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Ph.D. Thesis

Exploring immune cell populations: a lectin-based scanning approach

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Publications

Publications related to the subject of the Ph.D. thesis

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LIST OF ABBREVIATIONS

AAL	***Aurelia aurentia* lectin**
ACA	***Amaranthus caudatus* agglutinin**
AHA	***Arachis hypogea* agglutinin**
AFP-L3	**alpha-fetoprotein-L3**
AIA	***Artocarpus integrifolia* agglutinin**
Asn	**asparagine**
BCR	**B-cell receptor**
Gal	**galectin**
CA125	**carbohydrate antigen 125**
CRC	**colon carcinoma**
DNT	**double negative T-cells**
DolP	**dolichol phosphate**
DPT	**double positive T-cells**
ER	**endoplasmic reticulum**
Gal	**galactose**
GalNAc	**N-acetylgalactosamine**
Glc	**glucose**
GlcNAc	**N-acetylglucosamine**
GPI	**glycosylphosphatidylinositol**
GSA I	***Griffonia simplicifolia* agglutinin I**
HC	**healthy control**
HCC	**hepatocellular carcinoma**
HE4	**human epididymis protein 4**
IdoA	**iduronic acid**
LacNAc	**N-acetyllactosamine**
LCA	***Lens culinaris* lectin**
MAA	***Maackia amurensis* lectin**
Man	**mannose**
MO	**monocytes**
NKT	**natural killer T-cells**
NSCLC	**non-small cell lung cancer**
OST	**oligosaccharyltransferase**
PBMC	**peripheral blood mononuclear cells**
PD-1	**programmed cell death receptor-1**
Pro	**proline**
PSA	***Pisum sativum* lectin**
PWM	***Phytolacca americana* lectin**
SNA	***Sambucus nigra* lectin**
Gal1	**galectin-1**
Gal3	**galectin-3**
RBC	**red blood cell**
FUT	**fucosyltransferase**
Ser	**serine**
Sig1	**Siglec-1**
SLE	**systemic lupus erythematosus**
STn	**sialyl-Thomsen-nouveau antigen**
TCR	**T-cell receptor**
Tg	**thyroglobulin**
Thr	**threonine**
Tn	**Thomsen-nouveau antigen**
UEA I	***Ulex europaeus* agglutinin I**
WFA	***Wisteria floribunda* agglutinin**
Xyl	**xylose**

INTRODUCTION

In the process of glycosylation, sugar molecules, such as glycan chains or individual sugars, are covalently linked to organic molecules as a post-translational modification. Different compositions and quantities of these carbohydrate chains generate the glycan code, which is a cell and tissue-specific extremely complex pattern influenced by the cell's local milieu. Glycosylation has an important role in basic biological processes. For that reason, altering in glycan pattern modulates immunological responses. Studying altered glycosylation patterns provides exact information about the molecular mechanisms of diseases, offers potential targets for therapeutic interventions. It can contribute to the development of diagnostic tools to modulate specific glycan codes and develop personalized medicine. Therefore, understanding how glycosylation changes in individual diseases is essential.

Lectins can selectively recognize carbohydrate chains and bind them reversibly. Due to this characteristic feature, like high selective specificity, lectins are investigated in mapping unique carbohydrate chains. To analyse glycan code of immune cells, we built a fluorescent dye-labeled lectin-based multicolor flow cytometry panel. The individual carbohydrate-binding lectins were labeled with different fluorescent dyes, thus enabling parallel mapping of the glycan code.

In diseases where conditions characterized by either an overactive or highly suppressed immune system are present, changes in glycosylation patterns are particularly important. Autoimmune diseases and cancer-related diseases could help to understand altered glycosylation patterns. CT26 is a hypermutated murine colorectal carcinoma cell line that is the most commonly used preclinical model for human tumors. To establish a cancer model, CT26 colon carcinoma cells were injected into BALB/c mice's spleen in our current work. Another disease selected for characterizing the glycosylation code was systemic lupus erythematosus (SLE), an autoimmune condition characterized by the extreme overactivity of the immune system

AIMS

We aimed to investigate glycosylation changes in cancer-related and autoimmune conditions by using glycosylation pattern based technology. To achieve this goal we aimed to develop a fluorescent dye-labeled lectin based multicolor flow cytometry protocol.

Aim 1:
We intended to utilize the lectin-specific binding ability to map individual cell surface glycosylation patterns, therefore we aim to

- **build a fluorescent dye-labeled lectin-based multicolor flow cytometry panel and optimize this method**.

