Role of CCR4 in Regulating Cell Surface Glycan Expression on T-Lymphocytes

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ABSTRACT

Glycans (oligosaccharides) are one of the four fundamental structures that make up all living systems. Cell surface glycans have been shown to play an important role in bacteria and viral recognition, cell signaling, and cancer development. In previous studies, Chemokine Receptor 4 (CCR4) has been shown to be key components to T cell recruitment and it has been demonstrated that changes in glycan structure can act as a regulator of migration (Faustino et al., 2013; Gu & Taniguchi, 2008). For this reason, we characterized T-lymphocyte cell surface glycan structures from two different cell lines with a flow-based lectin array after the engagement of chemokine receptor CCR4. By further characterizing the role of chemokine receptors on glycans in T cells, we believe we can better understand the pathogenesis of immune-related diseases.

OBJECTIVES

The overall hypothesis is that chemokine receptor-ligand interactions change the T cell surface glycans through regulating the expression of glycosylation enzymes, which ultimately impact the T cell migratory functions. Specifically, our aim is to characterize T-lymphocyte cell surface glycan structures with a flow-based lectin array before and after the engagement of chemokine receptor CCR4 using Thymus and Activation Regulated Chemokine (TARC).

MATERIALS and METHODS

Cell Lines:

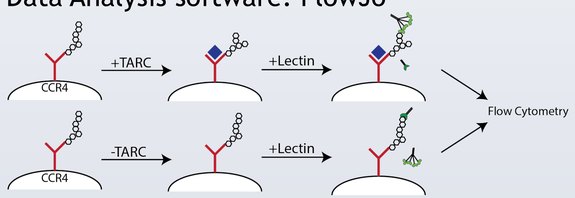
-HuT 78 (TIB-161) from ATCC

-MJ CRL-8294 from ATCC

C-type plant lectins: binding for GalNac, Galactose, a-methylglucoside, and L-Fucose

Flow Cytometer: BD LSR Fortessa

Data Analysis software: FlowJo



1) Cell lines are cultured and treated with or without TARC
2) Stained with fluorescently labeled antibodies CD4 (PE), CCR4 (PE-Cy7), and four different lectins (FITC)
3) Stained cells are analyzed by flow cytometry to determine the lectin-binding profile of each T-cell population expressing CD4 and CCR4

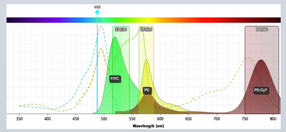


Figure 1. Fluorescent spectra viewer of emission wavelength for PE, PE-Cy7, and FITC

4) Fold change is calculated according to the below formula. MFI: Mean Flourescence Intensitiy, MFIR: Mean Fluorescence Intensity Ratio.

$$Fold\ Change = \frac{MFIR\ with\ TARC}{MFIR\ without\ TARC} = \frac{\frac{MFI\ of\ TARC(+)\ with\ Lectin}{MFI\ of\ Isotype\ Control}}{\frac{MFI\ of\ TARC\ (-)\ with\ Lectin}{MFI\ of\ Isotype\ Control}}$$

RESULTS

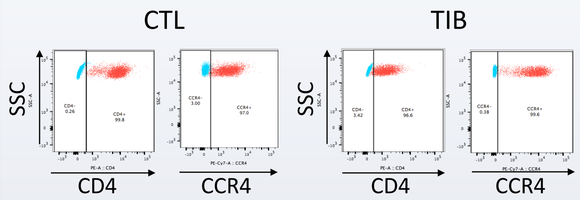


Figure 2. CD4 and CCR4 expression on CTL and TIB cell lines. Representative FACS profiles of SSC vs CD4 or CCR4 (red) compared to isotype controls (blue) are shown.

