Journal of Microbiology, Immunology and Infection 56 (2023) 729—738

Available online at www.sciencedirect.com

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Original Article

Nodal reactive proliferation of monocytoid B-cells may represent atypical memory B-cells

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Received 18 January 2023; received in revised form 7 March 2023; accepted 31 March 2023
Available online 11 April 2023

KEYWORDS
Age-associated B-cells;
Gene expression;
GeoMx;
Memory B-cells;
Monocytoid cells

Abstract *Background:* Reactive lymphadenopathies such as toxoplasmosis and cytomegalovirus lymphadenitis are associated with monocytoid cell proliferation. Monocytoid cells are B-lymphocytes with an undetermined subset.
Methods: Using digital spatial profiling whole transcriptome analyses, this study compared monocytoid and control B-cells. The B-cell subset of monocytoid cells was assigned according to gene expression profiles.
Results: This study identified 466 differentially expressed genes between monocytoid and control B-cells. The cellular deconvolution algorithm identified monocytoid cells as memory B-cells instead of as naïve B-cells. A comparison of the upregulated genes revealed that atypical memory B-cells had the largest number of genes overlapping with monocytoid cells compared with other memory B-cell subsets. Atypical memory B-cell markers, namely TBX21 (T-bet), FCRL4 (IRTA1), and ITGAX (CD11c), were all upregulated in monocytoid cells. Similar to atypical memory B-cells, monocytoid cells exhibited (1) upregulated transcription factors (TBX21, TOX), (2) upregulated genes associated with B-cell inhibition (FCRL5, FCRL4) and downregulated genes associated with B-cell activation (PIK3CG, NFKB1A, CD40), (3) downregulated cell cycle-related genes (CDK6, MYC), and (4) downregulated cytokine receptors (IL4R). This study also analyzed the expression of monocytoid cell signature genes in various memory B-cell subsets. Atypical memory B-cells exhibited a gene expression pattern similar to that of monocytoid cells, but other memory B-cell subsets did not. Furthermore, monocytoid cells and marginal zone lymphomas differed in gene expression profiles.

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https://doi.org/10.1016/j.jmii.2023.03.010

Conclusion: Spatial transcriptomic analyses indicated that monocytoid cells may be atypical memory B-cells.

Introduction

Monocytoid cells are a distinct B-cell population identified histopathologically in the lymph nodes.[1] Monocytoid cells are discernible when they accumulate along the subcapsular and trabecular sinuses. These cells, which have abundant clear cytoplasm, form pale band-like regions in the subcapsular or interfollicular areas. Monocytoid cell proliferation occurs in lymphadenopathies of various infections and autoimmune diseases. Well-known infectious agents causing monocytoid cell proliferation include *Toxoplasma gondii*,[1] human immunodeficiency virus (HIV),[2] and cytomegalovirus (CMV).[3]

Monocytoid cells are B-lymphocytes, as evidenced by the consistent expression of B-cell markers.[4,5] Mature B-lymphocytes are functionally classified into many types, including naïve, transitional, follicular, marginal zone, germinal center, and memory B-cells.[6] Each cell type can be further classified into many subsets; memory B-cell subsets include unswitched, pre-switched, switched-resting, switched, activated, and atypical memory B-cells.[6,7]

Because monocytoid cells are histomorphologically defined, the study of monocytoid cells requires the use of tissue sections and methods like staining or microdissection. Studies have examined protein expression using immunohistochemistry.[8–11] These studies have identified monocytoid cells as B-cells instead of as histiocytes but lacked the sufficient markers to determine B-cell types. Studies have also sequenced immunoglobulin genes with varying results and have failed to determine whether monocytoid cells are pre- or post-germinal center B-cells.[8–10]

The aim of this study was to determine the B-cell type of monocytoid cells. Unlike previous studies, this study assayed the gene expression of monocytoid cells. We attempted to assign a B-cell subset based on well-documented gene signatures. This study used digital spatial profiling, a multiplexed transcriptomic technology correlated with histomorphology.

We quantified the gene expression levels of monocytoid cells in four biopsy specimens. The monocytoid cells were compared with other interfollicular B-cells. We identified the B-cell subset closest to monocytoid cells in accordance with previously reported gene signatures.

Materials and methods

Case selection

We selected four lymph node biopsy specimens sampled from 2019 to 2021. We reviewed the archived slides to confirm the presence of monocytoid cell proliferation. Demographic characteristics and clinical information were obtained from medical records. Five additional specimens, sampled from 2022 to 2023, were selected as a validation cohort.

Digital spatial profiling whole transcriptome analyses

Tissue sections were cut from paraffin-embedded tissue blocks. The slides were baked at 60 °C for 1 h and then deparaffinized. The slides were incubated in 100 °C Tris EDTA for 15 min for target retrieval. The slides were then incubated in 1 μg/mL proteinase K for 5 min to expose the RNA targets. After postfix preservation, the slides were hybridized using GeoMx Human Whole Transcriptome Atlas probes (NanoString, Seattle, WA, USA) at 37 °C overnight. After blocking for 30 min, the slides were stained with morphology markers and the nucleic acid stain SYTO 13 (Thermo Fischer, Waltham, MA, USA) for 1 h. This study used morphology markers CD3 (1 mg/mL; clone: C3e/1308, conjugate: Alexa Fluor 647; Novus Biologicals, Centennial, CO, USA) and CD20 (4 μg/mL; clone: IGEL/773, conjugate: DyLight 594; Novus Biologicals, Centennial, CO, USA).

