine abscess model, in genetically modified cytokine-specific knockout mice.
**Results:** IL-12p40$^{-/-}$ mice exhibited more advanced tissue destruction and a reduced inflammatory cell infiltrate after subcutaneous *P. gingivalis* challenge. Deficiency of IL-4 or IL-10 did not result in increased susceptibility to *P. gingivalis*-mediated tissue destruction. Furthermore, TNF deficiency appeared to reduce local tissue destruction. Interestingly, serum-specific antibodies suggested a strong T helper type 2 response.
**Conclusion:** The results of our study indicate an important role for IL-12 in a primary *P. gingivalis* subcutaneous challenge.

Key words: cytokines; immune response; knockout mice; *Porphyromonas gingivalis*; skin lesion

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*Porphyromonas gingivalis* is a gram-negative, black-pigmented anaerobic bacterium that is highly associated with chronic periodontitis (39, 40). Periodontal tissue destruction occurs as a result of the host's inflammatory response to the plaque biofilm; however, plaque alone is insufficient to produce the disease in the non-susceptible host (25). Essentially, it is the nature of the immune response that determines disease outcome (16, 36).

Humoral and cell-mediated immune responses to *P. gingivalis* have been studied in rodent models (7) and in humans (29, 30). *P. gingivalis*-specific T cells have been shown to produce both T helper type 1 (Th1) and Th2 cytokines, regardless of the antigen-presenting cell population (12). It has also been suggested that the resultant cytokine profile may be genetically influenced (11).

Various animal models have been used to study the pathogenesis of periodontal disease, and these have been useful in elucidating the bacteria-mediated response (16). Gene knockout mice have more recently been used as models to test susceptibility to various pathogens and to identify the molecular pathways involved in host defence (27), although these have not been extensively used to elucidate the involvement of cytokines in periodontal pathogenesis.

Immunohistological studies have shown that a periodontitis lesion is characterized by a shift in the inflammatory infiltrate from a T-cell-dominant lesion with few B cells, to one with an increased proportion of B cells and plasma cells (36). The increased proportion of B cells in periodontitis suggests a role for Th2 cytokines (16). The development of a gingivitis lesion on the other hand, has shown similarities to that of a controlled delayed-type hypersensitivity response (37). Since interferon-γ (IFN-γ) is found at sites of delayed-type hypersensitivity reactions (43), the development of a gingivitis lesion has been suggested to be a Th1 response (37). Studies examining Th1 and Th2 cytokine profiles in periodontitis have been controversial and inconsistent (12, 42). Regardless of the various hypotheses for the role of Th1 and Th2 cytokines in

disease progression, T-cell-derived cytokines remain critical to our understanding of the immunoregulation of periodontal disease (38).

The aim of this study was to determine the role of Th1 and Th2 cytokines in the immune response to a subcutaneous challenge with *P. gingivalis* using a well-established murine abscess model in genetically modified cytokine-specific knockout mice. An enzyme-linked immunosorbent assay (ELISA) was used to determine the serum cytokine profile and to measure anti-*P. gingivalis* antibody levels. Splenic lymphocytes were analysed by dual-colour flow cytometry for CD4 and CD8 expression, and for intracytoplasmic cytokine synthesis.

## Materials and methods

### Mice

Specific pathogen-free female knockout mice and their respective wild-type controls, 6–8 weeks of age, were obtained from various sources as acknowledged below and bred at the Herston Medical Research Centre, Brisbane Australia, with the genotypes checked regularly by polymerase chain reaction. Knockout mice used in this study included interleukin-10 knockout (IL-$10^{-/-}$), IL-$12p40^{-/-}$, IFN-$\gamma^{-/-}$, and tumour necrosis factor knockout ($TNF^{-/-}$) on the C57BL/6J background, and IL-$4^{-/-}$ on the BALB/c background. Animal experiments were approved by the Animal Experimentation Ethics Committee of the University of Queensland, and were carried out in accordance with the National Health and Medical Research Council's Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 1997. Mice were housed in filter-top cages in a PC2 facility, and provided with food and water *ad libitum*.

### Bacteria

*P. gingivalis* W50 was used in this study and cultured anaerobically as described previously (5). Briefly, the bacterium was cultured on Wilkens Chalgrens agar plates (WCA; Oxoid Ltd, Basingstoke, UK) prepared from Wilken Chalgrens broth (WCB; Oxoid; 33 g/l) with the addition of agar (10 g/l) and 5% laked sheep blood. The plates were incubated for 4 days at 37°C in an atmosphere of 10% $H_2$, 10% $CO_2$ and 80% $N_2$ in an anaerobic cabinet (Katec Pty Ltd, Adelaide, South Australia). The black-pigmented colonies from plates were subcultured into WCB and incubated as above. Purity was monitored by Gram stain and by subculture to WCA plates. Both viable and non-viable bacteria in the broth cultures were determined by counting in a Helber bacterial counting chamber under phase-contrast microscopy. The bacteria were harvested from exponentially growing 48-h broth cultures, suspended in reduced phosphate-buffered saline (PBS) at the appropriate concentration for injection ($10^{10}$/ml) and transported in an anaerobic state for injection. All inoculation and sampling procedures were conducted under halothane anaesthesia (Veterinary Companies of Australia, Artarmon, NSW, Australia) using an inhalation apparatus (Fluortec, Mediquip, Brisbane, Qld, Australia) and a scavenging system (Omnicon Fresh Air Canister, Bickford Inc., NY).

