Thrombospondin-1 Inhibits Interleukin-10 Release from Monocytic Cells through Interaction with CD47

Yasushi YAMAUCHI, Motomu KUROKI*, Takayuki IMAKIIRE*, Koichi UNO*,
Yuichi YAMASHITA, Takayuki SHIRAKUSA and Masahide KUROKI*

*Second Department of Surgery and *Department of Biochemistry,*
Fukuoka University School of Medicine

Abstract: Interleukin-10 (IL-10), an immunosuppressive cytokine which plays a key regulatory role in immune responses, modulates inflammation, wound healing, and tumor growth. We have recently shown that thrombospondin-1 (TSP-1), a multifunctional extracellular matrix protein, inhibits the release of IL-10 from human monocytic cells by activating transforming growth factor-β_1 (TGF-β_1). We herein demonstrate an additional, novel inhibitory mechanism of IL-10 release by TSP-1. Human monocytic U937 cells were stimulated *in vitro* with phorbol myristate acetate and LPS in the presence of immobilized TSP-1 or proteolytic 70-kD fragments of TSP-1. The concentration of IL-10 in culture supernatants was determined by an enzyme-linked immunosorbent assay. Both TSP-1 and the 70-kD fragment inhibited the release of IL-10 from the U937 cells. Although the specific sequence to activate TGF-β_1 exists in both molecules, intact TSP-1 revealed a stronger inhibitory effect than the 70-kD fragment at the same molar concentration. CD47 engagement by anti-CD47 antibody or the 4N1K peptide, which corresponds to the CD47-binding site present on TSP-1 but not on the 70-kD fragment, also attenuated the release of IL-10. However, the CD47 engagement with anti-CD47 did not induce any TGF-β_1 release and the RGDS peptide did not affect the IL-10 release, thus suggesting that the inhibition of IL-10 release via CD47 is neither associated with TGF-β_1 nor dependent on $\alpha v \beta 3$ integrin. CD47 engagement by TSP-1 is therefore considered to be a novel pathway to downregulate IL-10 release from monocytic cells.

Key words: CD47, interleukin-10 (IL-10), transforming growth factor-β_1 (TGF-β_1), thrombospondin-1 (TSP-1), U937 cells