Aim 2:
Alterations in the function of the immune system play an important role in cancer progression, however, the immune cell's surface glycosylation role is not completely defined in cancer, so we aimed to

- **investigate different immunophenotypes surface glycosylation changes in murine CT26-induced colorectal carcinoma model with particular regard to sialylation and fucosylation**.

Aim 3:
Enzymes that are responsible for the creation of glycosylation patterns play an important role in cancer-related modifications, therefore our further aim was to

- **whole proteomic profiling of CRC-induced murine spleens and metastatic liver with particular regard to the enzymes involved in glycosylation**.

Aim 4:
Autoimmune diseases represent the opposite end of immune system dysregulation, wherein the immune system tends to overreact instead of suppressing its responses. Consequently our objective was

- **to investigate immune subsets in human systemic lupus erythematosus using the previously developed lectin-based multiparametric flow cytometry protocol**.

MATERIALS AND METHODS

Animals

Mice were kept in a sterile environment in Makrolon® cages at 22–24 °C (40–50% humidity), with light regulation of 12/12 h light/dark. The animals had free access to tap water and were fed by sterilized standard diet (VRF1, autoclavable, Akronom Kft., Budapest, Hungary) ad libitum. Animals used in our study were taken care of according to the "Guiding Principles for the Care and Use of Animals" based on the Helsinki Declaration, and they were approved by the ethical committee of the National Institute of Oncology. Animal housing density was according to the regulations and recommendations from directive 2010/63/EU of the European Parliament and the Council of the European Union on the protection of animals used for scientific purposes. Permission license for breeding and performing experiments with laboratory animals: PEI/001/1738-3/2015 and PE/EA/1461-7/2020.

Tissue culture

CT-26 cells were obtained from ATCC and cultured in RPMI-1640 Medium (BioSera, Boussens, France), supplemented with 10% Fetal bovine serum (BioSera, Boussens, France) and 1% Penicillin/Streptomycin (BioSera, Boussens, France) in sterile T75 tissue culture flasks with ventilation caps (Sarstedt, Nümbrecht, Germany) in a humidified 5% CO2 atmosphere at 37 ºC. Cells were cultured for a maximum of 25 passages or 60 days after thawing and were screened for mycoplasma infection.

Spleen injection

Single-cell suspensions were prepared from CT-26 monolayer cultures, washed with PBS (BioSera, Boussens, France), and diluted in Medium 199 (Lonza). 2×10^3 tumor cells were injected in a volume of 50 µl into the spleen of adult male inbred Balb/c mice from where metastatic colonies formed in the liver. Under anaesthetization, the skin was shaved and rubbed with ethanol pads. Next, a 6-8 mm incision was made adjacent to the spleen (a left flank incision approximately 2 cm left of the abdominal midline). The spleen was gently pulled out of the abdominal cavity and 50 µl of cell suspension was injected into the lower third of the spleen. After that, the spleen was carefully reinserted and the abdominal wall and skin were surgically closed. Control animals were injected with 50 µl medium. The treated animals were sacrificed on day 22 after tumor inoculations. Tumors were evaluated macroscopically for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry (IHC) was performed as described previously by our group with some modifications (Neuperger et al., 2021; Puskas et al., 2016). Briefly, mice were sacrificed at 22 days. Livers and spleens were removed and fixed overnight in 4% formalin, then embedded in paraffin, and cut into 3 μm sections with Rotary microtome (RM2235, Leica). IHC was performed on the platform of the Leica company, BOND-MAX Immunohistochemical staining machine. The primary antibodies used for IHC were rabbit polyclonal anti-arginase-1 (GenomeMe, IHC400-100, 1:800), rabbit polyclonal anti-CD45 antibody (Biocare, CM016B, 1:200), rabbit polyclonal anti-CDX2 antibody (Cellmarque, 235R-15, 1:200) rabbit polyclonal anti-pan-cytokeratin antibody (Cellmarque, 313M-14, 1:600, rabbit polyclonal anti-vimentin antibody (Novocastra, NCL-L-VIM-V9, 1:500) with overnight incubation. Labeling system (Bond Polymer Refine Detection, DS9800, Leica) containing anti-rabbit seconder antibody labeled with horseradish peroxidase (HRP), and DAB-3 (3′-diaminobenzidine) was used as the chromogen for antigen signal detection. Hematoxylin (ready to use, Leica) was used for contrast staining. The Zeiss Axio Imager Z1 microscope (ocular 10×, objectives 10×, 40×) was used for visualization with the Zeiss AxioCam MRm camera and AxioVision SE64 4.9.1 software (Carl Zeiss AG, Oberkochen, Germany).

