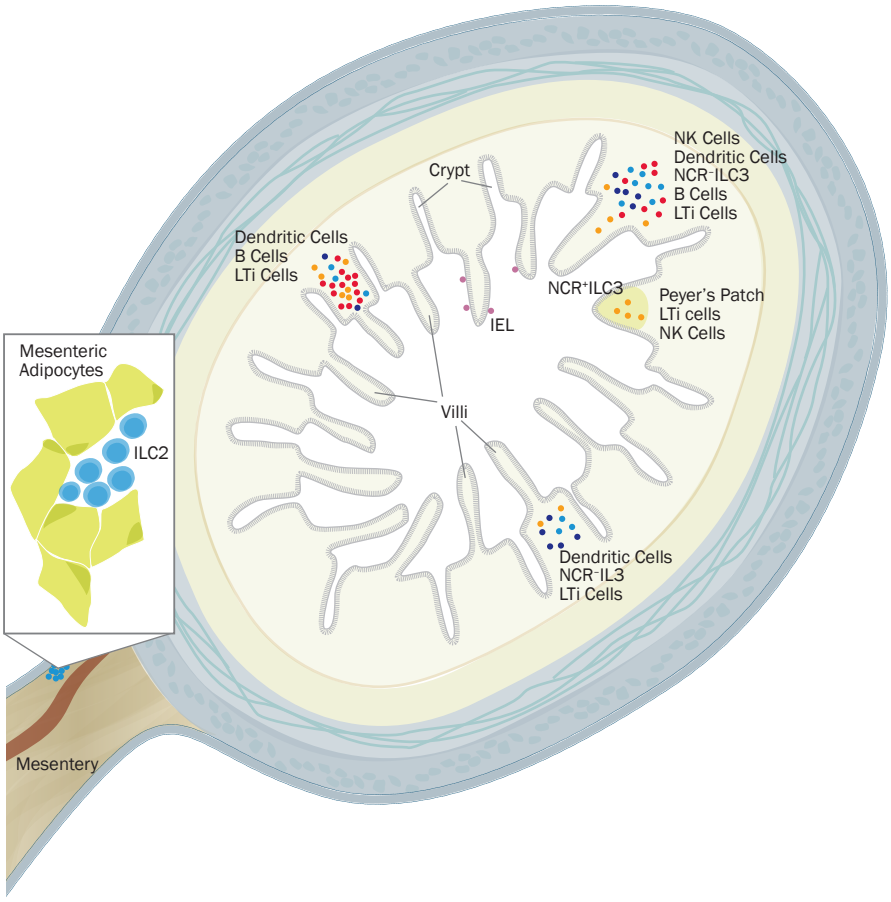
Innate Lymphoid Cells: ILCs

Tools for ILCs research



Cross-section of Small Intestine: Anatomical Localization of Innate Lymphoid Cells

