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Lectin flow cytometry for cell surface glycan profiling: an accessible approach to Glycophenotyping

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Lectins are widely used to detect glycan-associated features on the cell surface; however, their implementation in flow cytometry often requires empirical optimization and methodological considerations that are not always systematically described. Here, we provide a practical framework for implementing lectin-based glycophenotyping in flow cytometry, addressing both the experimental decisions involved in assay optimization and validation and its integration into conventional multiparametric workflows, highlighting its potential to address more complex biological questions. Using illustrative examples from murine splenocytes, we discuss key aspects of lectin titration, fluorochrome selection, negative controls, and specificity validation, and demonstrate the compatibility of lectin staining with commonly used flow cytometry applications, including viability assessment, immunophenotyping, intracellular cytokine detection, and proliferation analysis. By integrating methodological considerations with representative applications, this Technical Note provides a practical resource to guide experimental decision-making and facilitate the incorporation of lectin-based glycophenotyping into multiparametric flow cytometry workflows.

Keywords flow cytometry, lectins, glycan analysis, multiparametric analysis, cell surface glycans.

Introduction

Lectins are among the most accessible tools for investigating glycan-associated features. Their ability to recognize specific carbohydrate motifs has enabled the identification of glycosylation patterns in a wide variety of biological systems. Flow cytometry, in turn, offers several advantages for lectin-based analyses, including single-cell resolution, rapid multiparametric acquisition, and the possibility of combining glycan detection with phenotypic and functional markers.

Several studies have successfully used lectins in flow cytometry to analyze glycan-associated features in diverse biological systems, including bacteria (Hendrickson et al. 2019), mammalian cell lines (Kaku et al. 1993; Xiao et al. 2016), spermatozoa (Kekäläinen et al. 2015), and multiple leukocyte populations (Malin-Berdel et al. 1984; Amado et al. 2004; Gibadullin et al. 2017). More recently, multiparametric approaches have revealed associations between specific glycophenotypes, cellular activation states, and immune functions (Cabral et al. 2017; Sierra-Ulloa et al. 2024; Szabó et al. 2024), highlighting the value of flow cytometry as a platform for single-cell glycan analysis.

Although practical guidance for lectin-based flow cytometry has been previously described (Moriwaki and Miyoshi 2014; Ishigaki and Itoh 2022; Torres et al. 2023), many experimental considerations remain distributed across methodological reports and biologically focused studies. Consequently, investigators seeking to incorporate lectins into multiparametric flow cytometry experiments may face challenges not only in establishing appropriate staining conditions but also in identifying how lectin-based glycophenotyping can be integrated into conventional phenotypic and functional assays. To address these aspects, we provide a practical framework that brings together key considerations for assay optimization and validation (Fig. 1), alongside representative examples illustrating how lectin staining can be integrated with commonly used flow cytometry applications to expand the range of biological questions that can be addressed (Fig. 2). Using murine splenocytes and lymphocyte populations, we illustrate how lectin-based glycophenotyping can complement conventional flow cytometry workflows. Together, these recommendations aim to facilitate the incorporation of glycan-associated information into routine experimental designs.