Peripheral white blood cell isolation

Leukocytes were isolated as described previously (Balog et al., 2019). Briefly, from the blood via cardiac puncture from mice freshly. EDTA-treated whole blood was centrifuged 400 x g for 10 min, the supernatant was removed. Red blood cell lysis was carried out by the incubation of the cells with 5 mL ACK buffer (0.155 M NH4Cl, 10 mM KHCO3, 0.1 mM Na2EDTA, pH 7.3, GIBCO, Cat. A1049201, Thermo Fischer Scientific, New York, USA) solution at RT for 5 min. Samples were loaded on a cell strainer (70 μm in pore size) and washed twice with 10 mL PBS. Cells were resuspended and pipetted into 12 × 75 mm FACS tubes (VWR International, Pennsylvania, USA) and diluted with 100 μl staining buffer (PBS with 1% FBS, GIBCO, Life Technologies, Paisley, UK, and 0,1% sodium azide, Sigma-Aldrich, Saint Louis, USA).

Spleen cell isolation

Spleen was processed freshly as described previously with minor modification (Kotogany et al., 2020). Briefly, after the spleen was smashed on a 100 μm cell strainer, (VWR, Radnoe, PA, USA), washed with PBS, and centrifuged at 1400 rpm for 5 min, the pellet was resuspended in sterile PBS. Red blood cell lysis was carried out by the incubation of cells with 5 mL ACK (0.155 M NH4Cl, 10 mM KHCO3, 0.1 mM Na2 EDTA, pH 7.3, GIBCO, Cat.

A1049201, Thermo Fischer Scientific, New York, USA) solution for 5 min. Samples were loaded on a cell strainer (70 µm in pore size,) and washed twice with 20 mL PBS. After that cells were resuspended and pipetted into 12 × 75 mm FACS tubes (VWR International, Pennsylvania, USA) and diluted with 100 µl staining buffer (PBS with 1% FBS, GIBCO, Life Technologies, Paisley, UK, and 0,1% sodium azide, Sigma-Aldrich, Saint Louis, USA).

Lectin labeling

For fluorescent labeling of lectins, conjugation kits (Lightning-Link, Abcam, Cambridge, US) were used according to the manufacturer's instructions. Aleuria Aurantia lectin (AAL, Cat. L-1390-2, Vector Laboratories, Newark, USA) was labeled with APC (ab201807, Lot.GR3388854-1), Sambucus nigra lectin (SNA, Cat. 21510104 Glycomatrix, Ohio, USA) was labeled with PE/Cy7 (ab102903, Lot. GR3390407-1), human sialoadhesin, Siglec-1/CD169 recombinant protein (Siglec-1, Cat. 5197SL050, Fischer Scientific, Massachusetts, USA) were labeled with PE/Texas Red (ab269899, Lot. GR3395603-2), galectin-1 (Gal1, Cat. 450-39, PreproTech, London, UK) was labeled with Alexa Fluor 488 (ab236553, Lot. GR3394511-1), galectin-3 (Gal3, 450-38, PreproTech, London, UK) was labeled with APC/Cy7 (ab102859, Lot. GR3396716-1), Phytolacca americana lectin (PWM, Cat. L8777, Sigma-Aldrich, Saint Louis, USA) was labeled with Alexa Fluor 700 (ab269824, Lot. GR3393759-2) dye by the lectin amine groups. The list of the selected lectins for cytometry is listed in Table 3. Before experiments, we optimized the concentration of antibodies and labeled lectins by titration on a flow cytometer (C40313, CytoFLEX LX N3-V5-B3-Y5-R3-I0, Beckman Coulter, Indiana, USA).

Lectin agglutination test

After euthanizing the animals, the spleens were removed and homogenized freshly on a cell strainer (70 µm pore size, Merck Millipore Burlington, Massachusetts, USA) using the piston of a syringe and sterile PBS. Cells were pelleted by centrifugation at 1500 rpm for 5 min. The pellet was resuspended in 5 mL ACK lysis buffer for 5 min. Samples were loaded on a cell strainer (70 µm in pore size) and washed with 20 mL PBS. Cells were counted using a Bürker chamber and trypan blue viability dye (Thermo Fischer Scientific, Waltham, Massachusetts, USA), and 100.000 cells were plated on 96 well plates (Corning, New York, USA) for lectin agglutination assay and microscopically evaluation (HoloMonitor™ M3). Agglutination assay consisted of four different concentrations (0,1 µg/ml; 1 µg/ml; 10 µg/ml and 50 µg/ml) in RPMI (GIBCO, Life Technologies, Paisley, UK). Lectins were diluted in sterile PBS with 0,1% sodium azide (Sigma-Aldrich, Saint Louis, USA). The lectins involved in the study were used below the concentrations, where the agglutination activity of leukocytes were observed.