### Subcutaneous challenge

Mice were injected subcutaneously at two sites on the dorsal surface, approximately 1 cm on either side of the midline as described previously (14). Mice were injected with 100 µl/site of $10^{10}$/ml *P. gingivalis*, or the equivalent volume of sterile PBS. Lesion diameter was measured using a digital micrometer on days 1 and 10. Ten days after challenge, mice from each group were anaesthetized and blood samples were collected by direct heart puncture after which the mice were sacrificed. Serum from each group was pooled for the determination of cytokine and antibody levels. Spleens were removed and worked through cell strainers (Falcon, Becton Dickinson, Franklin Lakes, NJ); the resulting suspensions were washed and centrifuged on Ficoll–Paque (Pharmacia LKB, Uppsala, Sweden) gradients to obtain mononuclear cell suspensions. CD4 and CD8 T cells were then stained for intracytoplasmic cytokines and analysed by two-colour flow cytometry as described below.

### Flow cytometric analysis

The per cent of splenic CD4 and CD8 T cells staining positively for intracytoplasmic IL-4, IL-10 and IFN-γ were determined as described previously (14). Briefly, surface membrane staining of CD4 and CD8 cells was achieved using fluorescein isothiocyanate-conjugated rat anti-mouse CD4 or CD8 antibodies (Pharmingen, San Diego, CA), followed by fixation of these cells in paraformaldehyde, permeabilization using proteinase K and then incubation with phycoerythrin-conjugated rat anti-mouse IL-4, IL-10 or IFN-γ antibodies (Pharmingen). For the assessment of non-specific binding of the rat antibodies to the mouse cell surface antigens, phycoerythrin and fluorescein isothiocyanate-conjugated specific rat immunoglobulin isotypes (PharMingen) were used in place of the CD4 or CD8 antibodies and the anti-cytokine antibodies. Ten thousand stained cells from each sample were analysed using dual-colour flow cytometry on a FACSCalibur (Becton Dickinson, Mountain View, CA) and the percentages of CD4 and CD8 cells which were positive for IL-4, IL-10 and IFN-γ were determined.

### Detection of serum cytokines following subcutaneous challenge

Pooled serum was assayed for IL-4, IL-10, IL-12p40, IFN-γ and TNF levels by ELISA. Briefly, microtitre plates (Immunosorb, Nunc, Denmark) were coated with capture antibody (OptEIA, Pharmingen) and incubated overnight at 4°C. Following blocking with 10% newborn calf serum (1 h at room temperature), 100 µl aliquots of either sample or known concentrations of purified cytokine (for construction of a standard curve), were added to the appropriate wells for 2 h at room temperature. Following washing, plates were incubated with biotinylated anti-mouse monoclonal antibody and avidin–horseradish peroxidase conjugate (1 h at room temperature). Colour development was achieved by adding 150 µl 2.5 mM *o*-tolidine (Eastman Kodak, Rochester, NY) in 100 mM phosphate citrate buffer pH 3.5 containing 0.025 mM EDTA and activated with 3% $H_2O_2$. The resulting reaction was stopped after 10 min by adding 50 µl of 1 M HCl. Plates were then read in a Bio-Rad microplate reader (Bio-Rad Laboratories, Regents Park, NSW, Australia) at 450 and 655 nm. The results were expressed as cytokine titre (pg/ml).

### Detection of serum anti-*P. gingivalis* antibodies following subcutaneous challenge

Pooled serum was quantified for anti-*P. gingivalis* antibodies using an ELISA as described by Gemmell et al. (10). Microtitre plate wells (Immunosorb, Nunc, Denmark) were coated with 1 µg protein/ml suspension of *P. gingivalis* (Pierce, Rockford, IL). After blocking with 1% bovine serum albumin (Commonwealth Serum Laboratories, Melbourne, Australia), diluted serum samples were added followed by goat anti-mouse immunoglobulin G1-(IgG1), IgG2a- or IgM-specific horseradish peroxidase-labelled monoclonal antibody

(Caltag Laboratories, Burlingame, CA). Colour development and optical density were achieved as described above. *P. gingivalis*-specific IgG1, IgG2a and IgM antibody concentrations (μg/ml) in the serum samples were determined from a standard curve of known concentrations of purified mouse IgG1, IgG2a or IgM (Caltag Laboratories, Burlingame, CA).

### Histopathology

Skin lesions were removed from mice sacrificed on day 1 or day 10 following challenge; they were fixed in 10% neutral buffered formalin (pH 7.0), embedded in wax, sectioned at 5-μm thickness, stained with haematoxylin and eosin (H&E) in a routine manner, and examined by light microscopy.

### Statistics

Quantitative data were analysed using the statistical features of GRAPHPAD Prism Version 2.01 (GraphPad Inc., San Diego, CA). Student's *t*-test and one-way analysis of variance were used with $P < 0.05$ being significant unless otherwise indicated.

## Results

### Lesion size

At day 1 after *P. gingivalis* challenge, C57BL/6J mice exhibited a lesion that was significantly larger than that measured on day 10 (Fig. 1A) ($P < 0.05$). IL-12p40$^{-/-}$ mice exhibited a similar lesion size at day 1 compared to C57BL/6J mice wild-type mice, although unlike the wild-type mice, there was no significant reduction in lesion size by day 10. In addition, IL-12p40$^{-/-}$ mice had a significantly larger lesion diameter at day 10 when compared with wild-type mice (Fig. 1B) ($P < 0.01$). IL-10$^{-/-}$ and IFN-$\gamma^{-/-}$ mice exhibited a relatively smaller lesion diameter at day 1 compared with the wild-type strain, but by day 10, the lesion was not detectable in the IL-10$^{-/-}$ mice and was maintained with no significant change in the IFN-$\gamma^{-/-}$ mice. TNF$^{-/-}$ mice displayed no detectable lesion at day 1 or day 10.

