

Effects of the proinflammatory cytokine interleukin-33 on tissue factor expression and activity in monocyte subsets *in vitro*

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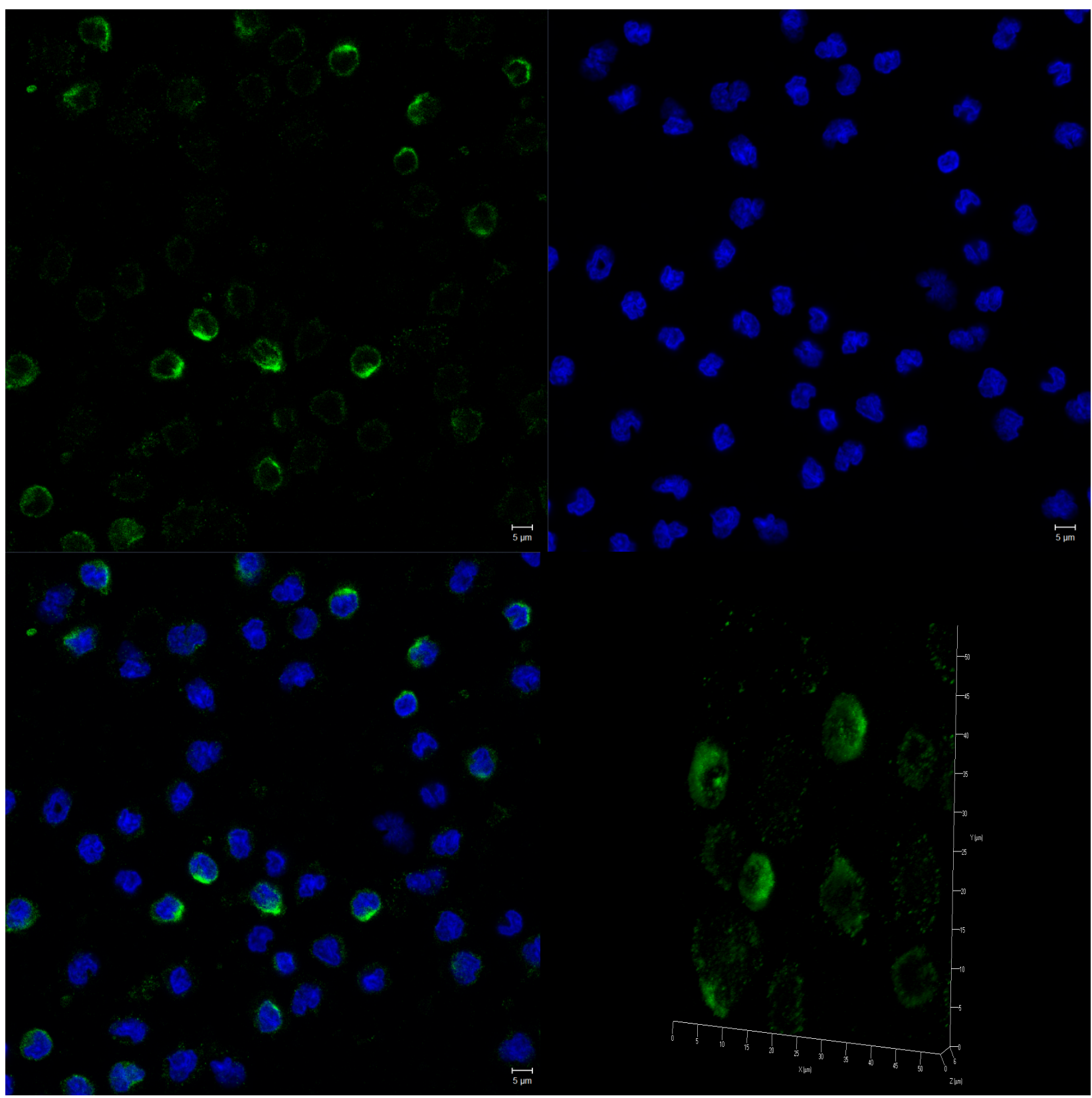
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Background. Monocytes play an important role in atherosclerotic plaque formation and development, and represent one of the predominant sources of blood-borne tissue factor (TF) upon proinflammatory activation. Interleukin (IL)-33 is a proinflammatory cytokine implicated in processes crucial for atherosclerotic plaque formation, progression and destabilization. Serum levels of IL-33 have been associated with increased mortality in patients with ST-elevation myocardial infarction.

