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SHORT COMMUNICATION

The Effects of Exogenous Interleukin-12 on the Induction of Immune Response to *Porphyromonas gingivalis In Vivo* in Mice

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SUMMARY **The effects of treatment with exogenous interleukin-12 (IL-12) on the induction of immune response to *Porphyromonas gingivalis*, a black pigmented periodontopathic oral bacterium in mice, were determined in the present study. An increased footpad swelling representing a delayed type hypersensitivity (DTH) response to *P. gingivalis* in IL-12-treated mice could be observed, although increasing doses of IL-12 did not produce cumulative effects on this cellular immune response. Multiple injections with IL-12 also resulted in elevated serum IFN-gamma levels. Treatment with this cytokine the day before, on and after immunization with heat-killed *P. gingivalis* augmented the levels of serum antigen-specific IgG2a and IgG3 antibodies, but had obviously little or no effects on those of serum antigen-specific IgG1 and IgG2b antibodies. The results of this study suggest that treatment with exogenous IL-12 in *P. gingivalis*-immunized mice may enhance DTH response and Th1 cell-associated antibody production.**

Porphyromonas gingivalis, a black pigmented oral bacterium, has been implicated in the development of chronic inflammatory periodontal disease (CIPD) because a higher number of this oral periodontopathogen has been detected in the dental plaque of periodontal diseased patients as compared to healthy subjects.[1] *P. gingivalis*-derived antigens, such as lipopolysaccharide (LPS) and outer membrane protein (OMP), not only stimulate the host immune response but also cause both periodontal soft and hard tissue destruction, indicating a crucial role of this periodontopathogen in the course of CIPD. [2-4]

Interleukin-12 (IL-12) is a 70 kDa cytokine, composed of two covalently linked proteins of 40 kD (p40) and 35 kD (p35), that stimulates T and NK cells to produce IFN-gamma; thus, the ability of this cytokine to upregulate IFN-gamma levels leads to preferentially induce development of Th1 cells (ref. 5 for review). The exact role of this cytokine in the course of CIPD remains far from clear. The levels of IL-12 (p35 and p40 subunits) mRNA expression detected from peripheral blood mononuclear cells of healthy and periodontal diseased subjects were similar.[6] However, Gemmel and Seymour[7] have found that after stimulation with OMP of *P. gingivalis*, IL-12-positive gingival B cells and macrophages of healthy or gingivitis subjects were much higher than those of patients with periodontitis, indicating that this cytokine may play a role in the early periodontal lesion. It has been hypothesized, furthermore, that the course of CIPD is regulated by distinct CD4+ cell subsets.[2,3] According to this hypothesis, the early or stable and the advanced or destructive periodontal lesions are mediated by Th1 and Th2 cells, respectively. If this is the case, it would seem plausible that IL-12 may act on the proliferation of periodonto-

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pathogenic bacteria-specific Th1 cells.[2] The aim of this study was, therefore, to determine the effects of exogenous IL-12 on the *P. gingivalis*-induced immune response in mice.

MATERIALS AND METHODS

P. gingivalis ATCC33274, a gift from Prof. Greg Seymour, Brisbane, was grown anaerobically as described elsewhere.[8] After purity was checked, the bacterial suspension was heat-killed by autoclaving at 105°C for 20 minutes[9] and the protein concentration was determined. Female 6 to 8 weeks old Balb/c mice were divided into 4 groups, each consisted of 3-5 mice. Groups I and II were intraperitoneally injected with 100 µl of PBS on day −1, 0 and +1. Using a similar protocol, groups III and IV were injected with 10 ng of recombinant mouse (rMu) IL-12 and 100 ng of rMuIL-12 (R & D System, Minneapolis, MN, USA), respectively.[10] At day 0, groups II-IV were intraperitoneally immunized with 100 µg of heat-killed *P. gingivalis* in 100 µl of PBS and the immunization was repeated one week later. Group I was sham-immunized with 100 µl of PBS only. One week after the last immunization, delayed-type hypersensitivity (DTH) response as judged by footpad swelling was measured.[11] Footpad thickness was measured before and 24 hours after intradermal injection in the right footpad with 1 µg of heat-killed *P. gingivalis*. After sacrificing the mice by asphyxiation, blood was collected by cardiac puncture and serum obtained. The levels of IFN-gamma were determined by an ELISA kit (R & D System) as described by the manufacturer. Serum anti-*P. gingivalis* IgG subclass antibodies were assessed by ELISA as previously described.[8] An one way-analysis of variance was used to analyze the data statistically.