The preprocessed slides were loaded onto the GeoMx Digital Spatial Profiler (NanoString, Seattle, WA, USA). We manually selected regions of interest (ROI) on the scanned immunofluorescence (IF) staining images according to IF and a serial sectioned hematoxylin-and-eosin (HE) staining. Each ROI was further segmented according to IF signals. The B-cell segments, which were profiled separately, were defined as CD20-positive, CD3-negative, and nuclear staining-positive cells.

The GeoMx Digital Spatial Profiler collected the oligonucleotide barcodes from the selected segments into a 96-well collection plate. NanoString SeqCode primers (NanoString, Seattle, WA, USA) were used for library preparation. The polymerase chain reaction products were pooled and purified with two rounds of AMPure XP beads (Beckman Coulter, Fischer Scientific, Waltham, MA, USA). Libraries were sequenced on a NextSeq 550 System (Illumina, San Diego, CA, USA) according to manufacturer instructions, with at least 27 × 27 paired-end reads. FASTQ files were converted to raw count files using NanoString NGS Pipeline (Version 2.3.3.10, NanoString, Seattle, WA, USA).

Data analysis

The data were analyzed using the R language (Version 4.2.1, the R Foundation, Vienna, Austria). The raw count data were processed using the GeomxTools package (Bioconductor version 3.15, NanoString, Seattle, WA, USA) in

Table 1 Clinicopathological features.

Case	Age (yr)	Sex	Underlying diseases	Biopsy site	Follicular hyperplasia	Microgranulomas	CMV IHC	EBER
1	51	F	Follicular lymphoma, mitral valve prolapse	Right neck	Present	Present	Negative	Negative
2	40	F	Aplastic anemia, post-transplant lymphoproliferative disorder	Right neck	Present	Absent	Positive	Scattered
3	65	M	Hodgkin lymphoma, diabetes mellitus, hypertension, gout	Left neck	Present	Present	Positive	Scattered
4	66	F	Hypertension, diabetes mellitus	Right neck	Present	Present	Positive	Scattered

CMV, cytomegalovirus; F, female; IHC, immunohistochemistry; M, male; yr, year.

accordance with developer introductions. The GeoMx raw count file and metadata were combined into a GeoMxSet file. The study removed outlier probes and segments that did not meet quality control cutoffs. Quartile 3 normalization was also implemented.

The differential expression analysis was conducted using a linear mixed-effects model. Statistical significance for differential gene expression was indicated at $p < 0.05$ and an absolute value of log2 (fold change) > 0.5.

The differentially expressed genes were further analyzed using the clusterProfiler package (Bioconductor version 3.15, Professor Guangchuang Yu, Southern Medical University, Guangzhou, Guangdong, China). We conducted gene set enrichment analysis using the Gene Ontology project, the Kyoto Encyclopedia of Genes and Genomes, and the Reactome database as resources.

The normalized data were also analyzed using the SpatialDecon package (Bioconductor version 3.15, NanoString, Seattle, WA, USA); we performed deconvolution using the cell matrix ImmuneTumor_safeTME (CellProfileLibrary GitHub Page, NanoString, Seattle, WA, USA).

The gene expression datasets, GSE149729, GSE79196, and GSE171059, were obtained from the Gene Expression Omnibus of the National Center for Biotechnology Information database (National Library of Medicine, Bethesda, MD, USA).

Results

Clinicopathological characteristics

The four cases analyzed are presented in Table 1. All specimens were neck lymph nodes exhibiting follicular hyperplasia and monocytoid cell proliferation; three exhibited microgranulomas. The three CMV + Epstein–Barr virus (EBV)+ cases had been reported in another study.[3]

Gene expression profile of monocytoid cells

We analyzed the gene expression profiles of monocytoid cells using digital spatial profiling. Monocytoid cell and other interfollicular regions were manually selected according to the histomorphology in IF and a serial sectioned HE staining (Fig. 1), and the B-cell compartments within these regions were segmented according to IF staining (CD20+/CD3−/nuclear staining+).

A comparison between monocytoid and control B-cells revealed 466 differentially expressed genes (Table S1 and