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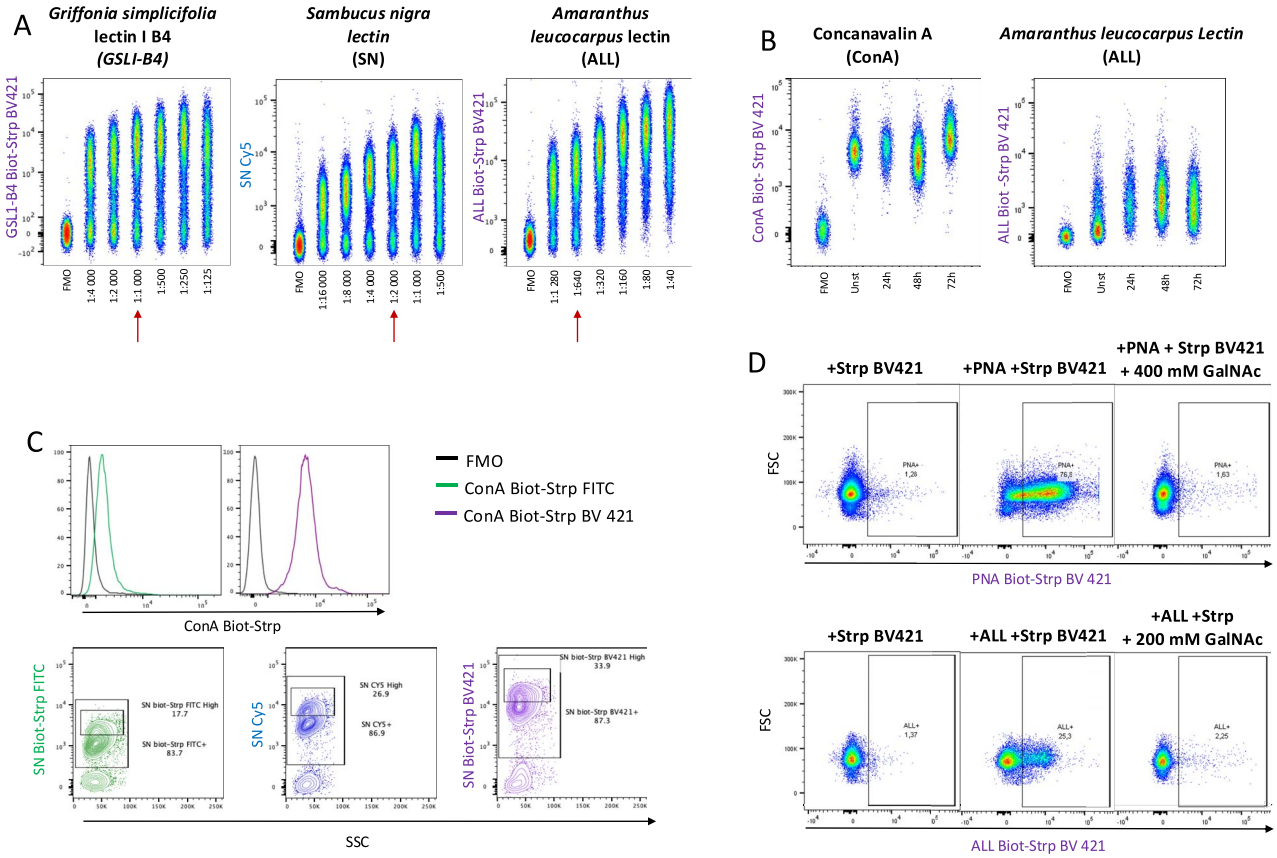

Figure 1 Optimization and validation of lectin-based flow cytometry. Representative examples illustrating key considerations for implementing lectin-based glycophenotyping by flow cytometry. (A) Titration of *Griffonia simplicifolia* lectin I B4 (GSLI-B4), *S. nigra* lectin (SN), and *A. leucocarpus* lectin (ALL) using serial dilutions. Concatenated data from viable, single lymphocytes are shown; arrows indicate the selected working dilutions. Each lectin was titrated independently using splenocytes from a different mouse ($n = 1$ per lectin). (B) Lectin binding in unstimulated (Unst) and activated murine splenocytes stained with concanavalin A (ConA) or ALL. Data shown were obtained from a single mouse ($n = 1$). (C) Comparison of fluorochromes and staining strategies using ConA and SN. Directly conjugated and indirect staining approaches are shown. Representative data from three biological replicates ($n = 3$ mice). (D) Competitive inhibition assays performed using peanut agglutinin (PNA) or ALL in the presence or absence of N-acetylgalactosamine (GalNAc). Data shown were obtained from a single mouse ($n = 1$). Lectin specificities are indicated in supplementary table 1, and detailed direct and indirect staining workflows are provided in supplementary protocols 1 and 2. Flow cytometry abbreviations: FMO, fluorescence minus one; FSC, forward scatter; SSC, side scatter; biot, biotinylated; Strp, streptavidin; FITC, fluorescein isothiocyanate; BV421, brilliant violet 421; Cy5, cyanine 5.

Materials and methods

Materials and methods can be found in Supplementary Data online.

Results and discussion

Optimization and validation of lectin-based flow cytometry

Successful implementation of lectin-based flow cytometry requires the same level of optimization and validation routinely applied to antibody-based assays (Bonilla et al. 2024). Although lectins differ from antibodies in their biochemical nature, when used as detection probes, their performance is influenced by reagent concentration, target abundance, fluorochrome selection, and experimental conditions. Therefore, assay optimization should be considered an essential step before integrating lectins into multiparametric analyses.

