

324 **ACKNOWLEDGMENTS**

325 This work was supported by Grants-in-Aid for Scientific Research 22390354 and 21659436
326 (to K.M.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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P. gingivalis interacts with E-selectin

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481 **FIGURE LEGENDS**

482 **Figure 1. Adherence of *P. gingivalis* to HUVECs was enhanced by stimulation with**
483 **TNF- α .** (A) HUVECs were incubated with TNF- α (10 ng/ml) for 0.5-3 h. Then *P.*
484 *gingivalis* ATCC 33277 cells (10^8 cells/ml/well) were added to the culture medium for 0.5-3
485 h. Cells were then washed and attachment of *P. gingivalis* to the cells was observed by
486 fluorescent microscopy. *P. gingivalis* was stained with FITC (green), and actin of endothelial
487 cells was visualized with TRITC (red). Scale bar is 10 μ m. (B) HUVECs were incubated
488 with TNF- α (10 ng/ml) for 0.5-3 h. Then *P. gingivalis* ATCC 33277 cells (10^8 cells/ml/well)
489 were added to the culture medium for 0.5-3 h. Cells were then washed and attachment of *P.*
490 *gingivalis* to the cells was observed by fluorescent microscopy. The attachment levels were
491 expressed as number of *P. gingivalis* cells per 60430 mm² (n = 3, means \pm SD; **P* < 0.01 vs
492 no TNF- α).

493

494 **Figure 2. Adherence of *P. gingivalis* to TNF- α -activated endothelial cells was mediated**
495 **by E-selectin.** (A) Inhibitory effect of anti-E-selectin antibodies. HUVECs were
496 incubated with TNF- α (10 ng/ml) for 3 h. Cells were then washed and incubated with *P.*
497 *gingivalis* ATCC 33277 (10^8 cells/ml/well) for 30 min in the presence of an antibody for
498 E-selectin or control IgG. Other procedures are described in the legend to Fig. 1B. (n = 3,
499 means \pm SD; **P* < 0.01 vs no TNF- α , †*P* < 0.01 vs. no Anti-E-selectin Abs). (B) Inhibitory
500 effect of sialyl Lewis X. HUVECs were incubated with TNF- α (10 ng/ml) for 30 min.
501 Cells were then washed and incubated with *P. gingivalis* ATCC 33277 (10^8 cells/ml/well) for
502 3 h in the presence of purified sialyl Lewis X (0-10 ng/ml). Other procedures are described in
503 the legend to Fig. 1B. (n = 3, means \pm SD; **P* < 0.01 vs. no TNF- α , †*P* < 0.01 vs. no sialyl
504 Lewis X). (C) Adherence of *P. gingivalis* was augmented in HEK293 cells transfected with
505 an expression with vector for E-selectin. *P. gingivalis* ATCC 33277 (10^8 cells/ml/well) was

506 incubated with 293 cells transfected with a human E-selectin-inserted vector for 3 h. Other
507 procedures are described in the legend to Fig. 1A. Scale bar is 10 μ m. (D) Adherence of *P.*
508 *gingivalis* was augmented in 293 cells transfected with an expression vector for E-selectin. *P.*
509 *gingivalis* ATCC 33277 (10^8 cells/ml/well) was incubated with 293 cells transfected with a
510 human E-selectin-inserted vector for 30 min. Other procedures are described in the legend
511 to Fig. 1B. (n = 3, means \pm SD; **P* < 0.01 vs. control).

