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Short Communication

CD5 Monoclonal Antibodies React with Human Peripheral Blood Dendritic Cells

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CD5 monoclonal antibodies (MAbs) define a 67,000 kd monomeric glycoprotein expressed predominantly by thymocytes, mature T cells and a subpopulation of B cells. CD5 is believed to be an alternative signaling molecules capable of increasing the supply of second messengers and thereby altering the cellular response threshold to other activation stimuli. Human peripheral blood dendritic cells (PBDC) are a circulating component of the immune dendritic cell family, which also includes Langerhans' cells in epithelia and interdigitating cells in the T-cell domains of lymphoid tissues. PBDC comprise less than 1% of the peripheral blood mononuclear cell fraction. They are morphologically, immunophenotypically, and functionally distinct from monocytes. In this study, we report that at least a subpopulation of PBDC react with the anti-CD5 MAbs Leu-1 and UCHT2, which define the two major non-crossblocking CD5 epitopes. In contrast, Langerhans' cells, interdigitating cells, monocytes, and macrophages were uniformly CD5$^-$. These findings suggest that PBDC can express the CD5 molecule. Furthermore, they define an additional feature of many enriched PBDC that distinguishes them from monocytes and certain other mononuclear leukocytes, and may provide insights into their activation pathways. (Am J Pathol 1992, 141:789–795)

The CD5 molecule is a monomeric glycoprotein with a molecular weight of approximately 67,000 kd. It is known to be expressed by thymocytes, mature peripheral T cells, many T-cell neoplasms, a subpopulation of B cells, some B-cell neoplasms, and an incompletely characterized population of CD8$^+$ peripheral blood cells (Table 1).[1] Although CD5 cDNA has been cloned[2] the physiologic functions and ligands of the CD5 molecule are still under investigation.[1,3] One recently described CD5 ligand is the pan–B-cell antigen CD72, which is the human homolog of murine Lyb-2 and exists as a 43 kd transmembrane glycoprotein homodimer.[4,5] Binding of CD72 by CD72 MAb results in increased B-cell proliferation, upregulation of class II MHC antigen expression and a transient increase in intracellular Ca^{++} concentration.[4,5] Certain CD5 MAbs are mitogenic or comitogenic or T cells, probably by increasing the pool of second messengers.[1,3,6–9] These observations suggest that the CD5 molecule may play an important role in T-cell activation by providing an alternate signaling pathway that would create a rheostat effect modifying the cellular response threshold to other activation signals.[9] There are at least two major non-crossblocking epitopes on the CD5 molecule recognized by Leu-1 and UCHT2 MAb.[10] Three additional minor epitopes have been defined by other CD5 MAbs that probably recognize amino-acid sequences overlapping between the two major antigenic determinants.[10–12]

Peripheral blood dendritic cells (PBDCs) are a circulating subset of the immune dendritic cell family that also includes Langerhans' cells in epithelia and interdigitating cells in the T-cell domains of lymphoid tissues such as tonsil, lymph node, and spleen.[13–15] These cells share several cytologic, immunophenotypic, and functional characteristics that suggest that they are more closely related to one another than to peripheral blood monocytes or their tissue counterparts, macrophages. For example, enriched PBDCs are larger than monocytes, and exhibit a bluntly lobated nucleus, clear cytoplasm, and

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Table 1. *$CD5^+$ Mononuclear Cell Subsets*

Cell type	Comment
Thymocytes	Medulla > cortex
Mature T cells	Vast majority of cells
T-cell lymphomas/ leukemias	Widely expressed in various subtypes but may be deficient
B-cell subset	Small subset in mantle zone of B-cell follicles
B-cell lymphomas/ leukemias	Predominantly chronic lymphocytic leukemia and small lymphocytic lymphoma
CD5-dim cells	CD5-dim $CD8^+$ $HNK\text{-}1^+$ $HLA\text{-}DR^+$ cells increased in HIV-infected patients. $CD8^+$ CD5-dim or $CD5^-$ cells increased in some patients with primary immunodeficiency

