Antibody neutralization of microbiota-derived circulating peptidoglycan dampens inflammation and ameliorates autoimmunit

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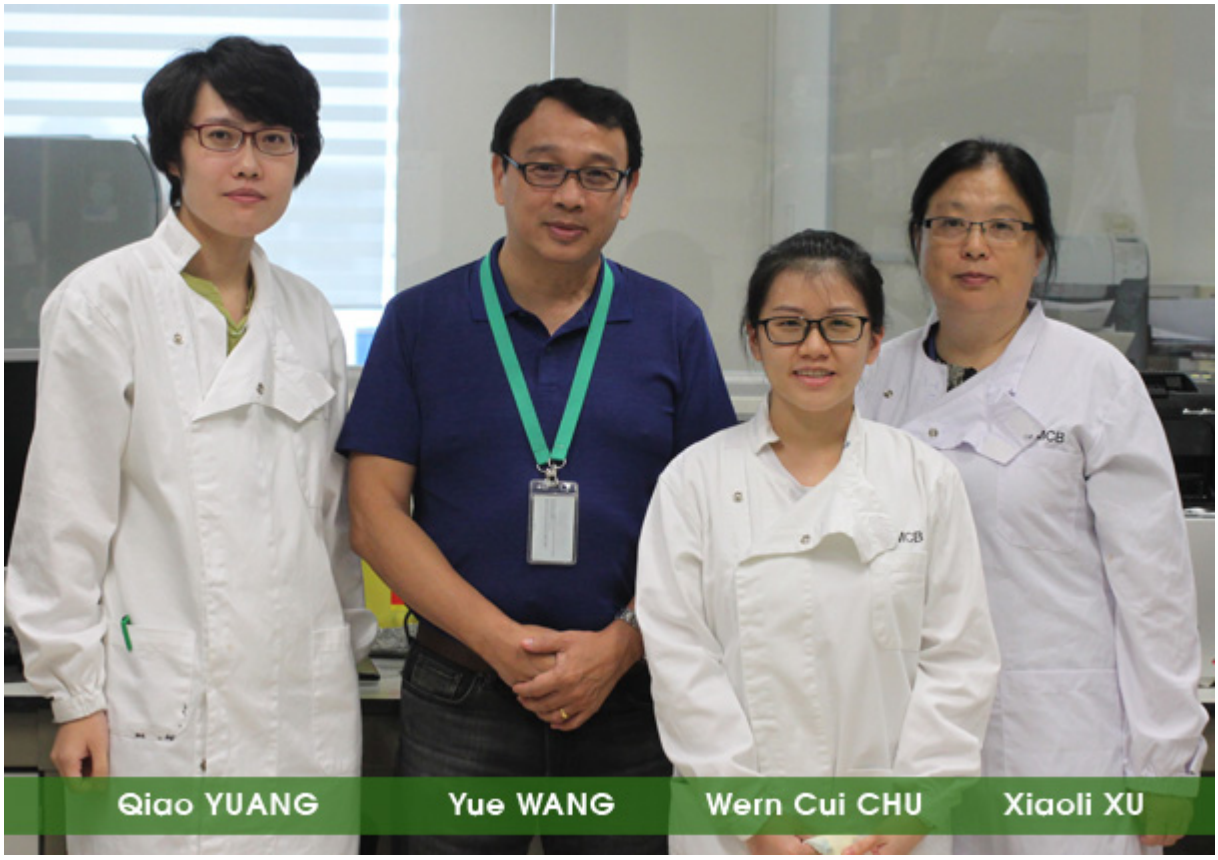

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Abstract

The human microbiota provides tonic signals that calibrate the host immune response, but their identity is unknown. Bacterial peptidoglycan (PGN) subunits are likely candidates, as they are well-known immunity-enhancing adjuvants, released by most bacteria during growth, and have been found in the blood of healthy people. We developed a monoclonal antibody, 2E7, that targets muramyl-L-alanyl-D-isoglutamine (MDP), a conserved and minimal immunostimulatory structure of PGN. Using 2E7-based assays, we detected PGN ubiquitously in human blood at a broad range of concentrations that is relatively stable in each individual. We also detected PGN in the serum of several warm-blooded animals. However, PGN is barely detectable in the serum of germ-free mice, indicating that its origin is the host microbiota. Neutralization of circulating PGN via intraperitoneal administration of 2E7 suppressed the development of autoimmune arthritis and experimental autoimmune encephalomyelitis in mice. Arthritic NOD2$^{-/-}$ mice lacking the MDP sensor did not respond to 2E7, indicating that 2E7 dampens inflammation by blocking Nod2-mediated pathways. We propose that circulating PGN acts as a natural immune potentiator that tunes the host immune response and altering its level is a promising therapeutic strategy for immune-mediated diseases.

