Transcriptional correlation analysis of the *CD180/MD-1* complex and toll-like receptors and signalling lymphocyte activation family receptors in CLL

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Introduction

- CD180 is an orphan toll-like receptor (TLR) which is highly co-expressed with the satellite molecule MD-1 on the surface of normal B cells and heterogeneously on the surface of CLL cells[1]. Higher levels of CD180 expression are associated with a positive prognosis in CLL [2].
- There is mounting evidence suggesting that CD180/MD-1 signals in concert with other surface receptors including signalling lymphocyte activation family (SLAMF) 1 in CLL[3] and TLR9 in healthy B cells[4].
- Little is known however about the interactions between CD180/MD-1 and TLRs and other members of the SLAMF receptors in CLL.
- We therefore set out to identify novel signalling partners for CD180/MD-1 by analysing the co-expression *CD180* and *MD-1* relative to *TLRs* and *SLAMF* receptor genes at the transcriptional (mRNA) level.

Methods

Data extraction

We accessed dataset GSE126595[5] from the gene expression omnibus which contained normalized gene expression for 726 treated and untreated CLL patients. We screened for the transcriptional expression of *TLRs* and *SLAMF* receptors.

Defining gene expression positivity

We considered the receptor to be expressed by CLL cells if the mRNA expression was higher than the *CD3G* gene which is exclusively expressed by T cells. The *CD19* gene, which is known to be highly expressed by CLL cells, was used as a positive control.

Correlation analysis

Pearson's correlation was applied to measure relationships between *CD180/MD-1* and *TLR*/*SLAMF* (mRNA) gene expression.

Results

1

- ***CD180* and *MD-1* mRNA were found to be highly expressed compared to *CD19*** (Figure 1).
- ***TLR7* was the most highly expressed TLR gene** followed by *TLR9*, *TLR6* and *TLR10*. *TLR2*, *TLR4* and the TLR4-associated satellite molecule *MD-2* were expressed at very low levels (Figure 1a). *TLR3* and *TLR8* were not expressed by CLL cells (Figure 1a).
- ***SLAMF2* was the most highly expressed *SLAMF* gene** by CLL cells followed by *SLAMF3*, *SLAMF6* and *SLAMF5* (Figure 1b). *SLAMF1* was also expressed albeit at lower levels (Figure 1b).
- The remaining *SLAMF* receptor genes were found to be negative or marginally positive and were excluded from the correlation analysis (Figure 1b).

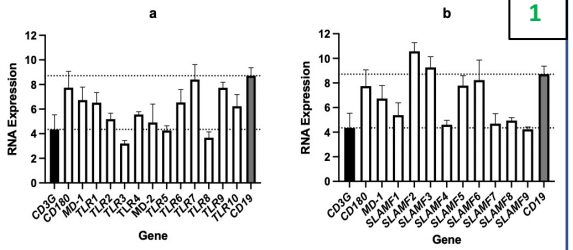

Figure 1. The expression of *TLRs* (a) and *SLAMF* (b) receptors in CLL cells. Levels of normalised mRNA gene expression were extracted from GEO under accession number GSE126595.

2

	CD180	MD-1
Vs		
TLR1	0.468	0.29
TLR2	0.151	0.184
TLR4	-0.507	-0.298
MD-2	0.783	0.55
TLR6	0.743	0.475
TLR7	0.813	0.512
TLR9	0.535	0.259
TLR10	0.853	0.617
SLAMF1	0.317	0.166
SLAMF2	0.708	0.542
SLAMF3	0.205	0.023[ns]
SLAMF5	0.685	0.384
SLAMF6	0.823	0.584

Table 1. The correlation between *CD180* and *MD-1* genes and *TLRs* and *SLAMF* receptors. All permutations were determined as significant ($p<0.001$) except where indicated ([ns] Non-significant).

- ***CD180/MD-1* mRNA was found to be highly correlated with *TLR1*, *TLR6* and *TLR10* mRNA** (Table 1) which encode for surface TLRs.
- There was a negative correlation between *CD180/MD-1* and *TLR4*, but a positive correlation with *MD-2* (Table 1), the satellite molecule which is co-expressed with TLR4 [6].
- **CD180/MD-1 were also both found to be highly correlated with *TLR7* and *TLR9* mRNA** which encode for TLRs expressed in the endosomes (Table 1).
- ***CD180/MD-1* was highly correlated with *SLAMF2*, *SLAMF5* and *SLAMF6*** (Table 1).

