# MACS® Cell Separation Strategies

Miltenyi Biotec

## Untouched isolation

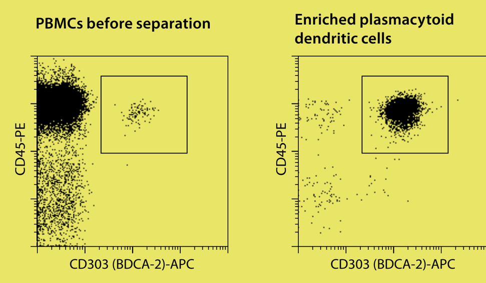
- Untouched isolation is performed by depletion of undesired cells
- Non-target cells are magnetically labeled and eliminated from the cell mixture
- The non-labeled, untouched cell fraction contains the target cells. Optionally, magnetically labeled cells can be eluted.
- For many different cell types MACS® Cell Isolation Kits for depletion of non-target cells are available

**Untouched isolation should be considered**

- for removal of unwanted cells,
- if no specific antibody is available for target cells,
- if binding of the antibody to the target cells is not desired, or
- for subsequent isolation of a cell subset by means of positive selection.

**Magnetic labeling**
Non-target cells are magnetically labeled with a biotinylated antibody cocktail and Anti-Biotin MicroBeads.

**Magnetic separation**
Undesired cells are retained in a MACS Column placed in a MACS Separator.
The target cells pass through the column and are collected as the enriched, unlabeled cell fraction, depleted of non-target cells.

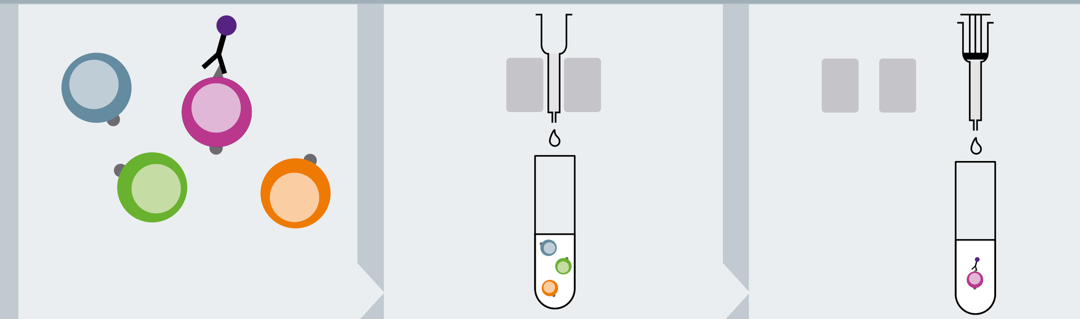



Isolation of human plasmacytoid dendritic cells from PBMCs by using the Plasmacytoid Dendritic Cell Isolation Kit, an LD Column, and a MidiMACS™ Separator.

## MACS® Technology—the gold standard in cell separation

MACS® Technology has become the standard method for cell separation. Numerous publications have proven its versatility for multiple applications: cell separations with consistent high-quality results from lab bench to clinical applications, from small scale to large scale separations, from frequently occurring cells to rare cells and sophisticated subsets. More than 250 MACS Cell Separation Reagents are available for the efficient separation of virtually any cell type.

MACS Cell Separation is based on MACS MicroBeads, specific monoclonal antibodies conjugated to superparamagnetic particles, which are only 50 nanometer in size. Cells labeled with MACS MicroBeads are retained in the magnetic field within a MACS Column placed in a MACS Separator, allowing fast and efficient separation. Separated cells—the positively labeled fraction as well as the non-labeled fraction—can directly be used for downstream applications, e.g., cell culture, expansion, or functional assays. MACS MicroBeads are extremely small and biodegradable and the whole separation procedure is fast and gentle. Thus, the separated cells remain viable and their functionality is not impaired.



**Magnetic labeling**
Cells are magnetically labeled with MACS MicroBeads in a short incubation step.

**Magnetic separation**
Cells are separated on a MACS Column placed in a MACS Separator. The flow-through can be collected as negative fraction depleted of the labeled cells.

**Elution of the labeled cells**
The MACS Column is removed from the magnetic field. The magnetically retained cells are flushed out as positively selected cells.

- Excellent purity, recovery, and viability of separated cells
- Efficient separation of frequently occurring cells as well as rare cells
- Flexible separation strategies: positive selection, untouched isolation, or sequential sorting
- From small-scale to large-scale cell separation
- Manual or automated separation

## Positive selection

- Most direct and specific way to isolate cells
- For isolation and depletion of cell subsets
- Cells of interest are magnetically labeled with MACS® MicroBeads
- Binding of MACS MicroBeads to the cell surface does not affect viability or function of the cells
- After separation, both fractions—labeled and unlabeled—can be recovered and used for downstream applications
- MACS MicroBeads are biodegradable and typically disappear after a few days in culture

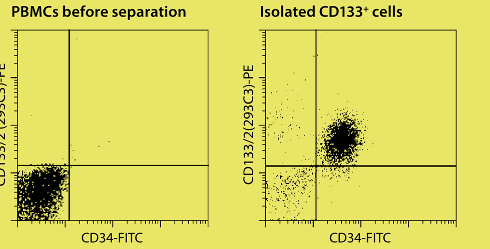
**The positive selection strategy should be considered for**

- excellent purity—even from rare cells,
- excellent recovery, and
- fast results.