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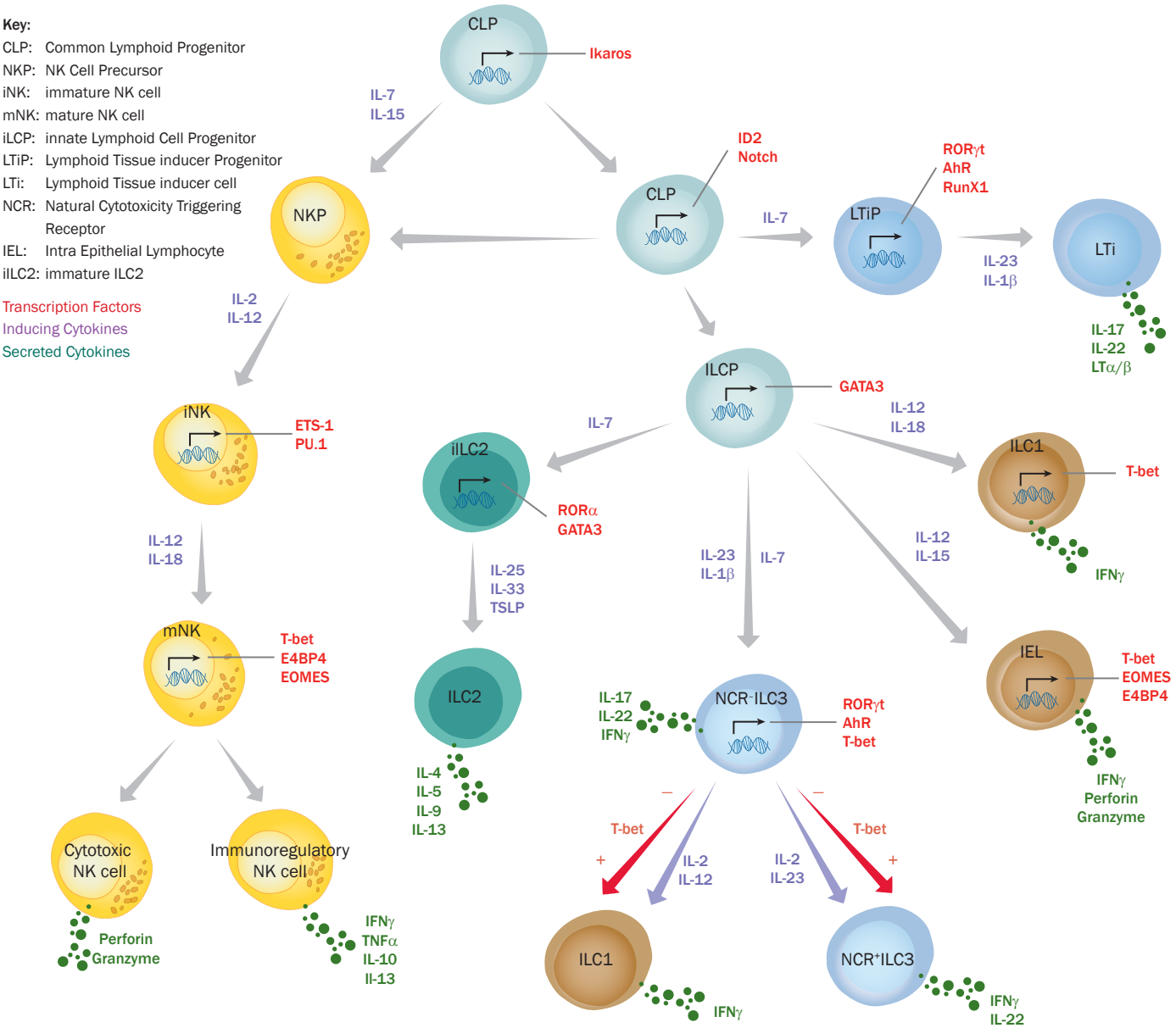
Innate Lymphoid Cell Development and Categorization

Innate lymphoid cells (ILCs) are newly described immune lymphoid cells that are morphologically similar to B cells and T cells but lack rearranged antigen receptors. ILCs secrete high concentrations of cytokines and are implicated in innate immunity, inflammation, lymphoid tissue formation, and tissue remodeling. Consistent with their role in immune surveillance and their involvement in early detection of pathogens, ILCs are localized to mucosal surfaces and respond to secreted molecules from the epithelium. All ILC populations differentiate from a common lymphoid progenitor (CLP), which is localized to the fetal liver or adult bone marrow, in response to the expression of specific transcription factors.[1] Because ILCs share developmental and functional similarities with helper T (Th) cells, nomenclature for ILCs has been established based on Th cell classification.[2] ILCs are categorized into three groups according to the transcription factors mediating their development and the cytokines they secrete. Group-1 ILCs are under the control of the T-bet transcription factor and include natural killer (NK) cells and ILC1 cells. They secrete type-1 cytokines such as IFN-γ and TNF-α in response to intracellular pathogens. Group-2 ILCs rely on the GATA3 and RORα transcription factors and produce type-2 cytokines (IL-5, IL-9, IL-13) in response to extracellular parasite infections.[3,4] Finally, Group-3 ILCs, including Lymphoid Tissues inducer cells (LTi) and ILC3 cells, are under the control of the RORγt transcription factor and produce IL-17 and/or IL-22.[5] LTi cells are required for the development of lymphoid tissues, while ILC3 cells mediate the balance between intestinal symbiotic microbiota and immunity.

References

1. Bendelac, A. *et al.* (2014) Nature. **508**:397.
2. Spits, H. *et al.* (2013) Nat. Rev. Immunol. **13**:145.
3. Hoyler, T. *et al.* (2012) Immunity. **37**:634.
4. Wong, S.H. *et al.* (2012) Nat. Immunol. **13**:229.
5. Luci, C. *et al.* (2009) Nat. Immunol. **10**:75.



Innate Lymphoid Cells Lineage-specific pathway. The illustration depicts a model of the hierarchy of innate lymphoid cell differentiation and should be considered neither comprehensive nor definitive.

