COLL2-1 peptide and its nitrated form initiate **pro-inflammatory pathways** of innate immunity

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PURPOSE. Osteoarthritis (OA) is characterized by degradation of the extracellular matrix associated with inadequate repair responses including pro-inflammatory pathways of nonspecific natural immune response. In this context, we evaluated the effect of type II collagen peptide (**Coll2-1**) and its nitrated form (**Coll2-1NO$_2$**) on oxidative stress and on the production of mediators of inflammation and angiogenesis by OA synoviocytes .

METHODS. Human synoviocytes from patients with knee OA (n=10) were treated for 24 hours in the presence or absence of Coll2-1 ([108]HRGYPGLDG[116]; 0.45 and 4.5 nmol) or Coll2-1NO$_2$ ([108]HRGY(NO$_2$)PGLDG[116]; 4 and 40 pmol) peptides. For competitive inhibitions, the peptides were pre-incubated overnight at 4°C on shaker in the presence of AS0619 and D37, 2 specific antisera respectively of Coll2-1 and Coll2-1NO$_2$. For the inhibition of TLR-4 receptor, synoviocytes are pre-treated 1 hour with CLI-095 (500 nM, 1 and 2.5 µM) before a 24 hours treatment with Coll2-1 at 4.5 nmol. Intracellular production of H_2O_2, GSH and NO was measured using fluorescent probes. The expression of Interleukins (IL)-6, -8, Vascular Endothelium Growth Factor (VEGF) and Thrombopondin-1 (TSP-1) was evaluated by quantitative real-time PCR. The phosphorylation of the IκB-α and p65, two key proteins in the NF-κB pathway were evaluated by Western blot in the presence or absence of oxidative stress inhibitors (apocynin; 0.2 mM and diphenyleneiodonium; 6.35 10^{-2} mM).

RESULTS

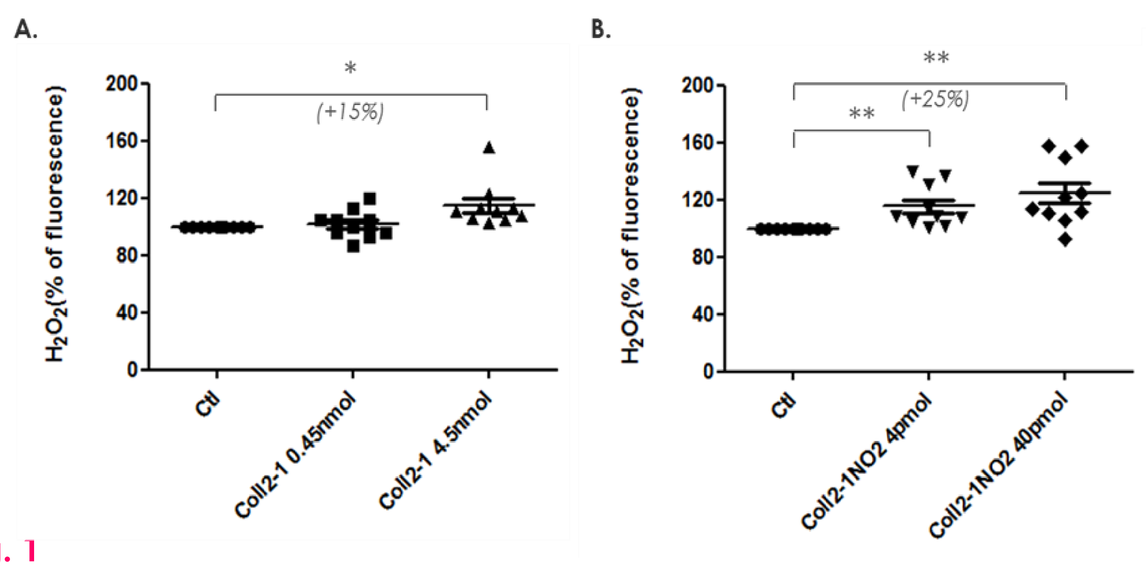

Fig. 1
Intracellular production of H_2O_2 by osteoarthritic synoviocytes in the presence of Coll2-1 (A) and Coll2-1NO$_2$ (B). N=10 patients, * *P*<0.05 and ** *P*<0.01