Staining and flow cytometry

Cell surface staining was performed as described previously by our group with some modifications (Honfi et al., 2022; Szebeni et al., 2022). Briefly, the antibody and lectin cocktail for extracellular staining was added to the freshly isolated white blood cells in 100 µl staining buffer (PBS, 1% fetal bovine serum, Gibco, Life Technologies, Paisley, UK, 0,1% sodium azide, Sigma-Aldrich, Saint Louis, USA). The fluorescently labeled lectins were titrated on healthy leukocytes in 2.5 µg/ml, 1 µg/ml, 0.5 µg/ml, and 0.1 µg/ml as shown in Supplementary Figure 1. The antibody cocktail consisted of 80× dilutions of eFluor 450 anti-mouse CD3 (Cat. 48-0032-82, Clone 17A2, Thermo Fischer Scientific, Waltham, Massachusetts, USA), BV605 anti-mouse CD11b(Cat. 101237, clone M1/70, BioLegend, San Diego, USA), BV650 anti-mouse CD19 (Cat. 115541, clone 6D5, BioLegend, San Diego, USA), PerCP/Cyanine5.5 anti-mouse CD45 (Cat. 103132, Clone 30-F11), 100× dilution of AAL, Gal1, Siglec-1, 10× dilution of SNA, 5× dilution of PWM, Gal3, HPA and 1000× dilution of Viability fixable dye eFluor 506 (Cat. 65-0866-14, Thermo Fischer Scientific, Waltham, Massachusetts, USA). Cells were incubated at 4°C for 30 min. All antibodies were used according to the manufacturer's instructions. Cells were washed with 500 µl PBS, and centrifuged at 500g for 5 min. Cells were resuspended in 400 µl PEB and were measured on Cytoflex LX fluorescence-activated cell sorter (C40313, CytoFLEX LX N3-V5-B3-Y5-R3-I0, Beckman Coulter, Indiana, USA) and analyzed using the CytExpert v.2.4.0.28 software (Beckman Coulter, Indiana, USA).

Proteomic characterization

Liver and spleen tissue samples were homogenized in 10% SDS / 50 mM TEAB using a TissueLyser (Qiagen). The resulting lysates were centrifuged (14,000 rcf for 10 min), the protein content of the supernatant was determined by the BCA colorimetric assay (Thermo Scientific) and 100 µg protein was digested according to the S-Trap mini protocol (https://protifi.com/pages/protocols). Briefly, protein disulfide bridges were reduced using TCEP (Tris(2-carboxyethyl)phosphine) and free thiol groups blocked by MMTS (S-methyl methanethiosulfonate) followed by a 2 h digestion with sequencing grade trypsin (2.5 µg/sample) at 47 °C. The resulting peptide mixtures were dried down and then labeled with TMTpro 16plex isobaric tags (Thermo Scientific) following the producer's protocol. Samples were combined in a 1:1 ratio and fractionated off-line using a high pH reversed-phase peptide fractionation kit (Thermo Scientific) according to the producer's protocol. Fractions were combined to yield four final fractions (fractions 1+5, 2+6, 3+7, 4+8), vacuum-dried, and approximately 1 ug total peptide amounts were loaded onto C18 EvoTips (Evosep) for LC-MS analysis. Reversed-phase separation of the peptides was performed using an

Evosep One HPLC (Evosep) applying the „15 SPD" 88-min gradient followed by SPS-MS3 data acquisition using an Orbitrap Fusion Lumos Tribrid (Thermo Scientific) instrument equipped with an FAIMS Pro ion mobility device. Data were collected using two compensation voltages (-70 and -50 V) in alternating 1.5 sec cycles. High resolution MS3 data were generated from the top 6 most abundant fragment ions isolated simultaneously from each CID MS2 acquired in the linear ion trap.
Peptide identification and quantitation were done using the Proteome Discoverer software (v3.0). Peptides and proteins were identified with the Sequest HT search engine using the mouse subset of the UniProt protein database (v2023-08-07, 17734 sequences). Relative quantitation was performed using the TMT reporter ion signal-to-noise (S/N) values extracted from MS3 HCD spectra. Unique and razor peptides were considered for protein abundance-based comparison of the tumor and control sample groups (acceptance parameters: Sequest HT Xcorr>1, precursor ion co-isolation threshold in MS1 <50%, average TMT reporter S/N in MS3 HCD spectra >10, SPS mass matches in MS2 >50%. Proteins identified with high confidence showing |log2FoldChange|>1 (adjusted p-Value≤0.05). were accepted as differently expressed.