RESULTS AND DISCUSSION

Footpad swelling representing DTH response was determined by measuring the footpad thickness before and 24 hours after intradermal injection of the antigen. Increased footpad swelling of the immunized mice (group II) compared to the sham-immunized mice (group I) was recorded ($p < 0.01$) (Fig. 1). Injection of 10 ng of exogenous rMuIL-12 (group III) resulted in significantly increased footpad swelling, 4 times higher than that of immunized animals (group II) ($p < 0.01$). Of interest, increasing doses of IL-12 did not necessarily augment footpad swelling when comparing groups III and IV ($p > 0.05$). Unlike DTH response *in vivo*, increasing doses of rMuIL-12 in groups III and IV resulted in gradually elevated levels of serum IFN-gamma ($p < 0.01$) (Fig. 1). Whilst no significant *in vivo* effects of rMuIL-12 (groups III and IV) on the levels of serum anti-*P. gingivalis* IgG1 and IgG2b antibodies were detected as compared to those of group II ($p > 0.05$), serum antigen-specific IgG2a and IgG3 antibody levels in the former groups were elevated ($p < 0.05$) (Fig. 2).

IL-12 is produced primarily by dedicated antigen-presenting cells, such as macrophages and Langerhans's cells and its main target cells are T and NK cells.[5] An interaction of IL-12 and its specific receptors on both T and NK cells leads ultimately to the production of IFN-gamma which in turn creates a microenvironment favorable to the development of Th1 cells. Previous reports have conclusively demonstrated that multiple injections of IL-12 in mice infected with intracellular bacteria such as *Leishmania major*[12] and *Mycobacterium tuberculosis*[13] induced elevated levels of IFN-gamma and inhibited

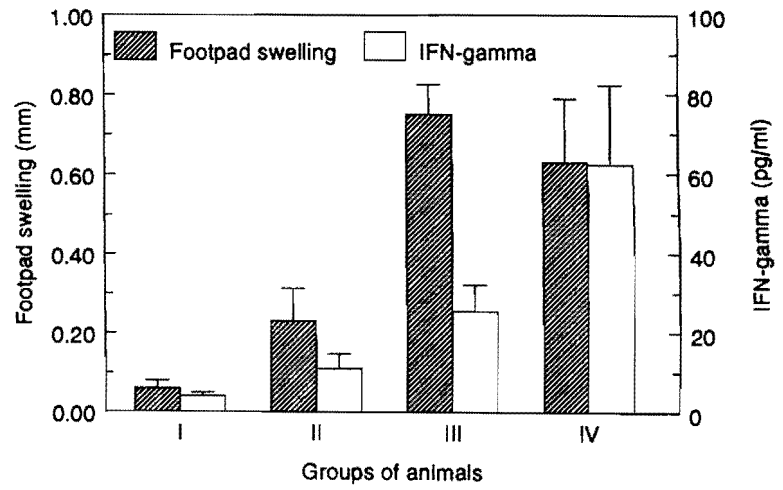

Fig. 1 Mean and standard deviation of footpad swelling and serum IFN-gamma levels in mice treated with or without IL-12 and *P. gingivalis*. Groups I and II were sham- and *P. gingivalis*-immunized mice, respectively. Groups III and IV were treated with 10 ng and 100 ng of IL-12, respectively, and immunized with *P. gingivalis*.