A critical first step in lectin-based staining begins with proper reagent titration. Because lectins differ in affinity, valency, and glycan specificity, optimal working concentrations cannot be extrapolated between reagents. To illustrate this, murine splenocytes were stained with serial dilutions of *Griffonia simplicifolia* lectin I B4 (GSLI-B4), *Sambucus nigra* lectin (SN), and *Amaranthus leucocarpus* lectin (ALL) (Fig. 1A). All lectins displayed concentration-dependent staining patterns; however, increasing concentrations did not invariably improve performance. Whereas insufficient concentrations failed to adequately resolve positive populations, excessive concentrations increased background staining and reduced the separation between positive and negative cells. High lectin concentrations may also promote cell aggregation. This effect is lectin- and concentration-dependent and has been experimentally observed at high concentrations, in some cases preventing subsequent flow cytometric analysis of the agglutinated cells. (Farago et al. 2024). Lectin exposure may also affect cell viability or initiate cellular responses, depending on the lectin and cell type. Such effects can occur within relatively short

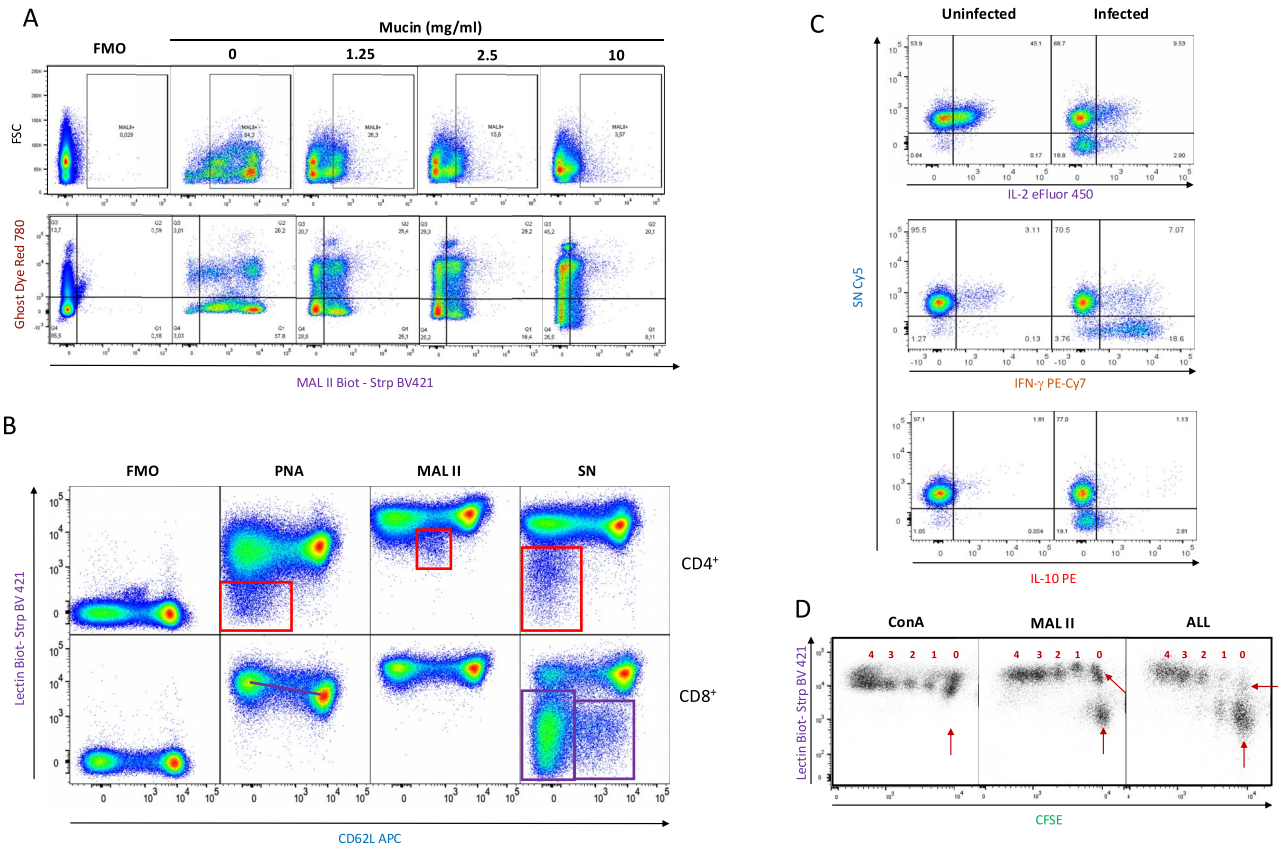

Figure 2 Integration of lectin staining into multiparametric flow cytometry workflows. Representative examples illustrating the compatibility of lectin-based glycophenotyping with conventional flow cytometry applications. (A) Co-staining of murine splenocytes with *Maackia amurensis* lectin II (MAL II) and the fixable viability dye Ghost Dye Red 780 in the presence of increasing concentrations of mucin. Representative data from three biological replicates ($n = 3$ mice). (B) Combined glyco- and immunophenotyping using lectins and antibodies against CD4, CD8, and CD62L; the complete gating strategy is shown in supplementary fig. 1. Data were obtained from a single mouse ($n = 1$). (C) Simultaneous detection of *S. nigra* lectin (SN) binding and intracellular IL-2, IFN-γ, and IL-10 in CD4$^+$ T cells from uninfected and *Toxoplasma gondii*-infected mice. Representative data from three independent experiments, including at least two mice per group in each experiment. Reproduced from fig. 4A of Sierra-Ulloa et al. (2024), originally published in Frontiers in Immunology under the terms of the creative commons attribution (CC BY) license. (D) Combination of lectin staining with CFSE-based proliferation analysis using ConA, MAL II, and ALL; data from viable and single lymphocytes are shown. Numbers indicate successive cell generations, with generation 0 representing undivided cells. Representative data from three biological replicates ($n = 3$ mice). Flow cytometry abbreviations: FMO, fluorescence minus one; FSC, forward scatter; biot, biotinylated; Strp, streptavidin; BV421, brilliant violet 421; Cy5, cyanine 5; CFSE, carboxyfluorescein succinimidyl ester; APC, allophycocyanin; PE, phycoerythrin; PE-Cy7, phycoerythrin-cyanine 7.