Aim. Here we aimed to investigate the effects of IL-33 on TF expression and activity in human monocyte subsets.

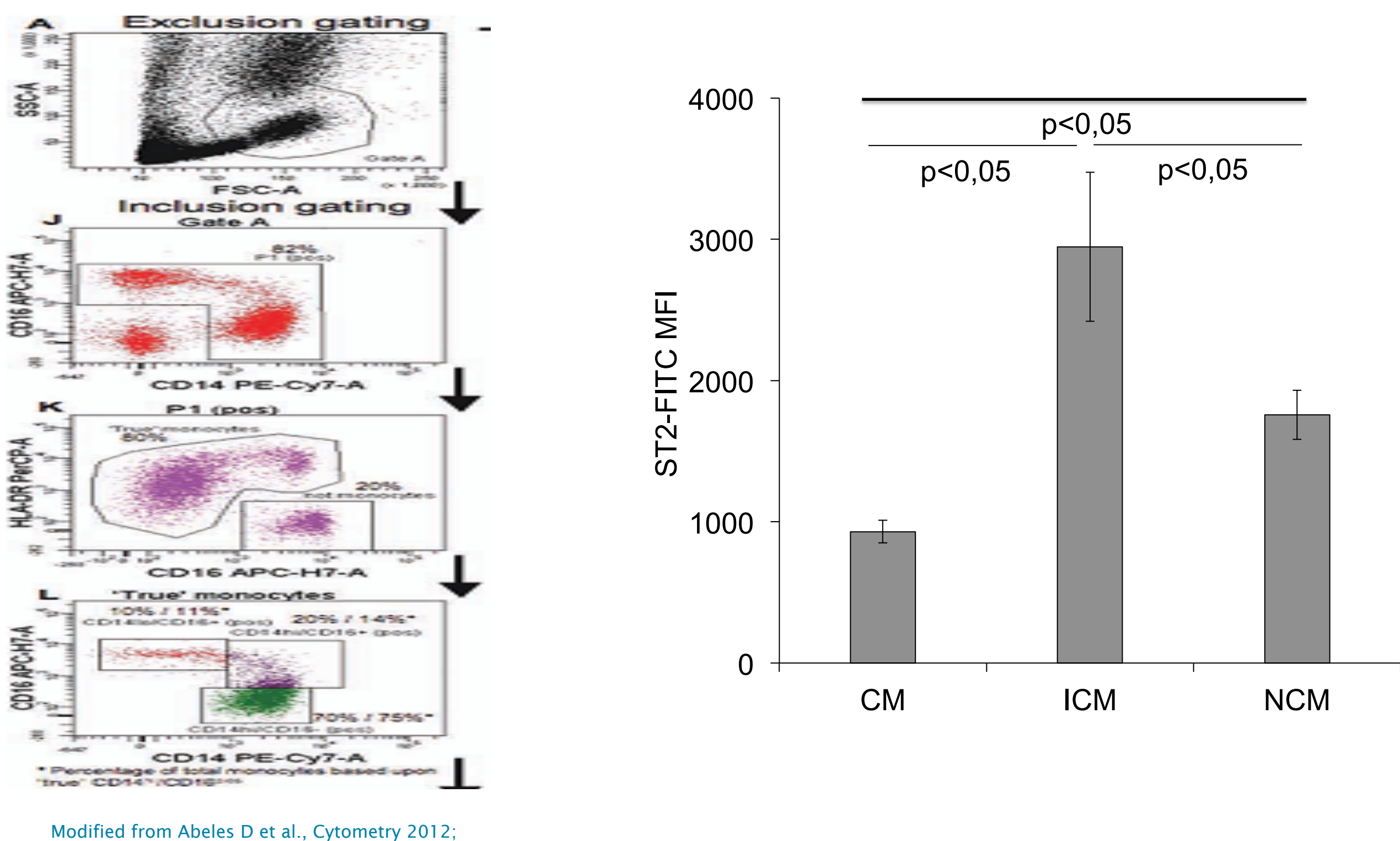
Methods and Results. Monocytes were isolated from whole blood of 3 healthy volunteers using a EasySep™ human monocyte enrichment kit without CD16 depletion according to manufacturer's instructions. Immediately after isolation, monocytes were seeded to adhesion slide's and stained with rabbit anti-human ST2 antibody. Figure 1 shows ST2-receptor expression of isolated human monocytes.