In the BALB/c mice, a small lesion was measured on day 1 after *P. gingivalis* challenge, and was the same size at day 10 (Fig. 1E). IL-4$^{-/-}$ mice on the other hand, exhibited a relatively larger lesion at day 1, which decreased by day 10, although this change was not significant (Fig. 1F).

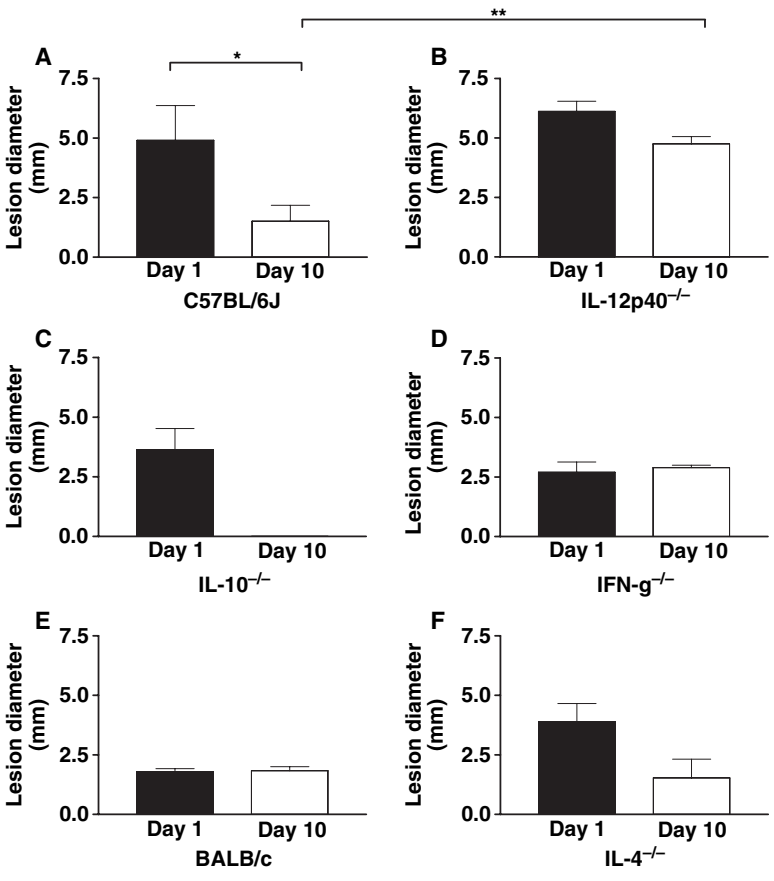


*Fig. 1.* Diameter of skin lesions (mean ± SEM) at day 1 and day 10 following *Porphyromonas gingivalis* subcutaneous challenge in (A) C57BL/6J, (B) IL-12p40$^{-/-}$, (C) IL-10$^{-/-}$, (D) IFN-$\gamma^{-/-}$, (E) BALB/c and (F) IL-4$^{-/-}$ mice. A minimum of three mice were used per group per time-point. *Significantly greater lesion diameter at day 1 compared to day 10 in C57BL/6J mice ($P < 0.05$). **Significantly greater lesion diameter in IL-12p40$^{-/-}$ mice compared to C57BL/6J mice at day 10 after *P. gingivalis* challenge ($P < 0.01$). Data for TNF$^{-/-}$ mice is not shown.

### Histopathology

C57BL/6J mice developed well-defined subcutaneous abscesses on day 1 following challenge with *P. gingivalis* (Fig. 2A), composed mainly of polymorphonuclear cells (PMNs) and macrophages (Fig. 2B). By day 10 the abscesses became more defined, and an attempt at wound healing was seen with the development of a semi-organized fibrotic matrix surrounding the central abscess area (Fig. 2C). The same pattern was seen in IL-12p40$^{-/-}$ mice on day 1 (Fig. 2D), but by day 10 the lesion was well-organized with a distinct lack of an inflammatory infiltrate (Fig. 2E). IL-10$^{-/-}$ and IFN-$\gamma^{-/-}$ mice displayed a similar pattern to C57BL/6J mice on day 1, although there tended to be a more intense and tightly packed inflammatory infiltrate seen in IL-10$^{-/-}$ mice (data not shown). TNF$^{-/-}$ mice did not exhibit an inflammatory infiltrate following challenge with *P. gingivalis* (data not shown).

IL-4$^{-/-}$ and BALB/c mice developed abscesses with similar characteristics that were comparable to those seen in C57BL/6J mice on day 1, although by day 10 the inflammatory pattern was more diffuse and not well organized in IL-4$^{-/-}$ mice (Fig. 2F).

### Splenic cytokine profiles

#### *C57BL/6J mice*

*P. gingivalis*-challenged C57BL/6J mice did not display any significant change in cytokine expression in either T-cell subset when compared with sham-challenged C57BL/6J mice (Figs 3A and 4A).