incubation periods: reduced viability has been observed after 1 h exposure to some, but not all, plant lectins, while early cellular responses to mitogenic lectins can be detected within minutes (Halliday et al. 1981; Ishiguro et al. 1992). Thus, lectin titration should consider not only population resolution and background fluorescence, but also sample integrity and the recovery of single-cell events. Each lectin should therefore be titrated independently under the experimental conditions intended for subsequent analyses, including cell type, incubation time and temperature, and staining buffer. In our laboratory, titration is routinely performed for each new lectin lot to establish appropriate staining conditions. These considerations are particularly important when viable cells will subsequently be used for functional analyses. Importantly, differences in staining behavior were observed not only between commercially available lectins and the in-house purified ALL, but also among lectins recognizing distinct glycan structures, reinforcing the importance of empirically determining optimal staining conditions for each lectin rather than extrapolating them from other reagents.

Cellular state represents an additional variable that may substantially influence lectin binding since glycan expression is highly dynamic and can change during activation, differentiation, or other biological processes. This is depicted in Fig. 1B, where Concanavalin A (ConA) displayed strong binding to both unstimulated and activated splenocytes, whereas ALL binding markedly increased following activation with the highest staining observed at 48 h of stimulation. These observations highlight the importance of performing assay optimization under conditions in which the glycan structures recognized by the lectin are expected to be present and accessible; otherwise, relevant glycan-associated populations may remain undetected or underestimated. Consequently, selecting appropriate biological controls may be as important as selecting the lectin itself.

Fluorochrome selection is another critical parameter that directly affects signal resolution. Using ConA and SN as examples, we compared different fluorochromes and detection strategies (Fig. 1C). In both cases, brighter fluorochromes improved population separation and facilitated the identification of lectin-binding

subsets. This effect was particularly evident for SN, where the use of very bright fluorochromes like Brilliant Violet 421 (BV 421) enabled the visualization of a distinct SN^{high} population. These results highlight that fluorochrome selection should be considered not only from a panel-design perspective but also as a factor influencing glycophenotyping sensitivity and resolution. Thus, optimal fluorochrome selection ultimately depends on the experimental objective; while in some applications discrimination of the major positive population may be sufficient, in others identification of minor lectin-defined subsets may justify the use of brighter fluorochromes or indirect staining strategies.