multiple dendritic processes or veils. They express increased cell surface levels of class II MHC antigens (HLA-DR, HLA-DP, and HLA-DQ), whereas monocytes express mainly HLA-DR. Furthermore, PBDCs are much more potent immune accessory cells than monocytes. These features are also characteristic of other immune dendritic cells.[15] PBDCs make up less than 1% of peripheral blood mononuclear cells and their enrichment requires a labor-intensive, multistep procedure lasting approximately 48 hours. Once enriched, the cells can be maintained *in vitro* for only a few days.[13,14]

During a search for additional features that distinguish PBDCs from monocytes, we discovered that CD5 MAbs react with PBDCs. We confirmed this result in additional samples, and then performed a systematic study of other types of immune dendritic cells, monocytes, and macrophages. Our results indicate that among these types of immune accessory cells, reactivity with CD5 MAbs is restricted to PBDCs. Other immunophenotypic differences between PBDCs and monocytes are also described.

Methods

Monoclonal Antibodies (MAbs)

A large panel of MAbs were employed as detailed in the Results and Table 2. This panel included the anti-CD5 MAb. Leu-1 and UCHT-2 which recognize the two major non-crossblocking CD5 epitopes.[10] All MAbs were purchased from Becton-Dickinson (Mountain View, CA) except UCHT-2, which was purchased from Accurate Scientific (Westbury, NY).

Cells and Tissues

PBDCs and monocytes were enriched from the peripheral blood of healthy donors using a 2-day, multistage cell separation protocol as described previously.[16] Skin, tonsils, and lymph nodes (see Results for diagnoses) were obtained from the Departments of Dermatology and Pathology at Case Western Reserve University or Stanford University. Tissues were snapfrozen, cryostat-sectioned, and acetone-fixed as described previously.[17]

Immunophenotypic Analysis

Live cell suspensions of PBDCs and monocytes were stained with a two-stage MAb/goat anti-mouse IgG-FITC procedure and analyzed cytofluorometrically using a FACScan instrument (Becton Dickinson, Mountain View, CA) as described previously.[16] Acetone-fixed cytocentrifuge preparations of PBDCs and monocytes, and cryostat sections of skin, tonsil, and lymph node were stained using a single-label three-stage MAb/goat anti-mouse IgG-biotin/avidin-HRP procedure as described previously.[17] Selected tissues were also stained using a double-label, five-stage Leu-1 biotin/avidin-FITC/biotin/HLA-DR-biotin/avidin-TRITC procedure as described previously.[18,19] Immunophenotypic controls included staining with various stages deleted and substitution of isotype controls for first-stage MAb.

Results

CD5 MAb Reactivity with PBDC

PBDCs enriched from healthy donors (N = 4) were tested for CD5 MAb reactivity using cytofluorometric

Table 2. *Immunophenotypic Differences Between PBDC and Monocytes*

Antigen	PBDC	Monocytes
Class II MHC		
HLA-DR	+++*	+/++/+++
HLA-DP	+++	0/tr/+/++/+++
HLPA-DQ	+++	0/tr/+/++/+++
T-cell		
CD1c	tr	0
CD4	0/tr	0/tr/+
CD5	0/tr/+/++/+++	0
B-cell		
CD24	tr	0
CD40	+/++	tr
Myeloid		
CD13	0	+
CD14	0	+/++
CD36	tr	++
Receptors		
CDw32	0	+
CD25	+/++	0
c-fms 0	+	0

* Semiquantitative staining intensity scale: 0, tr (trace), +, ++, +++.

analysis of cell-surface binding in live cell suspensions and immunoperoxidase detection of cell surface/cytoplasmic MAb localization to cytocentrifuge preparations. From 67% to 100% of PBDCs were variably CD5+ by both techniques in each case. In cytocentrifuge preparations, staining intensity varied from weak to strong with 33% to 50% of PBDCs strongly CD5+. CD5 MAb reactivity was observed with both Leu-1 and UCHT2, which define the two major non-crossblocking CD5 epitopes. Isotype controls were uniformly negative for PBDCs. Representative cytofluorometric and cytocentrifuge results are illustrated in Figures 1 and 2, respectively.