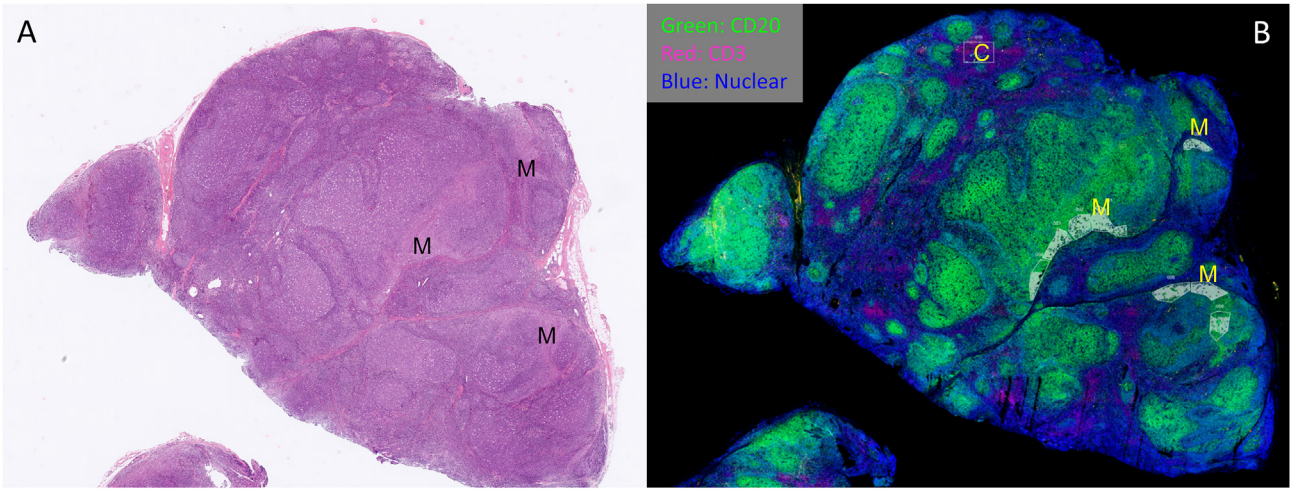

Figure 1. Monocytoid and control B-cell regions. (A) HE stain. (B) The scanned image of IF stain from the GeoMx digital spatial profiling system. C, control; M, monocytoid cells.

Fig. 2A). The gene set enrichment analysis did not indicate significantly associated pathways (Table S2–4).

Monocytoid cells may be memory B-cells

We used the SpatialDecon algorithm to classify the immune cell type according to gene expression profiles (Fig. 2B). Unsupervised hierarchical clustering divided almost all the monocytoid cell segments and the control B-cell segments into two groups. The majority of the monocytoid cell segments were estimated to be memory B-cells, and the control B-cell segments were not as memory B-cells.

However, studies have indicated that monocytoid cells do not express CD27,[12] a memory B-cell marker expressed in most memory B-cells.[6] According to surface markers CD27 and CD21, monocytoid cells (CD27−CD21−)[12] differed from classical memory B-cells (CD27 + CD21+) and activated memory B-cells (CD27 + CD21−) and more closely resembled atypical memory B-cells (CD27−CD21−).[13] According to surface markers CD27 and IgD, monocytoid cells constituted double-negative (CD27−IgD−) B-cells,[12] distinct from naïve B-cells (CD27−IgD+), unswitched memory B-cells (CD27 + IgD+) and switched memory B-cells (CD27 + IgD−).[14] Double-negative (CD27−IgD−) B-cells consist of many subsets; atypical memory B-cells are classified as double-negative 2 B-cells.[14] The immunophenotype of monocytoid cells appears to differ from that of most memory B-cell subsets but resembles that of atypical memory B-cells.

The characteristic markers of atypical memory B-cells are IRTA1 (FCRL4), T-bet (TBX21), and CD11c (ITGAX).[15–18] We noted that these genes were all upregulated in monocytoid cells (Table S1). Studies have also reported that monocytoid cells were positive for T-bet and IRTA1 immunostains.[8,12] Therefore, we postulated that monocytoid cells are atypical memory B-cells instead of classical memory B-cells.

Monocytoid cells may be atypical memory B-cells

We compared the upregulated genes between monocytoid cells, various memory B-cell subsets, and naïve B-cells (Fig. 3A).[19] We observed that the upregulated genes of atypical memory B-cells considerably overlapped with those of monocytoid cells (33 genes). The number of overlapping genes was much higher compared with other B-cell subsets (activated memory B-cells, 2 genes; classical memory B-cells, no genes; naïve B-cells, no genes). Among these B-cell subsets, atypical memory B-cells most closely resembled monocytoid cells.

We analyzed the expression of atypical memory B-cell signature genes in our samples. Similar to atypical memory B-cells,[18] monocytoid cells exhibited upregulation of (1) transcription factors, TBX21 and TOX, and (2) genes associated with B-cell inhibition, FCRL5 and FCRL4. They also exhibited downregulation of (1) genes associated with B-cell activation, PIK3CG, NFKB1A, and CD40, (2) cell cycle-related genes, CDK6 and MYC, and (3) cytokine receptors, IL4R. Unsupervised hierarchical clustering categorized the majority of monocytoid cell segments into one group (Fig. 3B).

Subsequently, we analyzed the differentially expressed genes of monocytoid cells in various B-cell subsets