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513 **Figure 3. Pgm6/7 in *P. gingivalis* mediated the interaction with activated endothelial**
514 **cells.** (A) *P. gingivalis* ATCC 33277 (wild type), FimA-deficient mutant (Δ FimA), and –
515 Pgm6/7-deficient mutant (Δ Pgm6/7) (10^8 cells/ml/well) were incubated with
516 TNF- α -pretreated HUVECs for 3 h, respectively. Other procedures are described in the
517 legend to Fig. 1A. Scale bar is 10 μ m. (B) *P. gingivalis* ATCC 33277 (wild type) and
518 FimA-deficient mutant (Δ FimA) (10^8 cells/ml/well) were incubated with TNF- α -pretreated
519 HUVECs for 30 min, respectively. Other procedures are described in the legend to Fig. 1A.
520 Scale bar is 10 μ m. (C) *P. gingivalis* ATCC 33277 (wild type) and Pgm6/7-deficient mutant
521 (Pgm6/7) (10^8 cells/ml/well) were incubated with TNF- α -pretreated HUVECs for 30 min,
522 respectively. Other procedures are described in the legend to Fig. 1B. (n = 3, means \pm SD;
523 **P* < 0.01 vs. no TNF- α). (D) Inhibitory effects of *P. gingivalis* envelopes on
524 TNF- α -induced adhesion of *P. gingivalis* to HUVECs. HUVECs were incubated with
525 TNF- α (10 ng/ml) for 30 min. Cells were then washed and incubated with *P. gingivalis* ATCC
526 33277 (10^8 cells/ml/well) for 30 min in the presence or absence of envelopes isolated from
527 wild-type or mutant *P. gingivalis*. Other procedures are described in the legend to Fig. 1B.
528 (n = 3, means \pm SD; **P* < 0.01 vs. no TNF- α , †*P* < 0.01 vs. control). (E) Effects of extracted
529 ~~OmpA-like protein~~ Pgm6/7 and FimA on TNF- α -induced adhesion of *P. gingivalis* to
530 HUVECs. HUVECs were incubated with TNF- α (10 ng/ml) for 30 min. Cells were then

531 washed and incubated with *P. gingivalis* ATCC 33277 (10^8 cells/ml/well) for 30 min in the
532 presence or absence of purified Pgm6/7 and FimA. Other procedures are described in the
533 legend to Fig. 1B. ($n = 3$, means \pm SD; $*P < 0.01$ vs. no TNF- α , $\dagger P < 0.01$ vs. Pgm6/7
534 fraction). (F) Inhibitory effect of *P. gingivalis* Pgm6/7 on TNF- α (10 ng/ml)-induced
535 adhesion of *P. gingivalis* to HUVECs. HUVECs were incubated with TNF- α (10 ng/ml) for
536 30 min. Cells were then washed and incubated with *P. gingivalis* ATCC 33277 (10^8 cells/
537 ml/well) for 30 min in the presence or absence of purified Pgm6/7. Other procedures are
538 described in the legend to Fig. 1B. ($n = 3$, means \pm SD; $*P < 0.01$ vs. no TNF- α , $\dagger P < 0.01$
539 vs. Pgm6/7 0 ng/ml). (G) Inhibitory effect of *P. gingivalis* Pgm6/7 on TNF- α -induced
540 adhesion of *P. gingivalis* to HUVECs. HUVECs were incubated with TNF- α (10 ng/ml) for
541 30 min. Cells were then washed and incubated with *P. gingivalis* ATCC 33277 (10^8
542 cells/ml/well) for 30 min in the presence or absence of purified Pgm6/7. Other procedures
543 are described in the legend to Fig. 1A. Scale bar is 10 μ m.

544

545 **Figure 4. Endothelial vWF exocytosis to *P. gingivalis* were augmented by pretreatment**
546 **with TNF- α .** HUVECs were incubated with TNF- α (10 ng/ml) for 3 h. Cells were then
547 washed and incubated with *P. gingivalis* ATCC 33277 (10^8 cells/ml/well) for 0-1 h. Then
548 the release of vWF into media was measured by ELISA. ($n = 3$, means \pm SD)