Lack of CD5 MAb Reactivity with Other Immune Accessory Cells

Peripheral blood monocytes enriched from the same donors as the PBDCs (N = 4) were uniformly CD5−. Similarly, in single-label immunoperoxidase stained sections, CD5 MAb reactivity appeared to be restricted to lymphocytes in 40 skin biopsies (23 mycosis fungoides, 4 B-cell lymphoma, 3 large plaque parapsoriasis, 3 pagetoid reticulosis, 2 peripheral T-cell lymphoma, 2 idiopathic erythroderma, 1 lymphomatoid papulosis, 1 cutaneous lymphoid hyperplasia, 1 monocytic leukemia), 9 lymph nodes (4 reactive, 4 follicular lymphoma, 1 mycosis fungoides), and 4 reactive tonsils. Representative findings are illustrated in Figure 3. This suggested that various immune accessory cells including Langerhans' cells/indeterminate cells, interdigitating cells, and tissue macrophages lacked detectable CD5 MAb reactivity. To confirm this observation, two-color immunofluorescence staining for CD5 and HLA-DR was performed on ten skin biopsies (seven mycosis fungoides, three pagetoid reticulosis) and seven lymph node biopsies (four follicular B-cell lymphoma, two reactive, one mycosis fungoides). In each case, there was no detectable CD5 MAb reactivity with HLA-DR+ cells exhibiting the morphologic features of Langerhans' cells/indeterminate cells, interdigitating cells, germinal center tingible body macrophages, or interstitial tissue macrophages (Figure 4).

Additional Immunophenotypic Analysis of PBDCs and Monocytes

PBDCs and monocytes were also analyzed with a large panel of MAbs to further define their similarities and dif-