In addition to fluorochrome selection, reliable discrimination of lectin-positive populations also requires appropriate negative controls**.** In multiparametric experiments, fluorescence-minus-one (FMO) controls provide an appropriate reference for defining positive populations, as they account for the fluorescence spread contributed by the remaining fluorochromes in the panel (Roederer 2001; Cossarizza et al. 2021). FMO controls should therefore contain all components of the staining panel except the lectin being evaluated. Unstained samples can additionally be used to assess cellular autofluorescence but do not reproduce the fluorescence spread present in a multicolor panel. When indirect lectin detection systems are used, a detection-reagent-only control, in which the lectin is omitted but the fluorescent secondary or detection reagent is retained, should also be included to assess background introduced by the detection system (Bowman et al. 2013).

A final recommendation is to validate lectin specificity whenever possible. Competitive inhibition assays using soluble carbohydrates provide a straightforward strategy to confirm glycan-dependent binding, particularly when working with novel applications or in-house reagents. As shown in Fig. 1D, the addition of GalNAc markedly reduced the binding of both Peanut Agglutinin (PNA) and ALL, returning fluorescence levels to those observed in control samples. Although lectin specificity has been extensively characterized by biochemical approaches, incorporating inhibition controls into flow cytometry workflows provides an additional level of confidence when interpreting lectin-binding patterns. While competition assays do not provide the structural resolution achieved by glycomic approaches such as MS or LC–MS, they represent an accessible validation strategy that can be readily incorporated into routine flow cytometry experiments.

Taken together, these observations demonstrate that successful lectin-based flow cytometry relies on a combination of reagent optimization, biological context, appropriate fluorochrome selection, and validation of binding specificity. Rather than independent technical considerations, these parameters should be viewed as complementary components of assay design, as each one can substantially influence data quality, interpretation, and ultimately the biological conclusions derived from glycophenotyping analyses. Importantly, lectin-based flow cytometry provides relative assessments of glycan-associated features at the single-cell level and should therefore be interpreted considering the binding specificity of each lectin and the biological context of the sample.

In addition to assay optimization, implementation details may also influence staining performance and reproducibility. To facilitate adoption of lectin-based approaches, Supplementary Protocols S1 and S2 provide representative workflows for direct and indirect lectin staining, respectively, including reagent order, incubation steps, and conditions. While individual laboratories may adapt these procedures to specific experimental requirements, they provide a practical starting point for incorporating lectins into routine flow cytometry analyses.

Integration of lectins into multiparametric flow cytometry workflows

One of the principal advantages of flow cytometry is its ability to simultaneously interrogate multiple cellular characteristics at the single-cell level. Therefore, beyond establishing appropriate staining conditions, an important consideration is whether lectin-based glycophenotyping can be readily integrated into conventional multiparametric workflows. To illustrate this, we present representative examples integrating lectin staining with commonly used flow cytometry applications, including viability assessment, immunophenotyping, intracellular cytokine detection, and proliferation analysis, highlighting the potential of these combinations to address more complex biological questions (Fig. 2).

The incorporation of viability dyes is an essential component of flow cytometry experiments, allowing the exclusion of non-viable cells and preventing potential misinterpretation of staining patterns. As illustrated in Fig. 2A, the combination of MAL II staining with Ghost Dye Red 780 enabled the simultaneous assessment of lectin binding and cell viability in murine splenocytes. Increasing concentrations of mucin progressively inhibited MAL II binding and were accompanied by a reduction in cell viability. Importantly, the inclusion of a viability dye allowed lectin binding to be evaluated separately in viable and non-viable populations, highlighting the importance of excluding non-viable cells for accurate data interpretation. We therefore recommend incorporating viability staining at the end of the surface staining sequence, when compatible with the viability reagent used (Pardo et al. 2026). This approach allows exclusion of non-viable cells after surface staining, thereby accounting for loss of viability that may occur during sample manipulation or incubation with the lectin or other assay reagents, including competitive inhibitors.

Lectin staining was also readily combined with conventional immunophenotyping markers. Simultaneous analysis of PNA, MAL II, and SN together with CD4, CD8, and CD62L revealed distinct lectin-binding patterns within defined T-cell subsets (Fig. 2B). In several cases, populations displaying differential lectin binding were associated with changes in CD62L expression, illustrating how glycophenotyping can complement traditional immunophenotypic analyses. Rather than replacing classical surface markers, lectins provide an additional layer of information that may reveal cellular heterogeneity not readily captured by protein expression alone. Importantly, these experiments also demonstrate that lectin staining can be incorporated into standard antibody panels while preserving the identification of conventional immunophenotypic populations. For applications requiring simultaneous staining with multiple lectins, specific approaches for the design and implementation of multicolor lectin panels have been described elsewhere (Torres et al. 2023).