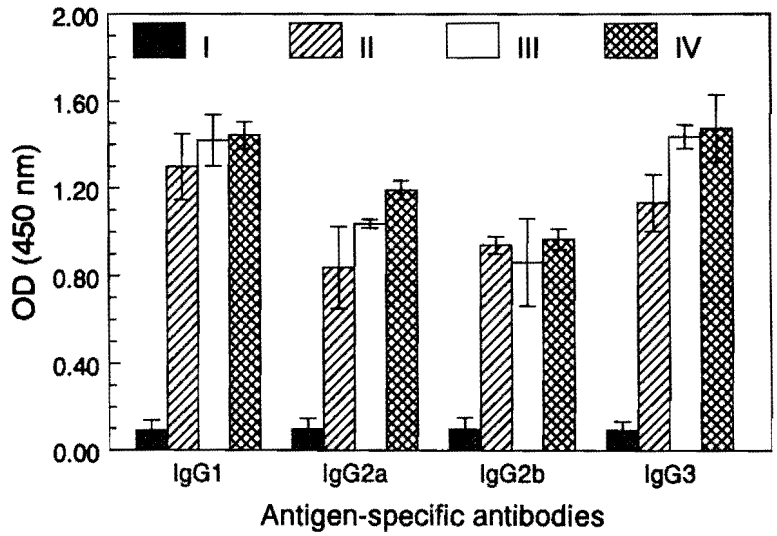

Fig. 2 Mean and standard deviations of serum IgG subclass antibodies specific to *P. gingivalis*. The groups of animals are the same as in Fig. 1.

the development of severe infection to these microorganisms. Since DTH response is mediated by IFN-gamma-producing Th1 cells, injections of IL-12 in mice seem to be an obvious way to up-regulate this cell-mediated immune response.[14] Thus, the results of the present study showing an increased DTH response to *P. gingivalis* in mice injected with exogenous IL-12 are not surprising. This elevated DTH response in IL-12-treated mice may most likely be due to the actions of augmented IFN-gamma levels. If so, via its ability to enhance IFN-gamma production, IL-12 may play a crucial role in the induction of DTH response to the periodontopathogen, *i.e. P. gingivalis*. An indirect support for this thesis may be drawn from the fact that increased DTH response to *P. gingivalis* and serum IFN-gamma could be detected in IL-10-depleted mice (Herminajeng E *et al.*, submitted). Since IL-10 acts as an inhibitory cytokine for IL-12 production,[5] it would seem plausible that IL-10 depletion *in vivo* may also elevate the levels of IL-12, thereby inducing increased DTH response and serum IFN-gamma in *P. gingivalis*-immunized mice.

Increased serum anti-*P. gingivalis* IgG2a and IgG3 antibodies in mice injected with antigen and rIL-12, particularly at doses up to 10 ng were observed in the present study but little effects on IgG1 and IgG2b, suggesting rIL-12 cytokine may play an important role in the elevation of anti-*P. gingivalis* IgG2a and IgG3 antibodies as also previously described.[15] Of interest, the levels of serum anti-*P. gingivalis* IgG1 and IgG2b antibodies as seen in the present study were relatively unaffected by IL-12-treatment. These results are in accordance with a previous report showing that intraperitoneal injections of exogenous IL-12 had little effects on IgG1 antibody levels.[15] On the other hand, Bliss and colleagues[16] have demonstrated that suppression of these Th2 cell-associated antibody isotypes occurred in IL-12-treated mice. The precise reason(s) to explain this discrepancy is still unknown but it may be associated with different routes of IL-12 injections. That suppressed IgG1 antibodies in IL-12-treated mice was due to subcutaneous injections of IL-12 as seen in the works of Bliss and colleagues[16] may support this speculation. Alternatively, continuous exposure to IL-12 may have different effects on IgG isotype antibody production. A study carried out by Metzger and colleagues[10] have shown that IL-12 initially enhances IFN-gamma and IgG2a antibody production and at the later stage, stimulates post-switched cells, such as IgG1 antibody-producing B cells, in an IFN-gamma-independent fashion. Thus, in the presence of exogenous IL-12, antigen-specific IgG1 antibody levels may be unaltered.

The results of the present study seem to indicate that treatment with exogenous IL-12 may preferentially provoke the development of a Th1-like response to *P. gingivalis* in mice. However, whether this treatment would eventually lead to induce a protective immune response to *P. gingivalis* in this murine model remains to be further investigated. Our recent findings have demonstrated that in IL-10-depleted mice, an increased Th1-like response following immunization with OMP of *P. gingivalis* was paralleled with rapid healing of live *P. gingivalis*-induced abcess formation (Herminajeng E *et al.*, submitted), suggesting that the induction of *P. gingivalis*-specific Th1-like response may be protective. Further support can also be drawn from the fact that the levels of IL-12 in gingivae of healthy or patients with gingivitis were much

higher than those in patients with periodontitis,[7] suggesting that Th1 cell development-associated IL-12 may maintain healthy gingival tissues or a minimal gingival inflammation under continuous periodontopathogenic bacterial challenge.

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