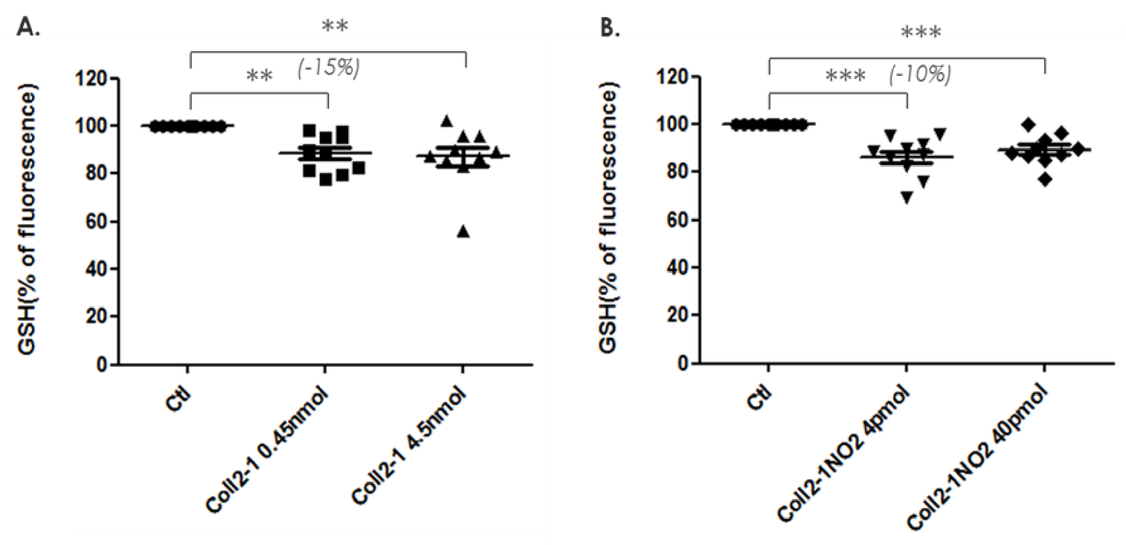

Fig. 2
Intracellular production of GSH by osteoarthritic synoviocytes in the presence of Coll2-1 (A) and Coll2-1NO$_2$ (B). N=10 patients, ** *P*<0.01 and *** *P*<0.001

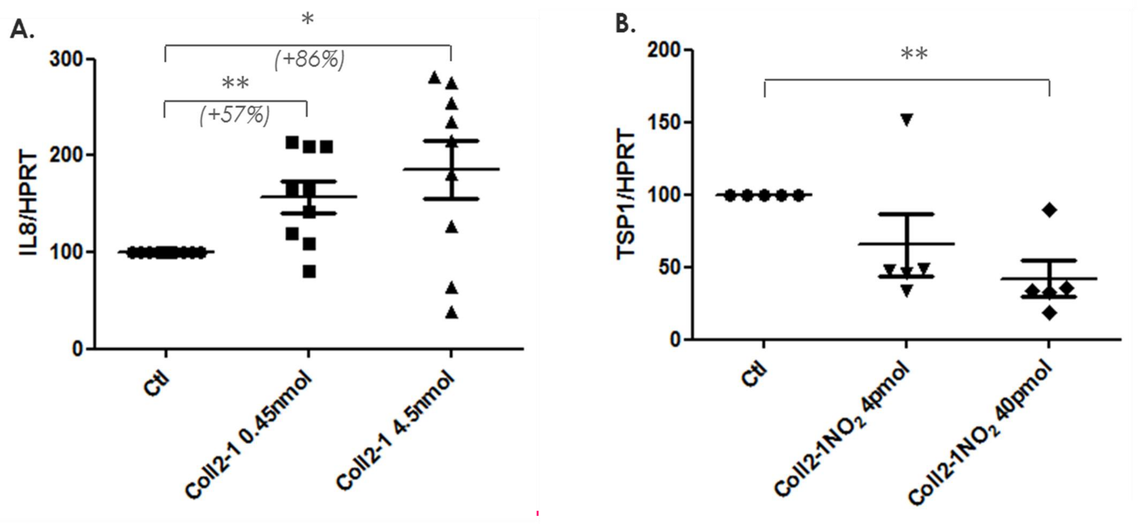

Fig. 3
IL-8 (A) and TSP1 (B) expression by osteoarthritic synoviocytes in the presence of Coll2-1 and Coll2-1NO$_2$. N= 5-10 patients, * *P*<0.05 and ** *P*<0.01