3

- **We found that almost all *TLR* (Figure 2a) and *SLAMF* (Figure 2b) genes were differentially expressed in treated cases compared to untreated.**
- We therefore re-analysed the correlations between CD180/MD-1 and TLR and SLAMF gene expression in the untreated cohort only.

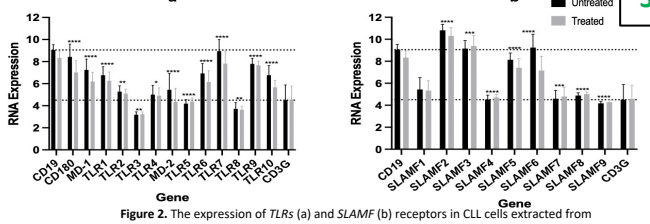

Figure 2. The expression of *TLRs* (a) and *SLAMF* (b) receptors in CLL cells extracted from untreated (n = 375) and treated (n = 351) patients. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

4

- Stronger correlations between ***CD180* and *MD-1* were found with endosomal *TLR9* and *TLR7* mRNA** expression in the untreated cohort (Table 2).
- Similarly, **there was a stronger correlation between *CD180/MD-1* and *TLR10* and *TLR6* mRNA**, however, the correlation between *CD180/MD-1* and *TLR1* was lost (Table 2). *CD180/MD-1* was highly correlated with *MD-2* mRNA expression.
- ***CD180/MD-1* was found to be most highly correlated with *SLAMF6* and then *SLAMF2 mRNA*** in the untreated cohort (Table 3).

Table 2. The correlation between *CD180* and *MD-1* genes and *TLRs* in untreated patients

	CD180 Vs		*MD-1 Vs*	
	r	P	r	p
TLR1	0.1867	0.0003	0.0472	0.3623
TLR2	0.0351	0.4984	0.1293	0.0122
TLR4	0.2627	< 0.0001	0.2544	< 0.0001
MD-2	0.7469	< 0.0001	0.4195	< 0.0001
TLR6	0.6455	< 0.0001	0.3466	< 0.0001
TLR7	0.7938	< 0.0001	0.3656	< 0.0001
TLR9	0.6932	< 0.0001	0.3111	< 0.0001
TLR10	0.8124	< 0.0001	0.4508	< 0.0001

Table 3. The correlation between *CD180* and *MD-1* genes and *SLAMFs* in untreated patients

	CD180 Vs		*MD-1 Vs*	
	r	P	r	p
SLAMF1	0.3848	0.0036	0.1530	0.0030
SLAMF2	0.6298	< 0.0001	0.3844	< 0.0001
SLAMF3	0.2998	0.6065	0.1202	0.0199
SLAMF5	0.5539	< 0.0001	0.1808	0.0004
SLAMF6	0.8400	< 0.0001	0.3925	< 0.0001

Discussion

- ***CD180/MD-1* was highly correlated with endosomal *TLR* genes**, indicating a potential role for CD180/MD-1 in modulating immune responses to intracellular pathogens.
- High correlation between *CD180/MD-1* and *MD-2* could be due to evolutionary relationships between MD-2 and MD-1 [6].
- CD180 can negatively regulate TLR9 [2,4], **thus CD180/MD-1 may prevent TLR9-mediated pro-migratory signaling in CLL** [7] and contribute to a positive prognostic outlook in the disease.
- **CD180/MD-1 could dimerize with TLR10 or TLR6** given the strong correlation between these TLRs at the mRNA level.
- Correlations between *CD180/MD-1* and *SLAMF2*, SLAM5, and *SLAM6* mRNA could **indicate a potential role for CD180/MD-1 in modulating the interaction between CLL cells and cellular components of the microenvironment.**

Conclusions

- **TLR6/TLR10 are rational novel targets for studying receptors which dimerize with CD180/MD-1 for ligand detection in CLL.**
- **CD180/MD-1 may interact/modulate endosomal TLR signaling in CLL.**
- **SLAMF2, SLAMF5 and SLAMF6 may be interacting with CD180/MD-1 in CLL to modulate the tumour microenvironment.**

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