POSTER 4: Characterization of LPS binding and subsequent activation of normal and malignant B cells through the CD180 receptor

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BACKGROUND. Our team identified a differential CD 180 membrane expression in various mature B cell neoplasms. While CD 180 is strongly expressed on normal B cells, its expression is decreased in most malignant B cells, except in the marginal zone lymphoma. (1•2•3) CD180 is an atypical Toll-like receptor (TLR). In mice, it's suggested to modulate the humoral response to LPS. (4,5,6,7) In humans, its ligand and signaling pathways are not well identified. This project aims to characterize the LPS binding on B cells through CD180 and the subsequent activation of normal and malignant B cells differentially expressing CD180. METHODS. Blood samples from healthy donors (HD), chronic lymphocytic leukemia (CLL) and marginal zone lymphoma (MZL) patients have been stimulated by an a-CD180 antibody (BV605) or LPS (FITC or Cy5) during 5 min, 15 min, 2h, 4h or 24h. The fixation of LPS has been measured by flow cytometry and observed by immunofluorescence. The expression of CD 180 has been measured by flow cytometry and R T-qPCR. The activation state of B cells has been assessed by the membrane expression of CD86 by flow cytometry.
RESULTS. In blood samples from HD, B cells could bind LPS in a time- and dose-dependent manner, but not T cells that don't express CD 180, Incubation of B cells with LPS or the a-CD 180 increased the membrane expression of CD 180. The membrane expression ofCD86 increased on healthy B cells after LPS incubation for 24h but not after a-CD 180 incubation. Co-incubation with LPS and a-CD 180 increased CD86 expression al a higher level than LPS alone. LPS incubation could activate B cells from HD and MZL but not from CLL.
DISCUSSION. LPS binding on lymphocytesis positively correlated to CD180 but not TLR4 membrane expression and led to the activation of the NF-KB pathway in B cells. Moreover, CD180 could be recruited to the membrane through LPS or a-CD180 stimulation, Since the incubation with a-CD180 did not activate B cells, co-incubation with LPS induced a stronger activation than with LPS alone. This synergetic effect could be attributed to the membrane overexpression of CD 180 mediated by the a-CD 180. On malignant B ce lis, LPS activated MZL but not CLL B cells. This differential sensitiveness is correlated with their CD 180 membrane expression. To pursue this study, we are trying to visualize the LPS/CD180 interaction through FRET microscopy and we are analyzing the transcriptome of normal and malignant B cells obtained after LPS stimulation by RNA-sequencing.
KEYWORDS. CD 180, Mature B cell neoplasm, Lipopolysaccharides, B cell activation, Human primary B cells
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