Figure 1. Human monocytes express ST2-receptor



In order to investigate if monocyte subsets show differential expression of ST2-receptor, we have stained isolated monocytes with ST2-FITC, CD14 PE, CD16 PE-Cy7 and HLA-DR APC antibodies and performed flow cytometry analysis. Cells were first visualized on forward- and side-scatter, and gate was drawn around the monocyte cloud to exclude the majority of debris and other cells. These cells were then viewed on a CD14 vs CD16 plot and a gate was drawn around the monocyte cloud. When cells of this gate are viewed on a CD16 vs HLA-DR plot the non-monocytes are distinguished from the true HLA+ monocytes (Figure 2). The monocytes population can now be viewed again on a CD14 vs CD16 plot and gated into CD14++CD16- (classical, CM), CD14++CD16+ (intermediates, IM) and CD14+CD16+ (non-classical, NCM) monocytes. This gating strategy revealed differential ST2 expression in human monocyte subsets. Intermediate monocytes showed significantly higher ST2-receptor mean fluorescent intensity, as compared to classical and non-classical monocytes (Figure 2).

Figure 2. Human monocyte subsets show differential ST2-receptor expression



Conclusion. IL-33 induces TF production and activity in human monocytes. Distinct monocytes subsets respond differently to IL-33 treatment, with intermediate monocytes showing the highest TF expression after stimulation. Interestingly, monocyte subsets also express ST2 receptor differently, and intermediate monocytes express highest levels of ST2. As danger signal and immune modulator, IL-33 could contribute to thrombotic response by inducing TF in human monocytes in the setting of acute or chronic inflammation and tissue damage. However, further investigation is required.