When lectins are combined with antibody-based immunophenotyping, potential interactions between the two staining systems should be considered. Antibodies are glycoproteins, and lectin binding to antibody-associated glycans has been experimentally

demonstrated in other assay formats such as antibody-lectin glycan assays, where it can contribute to background signal (Shang et al. 2016). However, this potential interaction does not necessarily translate into detectable interference in cell-based multiparametric staining, and several studies have successfully combined lectins and antibodies for flow cytometric immunophenotyping (Cabral et al. 2017; Ma et al. 2022; Sierra-Ulloa et al. 2024). To minimize the possibility that antibody-associated glycans contribute to lectin binding, we recommend sequential staining with the lectin before addition of the antibody panel, with appropriate washing between steps, as described in Supplementary Protocols S1 and S2.

Because many flow cytometry studies seek to evaluate cellular function in addition to phenotype, compatibility with intracellular staining protocols is another important consideration. To illustrate this, previously published data from *Toxoplasma gondii*-infected mice are reproduced (Sierra-Ulloa et al. 2024). As shown in Fig. 2C, SN staining was successfully combined with intracellular detection of IL-2, IFN-γ, and IL-10, revealing distinct cytokine profiles associated with SN-defined subpopulations. While the biological significance of these patterns was explored in the original study, the present example illustrates a broader technical principle: lectin-defined populations can be interrogated using the same functional assays routinely employed in immunological studies. This expands the utility of glycophenotyping beyond descriptive analyses and facilitates the incorporation of glycan-associated information into conventional experimental workflows.

Finally, lectin staining was combined with CFSE-based proliferation assays to evaluate whether glycan-associated features could be monitored during cell division (Lyons 2000). ConA, MAL II, and ALL each displayed distinct binding patterns throughout the proliferative response (Fig. 2D). Whereas ConA staining remained relatively stable across successive generations, ALL and MAL II revealed more dynamic patterns associated with the proliferative response. These observations demonstrate that lectin binding can be analyzed simultaneously with cell tracking dyes and that changes in lectin-binding patterns can be monitored throughout the course of a proliferative response. From a technical perspective, this approach expands the range of biological questions that can be addressed using lectin-based flow cytometry without requiring substantial modifications to established proliferation protocols.

Collectively, these examples demonstrate that lectin staining can be integrated into many of the assays routinely performed by flow cytometry. Although lectin-based approaches do not provide the structural resolution achieved by other glycomic methodologies, they offer a practical means of incorporating glycan-associated information into single-cell level phenotypic and functional analyses. This combination represents one of the major strengths of lectin-based flow cytometry and contributes to its value as an accessible tool for glycophenotyping studies.

Concluding remarks

Flow cytometry has transformed the way cellular heterogeneity is studied by allowing the simultaneous analysis of multiple phenotypic and functional parameters at the single-cell level. However, glycan-associated features remain underrepresented in many experimental designs despite their recognized role in cellular communication, activation, differentiation, and immune regulation. One contributing factor may be the methodological considerations involved in optimizing lectin staining and integrating it into established workflows.

Lectin-based flow cytometry provides a valuable opportunity to investigate glycan-associated features in the context of cellular identity and function. By combining lectin staining with conventional immunophenotyping and functional assays, glycophenotyping can be performed within well-defined cell populations, including minor subsets that may be overlooked in bulk analyses. This is particularly relevant in immunology, where closely related cell populations can display distinct differentiation states and functional properties. Moreover, the analysis of large numbers of individual cells enables detailed characterization of lectin-binding distributions and cellular heterogeneity, providing information that may be obscured when heterogeneous samples are analyzed as a whole.

Nevertheless, the interpretation of lectin-based flow cytometry data should consider that this approach does not determine complete glycan structures, distinguish between different types of glycosylation or identify the molecular carriers of the recognized motifs. While lectin specificity provides information about the glycan motifs recognized, these motifs may be carried by glycoproteins, glycolipids, or glycosylated RNA. Furthermore, differences in lectin binding may reflect changes in the abundance, accessibility, or presentation of the recognized glycan motifs, rather than changes in glycan abundance alone. These considerations are essential when interpreting lectin-defined glycophenotypes and assigning biological significance to differences in staining.