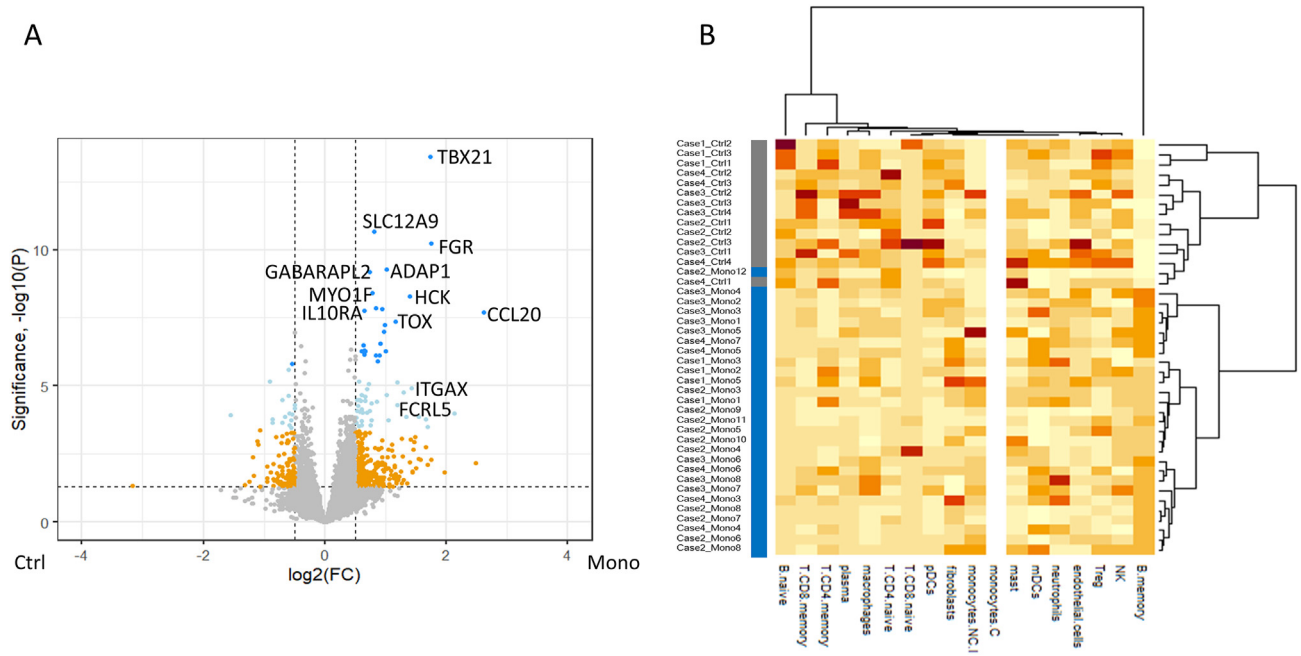

Figure 2. Differentially expressed genes and immune cell type estimation. (A) Volcano plot of the differentially expressed genes between monocytoid cells (Mono) and control B-cells (Ctrl). X-axis: log2 (fold change) of gene expression, Y-axis: −log10 (*p*-value). Each dot represents a gene. Genes with a false discovery rate (FDR)-adjusted *p*-value < 0.001 are indicated by blue dots; those with an FDR-adjusted *p*-value <0.05 are indicated by light blue dots; those with a *p*-value <0.05 are indicated by orange dots. Other genes are indicated by gray dots. (B) Heatmap illustrating the estimated cell abundance of the samples. Monocytoid and control B-cell segments are labeled as blue and gray boxes, respectively.