#### *IL-10$^{-/-}$ mice*

There were no significant differences in cytokine expression in *P. gingivalis*-challenged IL-10$^{-/-}$ mice compared with control unchallenged IL-10$^{-/-}$ mice, although the general trend was one of down-regulation in both CD4 and CD8 T-cell subsets (Figs 3B and 4B). When compared with challenged wild-type mice, there was a

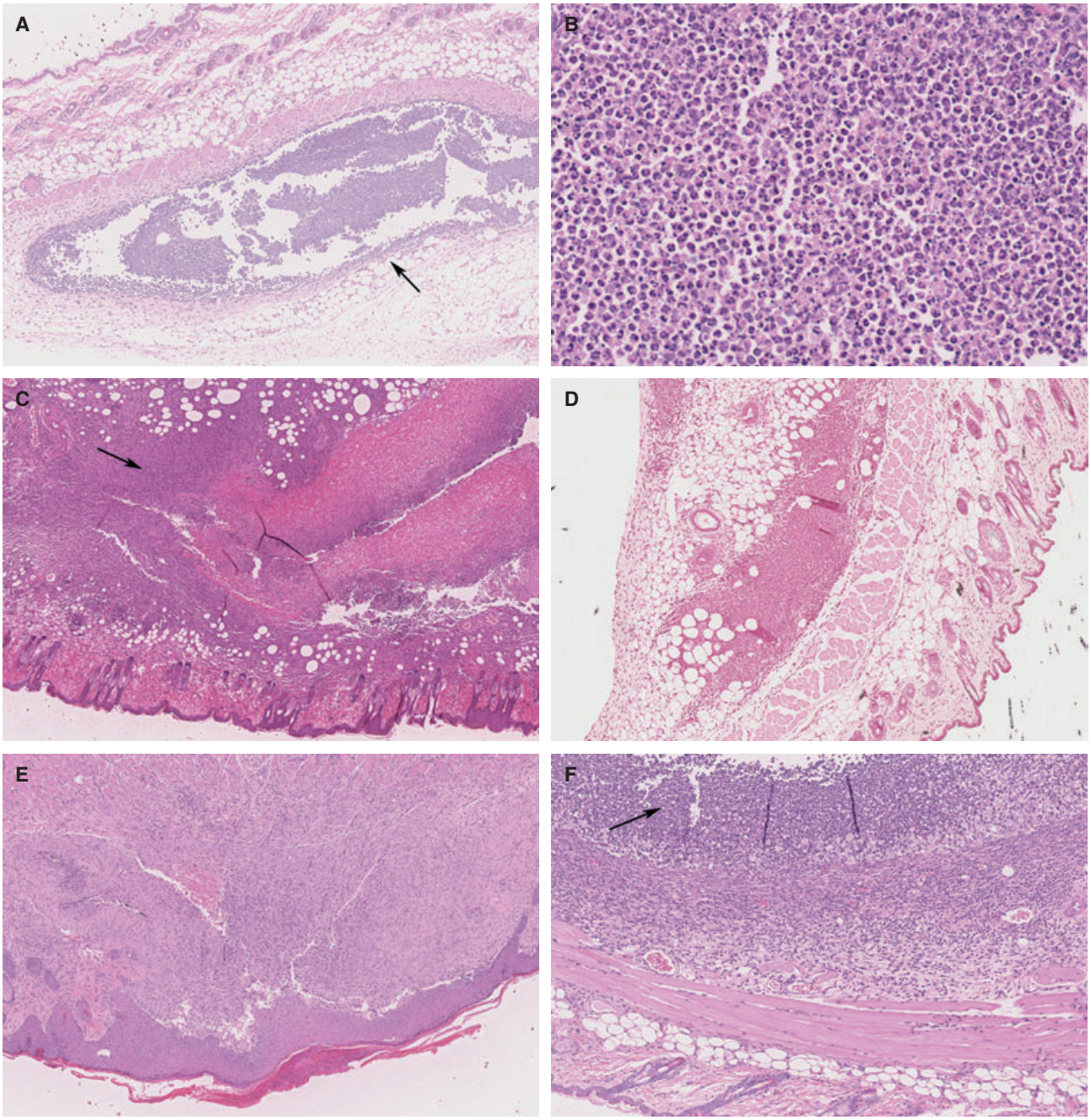


*Fig. 2.* A well-defined subcutaneous abscess (arrow) is seen in a C57BL/6J mouse on day 1 following challenge with *Porphyromonas gingivalis* (A; H&E ×6), composed mainly of PMNs and macrophages (B; H&E ×40). A semi-organized fibrotic matrix (arrow) surrounds the central abscess on day 10 in C57BL/6J mice (C; H&E ×4). The same subcutaneous pattern was seen in IL-12p40$^{-/-}$ mice on day 1 (D; H&E ×8), but by day 10 the lesion was well organized with a distinct lack of an inflammatory infiltrate (E; H&E ×6). The inflammatory infiltrate (arrow) was more diffuse and not well organized in IL-4$^{-/-}$ mice on day 10 (F; H&E ×10).

significant down-regulation of IFN-$\gamma^{+}$ CD8 cells ($P < 0.05$) and IL-10$^{+}$ CD8 cells ($P < 0.01$). No other significant differences were noted between IL-10$^{-/-}$ and wild-type mice, although there was a down-regulation in IL-10 and IFN-$\gamma$ expression in CD4 and CD8 T cells following *P. gingivalis* challenge.