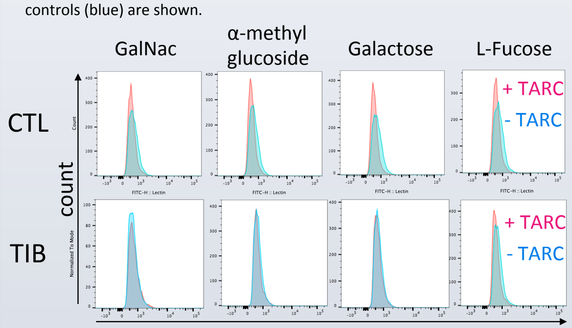


Figure 3. Lectin binding profiles of CTL and TIB cell lines. Representative histograms of lectin binding on CTL or TIB cells with (red) or without (bue) TARC treatment are shown.

Table 1. Summary of fold change of the MFI for each lectin binding on CTL and TIB cells with and without TARC treatment

CTL - FITC-LECTIN BINDING	Lectin for GalNac	Mean Fluorescence Intensity Ratio	Lectin for Galactose	Mean Fluorescence Intensity Ratio	Lectin for α-methylglucoside	Mean Fluorescence Intensity Ratio	Lectin for L-Fucose	Mean Fluorescence Intensity Ratio	MFI of Isotype Control
CCL17(-)MFI	788	0.845493562	806	0.864806867	817	0.876609442	892	0.957081545	932
CCL17(+) MFI	697	0.998567335	670	0.959885387	663	0.949856734	709	1.015759312	698
% or fold change:	-11.54822335	**1.181046645**	-16.87344913	**1.109941911**	-18.8494492	**1.083557498**	-20.51569507	**1.061309057**	

CTL - PE-Cy7-CCR4	Lectin for GalNac	Mean Fluorescence Intensity Ratio	Lectin for Galactose	Mean Fluorescence Intensity Ratio	Lectin for α-methylglucoside	Mean Fluorescence Intensity Ratio	Lectin for L-Fucose	Mean Fluorescence Intensity Ratio	MFI of Isotype Control
CCL17(-)MFI	1399	17.98200514	1196	15.37275064	1227	15.77120823	1268	16.29820051	77.8
CCL17(+) MFI	1049	17.74957699	866	14.65313029	926	15.66835871	857	14.50084602	59.1
% or fold change:	-25.01786991	**0.987074403**	-27.59197324	**0.953188576**	-24.53137734	**0.993478654**	-32.41324921	**0.889720679**	

TIB - FITC-LECTIN BINDING	Lectin for GalNac	Mean Fluorescence Intensity Ratio	Lectin for Galactose	Mean Fluorescence Intensity Ratio	Lectin for α-methylglucoside	Mean Fluorescence Intensity Ratio	Lectin for L-Fucose	Mean Fluorescence Intensity Ratio	MFI of Isotype Control
CCL17(-)MFI	838	1.309375	786	1.228125	781	1.2203125	871	1.3609375	640
CCL17(+) MFI	934	1.216145833	769	1.001302083	755	0.983072917	722	0.940104167	768
% or fold change:	11.45584726	**0.928798727**	-2.162849873	**0.815309584**	-3.329065301	**0.805591122**	-17.10677382	**0.690776885**	

TIB - PE-Cy7-CCR4	Lectin for GalNac	Mean Fluorescence Intensity Ratio	Lectin for Galactose	Mean Fluorescence Intensity Ratio	Lectin for α-methylglucoside	Mean Fluorescence Intensity Ratio	Lectin for L-Fucose	Mean Fluorescence Intensity Ratio	MFI of Isotype Control
CCL17(-)MFI	1733	31.85661765	1438	26.43382353	1404	25.80882353	1293	23.76838235	54.4
CCL17(+) MFI	504	10.14084507	1364	27.44466801	1447	29.11468813	1408	28.32997988	49.7
% or fold change:	-70.91748413	**0.318327739**	-5.146036161	**1.03824057**	3.062678063	**1.12809048**	8.894044857	**1.19191872**	

CONCLUSIONS

CCR4 stimulated by TARC did not cause significant changes (1.06 – 1.1 fold change) in the CTL cell line whereas a slight decrease in L-Fucose (0.7 fold change) was found in the TIB cell line. The different response between two cell lines may be related to the expression levels of CCR4. Future directions include testing the assay with longer treatment of TARC, expanding the lectin panels to detect other types of glycan changes, and characterizing glycosylation gene expressions with targeted glycomic gene qPCR analysis.

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