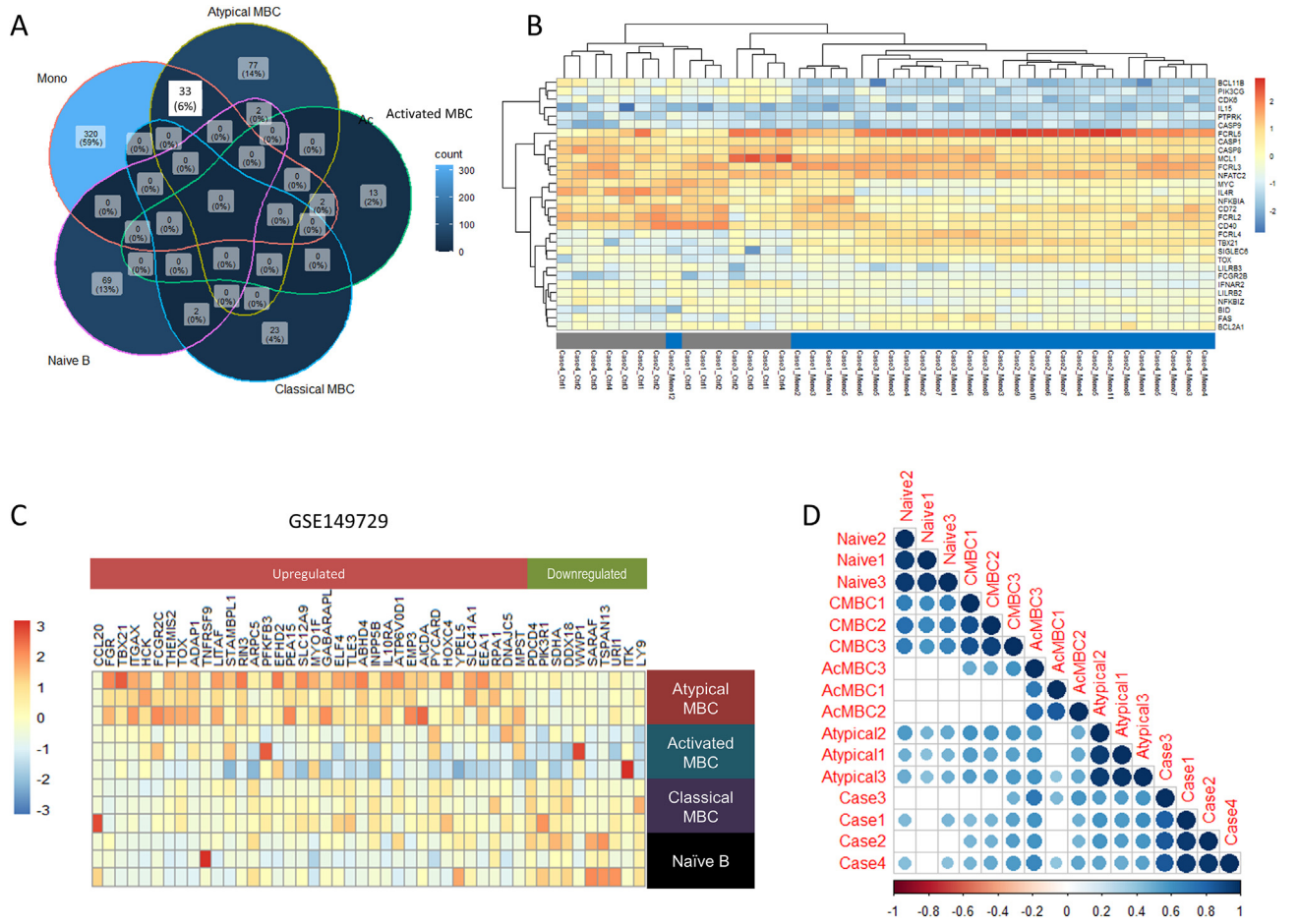

Figure 3. Comparison with various B-cell subsets. (A) Venn diagram comparing the upregulated genes between monocytoid cells and various B-cell subsets. (B) Heatmap illustrating the expression of atypical memory B-cell signature genes. Monocytoid and control B-cell segments are labeled as blue and gray boxes, respectively. (C) Heatmap illustrating the gene expression of various B-cell subsets (GSE149729). The upregulated and downregulated genes in monocytoid cells are labeled as red and green boxes, respectively. MBC, memory B-cells; Mono, monocytoid cells. (D) Correlation matrix of Spearman coefficients. Variables are hierarchically clustered, and coefficients with significant correlation ($p < 0.01$) are plotted.

(GSE149729). Compared with other B-cell subsets, atypical memory B-cells exhibited a gene expression pattern like that of monocytoid cells (Fig. 3C). Monocytoid cells and atypical memory B-cells upregulated and downregulated the same genes. Additionally, Spearman correlation analyses showed that the correlation between monocytoid cells and the three types of memory B-cells was higher compared to naïve B-cells, with the highest correlation observed in atypical memory B-cells (Fig. 3D).

Monocytoid cells and atypical memory B-cells shared similar gene expression profiles, indicating that monocytoid cells are atypical memory B-cells.

Immunohistochemical staining

We also included immunohistochemical staining results from five additional cases (Table 2). In all cases, monocytoid cell regions displayed CD20+/CD3−/T-bet + expression patterns, which were consistent with atypical memory B-cells (representative photographs in Fig. 4). Our findings are in line with previous studies that have reported similar staining patterns in toxoplasmosis,[12] suggesting that monocytoid cells in various clinical contexts may represent atypical memory B-cells.

Comparison with B-cell lymphomas

This study also compared monocytoid cells with B-cell lymphomas. We first compared the gene signatures of monocytoid cells and indolent B-cell lymphomas.[20] The monocytoid cells shared rare upregulated genes with the following indolent B-cell lymphomas: 2 with extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue, 1 with splenic marginal zone lymphoma (Fig. 5A), 5 with hairy cell leukemia-variant, 2 with splenic diffuse red pulp small B-cell lymphoma (Fig. 5B), 2 with hairy cell leukemia, 1 with follicular lymphoma, 1 with chronic lymphocytic leukemia, and none with mantle cell lymphoma or lymphoplasmacytic lymphoma.

We analyzed the differentially expressed genes of monocytoid cells in various indolent B-cell lymphomas (GSE79196; Fig. 5C). These lymphomas, including marginal zone lymphomas, were not similar to monocytoid cells. Spearman correlation analysis showed that while there was

Table 2 Clinicopathological features of the validation cohort.

Case	Age (yr)	Sex	Underlying diseases	Biopsy site	Follicular hyperplasia	Microgranulomas	CMV IHC	EBER
5	64	M	Diffuse large B-cell lymphoma, diabetes mellitus, coronary artery disease, chronic hepatitis B	Right neck	Present	Present	Positive	Negative
6	42	M	Nil	Right neck	Present	Absent	Positive	Scattered
7	30	F	Hodgkin lymphoma	Right neck	Present	Absent	Positive	Negative
8	40	F	Mycosis fungoides, status post allogeneic PBSCT	Right inguinal	Present	Present	Negative	Scattered
9	41	F	Follicular lymphoma	Left inguinal	Present	Absent	Negative	Scattered

CMV, cytomegalovirus; F, female; IHC, immunohistochemistry; M, male; PBSCT, peripheral blood hematopoietic stem cell transplant; yr, year.

some correlation between monocytoid cells and various indolent B-cell lymphomas, there was not a particularly high correlation with marginal zone lymphoma (Fig. 5D).

In addition, we analyzed another dataset with diffuse large B-cell lymphoma (GSE171059; Fig. 5E). These lymphomas were not similar to monocytoid cells.

Discussion

Lennert et al. first noted monocytoid cell proliferation in toxoplasmosis lymphadenitis.[1] Monocytoid cell proliferation is typically accompanied by follicular hyperplasia and microgranulomas.[1] This cell population accumulates in the lymph node sinuses and morphologically resembles histiocytes.[1] However, histochemical and immunohistochemical stains have identified monocytoid cells as mature B-cells and not as sinus histiocytes.[1,4,5]

In addition to toxoplasmosis, monocytoid cell proliferation occurs in various reactive lymphadenopathies. Pattern A of HIV lymphadenopathy is characterized by follicular hyperplasia, follicle lysis, and monocytoid cell proliferation[2]; this morphology is believed to indicate the early-stage lymph node changes of HIV infection.[2] CMV lymphadenitis also presents with follicular hyperplasia and monocytoid cell proliferation; CMV-positive cells are typically located in the monocytoid cell regions.[3] In addition, monocytoid cell proliferation occurs in nonspecific reactive lymphoid hyperplasia, suppurative lymphadenitis, and granulomatous lymphadenitis.[21] One study has also reported an association between monocytoid cell proliferation and EBV infection.[22]

Researchers initially classified monocytoid cells as nodal marginal zone B-cells.[23] However, Stein et al. published a series of studies indicating that monocytoid cells are distinct from marginal zone B-cells.[10,12] Nevertheless, the B-cell subset of monocytoid cells remains unclear.