The examples presented here illustrate that glycophenotyping can be incorporated into conventional flow cytometry analyses using the same experimental logic routinely applied to antibodies and other fluorescent probes. By integrating optimization strategies, validation approaches, and representative applications within a single framework, we aim to reduce some of the practical barriers that currently limit the incorporation of glycan-associated information into routine flow cytometry studies. Ultimately, incorporating lectin-defined glycophenotypes into conventional flow cytometry analyses may contribute to a more comprehensive understanding of cellular heterogeneity in both basic and translational research.

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Author contributions

Eda Patricia Tenorio (Conceptualization [lead], Formal analysis [lead], Funding acquisition [lead], Methodology [lead], Project administration [lead], Supervision [lead], Visualization [lead],

Writing—original draft [lead], Writing—review & editing [lead]), Vanessa Ángeles-Quevedo (Formal analysis [equal], Visualization [equal]), María Cacelín (Investigation [equal]), Wilton Gómez-Henao (Investigation [equal], Methodology [supporting]), Roberto González-Rodríguez (Visualization [supporting], Writing—review & editing [supporting]).

Supplementary material

Supplementary material is available at *Glycobiology Journal* online.

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Conflicts of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be considered a potential conflict of interest.

Data availability statement

The flow cytometry data supporting the findings of this study are available in the Zenodo repository at https://doi.org/10.5281/zenodo.23787774. Additional information is available from the corresponding author upon reasonable request.

References

Amado M, Yan Q, Comelli EM, Collins BE, Paulson JC. Peanut agglutinin high phenotype of activated $CD8^+$ T cells results from de novo synthesis of CD45 glycans. J Biol Chem. 2004:279(35):36689–36697. https://doi.org/10.1074/jbc.M405629200.

Bonilla DL, Paul A, Gil-Pulido J, Park LM, Jaimes MC. The power of reagent titration in flow cytometry. Cells. 2024:13(20):1677. https://doi.org/10.3390/cells13201677.

Bowman AS, Nelson SW, Edwards JL, Hofer CC, Nolting JM, Davis IC, Slemons RD. Comparative effectiveness of isolation techniques for contemporary influenza a virus strains circulating in exhibition swine. J Vet Diagn Invest. 2013:25(1):82–90. https://doi.org/10.1177/1040638712470449.

Cabral J, Hanley SA, Gerlach JQ, O'Leary N, Cunningham S, Ritter T, Ceredig R, Joshi L, Griffin MD. Distinctive surface glycosylation patterns associated with mouse and human $CD4^+$ regulatory T cells and their suppressive function. Front Immunol. 2017:8:987. https://doi.org/10.3389/fimmu.2017.00987.

Cossarizza A, Chang HD, Radbruch A, Abrignani S, Addo R, Akdis M, Andra I, Andreata F, Annunziato F, Arranz E, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition). Eur J Immunol. 2021:51(12):2708–3145. https://doi.org/10.1002/eji.202170126.

Farago A, Zvara A, Tiszlavicz L, Hunyadi-Gulyas E, Darula Z, Hegedus Z, Szabo E, Surguta SE, Tovari J, Puskas LG, et al. Lectin-based immunophenotyping and whole proteomic profiling of CT-26 colon carcinoma murine model. Int J Mol Sci. 2024:25(7):4022. https://doi.org/10.3390/ijms25074022.

Gibadullin R, Farnsworth DW, Barchi JJ Jr, Gildersleeve JC. GalNAc-tyrosine is a ligand of plant lectins, antibodies, and human and murine macrophage galactose-type lectins. ACS Chem Biol. 2017:12(8):2172–2182. https://doi.org/10.1021/acschembio.7b00471.

Halliday GM, Nairn RC, Rolland JM. Lymphocyte stimulation by concanavalin a studied by the fluorescent probe acridine orange. Cell Tissue Res. 1981:217(1):117–126. https://doi.org/10.1007/BF00233831.

Hendrickson OD, Nikitushkin VD, Zherdev AV, Dzantiev BB. Lectin-based detection of *Escherichia coli* and *Staphylococcus aureus* by flow cytometry. Arch Microbiol. 2019:201(3):313–324. https://doi.org/10.1007/s00203-018-1613-0.