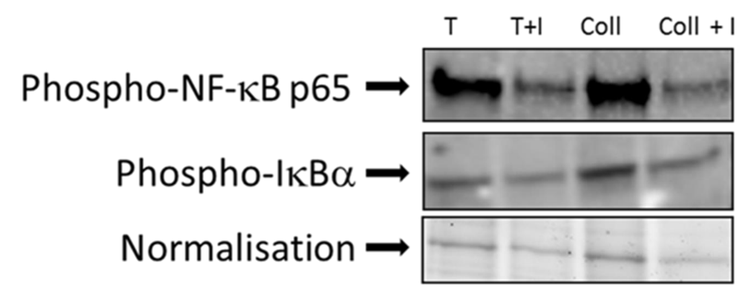

Fig 4
Effect of Coll2-1, in the presence or absence of oxidative stress inhibitors (I), on the phosphorylation of p65 and IκB-α.

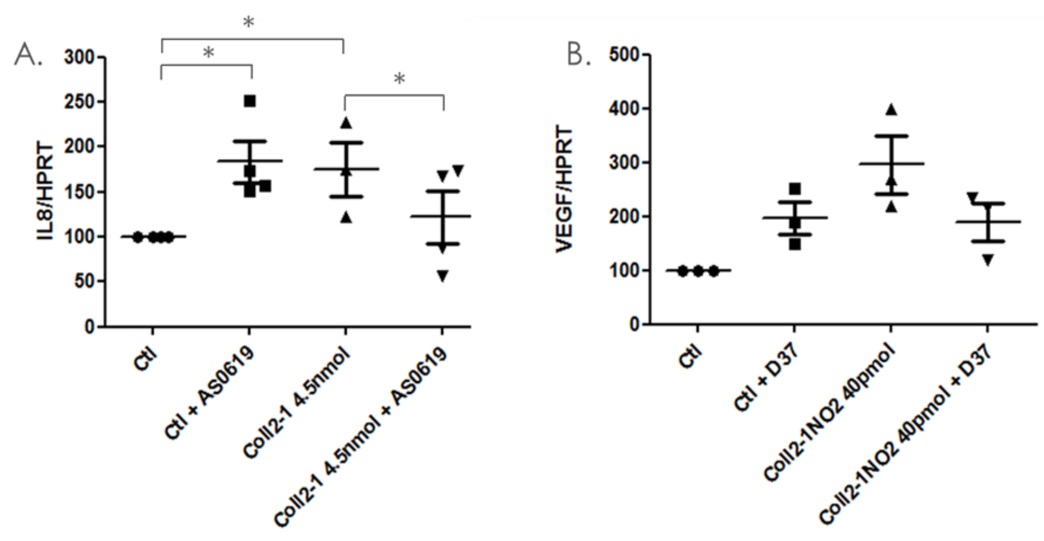

Fig 5
Effect of AS0619 (A) and D37 (B) on the IL-8 and VEGF expression by osteoarthritic synoviocytes in the presence of Coll2-1 (A) and Coll2-1NO$_2$ (B). * *P*<0.05

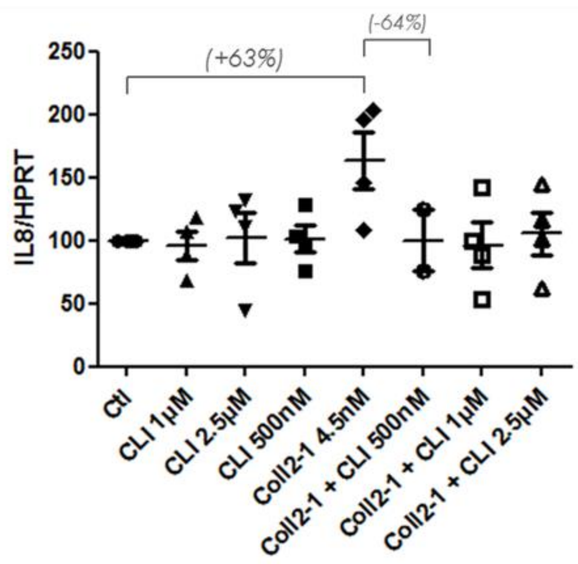

Fig 6
Effect of CLI-095 on the IL-8 expression by osteoarthritic synoviocytes in the presence of Coll2-1.

CONCLUSION. **For the first time, we have shown that type II collagen peptides have pro-inflammatory, pro-angiogenic and immunomodulatory properties directly related with OA. These findings indicate that Coll2-1, a marker of cartilage degradation, could be a target for immunotherapy.**

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