Flow cytometry markers like CD10, CD21, CD24, CD27, CD38, and Ig isotypes are used to assign B-cells to subsets.[6] Many researchers have employed immunohistochemistry to study these markers in monocytoid cells. Monocytoid cells are consistently negative for CD21 and CD10,[9,10,12] but the results of CD27 and Ig isotypes are mixed.[8–11] The incomplete and unreproducible immunophenotypes were inconclusive for B-cell classification.

Because somatic hypermutation is a hallmark of germinal center reaction, studies have analyzed immunoglobulin genes to distinguish pre- and post-germinal center B-cells. Some studies have detected somatic mutations, but

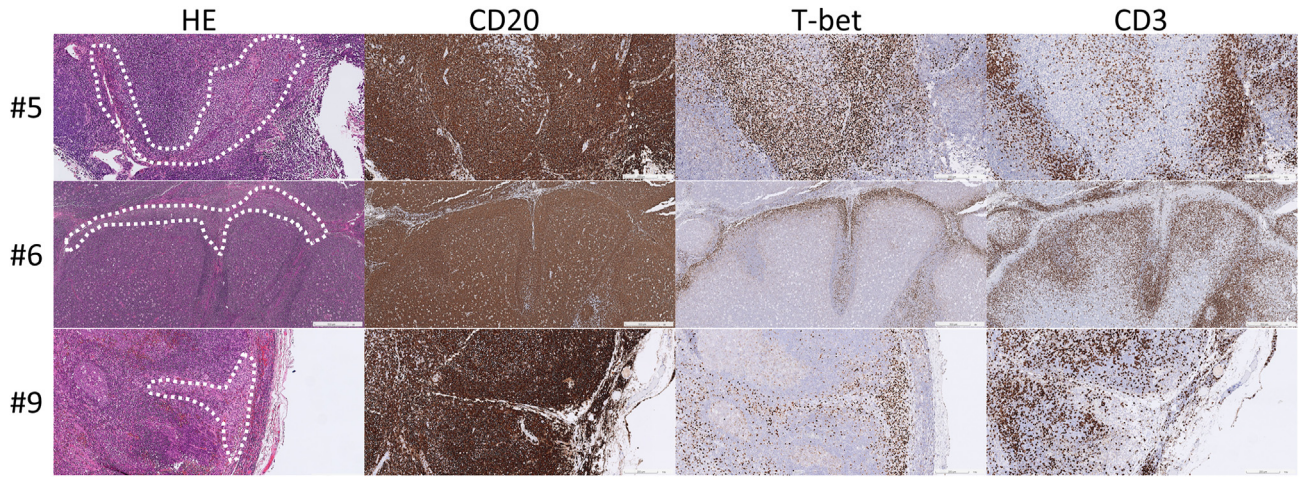

Figure 4. Immunohistochemical staining of monocytoid cell regions in three representative cases (cases 5, 6, and 9). White dashed lines indicate the location of monocytoid cell regions in the HE staining.