Figure

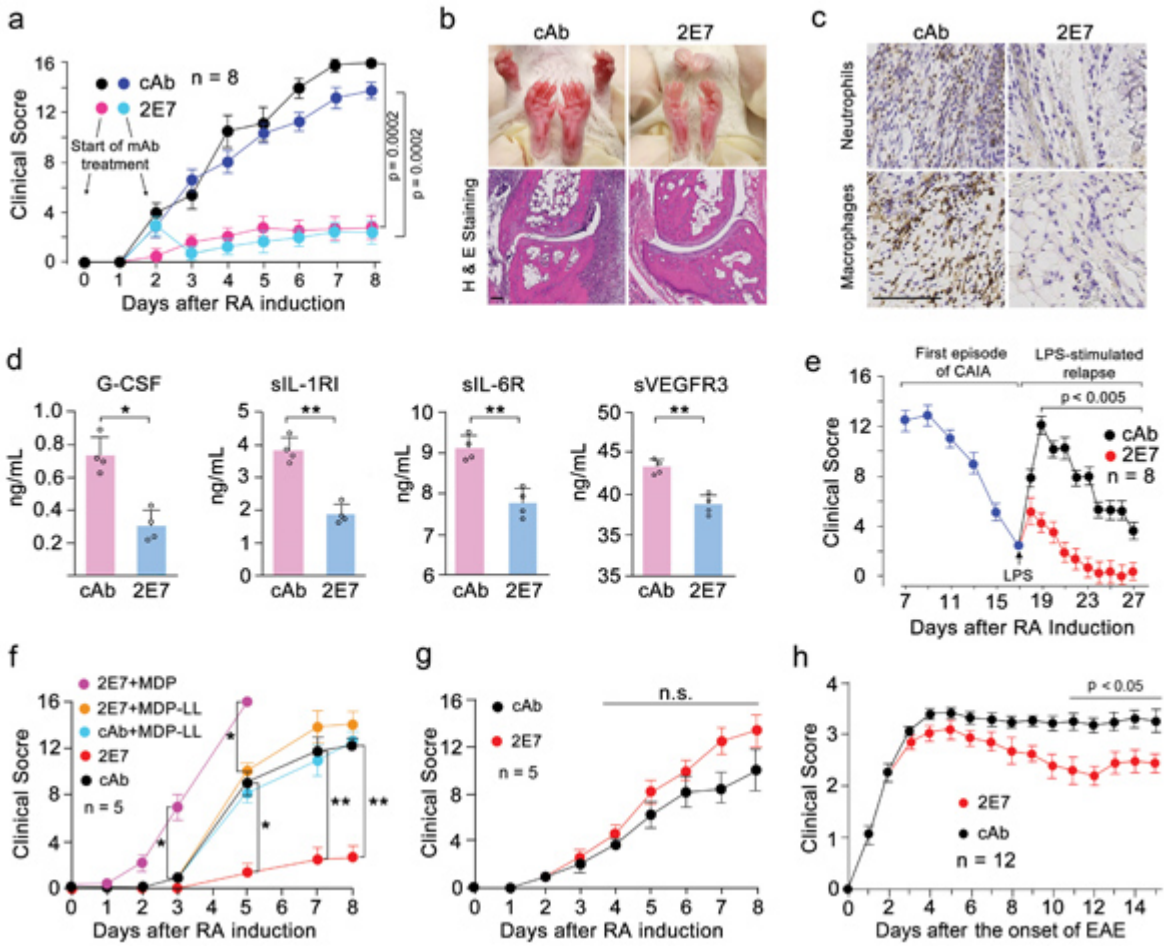

Figure Legend:

Therapeutic effect of 2E7 on CAIA. (**a**) Each mouse was injected intraperitoneally with one dose (160 mg/kg) of cAb or 2E7 6 h before or 48 h after CAIA induction; n = 8. P-values for clinical scores on day 8 were calculated by a two-sided Mann-Whitney's non-parametric test. (**b**) On days 8-10, paws of the mice were harvested and joint sections were stained with H&E (scale bar = 500 μm) and (**c**) with NIMP-R14 or anti-F4/80 antibody to stain neutrophils and macrophages. Scale bar = 200 μm. The experiment was repeated at least three times independently with similar results. (**d**) Serum samples from CAIA mice (n = 4) on day 7-8 of treatment were analyzed by Luminex assay. P-values were determined by a two-sided Mann-Whitney test. *P < 0.05 and **P < 0.01. (**e**) Mice were allowed to recover naturally and on day 17 were injected with 25 μg of LPS to re-stimulate the disease. The mice were then divided into two groups, one injected with one dose (160 mg/kg/mouse) of 2E7 and the other cAb. P-values were calculated by a two-sided Mann Whitney's non-parametric test. (**f**) Mice were injected with one dose of 160 mg/kg 2E7 + 1 mg/kg MDP (2E7+MDP), 160 mg/kg 2E7 + 2 mg/kg MDP-LL, 160 mg/kg cAb + 2 mg/kg MDP-LL, 160 mg/kg 2E7 or 160 mg/kg cAb 6 h before CAIA induction. P-values were calculated using two-sided Mann Whitney's non-

parametric test. *p < 0.05; **p < 0.01. (**g**) CAIA was induced in NOD2$^{-/-}$ mice. Each mouse received one injection (160 mg/kg) of cAb or 2E7 6 h before disease induction. Significance was analyzed by a two-sided Mann Whitney's non-parametric test. n.s., not significant with P > 0.05. No difference was observed in the levels of circulating PGN between NOD2$^{-/-}$ and wild-type mice (Supplementary Figure 8). (**h**) EAE was induced as described in Methods. Each mouse was injected intraperitoneally with one dose (40 mg/kg) of 2E7 or cAb on day 1, 4 and 7 of the clinical disease. Disease severity was scored as described Supplementary Table 2, and P-values for end scores were calculated by a two-sided Wilcoxon's non-parametric test. Error bars: mean ± SEM.