### *IL-12p40$^{-/-}$ mice*

There was a significant up-regulation in the percentage of T cells positive for IL-4 ($P < 0.05$ for CD4 and CD8) and IFN-$\gamma$ ($P < 0.05$ for CD4 and $P < 0.01$ for CD8) in *P. gingivalis*-challenged IL-12p40$^{-/-}$ mice in both T-cell subsets when compared with unchallenged IL-12p40$^{-/-}$ mice (Figs 3C and 4C). When compared with *P. gingivalis*-challenged wild-type mice, challenged IL-12p40$^{-/-}$ mice exhibited significantly greater expression of IL-4 ($P < 0.05$ for CD4 and $P < 0.01$ for CD8), IL-10 ($P < 0.05$ for CD4 and CD8) and IFN-$\gamma$ ($P < 0.01$ for CD4 and $P < 0.05$ for

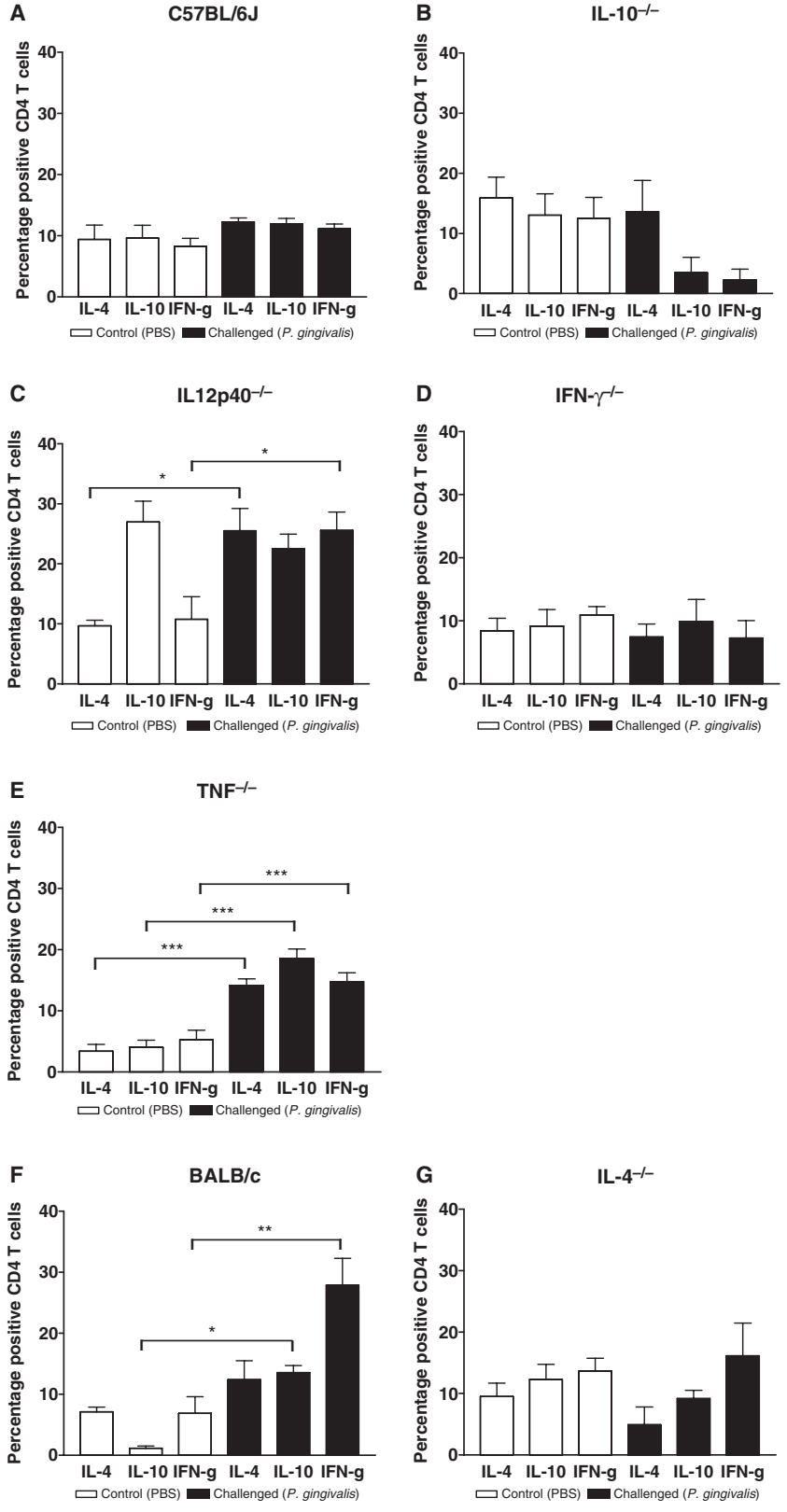


*Fig. 3.* Percentage (mean ± SEM) of IL-4-, IL-10- and IFN-γ-positive splenic CD4 T cells in (A) C57BL/6J, (B) IL-10$^{-/-}$, (C) IL-12p40$^{-/-}$, (D) IFN-γ$^{-/-}$, (E) TNF$^{-/-}$, (F) BALB/c and (G) IL-4$^{-/-}$ mice at day 10 following *Porphyromonas gingivalis* subcutaneous challenge. A minimum of five mice were used per group. Significant differences are shown for comparisons made between *P. gingivalis*-challenged and PBS control mice in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

CD8) in both T-cell subsets. In addition, there was a significant increase of IL-10-positive CD4 cells ($P < 0.01$) in unchallenged IL-12p40$^{-/-}$ mice compared to unchallenged wild-type mice.

### *IFN-γ$^{-/-}$ mice*

There were no significant differences in cytokine expression in challenged IFN-γ$^{-/-}$ mice compared with either unchallenged IFN-γ$^{-/-}$ mice or wild-type C57BL/6J mice (Figs 3D and 4D).