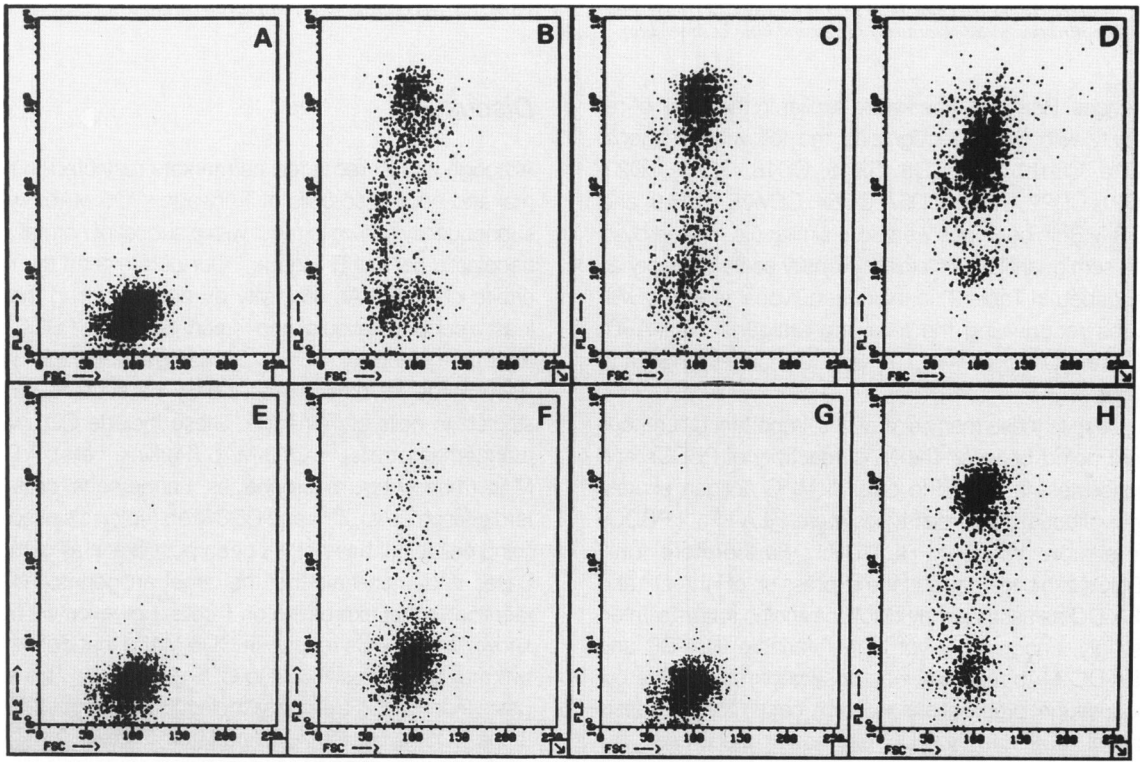

Figure 1. *Representative dot-plot cytofluorometric analysis of PBDC stained with a two-stage immunolabeling protocol (Methods). X axis shows forward light scatter (linear scale). Y axis shows fluorescence intensity (logarithmic scale). PBDC are unstained by a representative isotype matched MAb control* (A); *however, they are HLA-DR+* (B), *CD5+(Leu-1+)* (C), *CD5+(UCHT-2+)* (D), *CD3−(Leu-4−)* (E), *CD19−/CD20−(Leu-12−/Leu-16−)* (F), *CD14−(Leu-M3−)* (G), *and CD11c+(Leu-M5+)* (H). *The occasional stained cells in* (E), (F), *and* (G) *represent contaminating T cells, B cells, and monocytes, respectively.*