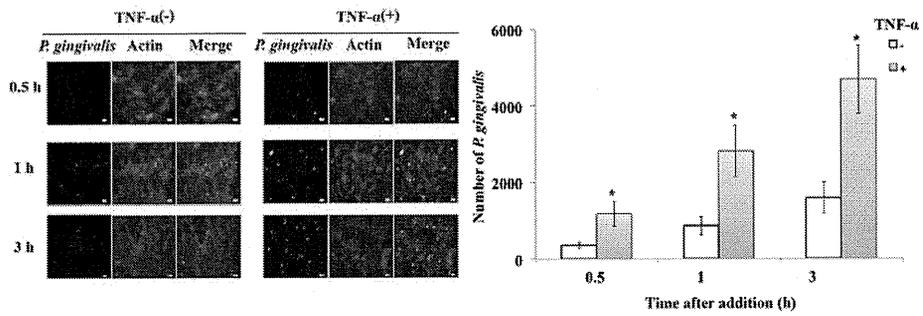
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550 **Figure 5. *P. gingivalis*-induced nitric oxide release from activated endothelial cells was**
551 **mediated by E-selectin.** HUVECs were incubated with TNF- α (10 ng/ml) for 3 h. Cells
552 were then washed and incubated with *P. gingivalis* ATCC 33277 (10^8 cells ml⁻¹/well) for 30