**Magnetic labeling**
Cells of interest are magnetically labeled with MACS MicroBeads.

**Magnetic separation**
Cells are separated in a MACS Column placed in a MACS Separator.
The flow-through fraction can be collected as negative fraction depleted of the labeled cells.

**Elution of the labeled cell fraction**
The column is removed from the separator. The retained cells are eluted as the enriched, positively selected cell fraction.




Isolation of CD133+ cells from human PBMCs using the CD133 MicroBead Kit, two MS Columns, and a MiniMACS™ Separator.

## Sequential sorting

### Depletion followed by positive selection

- Cell subsets can be isolated by first depleting the non-target cells and then positively selecting the cell subsets of interest

**Depletion followed by positive selection should be considered**

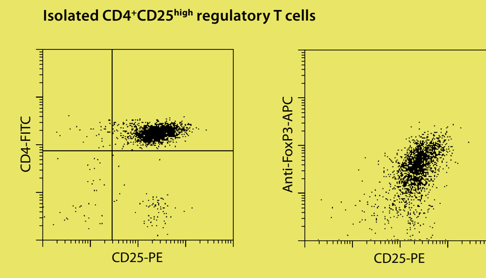
- if undesired cells in the cell suspension express the same antigen which is used for positive selection of the target cells.
- for isolation of extremely rare cells, it also can be useful to deplete non-target cells from the suspension prior to positive selection to obtain very pure cells, e.g., the isolation of human plasmacytoid dendritic cells using the Diamond Plasmacytoid Dendritic Cell Isolation Kit.

**1st magnetic labeling**
Non-target cells are magnetically labeled with a biotinylated antibody cocktail and Anti-Biotin MicroBeads.

**1st magnetic separation**
Undesired cells are retained in a MACS Column placed in a MACS Separator while the unlabeled cells pass through.

**2nd magnetic labeling**
Target cells are magnetically labeled with MicroBeads according to a subset marker.

**2nd magnetic separation**
Target cells are retained in the column while unlabeled cells pass through.
After the column is removed from the separator, the target cells are eluted as the enriched, positively selected cell fraction.




Isolation of human peripheral blood CD4+CD25high regulatory T cells using the CD4+CD25+ Regulatory T Cell Isolation Kit.
Cells were stained with CD4-FITC, CD25-PE, and intracellularly with Anti-FoxP3-APC.

## MultiSort strategy

- Sequential sorting with MACS® MultiSort Kits allows consecutive positive selections of cells
- Even rare cells can be enriched in a time-efficient way

**MACS® MultiSort strategy should be considered for**

- isolation of cells which are characterized by multiple cell surface markers

**1st magnetic labeling**
Cells of interest are magnetically labeled with MultiSort MicroBeads.

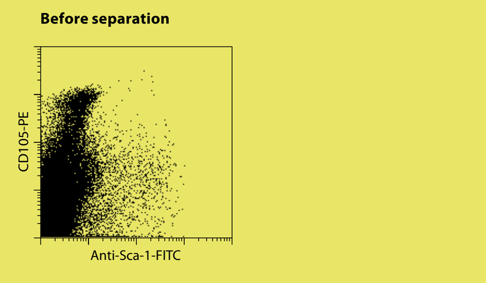
**1st magnetic separation**
Target cells are magnetically isolated by positive selection.

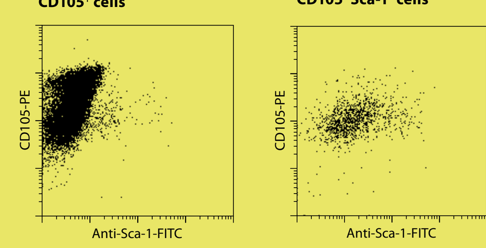
**Release of magnetic particles**
MultiSort MicroBeads are enzymatically released.

STOP
Inhibition of release reaction

**2nd magnetic labeling**
Cell subset of interest is labeled with MACS MicroBeads according to a second marker.

**2nd magnetic separation**
Target cells are separated.




Separation of CD105+ Sca-1+ cells from mouse bone marrow using the CD105 MultiSort Kit (PE), mouse, and two LS Columns for the first positive selection followed by the Anti-Sca-1 MicroBead Kit (FITC), mouse, and two MS Columns in the second positive selection step.

## MACS® MicroBeads

MACS MicroBeads are superparamagnetic particles that bind to specific antigens on the cell surface and magnetically label these cells. The MicroBeads do not alter structure, function, or activity status of labeled cells.

MACS MicroBeads are nanosized particles which are not detectable via scanning electron micrograph. This image shows a CD8+ T cell isolated with CD8 MicroBeads.´

Image Courtesy of Prof. Groscurth/Zürich/Switzerland

Transmission electron micrograph of an isolated CD8+ T cell shows CD8 MicroBeads (arrow) on the cell surface.