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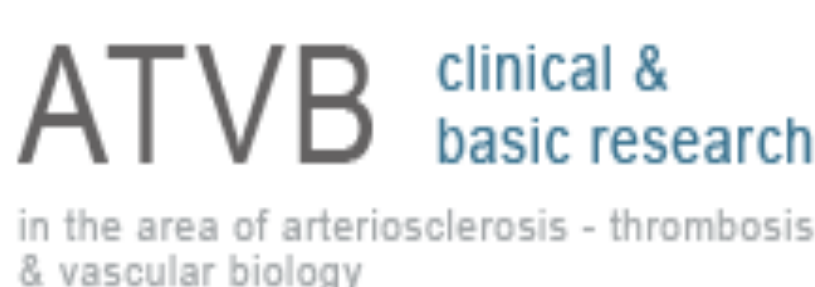
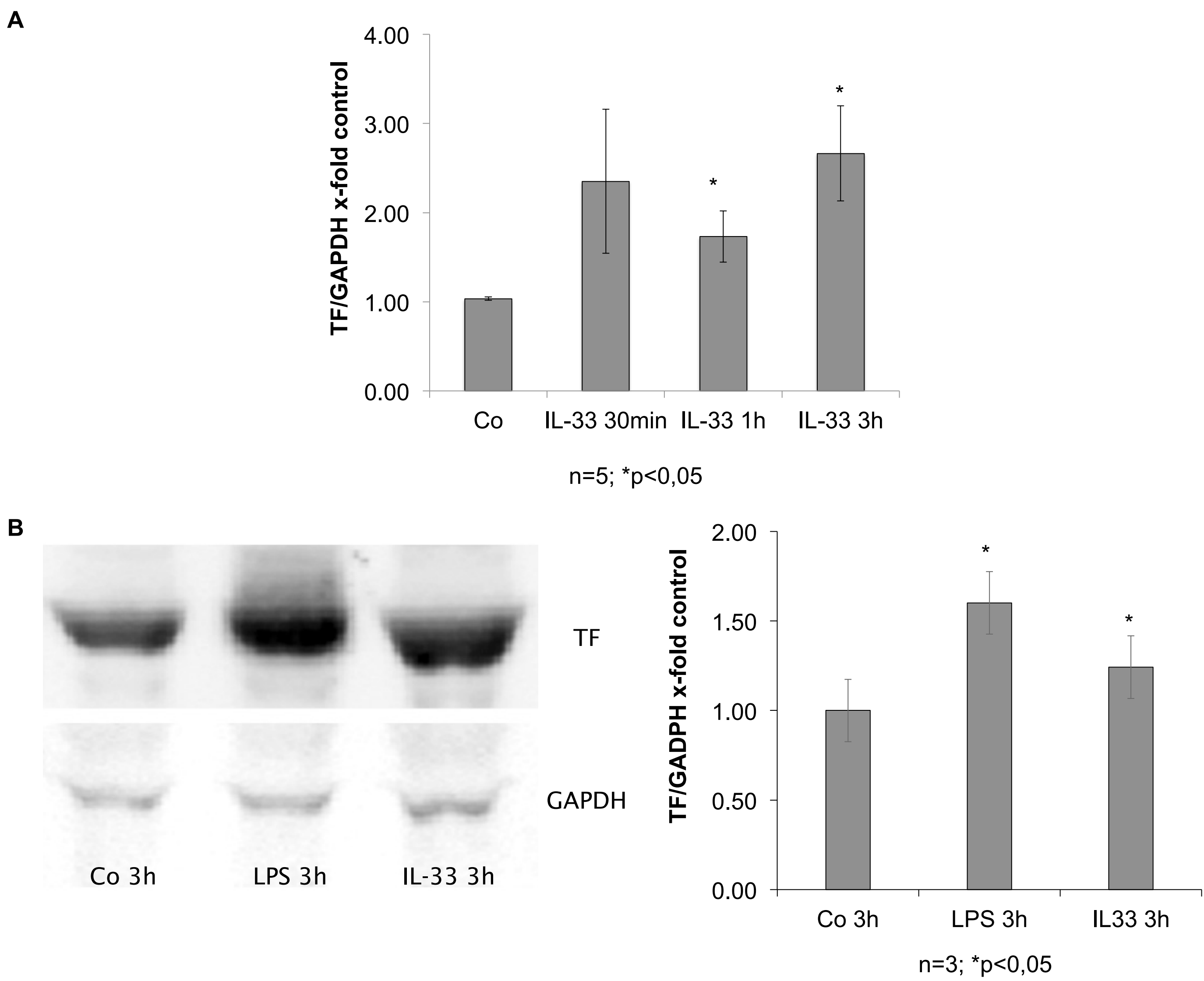
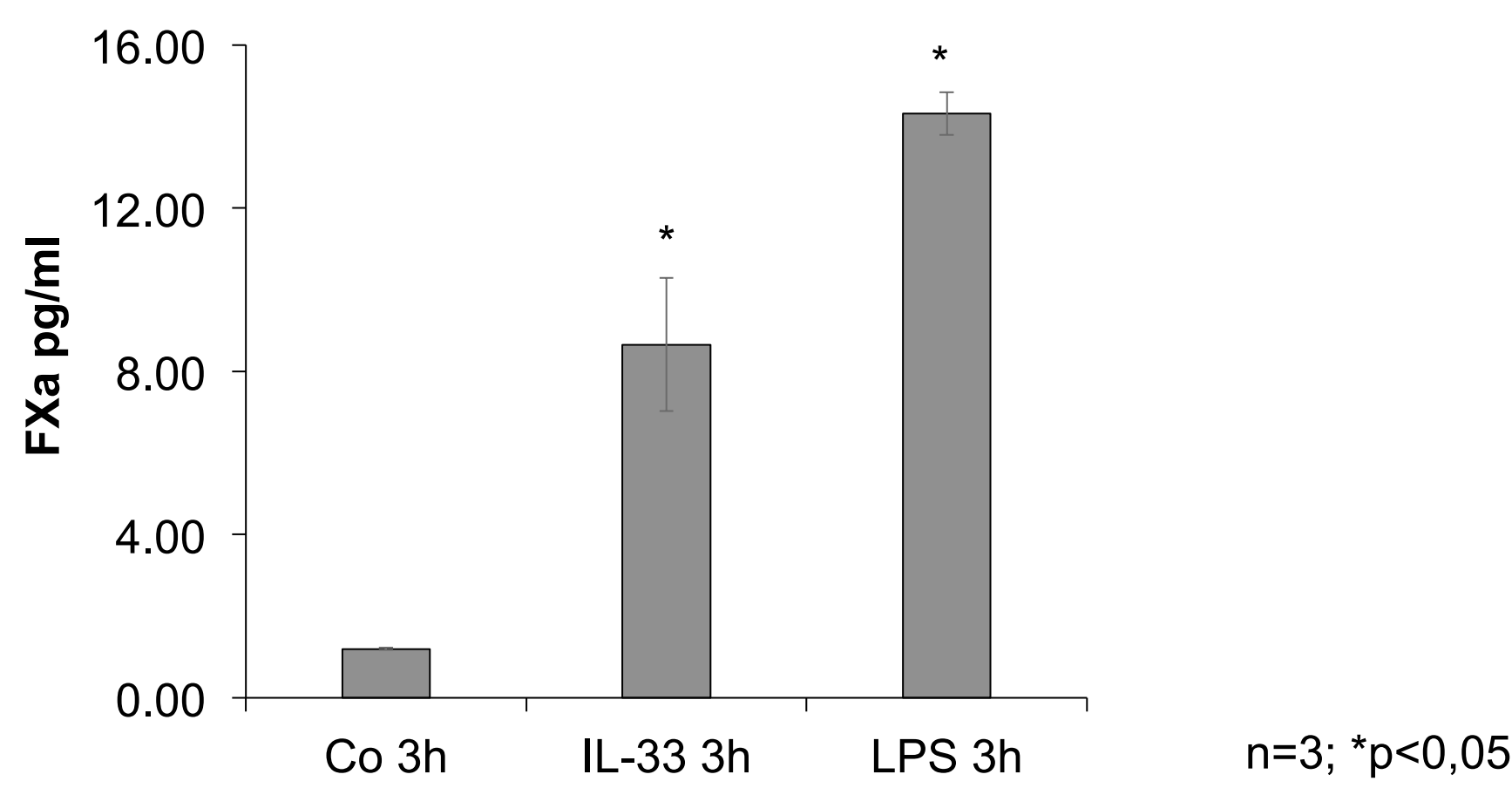