553 min in the presence or absence of an antibody for E-selectin. Then the release of nitric oxide
554 into media was measured by DAN assay ($n = 3$, means \pm SD; $*P < 0.01$ vs no TNF- α).
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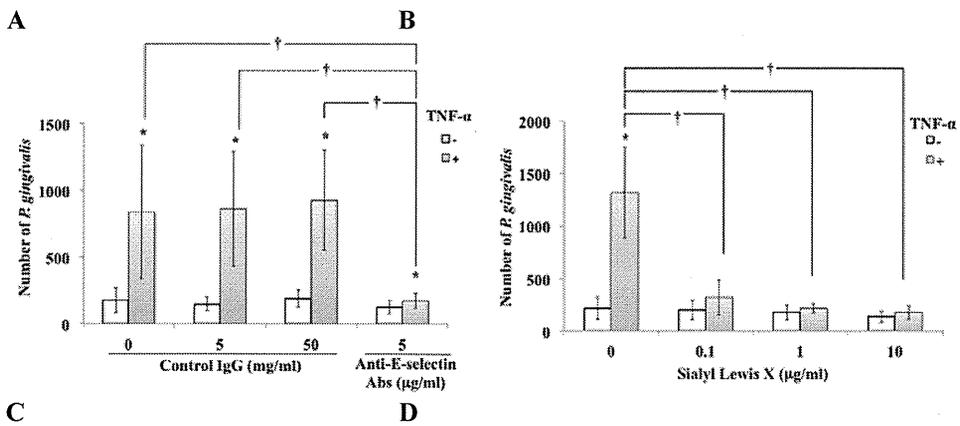
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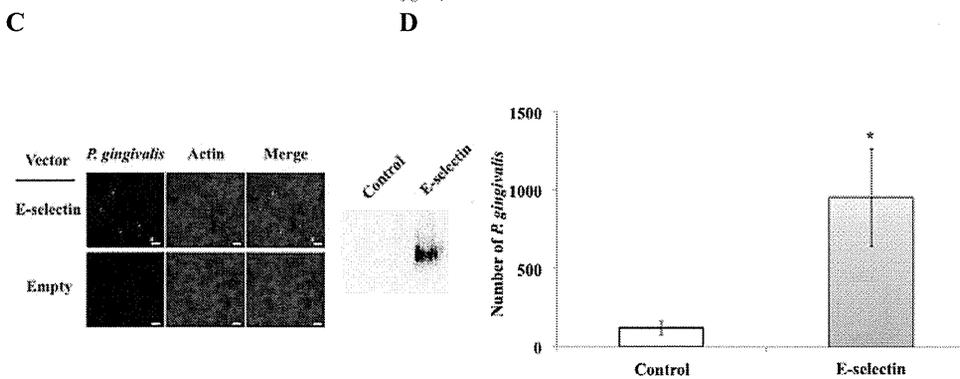
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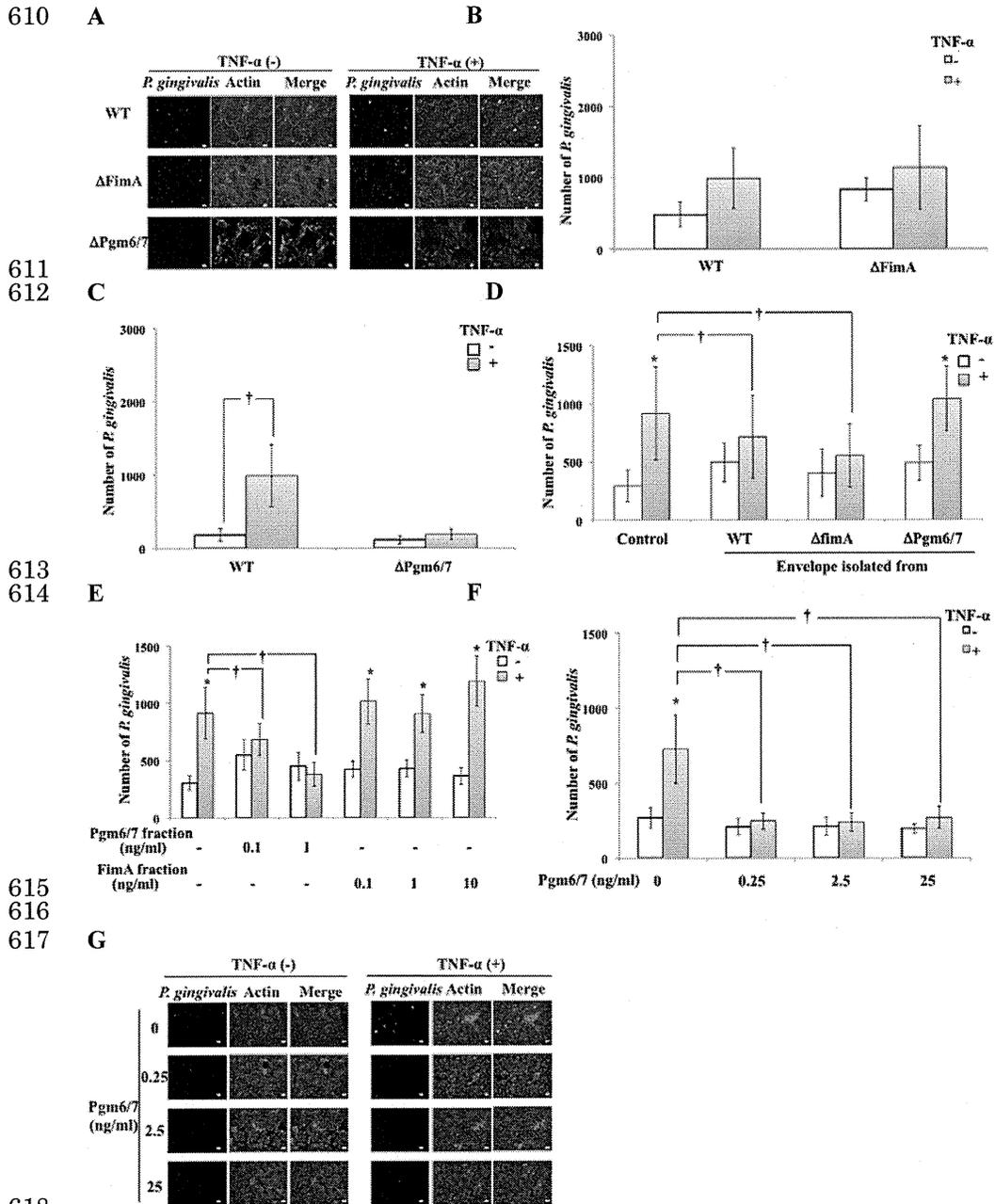