Ishigaki H, and Itoh Y. 2022. Detection of sialic acids on the cell surface using flow cytometry. Methods Mol Biol 2556:31–35, https://doi.org/10.1007/978-1-0716-2635-1_3, New York, NY, Springer US.

Ishiguro M, Nakashima H, Tanabe S, Sakakibara R. Interaction of toxic lectin ricin with epithelial cells of rat small intestine in vitro. Chem Pharm Bull (Tokyo). 1992:40(2):441–445. https://doi.org/10.1248/cpb.40.441.

Kaku H, Mori Y, Goldstein IJ, Shibuya N. Monomeric, monovalent derivative of *Maackia amurensis* leukoagglutinin. Preparation and application to the study of cell surface glycoconjugates by flow cytometry. J Biol Chem. 1993:268(18):13237–13241. https://doi.org/10.1016/S0021-9258(19)38642-9.

Kekäläinen J, Larma I, Linden M, Evans JP. Lectin staining and flow cytometry reveals female-induced sperm acrosome reaction and surface carbohydrate reorganization. Sci Rep. 2015:5(1):15321. https://doi.org/10.1038/srep15321.

Lyons AB. Analysing cell division in vivo and in vitro using flow cytometric measurement of CFSE dye dilution. J Immunol Methods. 2000:243(1–2):147–154. https://doi.org/10.1016/S0022-1759(00)00231-3.

Ma T, McGregor M, Giron L, Xie G, George AF, Abdel-Mohsen M, Roan NR. Single-cell glycomics analysis by CyTOF-Lec reveals glycan features defining cells differentially susceptible to HIV. eLife. 2022:11:e78870. https://doi.org/10.7554/eLife.78870.

Malin-Berdel J, Valet G, Thiel E, Forrester JA, Gurtler L. Flow cytometric analysis of the binding of eleven lectins to human T- and B-cells and to human T- and B-cell lines. Cytometry. 1984:5(2):204–209. https://doi.org/10.1002/cyto.990050215.

Moriwaki K, and Miyoshi E. 2014. Basic procedures for lectin flow cytometry. Methods Mol Biol 1200:147–152, https://doi.org/10.1007/978-1-4939-1292-6_13, New York, NY, Springer New York.

Pardo JP, Tenorio EP, and Romero-Aguilar L. 2026. Viability of *Ustilago maydis* by flow cytometry. Methods Mol Biol 3050:191–199, https://doi.org/10.1007/978-1-0716-5364-7_19, New York, NY, Springer US.

Roederer M. Spectral compensation for flow cytometry: visualization artifacts, limitations, and caveats. Cytometry. 2001:45(3):194–205. https://doi.org/10.1002/1097-0320(20011101)45:3<194::AID-CYTO1163>3.0.CO;2-C.

Shang Y, Zeng Y, Zeng Y. Integrated microfluidic lectin barcode platform for high-performance focused Glycomic profiling. Sci Rep. 2016:6(1):20297. https://doi.org/10.1038/srep20297.

Sierra-Ulloa D, Fernandez J, Cacelin M, Gonzalez-Aguilar GA, Saavedra R, Tenorio EP. α2,6 sialylation distinguishes a novel active state in $CD4^+$ and $CD8^+$ cells during acute *toxoplasma gondii* infection. Front Immunol. 2024:15:1429302. https://doi.org/10.3389/fimmu.2024.1429302.

Szabó E, Faragó A, Bodor G, Gémes N, Puskás LG, Kovács L, Szebeni GJ. Identification of immune subsets with distinct lectin binding signatures using multi-parameter flow cytometry: correlations with disease activity in systemic lupus erythematosus. Front Immunol. 2024:15:1380481. https://doi.org/10.3389/fimmu.2024.1380481.

Torres NI, Manselle Cocco MN, Perrotta RM, Mahmoud YD, Salatino M, Mariño KV, Rabinovich GA. A single-step, rapid, and versatile method for simultaneous detection of cell surface glycan profiles using fluorochrome-conjugated lectins. Glycobiology. 2023:33(11):855–860. https://doi.org/10.1093/glycob/cwad065.

Xiao X, Fischbach S, Fusco J, Zimmerman R, Song Z, Nebres P, Ricks DM, Prasadan K, Shiota C, Husain SZ, et al. PNA lectin for purifying mouse acinar cells from the inflamed pancreas. Sci Rep. 2016:6(1):21127. https://doi.org/10.1038/srep21127.

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