Interact with this graphic | RnDSystems.com/ILCpathway

Innate Lymphoid Cell Gating Strategies for Flow Cytometry

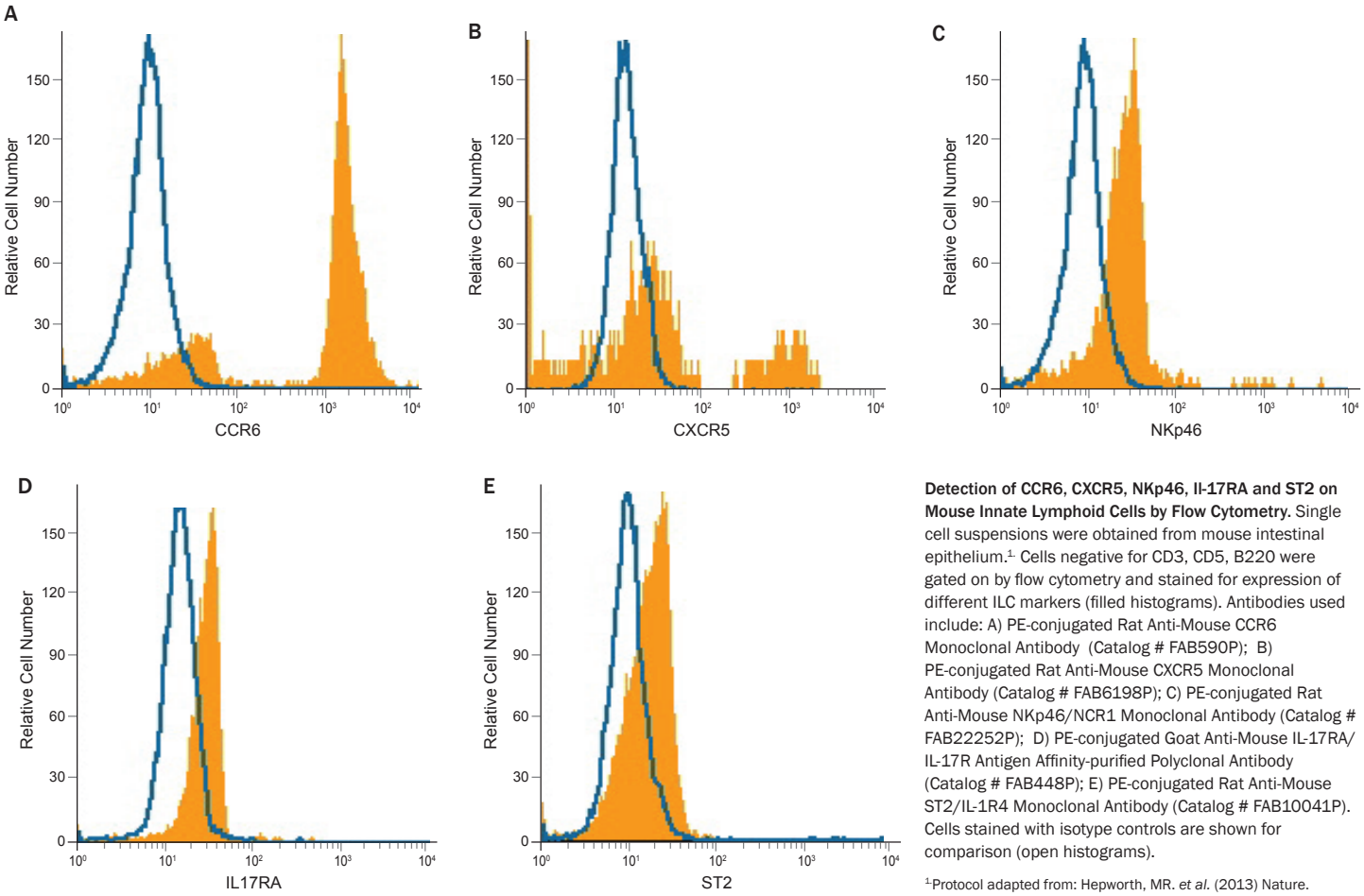
Group 1 ILCs	
Natural killer cells	
Mouse	$CD3^-$ $CD49b^+$ $CD161^+$ $NKp46^+$ $CD27^{high/low}$
Human	$CD3^-$ $CD57^+$ $CD56^{dim/bright}$ $CD16^{dim/-}$
ILC1	
Mouse/Human	$CD34^-$ $CD127^+$ $NKp44^-CD117^-$
IEL	
Mouse	$CD45^+$ $CD3\varepsilon^-$ $CD19^-$ $CD161^+$ $NKp46^+$ $CD160^+$
Human	$CD45^+$ $CD3\varepsilon^-$ $CD19^-$ $NKp44^+$ $NKp46^+$ $CD103^+$
Group 2 ILCs	
ILC2	
Mouse/Human	$CD45^+$ $CD3\varepsilon^-$ $CD4^-$ $CD8^-$ $CD19^-$ $CD11b^-$ $CD161^-$ $CD90.2^+$ T1/$ST2^+$ $GATA3^{high}$
Group 3 ILCs	
LTi cells	
Mouse/Human	$CD4^{+/-}$ $CD127^+$ Integrin $\alpha4\beta7^+$ $ROR\gamma t^+$ $NKp46^-$ $CCR6^+$
NCR^- ILC3	
Mouse	$CD45^+$ $CD3^-$ $CD19^-$ $ROR\gamma t^+$ $NKp46^-$ $CD161^-$T-$bet^{+/-}$
Human	$CD45^+$ $CD3^-$ $CD19^-$ $CD127^+$ $NKp44^+$ $CCR6^-$ T-$bet^{+/-}$
NCR^+ ILC3	
Mouse	$CD45^+$ $CD3^-$ $CD19^-$ $ROR\gamma t^+$ $NKp46^+$ $CD161^{low/-}$ T-bet^{high}
Human	$CD45^+$ $CD3^-$ $CD19^-$ $ROR\gamma t^+$ $CD127^+$ $NKp44^+$ $CCR6^+$T-bet^{high}