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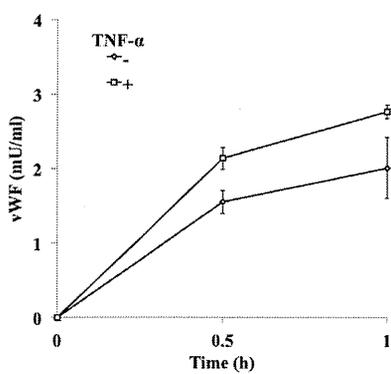
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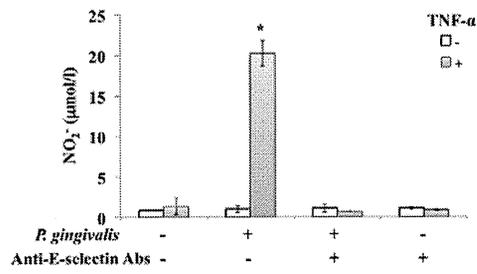
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Komatsu *et al.* Fig. 4



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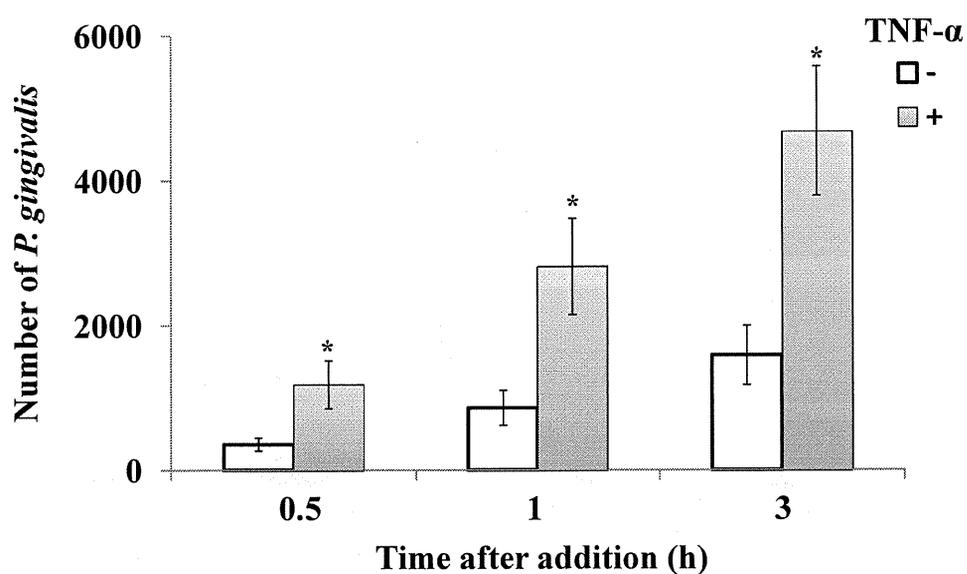


Figure 1. Adherence of *P. gingivalis* to HUVECs was enhanced by stimulation with TNF- α . (B) HUVECs were incubated with TNF- α (10 ng/ml) for 0.5-3 h. Then *P. gingivalis* ATCC 33277 cells (10^8 cells/ml/well) were added to the culture medium for 0.5-3 h. Cells were then washed and attachment of *P. gingivalis* to the cells was observed by fluorescent microscopy. The attachment levels were expressed as number of *P. gingivalis* cells per 60430 mm^2 ($n = 3$, means \pm SD; * $P < 0.01$ vs no TNF- α).