SLE patients

SLE patients meeting the 2019 European League Against Rheumatism/American College of Rheumatology classification criteria were included if they had active disease (either newly diagnosed or relapsing) as defined as a SLEDAI-2K score ≥ 6 (Aringer et al., 2019; Gladman et al., 2002). Patients with a concurrent other inflammatory condition (e.g. infection) or overlapping systemic autoimmune disease except antiphospholipid syndrome or Sjögren's syndrome with clinically active extra glandular manifestations were excluded. Newly diagnosed patients were therapy-naïve, whereas relapsing patients were on maintenance immunosuppressive therapy, but corticosteroid dose at least 5 mg/day prednisolone was also an exclusion.

Ethical statement

Patients were recruited during visits at the Department of Rheumatology and Immunology (University of Szeged). Healthy controls were voluntary employees of the BRC or University of Szeged. Subjects were informed about the research by a physician. Written informed consent was obtained from all subjects, and our study was reviewed and approved by an independent ethical committee of the university. Details about the study design and handling of biological materials were submitted to the Human Investigation Review Board of the University of Szeged under the 21/2011 and 149/2019-SZTE Project Identification codes. Laboratory studies and interpretations were

performed on anonymized samples lacking personal identifiers. The study adhered to the tenets of the most recent revision of the Declaration of Helsinki.

PBMC isolation from SLE patients

PBMCs were isolated as described previously by our group (Gemes et al., 2023; Neuperger et al., 2023). Briefly, after the collection of 20 mL peripheral blood into an EDTA vacutainer (Becton Dickinson, Franklin-Lakes, USA), PBMCs were isolated by standard density gradient centrifugation using Leucosep tubes (Greiner Bio-One, Kremsmünster, Austria). Residual red blood cells were lysed suspending the PBMC pellet in 2 mL Ammonium-Chloride-Potassium Lysis Buffer (ACK: 0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.3; Merck, Darmstadt, Germany) and incubating at room temperature (RT) for 2 min. Samples were washed twice with 10 mL phosphate-buffered saline (PBS, Merck), and subsequently, cell count and viability were determined with a trypan blue (Merck) exclusion test. Aliquots of 4×10^6 PBMCs were subjected to cryopreservation in Fetal Bovine Serum (FBS, Capricorn Scientific, Ebsdorfergrund, Germany) containing 10% (v/v) dimethyl sulfoxide (DMSO, Merck) in liquid nitrogen (Messer, Bad Soden, Germany).

Data visualization and statistics

GraphPad Prism software (version 8.0.1 for Windows, GraphPad Software) was used for FACS data visualization. For flow cytometry statistical analysis was performed using two-tailed, heteroscedastic Student's t-test to evaluate the statistical significance (set at * $p < 0.05$) between two given experimental groups: Pairwise comparison of each sample. Figure illustrations and visualizations were created with the BioRender web-based platform.

RESULTS

Lectin pattern of murine CRC

There were significant differences in median fluorescence intensity (MFI) in the binding of the lectins to three different types of immune cells of the CRC-bearing mice compared to healthy controls. Binding of AAL to fucose/arabinose structures was significantly higher on the cell surface of all three immune cell types of mice's spleen with induced CRC versus healthy control. However, binding of AAL to PBMCs was significantly lower in the case of CD11+ myeloid cells. Binding of SNA to sialic acid moiety was significantly higher on the CD3+ T cells and CD11b+ myeloid cells surface of mice with CRC versus healthy control. PWM, N-Acetylglucosamine binding lectin showed significant differences in binding to cell surfaces. PWM binding was significantly higher in the case of the spleen and blood on CD11b+ myeloid cells. Binding of siglec-1 to sialic acid was significantly lower on the CD11b+ myeloid cells surface on the CD19+ B cells surface of spleen-derived cells vs. controls. N-acetyllactosamine binding galectin-1 was significantly lower on the CD11b+ myeloid cells of the blood