### *TNF$^{-/-}$ mice*

*P. gingivalis*-challenged TNF$^{-/-}$ mice exhibited a significant up-regulation of T cells expressing IL-4 ($P < 0.001$ for CD4 and $P < 0.05$ for CD8), IL-10 ($P < 0.001$ for CD4 and CD8) and IFN-γ ($P < 0.001$ for CD4 and $P < 0.01$ for CD8) in both T-cell subsets when compared with unchallenged TNF$^{-/-}$ mice (Figs 3E and 4E). When compared with wild-type mice, TNF$^{-/-}$ mice showed no significant differences with respect to cytokine expression.

### *BALB/c mice*

There was a significant up-regulation of IL-10$^{+}$ CD4 T cells ($P < 0.05$) and IFN-γ$^{+}$ cells ($P < 0.01$ for CD4 and $P < 0.001$ for CD8 T cells) in BALB/c mice challenged with *P. gingivalis* when compared with unchallenged BALB/c mice (Figs 3F and 4F).

### *IL-4$^{-/-}$ mice*

There were no significant differences in cytokine expression in *P. gingivalis*-challenged IL-4$^{-/-}$ mice when compared with unchallenged IL-4$^{-/-}$ mice (Figs 3G and 4G). However, unchallenged IL-4$^{-/-}$ mice exhibited a significant up-regulation of IL-10$^{+}$ T cells ($P < 0.001$ for CD4 and $P < 0.05$ for CD8) compared with unchallenged BALB/c mice. *P. gingivalis*-challenged IL-4$^{-/-}$ mice exhibited a significant down-regulation of IFN-γ$^{+}$ CD8 T cells ($P < 0.05$) compared with challenged BALB/c mice.

### Serum cytokine profile

IL-12 was detected in relatively large amounts in the serum of IL-10$^{-/-}$ mice 10 days after *P. gingivalis* challenge. Moderate amounts were detected in the C57BL/6J, IFN-γ$^{-/-}$ and IL-4$^{-/-}$ mice but none was detected in the IL-12p40$^{-/-}$, TNF$^{-/-}$ or BALB/c mice (Table 1).

