Follicular Dendritic Cells Contain a Unique Gene Repertoire Demonstrated by Single-Cell Polymerase Chain Reaction

By Folke Schriever, Gordon Freeman, and Lee M. Nadler

Follicular dendritic cells (FDCs) form a dense network between B cells within the germinal center and are thought to be an important component of this B-cell microenvironment. Previous immunophenotypic studies have been inconclusive in determining the cellular origin of FDCs. Gene coexpression within individual and highly enriched FDCs was determined using polymerase chain reaction. FDCs contain a very restricted mRNA pattern with high levels of message for the C3d receptor (CR2, Epstein-Barr-virus/EBV receptor, CD21) and lack of mRNA for CD20, CD45, CD4, fibronectin, and platelet-derived growth factor receptor α and β. These observations are consistent with the hypothesis that FDCs may not be of classical hematopoietic or fibroblastic origin. The absence of interferon-γ, tumor necrosis factor-α, interleukin-3, and interleukin-6 mRNA provides preliminary evidence that these cells might produce only a very restricted set of cytokines limited to the germinal center.

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Moloney murine leukemia virus (M-MLV) reverse transcriptase (Bethesda Research Labs, Gaithersburg, MD) in 10 μL of buffer containing 1 μmol/L antisense primer, 0.5 mmol/L dNTP mix, 50 mmol/L Tris-HCl (pH 8.3), 75 mmol/L KCl, 10 mmol/L dithiothreitol, 3 mmol/L MgCl₂, and 20 U human placental ribonuclease inhibitor (Promega, Madison, WI). cDNA was heated for 10 minutes at 94°C and quick chilled on ice. PCR was performed by adding 40 μL of buffer containing 1 μmol/L antisense primer, 2 μmol/L sense primer, 1 U AmpliTaq DNA Polymerase (Perkin Elmer Cetus, Norwalk, CT), 8 mmol/L Tris-HCl pH 8.3, 40 mmol/L KCl, 2 mmol/L MgCl₂, 0.008% gelatin, and 2 mmol/L dNTP mix. Forty-five PCR cycles were performed at 94°C (1 minute), 55°C (1 minute), and 72°C (1 minute) using a Techne Thermocycler (Techne Ltd, Cambridge, UK). To further increase sensitivity and specificity, a second PCR (25 cycles) was performed using 3 μL of the first PCR product as template and 2 μmol/L of primers specific for the cDNA fragment. PCR products were electrophoresed in a 1% Agarose/3% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME) and Southern transferred onto Zeta-Probe membranes (Bio-Rad, Richmond, CA). Hybridization was performed with 32P-labeled oligonucleotide probes specific for the amplified DNA fragment (Table 1).

RESULTS

FDCs were examined for the expression of genes characteristic of hematopoietic or fibroblastic origin. FDC mRNA was PCR amplified with oligonucleotide primers specific for CD20 and CD21 (B-cell-associated antigens), CD4 (T-cell-associated antigen), common leukocyte antigen

Table 1. Primers and Probes Used for Reverse Transcription, PCR, and Southern Analysis

<table>
<thead>
<tr>
<th>Gene (reference)</th>
<th>Primers Probes</th>
<th>Nucleotide Position (bp)</th>
<th>PCR Product</th>
<th>Shown in Fig</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 (12)</td>
<td>SI</td>
<td>722-745</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD20 (13)</td>
<td>S</td>
<td>507-530</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD21 (14)</td>
<td>SI</td>
<td>2536-2559</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45 (15)</td>
<td>S</td>
<td>925-948</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibronectin (16)</td>
<td>SI</td>
<td>3432-3455</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF-Rα (17)</td>
<td>S</td>
<td>1084-1107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF-Rβ (18)</td>
<td>S</td>
<td>1367-1390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ (19)</td>
<td>SI</td>
<td>147-170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (20)</td>
<td>SI</td>
<td>232-255</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

cDNA sequences were derived from published reports. Sense (SI, II) and antisense (AI, I) primers were chosen on different exons, or, if the genomic structure was unknown (PDGF-Rα and PDGF-Rβ), were widely separated so as to be likely on different exons. Therefore, PCR products of the appropriate size could only be generated from mRNA and not from genomic DNA. Primers for CD45 were within constitutively used exons. Probes (PI, II) were used for Southern blot analyses (Fig 1).
CD45 (hematopoietic lineage marker), and fibronectin and platelet-derived growth factor receptors α and β (fibroblast-associated antigens). Single unclustered tonsillar FDCs were sorted by their strong expression of CD146 and by their characteristic large cell size. Individual FDCs were identified under light microscopy by their unique cell size, bilobated nuclei, and fine cytoplasmic processes, and were picked as single cells. To analyze coexpression of mRNAs of a single cell, RNAs of individual cells were split into three equal parts and examined by PCR with the primers and probes detailed in Table 1.