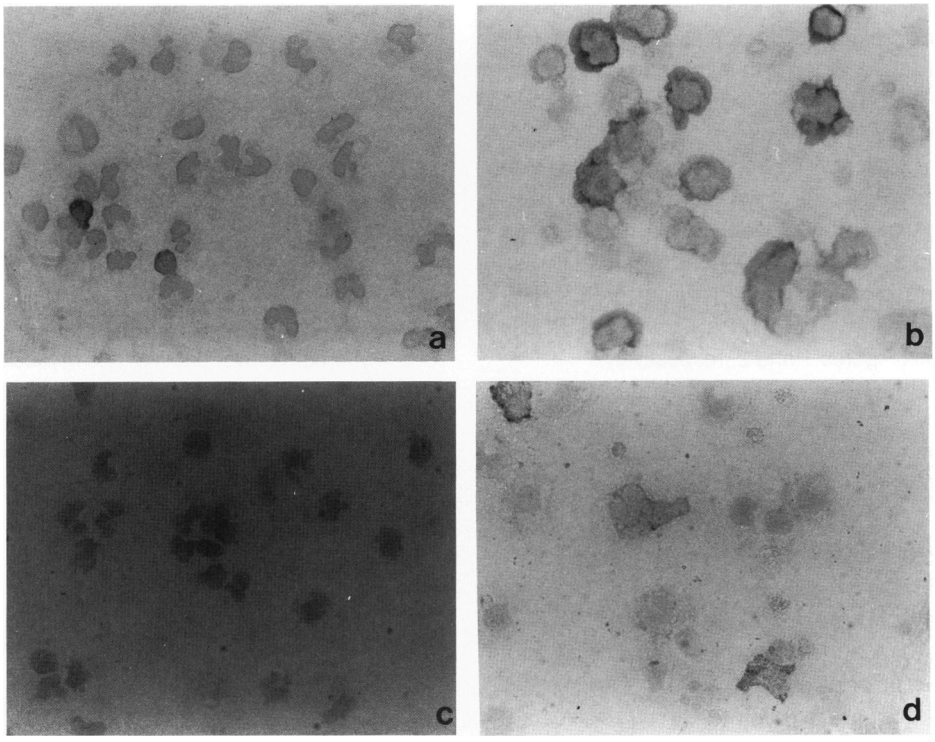

Figure 2. *Cell surface/cytoplasmic reactivity of PBDC in cytocentrifuge preparations with MAb recognizing the two major non–cross-blocking CD5 epitopes. In all fields, PBDC appear as large cells with abundant cytoplasm and bean-shaped or multilobed nuclei. Rare, smaller mononuclear leukocytes are present in some fields. These contaminants can serve as internal positive controls for MAb reactivity. Isotype matched control for Leu-1(CD5) shows CD7$^-$ PBDC and rare CD7$^+$ contaminants* **(a)**. *In contrast, most PBDC are variably Leu-1$^+$(CD5$^+$)* **(b)**. *Similarly, an isotype matched control for UCHT-2 (CD5) shows CD8$^-$ PBDC* **(c)**. *In contrast, many PBDC are variably UCHT-2$^+$(CD5$^+$)* **(d)**. *Immunoperoxidase stainwith methylene blue counterstain, ×320.*

ferences. Both cell types were similar in their lack of reactivity with MAbs recognizing the following antigens: CD1a, CD1b, CD3, CD8, CD15, CD16, CD19, CD20, CD21, CD22, CD56, CD57, CD64, CDw65, TCR-β, and TCR-δ. Both cell types were also similar (i.e., varied by ≤ one semiquantitative staining intensity scale category as described in Table 2) in their unequivocal reactivity with MAbs recognizing the following antigens: HLA-ABC, CD11a, CD11b, CD11c, CD18, CD29, CD39, CD54, and CD58.

Despite these many similarities, important differences were noted between the MAb reactivity of PBDCs and monocytes. In regard to class II MHC antigen expression, although both cell types were HLA-DR$^+$, PBDCs were more intensely HLA-DR$^+$. Furthermore, only PBDCs exhibited cell surface expression of HLA-DP and HLA-DQ as assessed by cytofluorometric analysis. Interestingly, unequivocal yet highly variable HLA-DP and HLA-DQ MAb reactivity was observed in monocyte cytocentrifuge preparations in each case. This suggests that HLA-DP and HLA-DQ expression are present but predominantly cytoplasmic in a subset of monocytes enriched according to our protocol. These and other immunophenotypic differences between PBDCs, and monocytes are summarized in Table 2.

Discussion

Although initially regarded as markers restricted to normal and neoplastic cells of T lineage, CD5 MAbs were subsequently shown to react with a subset of normal and neoplastic cells of B lineage.[1] Our data extend the repertoire of CD5 MAb reactivity by demonstrating that at least a subpopulation of non–T/non–B-lineage PBDC are also unequivocally CD5$^+$. Similar observations have been made for other MAbs initially believed to be restricted to cells of T lineage. These include CD1 MAb (Langerhans' cells),[20] CD3 MAb (Purkinje cells),[21] CD4 MAb (monocytes, macrophages, Langerhans' cells, interdigitating cells),[15,22] and CD8 MAb (splenic sinusoidal lining cells).[23] It has not yet been possible in all of these cases to demonstrate that the target antigen is, in fact, identical to that expressed on T cells. However, we have shown that PBDCs react with CD5 MAbs that define the two major non-crossblocking CD5 epitopes.[10] This suggests that PBDCs express a molecule similar or identical to the CD5 glycoprotein isolated from T cells.

The pattern of CD5 MAb reactivity with cortical thymocytes, medullary thymocytes, and peripheral T cells suggests that CD5 expression increases during T-cell maturation.[1] The range in intensity of CD5 MAb reactivity ob-