Figure 3. IL-33 induces TF mRNA and protein expression in human monocytes



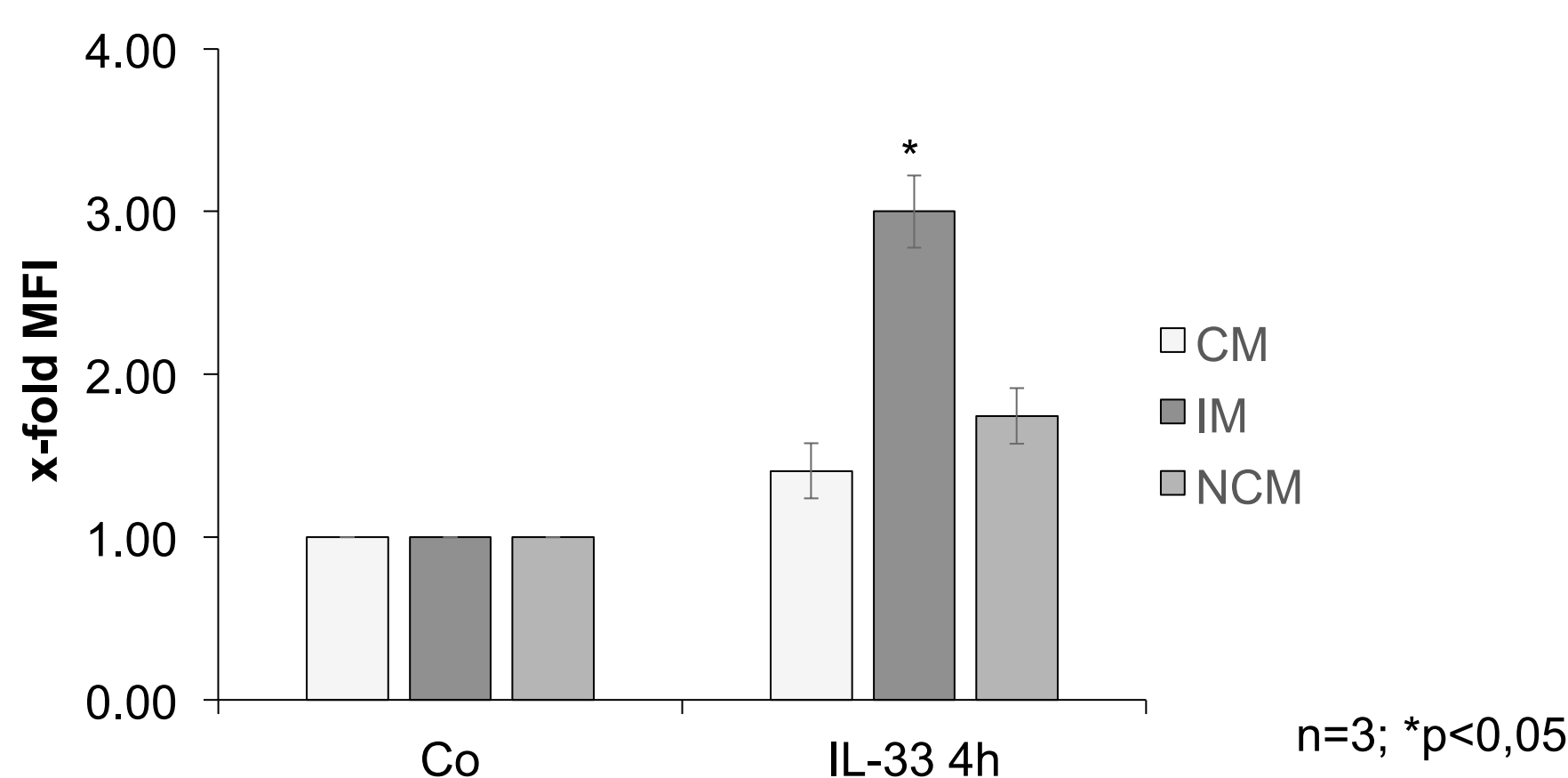
Monocytes were isolated from 5 different healthy donors, and incubated with 100 ng/ml IL-33 for 30 min, 1 h or 3 h, or were left untreated (Co). IL-33 significantly induced TF mRNA expression in a time-dependent manner (Figure 3A). Furthermore, after 3h of incubation with IL-33, we have detected a significant increase of total TF protein in monocyte cell lysates using western blot (Figure 3B).

Figure 4. IL-33 induces TF activity in human monocytes



We have further investigated the effects of IL-33 on TF activity levels as these are crucial in the blood coagulation cascade. Cell surface TF activity levels were increased after stimulation of monocytes with 100 ng/ml IL-33 for 3h. (Figure 4)

Figure 5. Human monocyte subsets differently express TF upon IL-33 stimulation



Using flow cytometry analysis, monocytes were divided into 3 subsets according to their distinct surface expression of CD14 and CD16, as described above. After 4h of stimulation with IL-33 a significant increase of TF mean fluorescence intensity (MFI) was observed only in CD14++CD16+ monocytes, whereas no significant changes of TF MFI were seen in CD14++CD16- and CD14+CD16++ monocytes, respectively.