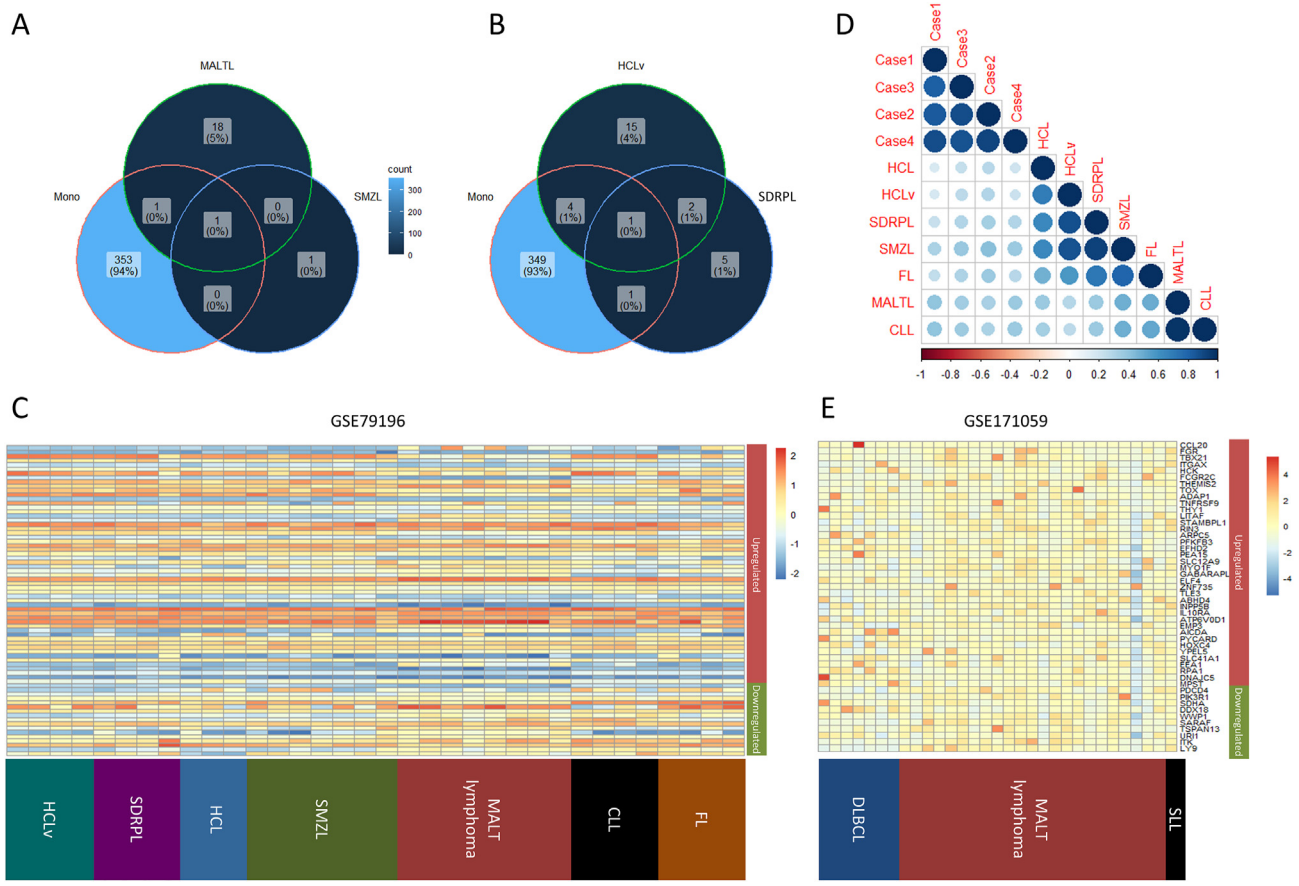

Figure 5. Comparison with B-cell lymphomas. (A) Venn diagram comparing the upregulated genes between monocytoid cells and marginal zone lymphomas. (B) Venn diagram comparing the upregulated genes between monocytoid cells, hairy cell leukemia-variant, and splenic diffuse red pulp small B-cell lymphoma. (C) Heatmap illustrating the gene expression of various indolent B-cell lymphomas (GSE79196). (D) Correlation matrix of Spearman coefficients. Variables are hierarchically clustered, and coefficients with significant correlation ($p < 0.01$) are plotted. (E) Heatmap illustrating the gene expression of diffuse large B-cell lymphoma and indolent B-cell lymphomas (GSE171059). The upregulated and downregulated genes in monocytoid cells are labeled as red and green boxes, respectively. CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HCL, hairy cell leukemia; HCLv, hairy cell leukemia-variant; MALTL, MALT lymphoma; Mono, monocytoid cells; SDRPL, splenic diffuse red pulp small B-cell lymphoma; SLL, small lymphocytic lymphoma; SMZL, splenic marginal zone lymphoma.

others have not.[8–10] The mixed data has thus generated further debates.[10,23]

Based on the spatial transcriptomic analyses, we hypothesized that monocytoid cells are atypical memory B-cells. This is not a new theory; some immunologists considered monocytoid cells and atypical memory B-cells to be interchangeable terms,[24,25] but their opinions were solely based on T-bet and IRTA1 expression. Our high-throughput transcriptomic data have corroborated this hypothesis.

Memory B-cells are persistent antigen-specific B-cells generated during immunization or infection. CD27 can be used to distinguish memory B-cells from naïve and germinal center B-cells and is therefore considered a memory B-cell marker.[26]

Unlike most memory B-cells, atypical memory B-cells are CD27-negative.[16] They can be quantified using flow cytometry or single-cell sequencing based on various markers and gene panels.[16,27] A growing number of studies have investigated the role of atypical memory B-cells in human diseases and immune responses.[15–18]

Although atypical memory B-cells lack a histological definition, we hypothesize that tonsillar epithelial-associated B-cells are their prototype. Atypical memory B-cells were initially defined as FCRL4 (IRTA1)-positive B-cells in the tonsil.[28] Similarly, the B-cells infiltrating the tonsillar epithelium are IRTA1-positive.[11,29,30] Apart from intraepithelial IRTA1-positive cells, no major IRTA1-positive B-cell population exists in the tonsils.[29] The tonsillar epithelial-associated B-cells observed in tissue sections may be the same atypical memory B-cells identified by flow cytometry.

Prior to our study, an identical immunophenotype indicated that monocytoid cells, tonsillar epithelial-associated B-cells, and atypical memory B-cells belong to one cell population. According to flow cytometry, atypical memory B-cells are IRTA1+/T-bet+/CD21−/CD27−/IgD−.[15–18] By immunohistochemistry, monocytoid cells are also positive for IRTA1 and T-bet[8,12]; many studies have also reported that monocytoid cells are CD21−/CD27−/IgD−.[9,10,12] Tonsillar epithelial-associated B-cells are also IRTA1+/T-bet+/CD21−/CD27−/IgD−.[11,12]

The gene expression profile is widely used to identify atypical memory B-cells. Studies have described the characteristic gene expression pattern of atypical memory B-cells as follows: (1) upregulation of B-cell inhibition-related genes and downregulation of B-cell activation-related genes; (2) upregulation of apoptosis-associated genes and downregulation of genes associated with cell survival and proliferation; (3) downregulation of cytokine and cytokine receptors associated with cell proliferation and upregulation of tumor necrosis factor receptors and interferon receptors; and (4) upregulation of transcription factors crucial for atypical memory B-cells.[18]

Monocytoid cells exhibited a similar gene signature to that of atypical memory B-cells (Table S1). Monocytoid cells exhibited upregulated FCRL5, FCRL3, SIGLEC6, and FCGR2B, which were associated with B-cell inhibition, and downregulated PIK3CG, which was associated with B-cell activation. The apoptosis-associated genes FAS and BCL2A1 were upregulated. IL4R, a cytokine receptor associated with cell proliferation, was downregulated. Transcription factors TBX21, TOX, and NFATC2 were upregulated.