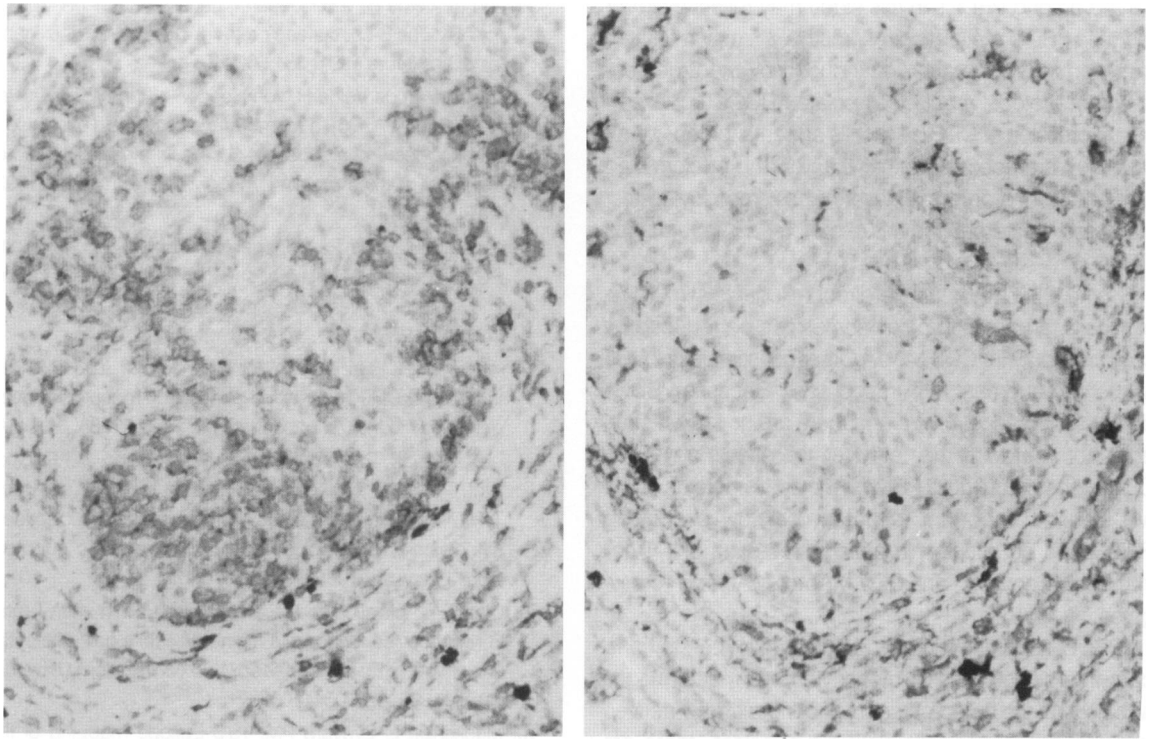

Figure 3. *Epidermal HLA-DR+ dendritic cells are CD5−. Semi-serial cryostat sections of skin involved by mycosis fungoides (cutaneous T-cell lymphoma). Most of this field shows a portion of epidermis heavily infiltrated by Leu-1+ (CD5+) T cells (left). Note that these CD5+ cells are small and round consistent with their T-cell lineage. CD5+ epidermal dendritic cells are not present. In contrast, several larger dendritic cells (mostly Langerhans cells) are evident in this same region when stained with MAb recognizing HLA-DR (right). Immunoperoxidase stain with methylene blue counterstain, ×160.*