Flow cytometry is essential for the study of innate lymphoid cells. It allows multiparameter analysis of cell populations based on the expression of cell surface and/or intracellular molecules.

Consensus gating strategies were established in collaboration with researchers in the ILC field.[1,2]

References

1. Fuchs, A. *et al.* (2013) Immunity. **38**:769.
2. Molofsky, A.B. *et al.* (2013) J. Exp. Med. **210**:535.

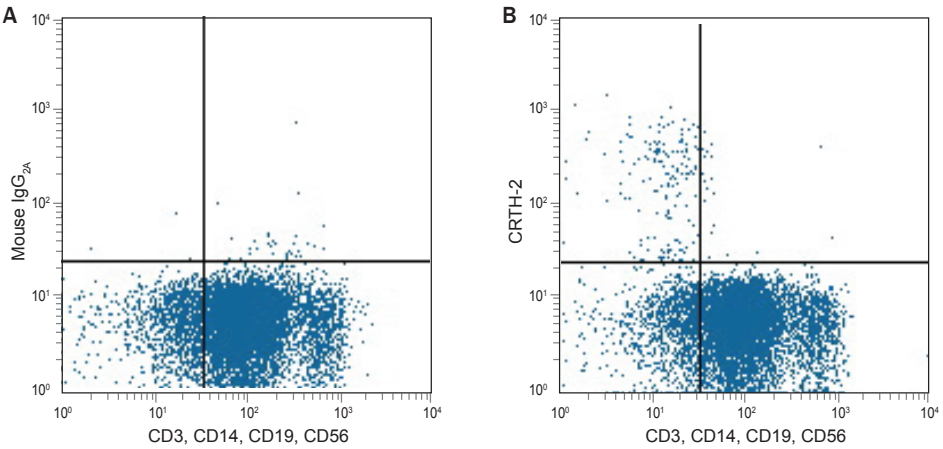


Detection of CCR6, CXCR5, NKp46, Il-17RA and ST2 on Mouse Innate Lymphoid Cells by Flow Cytometry. Single cell suspensions were obtained from mouse intestinal epithelium.[1] Cells negative for CD3, CD5, B220 were gated on by flow cytometry and stained for expression of different ILC markers (filled histograms). Antibodies used include: A) PE-conjugated Rat Anti-Mouse CCR6 Monoclonal Antibody (Catalog # FAB590P); B) PE-conjugated Rat Anti-Mouse CXCR5 Monoclonal Antibody (Catalog # FAB6198P); C) PE-conjugated Rat Anti-Mouse NKp46/NCR1 Monoclonal Antibody (Catalog # FAB22252P); D) PE-conjugated Goat Anti-Mouse IL-17RA/IL-17R Antigen Affinity-purified Polyclonal Antibody (Catalog # FAB448P); E) PE-conjugated Rat Anti-Mouse ST2/IL-1R4 Monoclonal Antibody (Catalog # FAB10041P). Cells stained with isotype controls are shown for comparison (open histograms).