Atypical memory B-cells were morphologically described as large cells in Giemsa staining.[28,31] The study images revealed these cells have abundant cytoplasm or cytoplasmic processes. We consider their cytological features similar to those of the monocytoid cells in the HE staining.

Similarities in morphologies, immunophenotypes, and gene expression profiles indicate that monocytoid cells may be atypical memory B-cells. Monocytoid cell proliferation may be equivalent to increased atypical memory B-cells in the lymph nodes. Although the two compartments, lymph nodes and peripheral blood, have not been correlated, they share similar changing patterns. Studies have reported increased atypical memory B-cells in peripheral blood in HIV and CMV infections.[15,32] Monocytoid cell proliferation is also frequently observed in these diseases.[2,3]

Many researchers believe that lymph node tissue is devoid of atypical memory B-cells.[17,18] Most atypical memory B-cells are in the peripheral blood, spleen, and bone marrow, not in the lymph nodes and tonsils.[33] In influenza-infected murine models, atypical memory B-cells have been transiently observed in mediastinal lymph nodes but do not persist in the lymph nodes.[33] Although one IF study demonstrated increased T-bet-positive atypical memory B-cells in HIV lymphadenopathy,[25] this finding was considered an exception.[33]

During infections, atypical memory B-cells increase sharply in the peripheral blood and increase slightly in the lymph nodes. Atypical memory B-cells comprise 3—5% of peripheral blood B-cells in healthy adults and increase to up to 50% of circulating B-cells in individuals infected with HIV, malaria, tuberculosis, and hepatitis C virus.[27] By contrast, the baseline of atypical memory B-cells is near 0% in the lymph nodes, and the peak after influenza vaccination is approximately 5%.[27]

We believe that the histological findings do not conflict with the quantitative measurements. Monocytoid cells typically account for $<$5% of cells in the lymph nodes. They are recognized for their unique morphology, not their quantity. What is considered prominent monocytoid cell proliferation to pathologists may be deemed a negligible increase by immunologists.

Because monocytoid cells selectively accumulate in lymph node sinuses, we speculate that the sinus lining cells practice cell homing. We noted minor differences between monocytoid cells and atypical memory B-cells, e.g. CCL20. These genes may be chemotactic signals leading to monocytoid cell accumulation in the lymph node sinuses.

The coexistence of follicular hyperplasia with monocytoid cell proliferation constitutes another unanswered question. The monocytoid cells may arise from germinal centers, or some factors may trigger the proliferation of both monocytoid and germinal center cells. Further studies are required to clarify the associated mechanism.

Studies have investigated atypical memory B-cells in a range of clinical conditions, including infections, autoimmune diseases, organ transplantation, primary immunodeficiency, and aging.[15—18] The associated microbes include viruses, parasites, and bacteria, and the infection types may be acute, chronic, or latent viral infections.[15—18]

This study identified *T. gondii* as another microbe associated with increased atypical memory B-cells. Most studies focus on innate immunity and T cells[34] and may overlook the role of B-cells in toxoplasmosis.

Several issues regarding CMV lymphadenitis warrant further discussion. Studies have only reported increased peripheral blood atypical memory B-cells in primary CMV infection.[32] An earlier study demonstrated that most patients with CMV lymphadenitis experienced CMV reactivation, not primary infection.[3] Monocytoid cell proliferation indicates increased atypical memory B-cells during CMV reactivation.

CMV-positive cells are typically found in monocytoid cell regions.[3,35] Although the nature of CMV-positive cells is undetermined, most researchers believe they are not B-lymphocytes.[35] We believe that CMV-infected cells may recruit or interact with atypical memory B-cells.

One study reported high glucose uptake of CMV lymphadenitis in positron emission tomography[3]; the mechanism may involve atypical memory B-cells. Because studies have noted upregulation of the mTORC1 pathway in atypical memory B-cells,[36] these cells may increase glucose uptake in CMV lymphadenitis through the mTORC1 pathway.

In addition, this study also indicates that monocytoid cells are distinct from marginal zone lymphomas. The crucial pathways in marginal zone lymphomas, namely NF-κB, NOTCH, BCR, PI3K, and TLR,[37] were not upregulated in monocytoid or atypical memory B-cells.[18] Monocytoid cells and marginal zone lymphomas are cytologically similar, and they both express IRTA1 and T-bet.[30,38] However, markers like MNDA and BCL2 were differentially expressed,[29,39] and monocytoid cells and marginal zone lymphomas had different gene expression profiles.

Limitations of our study include a small sample size and the use of tissue samples, which inherently contain a mixture of cell types.

In conclusion, gene expression profiles suggest that monocytoid cells are atypical memory B-cells. Our findings suggest that histopathological examination of lymph node biopsy specimens could complement research on the role of atypical memory B-cells in human diseases.

Acknowledgements

This study was supported by National Taiwan University Hospital (Grant 112-S0012).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.03.010.