As seen in Fig 1A, FDCs showed a characteristic pattern of high level of CD21 mRNA and lack of message for CD20 and CD45. Single tonsillar B cells, in contrast, contained mRNAs for CD20, CD21, and CD45 (Fig 1A). As seen in Fig 1B, FDCs did not contain the message for the fibroblast-specific markers fibronectin and platelet-derived growth factor receptor α and β (PDGF-R α and PDGF-R β) or the T-cell marker CD4. This very unique mRNA pattern is consistent with the hypothesis that FDCs may not be, as previously thought, derived from classical hematopoietic or fibroblastic cells.

To investigate whether FDCs express the message for one or more cytokines known to affect B-cell growth and differentiation, we examined whether unstimulated or in vitro-activated FDCs contain cytokine mRNAs. FDCs, isolated by our method, did not appear to produce tumor necrosis factor-α (TNF-α) or IFN-γ because the corresponding mRNAs were not detected in unstimulated FDCs (Fig 1B and D) and with IFN-γ or T-cell–conditioned medium-stimulated FDCs (data not shown). In addition, unstimulated FDCs did not express mRNAs for interleukin-3 (IL-3) and IL-6 (data not shown).

**DISCUSSION**

Previous immunostaining studies of FDCs led to conflicting results with regard to expression of antigens defining the lineage of these cells. Antigenic phenotype, morphology, and consistent mRNA profile of the cells isolated by our method clearly identified them as a homogeneous group of FDCs. However, it is presently unknown if these cells are representative for all FDCs of the germinal center or if they define a subpopulation of FDCs.

The present report has attempted to overcome the ambiguity of cell-surface staining by coexamining gene expression in single and highly enriched FDCs. The observation that FDCs are positive for CD21 mRNA, but negative for the CD20 or CD45 genes, is consistent with our prior immunophenotypic observations and is convincing because they were probed on single cells. The finding that...
FDCs do not contain mRNAs for the B-cell–associated marker CD20, the T-cell–associated marker CD4, the hematopoietic cell antigen CD45, and the fibroblast-associated markers fibronectin and PDGF-R α and β strongly suggests that FDCs are not of classical hematopoietic or fibroblastic derivation. These results further extend previous immunostaining data, which show that FDCs are also not of epithelial or endothelial origin. These data support the hypothesis that FDCs might belong to a unique cellular lineage.

The high amount of mRNA for the complement receptor CR2 in FDCs could reflect that this molecule is an important mediator of FDC function. CR2, together with CR1 and CR3, may be involved in the antigen-driven B-cell selection within the germinal center by presenting immune complexes to germinal center cells. Another role of CR2 could be to provide B cells with bound C3d, which represents a positive growth signal to B cells. Another mechanism responsible for the FDC–B-cell interaction has been recently defined as the unique binding of activated B cells to FDCs within the germinal center via the receptor-ligand interaction of VLA-4 and INCAM-110.

The close proximity of FDCs with B cells provides the basis for the hypothesis that FDCs might also secrete cytokines to regulate the B-cell microenvironment. This idea was strengthened by the observation that FDCs support the survival of B cells in culture. In addition, it was shown that TNF-α and IL-6 are secreted by FDC-enriched preparations but not by FDC-depleted populations. However, FDCs do not contain IL-6 mRNA as determined by PCR and by in situ hybridization. Our present report indicates that FDCs also do not express mRNAs for TNF-α and INF-γ, which are also potent B-cell stimulating agents. One possible explanation for these observations could be that FDCs induce surrounding lymphocytes to produce TNF-α and IL-6, rather than secrete these cytokines. Moreover, it cannot be excluded that FDCs secrete these or other cytokines following other appropriate activating stimuli. Alternatively, FDCs might produce a very restricted set of cytokines limited to the specialized germinal center microenvironment.

Immunophenotypic analyses have also yielded contradictory results with regard to the expression of CD4. This is relevant to explain the binding of human immunodeficiency virus (HIV) particles to FDCs in acquired immunodeficiency syndrome-related lymphadenopathy. Although not pathognomonic, continuous lysis of FDCs within degenerating germinal centers is a cardinal feature of this disease and is directly correlated with clinical deterioration. Lack of CD4 message, together with negative antigen expression, clearly shows that FDCs do not synthesize the CD4 molecule. It is possible that adherence of HIV to FDCs may involve an alternative mechanism other than classical binding of HIV to the CD4 receptor.

In summary, this study shows that FDCs express a unique mRNA pattern that is not characteristic of any classical cellular derivative. Resolution of the origin of FDCs and isolation of their precursors will aid us in studying the function of these cells both in normal and neoplastic microenvironments.

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