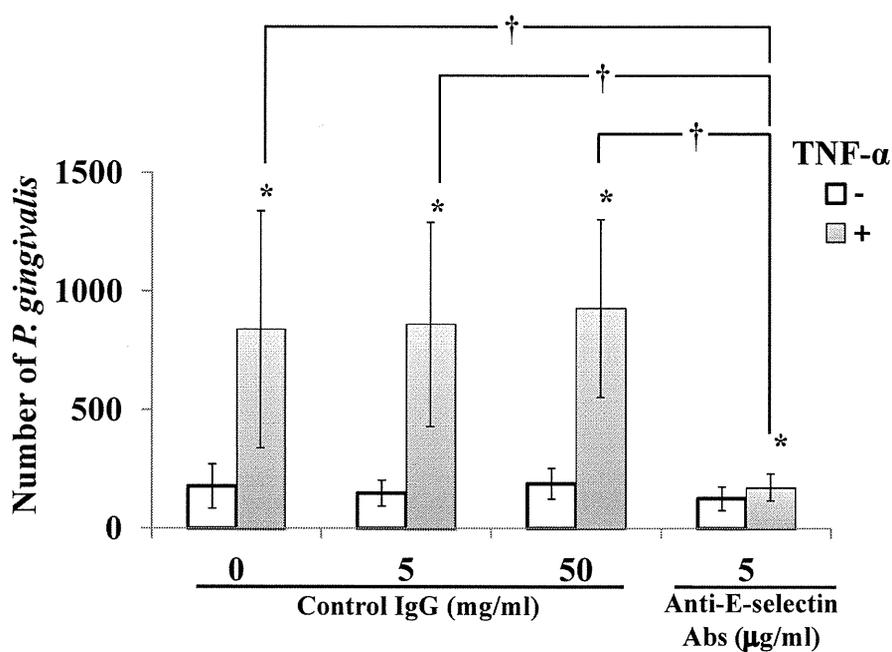


Figure 2. Adherence of *P. gingivalis* to TNF- α -activated endothelial cells was mediated by E-selectin. (A) Inhibitory effect of anti-E-selectin antibodies. HUVECs were incubated with TNF- α (10 ng/ml) for 3 h. Cells were then washed and incubated with *P. gingivalis* ATCC 33277 (10^8 cells/ml/well) for 30 min in the presence of an antibody for E-selectin or control IgG. Other procedures are described in the legend to Fig. 1B. (n = 3, means \pm SD; * P < 0.01 vs no TNF- α , † P < 0.01 vs. no Anti-E-selectin Abs).

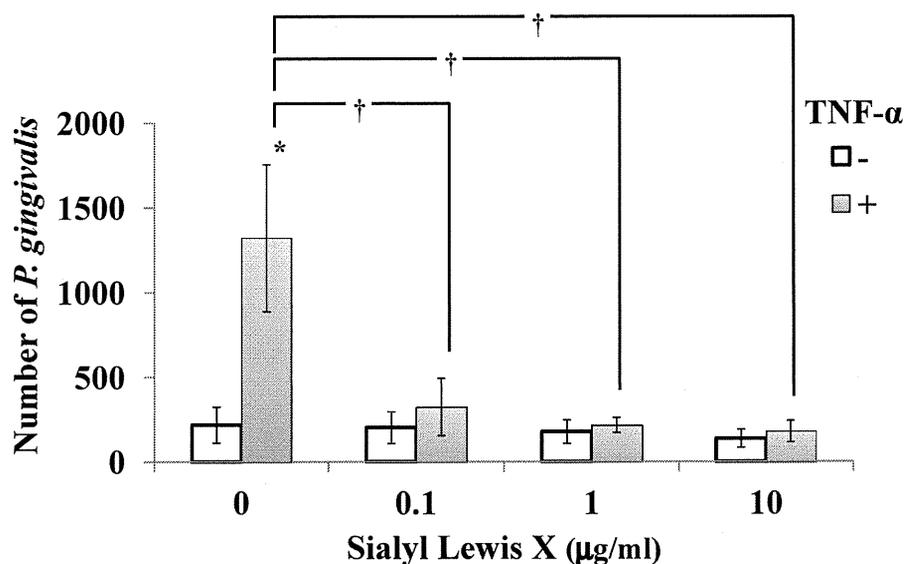


Figure 2. Adherence of *P. gingivalis* to TNF- α -activated endothelial cells was mediated by E-selectin. (B) Inhibitory effect of sialyl Lewis X. HUVECs were incubated with TNF- α (10 ng/ml) for 30 min. Cells were then washed and incubated with *P. gingivalis* ATCC 33277 (10^8 cells/ml/well) for 3 h in the presence of purified sialyl Lewis X (0-10 ng/ml). Other procedures are described in the legend to Fig. 1B. (n = 3, means \pm SD; * P < 0.01 vs. no TNF- α , † P < 0.01 vs. no sialyl Lewis X).