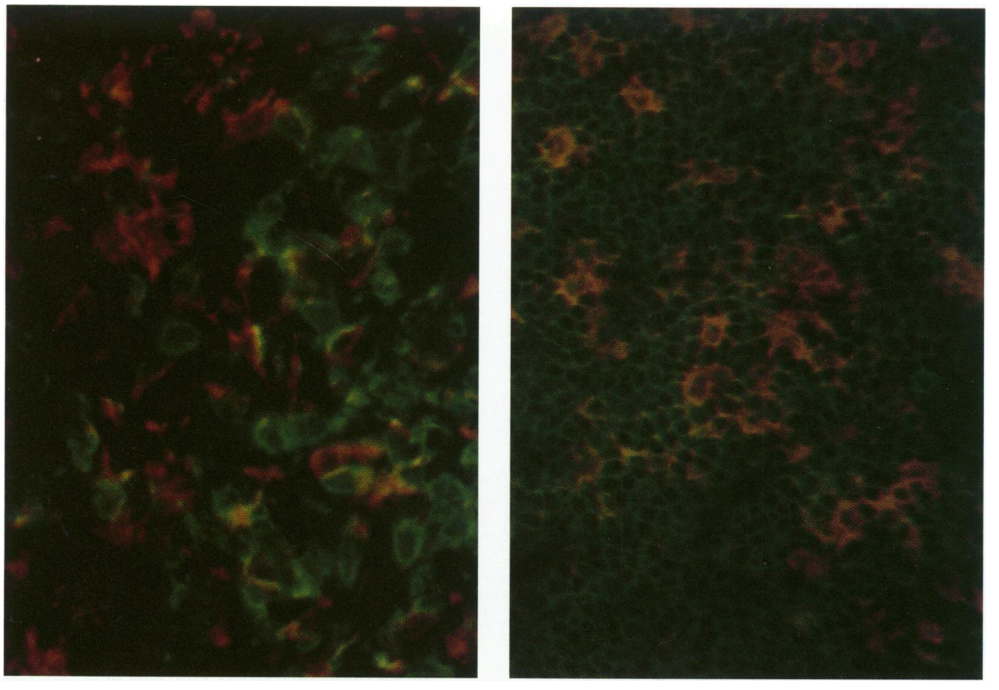

Figure 4. *Dermal and lymph-node paracortical HLA-DR+ dendritic cells are CD5−.* **Left:** *Two-color immunofluorescence analysis of CD5 and HLA-DR expression by cells in a cryostat section of skin involved by mycosis fungoides. A double-exposed photomicrograph of the same field shows round, green Leu-1+(CD5+) cells and larger, more irregularly shaped, red-orange HLA-DR+ cells (Langerhans cells/ indeterminate cells, macrophages, etc).* **Right:** *Double exposure of a similarly stained cryostat section of reactive lymph node paracortex shows large, dendritic red-orange HLA-DR+ interdigitating cells and small, round green Leu-1+(CD5+) T-cells. In both photomicrographs, if the HLA-DR+ cells were also Leu-1+ (CD5+), they would appear yellow rather than red-orange.* **Left,** *×320.* **Right,** *×160.*

served among PBDCs in this study may reflect a similar range in PBDC maturation. Alternatively, it may reflect an alteration in CD5 expression induced by the 2-day enrichment protocol required to isolate PBDCs. For example, expression of CD5 by B cells appears to depend on how they are activated rather than on a predetermined differentiation program.[9] Nevertheless, monocytes that were also isolated during this same procedure remained $CD5^-$.

Some CD5 mAbs cause direct mitogenic stimulation of T cells, an effect that can sometimes be reduced by coincubation with CD3 MAb.[3] Other nonstimulatory CD5 MAbs can augment PHA responses.[3] Because PBDCs do not survive long *in vitro* and there are no known strategies for improving their survival, the effects of various CD5 MAbs on cultured PBDCs should be determined. It will also be important to determine how circulating PBDCs will be affected in patients receiving anti–T-cell immunotherapy with anti-CD5 MAb or MAb-toxin conjugates.

PBDC, tissue immune dendritic ells (e.g., Langerhans' cells, interdigitating cells), monocytes, and macrophages all belong to the broad category of bone-marrow–derived immune accessory cells.[13–15] Nevertheless, there are numerous morphologic, immunophenotypic, and functional characteristics that indicate that PBDCs and other immune dendritic cells form a lineage distinct from monocytes and macrophages.[13–15] Among the dendritic cells, those associated with epithelia (Langerhans' cells/indeterminate cells) exhibit a $CD1a^+$ immunophenotype.[15] Our data have documented a feature apparently unique to a subpopulation of dendritic cells derived from the peripheral blood, i.e., CD5 MAb reactivity. This provides another characteristic that distinguishes enriched PBDCs from monocytes and several other peripheral blood cells, and may provide insights into PBDC activation mechanisms.

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