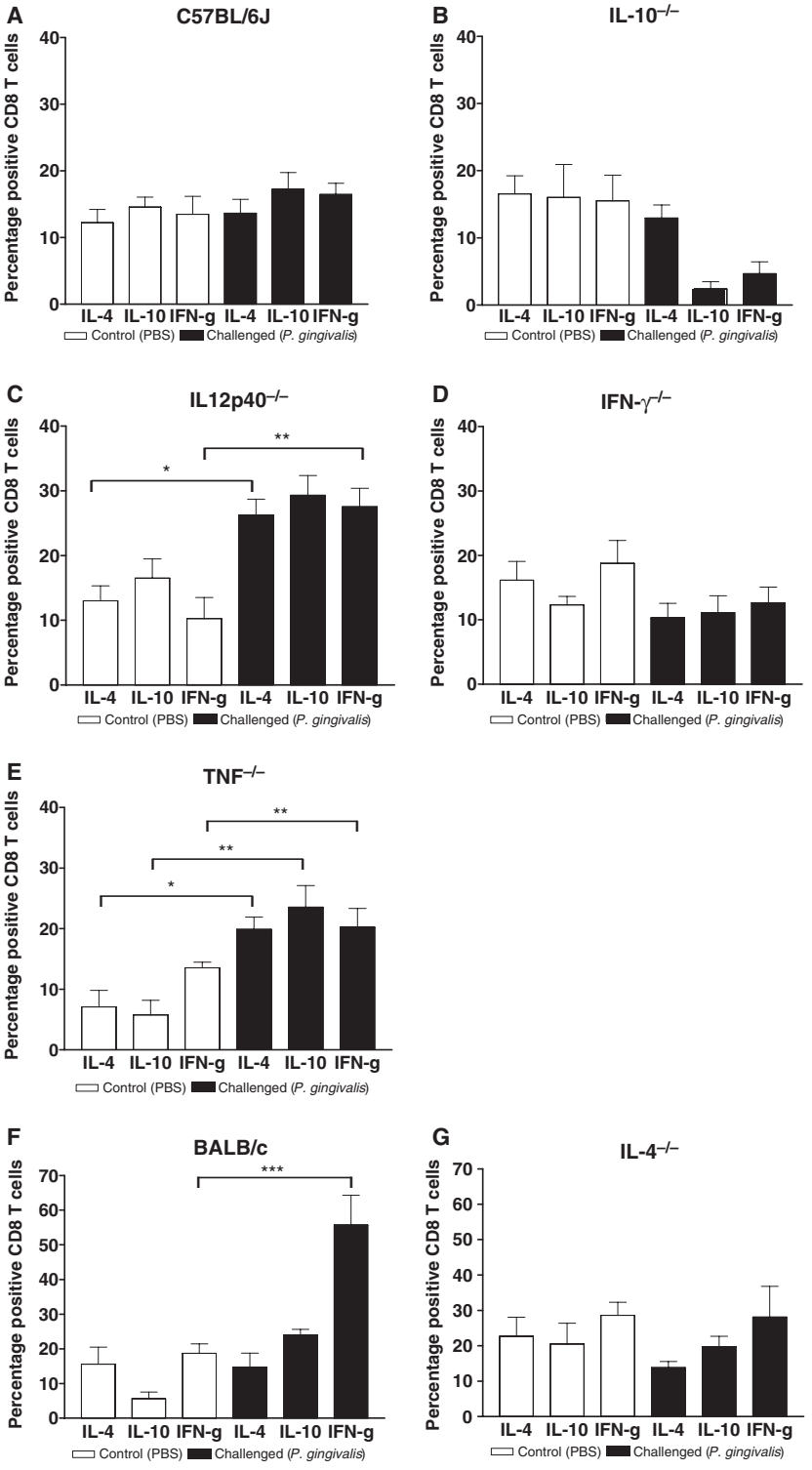


*Fig. 4.* Percentage (mean ± SEM) of IL-4-, IL-10- and IFN-γ-positive splenic CD8 T cells in (A) C57BL/6J, (B) IL-10$^{-/-}$, (C) IL-12p40$^{-/-}$, (D) IFN-$\gamma^{-/-}$, (E) TNF$^{-/-}$, (F) BALB/c and (G) IL-4$^{-/-}$ mice at day 10 following *Porphyromonas gingivalis* subcutaneous challenge. A minimum of five mice were used per group. Significant differences are shown for comparisons made between *P. gingivalis*-challenged and PBS control mice in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

On the other hand, IL-10 was detected in relatively large amounts in BALB/c and TNF$^{-/-}$ mice, with low levels in IFN-$\gamma^{-/-}$ mice. Negligible or no IL-10 was detected in C57BL/6J, IL-10$^{-/-}$, IL-12p40$^{-/-}$ and IL-4$^{-/-}$ mice. TNF was detected in moderate amounts in IL-10$^{-/-}$ mice, while IFN-$\gamma$ was only found in C57BL/6J mice. IL-4 was not detected in any of the groups.

*Table 1.* Serum cytokine profile at day 10 after *Porphyromonas gingivalis* subcutaneous challenge[1]

| | Serum cytokine levels (pg/ml) | | | |
|---|---|---|---|---|
| Mouse strain | IL-12 | IL-10 | IFN-γ | TNF |
| C57BL/6J | 65.9 | 0.0 | 53.07 | 0.0 |
| IL-10$^{-/-}$ | 713.0 | 0.0 | 0.0 | 205.8 |
| IL-12p40$^{-/-}$ | 0.0 | 0.0 | 0.0 | 0.0 |
| IFN-$\gamma^{-/-}$ | 126.0 | 25.33 | 0.0 | 0.0 |
| TNF$^{-/-}$ | 0.0 | 247.3 | 0.0 | 0.0 |
| BALB/c | 0.0 | 468.5 | 0.0 | 0.0 |
| IL-4$^{-/-}$ | 75.0 | 0.0 | 0.0 | 0.0 |

[1]The data represent cytokine levels for pooled serum from five mice/strain.

*Table 2.* Serum anti-*Porphyromonas gingivalis* antibody levels at day 10 after *P. gingivalis* subcutaneous challenge[1]

| | Serum antibody levels (μg/ml) | |
|---|---|---|
| Mouse strain | IgG1 | IgG2a |
| C57BL/6J | 139.6 | 1.7 |
| IL-10$^{-/-}$ | 190.4 | 1.0 |
| IL-12p40$^{-/-}$ | 133.0 | 3.0 |
| IFN-$\gamma^{-/-}$ | 68.0 | 0.6 |
| TNF$^{-/-}$ | 64.2 | 1.2 |
| BALB/c | 86.0 | 1.3 |
| IL-4$^{-/-}$ | 52.0 | 16.2 |

[1]The data represent antibody levels for pooled serum from five mice/strain.

### Serum anti-*P. gingivalis* antibody levels

Anti-*P. gingivalis* IgG1 antibodies were detected in all groups of mice, while low levels of anti-*P. gingivalis* IgG2a antibodies were also found. High levels of IgG1 antibody subclass were demonstrated in C57BL/6J, IL-10$^{-/-}$ and IL-12p40$^{-/-}$ mice, with lower levels in IFN-$\gamma^{-/-}$, TNF$^{-/-}$, BALB/c and IL-4$^{-/-}$ mice (Table 2). Anti-*P. gingivalis* IgM antibodies were not detected in any of the groups.

## Discussion

Protection against a primary *P. gingivalis* challenge in a murine abscess model has been shown to be mediated by innate immune mechanisms (PMN/natural killer cells), whereas T-cell-dependent and/or specific antibodies are critical in subsequent challenges (21). In the absence of particular cytokines critical to the early control of infection, we observed an increased susceptibility to a local subcutaneous *P. gingivalis* infection. This is highlighted by our observation of the

persistent subcutaneous lesions in the IL-12p40$^{-/-}$ mice and of more intense tissue destruction 10 days post-challenge, in the absence of an appreciable inflammatory infiltrate. IL-12 is well known for its critical role in innate resistance and in immunoregulation between the innate and adaptive systems (23, 26). Furthermore, the absence of both the Th1 and Th2 cytokines examined in the serum of infected IL-12p40$^{-/-}$ mice highlights this defective adaptive response, and reinforces the central role of IL-12 in the development of appropriate cell-mediated immunity (35). Humoral responses, however appeared unaffected by the absence of IL-12, with elevated levels of IgG1 anti-*P. gingivalis* antibodies produced in IL-12p40$^{-/-}$ mice similar to that seen in wild-type mice.

Experimental evidence indicates that TNF plays an important role in the resistance to *P. gingivalis* infections (9, 41, 44), most likely via its effects on the innate response (23). TNF deficiency has been shown to impair the innate response to *P. gingivalis* infection by substantially decreasing leucocyte recruitment (2). The complete absence of an initial inflammatory response to subcutaneous *P. gingivalis* challenge in TNF$^{-/-}$ mice in our study supports this observation. Moreover, the lack of any appreciable tissue destruction in the skin model in mice deficient for TNF further highlights its essential role in host-mediated tissue destruction. It is well known that tissue damage caused by *P. gingivalis* is the result of stimulation of the host response as well as of the direct effects of the bacteria (23). Association between elevated titres of TNF and the occurrence or progression of host tissue destruction was recently reported in a study of subcutaneous *P. gingivalis* infection (23). More importantly, when the mice were injected with thalidomide, a TNF-inhibitor, significantly less tissue destruction was evident.