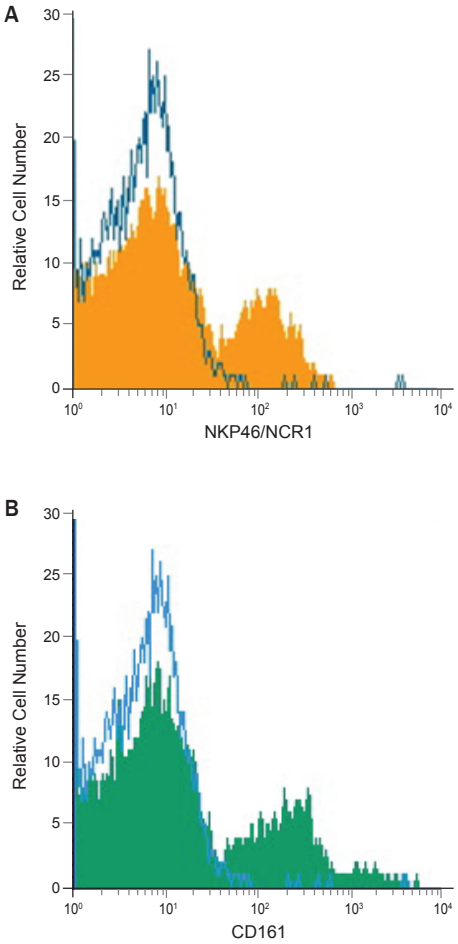
[1]Protocol adapted from: Hepworth, MR. *et al.* (2013) Nature. **498**:113.

Detection of Intracellular Cytokines for Flow Cytometry

Molecule	Species	Fluorochrome-conjugated Antibodies for Flow Cytometry					
		APC	Fluores-cein	PE	PerCP	Alexa Fluor	
						488	700
Group 1 ILCs: NK, ILC1, IEL							
IFN-γ	Human	IC285A	IC285F	IC285P	IC285C	IC285G	
	Mouse	IC485A	IC485F	IC485P	IC485C		IC485N
TNF-α	Human		IC210F	IC210P			
	Mouse		IC410F	IC410P			
IL-10	Human	IC2172A	IC2172F	IC2172P	IC2172C		
	Mouse						
Group 2 ILCs: ILC2							
IL-4	Human	IC304A	IC304F	IC304P	IC304C		
	Mouse						
IL-5	Human		IC605F	IC605P			
	Mouse			IC405P			
IL-9	Human	IC209A					
	Mouse						
IL-13	Human	IC2131A	IC2131F	IC2131P			
	Mouse						
Group 3 ILCs: LTi, NCR$^+$ILC3, NCR$^-$ILC3							
IL-17/IL-17A	Human	IC3171A		IC3171P	IC3171C	IC317G	IC317N
	Mouse		IC421F	IC421P	IC421C		
IL-17E/IL-25	Human	IC1258A	IC1258F	IC1258P			
	Mouse			IC13991P			
IL-22	Human	IC7821A	IC7821F	IC7821P			
	Mouse	IC582A	IC582F	IC582P	IC582C		
Lymphotoxin-α/ TNF-β	Human		IC2111F				
	Mouse						



Detection of CRTH-2 on Innate Lymphoid Cells isolated from Human Peripheral Blood Mononuclear Cells (PBMCs). T cells, B cells and monocytes were removed from human PBMCs using the MagCellect Human NK Cell Isolation Kit (Catalog # MAGH109). Cells were stained with CD3, CD14, CD19 and CD56 APC and either A) PE-conjugated Mouse IgG_{2A} Isotype Control (Catalog # IC003P) or a B) PE-conjugated Mouse Anti-Human CRTH-2 Monoclonal Antibody (Catalog # FAB33381P). Quadrants were set based on isotype controls.



Detection of NKp46/NKR1 and CD161 on Innate Lymphoid Cells Isolated from Human Peripheral Blood Mononuclear Cells (PBMCs). T cells, B cells and monocytes were removed from human PBMCs using the MagCellect Human NK Cell Isolation Kit (Catalog # MAGH109). Cells negative for CD3, CD14, CD19 and CD56 were gated on by flow cytometry and stained for expression of different ILC makers (filled histograms). Antibodies used include: A) PE-conjugated Mouse Anti-Human NKp46/NCR1 Monoclonal Antibody (Catalog # FAB1850P); B) PE-conjugated Mouse Anti-Human CD161 Monoclonal Antibody (Catalog # FAB7448P). Cells stained with isotype controls are shown for comparison (open histograms).

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