We also found significant differences in the percentage of reactive cells in three different types of immune cells of the CRC-bearing mice compared to healthy controls. The number of siglec-1 binding cells was significantly higher in all three types of immune cells in the case of spleen samples compared to the healthy controls. Besides, the SNA binding cell number was significantly higher at CD11b+ and CD19+ cells from the spleen of CRC-bearing mice vs. controls, but in the case of PBMCs, the number of SNA reactive cells was significantly lower at CD3+ T cells of CRC bearing mice compared to the healthy controls. PWM binding CD11b+ myeloid cells and CD19+ B-lymphocytes showed a significant increase in PBMCs of CRC mice compared to healthy control. Gal1 binding showed a significantly increased number of cells on CD3+ T cells in the case of spleen samples, however, Gal3 binding was lower on CD3+ T cells of PBMC samples in mice with CRC vs. controls.

Proteomic analysis of murine CRC samples

The proteomic analysis was performed at the Laboratory of Proteomics Research, HUN-REN Biological Research Centre. For whole proteome analysis, The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used and The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis revealed that most metabolic traits were changed in CRC such as biosynthesis of amino acid, glycolysis/gluconeogenesis, carbon metabolism, complement and coagulation cascade. In metastatic liver total changes affected 2164 proteins, where 730 decreased and 1434 increased significantly in relation to healthy control liver. From this, we focused on the proteins involved in glycosylation and we collected endogenous lectins, that showed significant changes compared to healthy controls. Galectin-1, galetin-3, and C-type mannose receptor 2 showed a significantly increased amount in both the liver and spleen, however, galectin-9 decreased significantly in both organs compared to the healthy control. Among the enzymes directly involved in forming the glycosylation pattern, elevated levels were observed in both the glucosyltransferases, galactosyltransferases, mannosyltransferases, and fucosyltransferases.

Flow cytometry analysis results of SLE patients

There was a significant increase in the binding of AAL and Gal3 binding in CD3 T-cells and CD4 T-cells. Additionally, SLE CD8high T-cells exhibited heightened Gal3 binding compared to their HC counterparts. Total CD3 T-cells including CD4 T-cells displayed significantly increased AAL, Gal1, SNA and Siglec 1 binding in SLE. CD8 T-cells including their major subset CD8high T-cells were characterized by significantly elevated AAL, SNA and Siglec 1 binding in SLE whereas SLE CD8dim T-cells also bound significantly higher amounts of SNA and Siglec 1. DNT-cells only showed a notable increase in Siglec 1 binding in SLE. In the case of NK cell subsets in a quiescent state, significant elevations were observed in Gal3 binding in total CD56 NK-cells and CD56high NK-cells. In resting classical monocytes (MO) of SLE patients, there was a significant reduction in Gal1, Gal3 and SNA binding.

SUMMARY

Altering in glycan patterns modulates immunological responses, such as inflammation, and immune response in different diseases, including cancer. Lectins can selectively recognize carbohydrate chains and bind them reversibly. Due to this characteristic feature, like high selective specificity, lectins are investigated in mapping unique carbohydrate chains. To analyse glycan code of immune cells, we built a fluorescent dye-labeled lectin-based multicolor flow cytometry panel and optimized it successfully. We established a murine CRC model to investigate the immune cell population's altered glycosylation pattern in cancer. Immunophenotyping of leukocytes isolated from CRC-bearing BALB/c mice or healthy controls, such as CD19+ B-cells, CD11+ myeloid cells, and CD3+ T-cells was carried out using fluorochrome-labeled lectins. The individual carbohydrate-binding lectins were labeled with different fluorescent dyes, thus enabling parallel mapping of the glycan code. The binding of previously selected six lectins to white blood cells, such as galectin-1 (Gal1), siglec-1 (Sig1), *Sambucus nigra* lectin (SNA), *Aleuria aurantia* lectin (AAL), *Phytolacca americana* lectin (PWM), and galectin-3 (Gal3) was assayed. Flow cytometric analysis of the splenocytes revealed the increased binding of SNA, and AAL to CD3+T-cells and CD11b myeloid cells; and increased siglec-1 and AAL binding to CD19 B-cells of the tumor-bearing mice. The whole proteomic analysis of the established CRC liver and spleen identified differentially expressed proteins. We focused on endogen lectins and enzymes participating in glycosylation, and we identified numerous significantly changed enzyme abundances in CRC tissues compared to healthy controls. On the other hand, we successfully utilized the lectin-based multicolor panel to investigate the glycosylation patterns of immune cell populations in human patients with systemic lupus erythematosus. This study showed that altered core fucosylation emerged as a prominent feature of SLE immune subsets.

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