Previous studies have reported that IFN-$\gamma^{-/-}$ mice exhibit a reduced local proinflammatory response, as well as higher baseline levels of the anti-inflammatory cytokine IL-10 (18). The results of our study support these findings whereby IFN-$\gamma^{-/-}$ mice exhibited a reduced initial inflammatory response at the local level that remained unchanged 10 days after bacterial challenge, although low levels of both IL-10 and IL-12 were detected in serum.

The results of the current study suggest that the local innate and adaptive responses in IL-10$^{-/-}$ mice were not impaired. This is highlighted by earlier resolution of skin lesions in these mice relative to the wild-type control mice post-infection. A study into the effects of IL-10 depletion on the immune response to *P. gingivalis* in a similar model, reported significantly improved healing of skin lesions in the IL-10-depleted mice relative to controls (17). This same study also reported that IL-10 depletion led to a Th1-like immune response, similar to that seen in our study with elevated titres of IL-12 and TNF. The serum antibody response in these mice demonstrated a Th2 phenotype with almost all anti-*P. gingivalis* IgG antibodies produced being of the IgG1 subclass.

Although skin lesions in IL-4$^{-/-}$ mice improved in a fashion similar to that in the wild-type BALB/c mice, there were no observed differences in the splenic response. IL-4-deficient mice expressed only low levels of IL-12 in serum, while BALB/c mice showed a high titre of IL-10. IL-4 plays an important role in regulating the differentiation of naive CD4 T cells towards Th2 cells (20), and in its absence, proinflammatory cytokines such as IL-12 and IFN-$\gamma$ may help to mediate the resolution of the *P. gingivalis* subcutaneous lesion.

Except for IL-12p40$^{-/-}$ and TNF$^{-/-}$ mice, the remaining knockout mice were characterized by a weak splenic CD4 and CD8 T-cell cytokine response to *P. gingivalis*. Multiple factors could have contributed to this result, including *P. gingivalis*-mediated T-cell suppression. In fact, CD4 and CD8 T-cell suppression was the predominant finding of a recent study into gene expression in T cells in response to *P. gingivalis* in mice (13). Many of the genes down-regulated were involved in innate and specific immunity. These findings add to the increasing evidence implicating *P. gingivalis* lipopolysaccharide as a down-regulator of host defence (4, 22). *P. gingivalis* lipopolysaccharide has been previously shown to be a poor activator of monocytes (3, 31, 32), a poor binder to lipopolysaccharide-binding protein (8), and it possesses a much lower potency to stimulate cytokine production (1, 24, 32, 34).

The genetic background of the host is another factor that may have contributed to the weak splenic T-cell response observed in this study. The importance of the host response to bacterial plaque is frequently cited as being fundamental in the development of periodontal disease (16, 25, 36). Previous studies have highlighted the influence of the host's genetic background on splenic T-cell and antibody responses to *P. gingivalis* in mice. Specifically, C57BL/6J mice have been shown to be high responders in terms of anti-*P. gingivalis* antibodies, whereas BALB/c mice were shown to be poor responders (15). On the other hand, BALB/c mice demonstrated a strong T-cell response, but a very weak response was demonstrated by the C57BL/6J mice (15). The data from the current study support these observations, because a greater splenic T-cell response was observed in BALB/c compared to C57BL/6J mice.

The specific antibody response to subcutaneous *P. gingivalis* challenge has been shown to require the presence of T cells (5). Anti-*P. gingivalis* antibodies were detected in the serum of all infected groups, indicating that subcutaneous challenge of *P. gingivalis* led to activation of the humoral immune response. This response was clearly polarized towards a Th2 phenotype, as indicated by the elevated levels of IgG1 antibodies (28). Previous studies have shown that lipopolysaccharide from *P. gingivalis* polarizes both murine (33) and human dendritic cell responses towards a Th2 phenotype (19). Moreover, the *P. gingivalis*-induced polarization seen in our study was not altered by the absence of the Th1 or Th2 cytokine-specific genes examined, and reinforces the role of this periodontopathic pathogen in altering the immune response (6, 19, 33). However, the tendency towards a serum Th2 response may have been the result of the action of other Th2-inducing cytokines, such as IL-13. This cytokine is a potent modulator of human monocyte and B-cell function similar to IL-4. It is produced early after activation and over prolonged periods of time, which is in contrast to IL-4, which is secreted much later and occurs transiently (45). Further studies are required to determine whether IL-13 does in fact influence the serum Th2 response to *P. gingivalis* infection.

## Conclusion

The results of our study indicate an important role for innate mechanisms when responding to a primary *P. gingivalis* challenge. We found that IL-12p40$^{-/-}$ mice exhibited more advanced tissue destruction and an impaired immune response after subcutaneous *P. gingivalis* challenge. Deficiency in IL-4 or IL-10 did not appear to contribute to a defective immune response to *P. gingivalis*. Furthermore, TNF deficiency appears to reduce tissue destruction at the local level. While the tendency towards a serum Th2

response is at odds with these results, it is likely that this may be the result of the action of other Th2-inducing cytokines such as IL-13.


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