Image Courtesy of Prof. Groscurth/Zürich/Switzerland

- Small (50 nm) superparamagnetic particles coupled to highly specific antibodies
- Short incubation times (15 minutes)
- Non-toxic and biodegradable
- No bead detachment required:
  Labeled cells can go straight to flow cytometric analysis, cell culture, or other downstream applications

## MACS® Columns and Separators

MACS Columns and MACS Separators are designed for fast and easy cell separation of cells which are labeled with MACS MicroBeads.
MACS Separators consist of strong permanent magnets.

MACS Columns are composed of a spherical steel matrix. When a column is placed in a MACS Separator, a high-gradient magnetic field is induced within the column.

The high-gradient magnetic field efficiently retains the cells that are magnetically labeled with minimal amounts of MACS MicroBeads.

- Gentle to cells
- Thorough rinsing procedure
- Convenient for working under sterile conditions

**A MiniMACS™ Separator** holds one MS Column.

**A MidiMACS™ Separator** shown with an LD Column.

**The QuadroMACS™ Separator** attached to a MultiStand and shown with LS Columns and the 15 mL Tube Rack.

**The autoMACS® Pro Separator** allows walk-away cell sorting of multiple samples, even directly from whole blood and bone marrow.

MACS Separators are available for manual and automated cell separation.
The MACS Separator portfolio allows the convenient processing of a wide range of sample sizes and cell numbers.

## MACS® Whole Blood Cell Sorting

- Cells are labeled with Whole Blood MicroBeads to be separated by using the autoMACS® Pro Separator, the autoMACS Separator, or MACS® Whole Blood Columns
- No density gradient centrifugation necessary
- Short procedure
- Small sample volumes and high yields
- Excellent purity
- Reproducible results
- Safe handling of hazardous samples

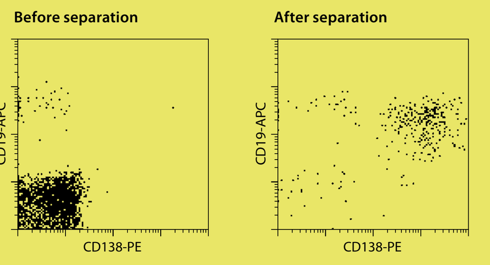
**MACS® Whole Blood Cell Sorting should be considered for**

- small sample volumes,
- time-efficient handling, or
- safe handling

1. Magnetic labeling is performed for 15 minutes by adding Whole Blood MicroBeads directly to the whole blood sample.

2. Cells are washed in a 10 minutes centrifugation step.

Cells are obtained with a high purity (90 – 95% on average).




CD138+ cells were separated from whole blood using Whole Blood CD138 MicroBeads and the autoMACS® Pro Separator with program Posselwb. Cells were fluorescently stained with CD138-PE and CD19-APC.

## Cytokine Secretion Assay

Cytokine-producing cells can be enriched by MACS® Cell Separation Technology. The secreted cytokine is captured by an affinity matrix on the cell surface. Subsequently, the cytokine is fluorescently and magnetically labeled for enrichment and detection of the cytokine-secreting cells.

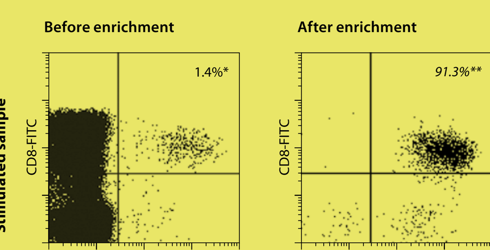
**Unique technology for**

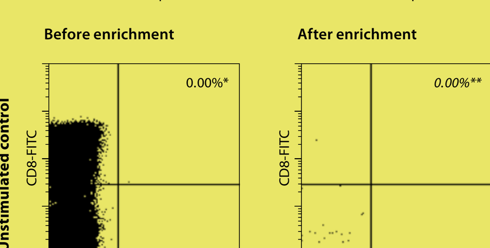
**Separation of cytokine-secreting cells**
- Cells remain viable and functional
- Separated cells can be expanded and used for functional assays, e.g., cytotoxicity assays

**Analysis of cytokine-secreting cells**
- Extraordinary sensitivity of detection (up to 1 cell in $10^6$ cells)
- For multiparameter flow cytometric analyses

**Magnetic labeling**
Cytokine-secreting cells are magnetically labeled via a cytokine-specufic affinity matrix.

**Magnetic separation**
The cytokine-secreting cells can be efficiently magnetically enriched using MACS Columns and MACS Separators.




PBMCs from a CMV+ donor were stimulated with PepTivator® – CMV pp65 or left untreated. Cells were stained and isolated according to secretion of IFN-γ using the IFN-γ Secretion Assay – Cell Enrichment and Detection Kit. The cells were counterstained with CD8-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

* Percentage represents frequency among CD8+ T cells.
** Percentage represents frequency among enriched cells.

## MACS® Labeling Strategies

### Direct magnetic cell labeling

- Fast way of magnetic labeling
- Requires only one labeling step
- Specific antibody is directly coupled to the magnetic particle
- Reduces the number of washing steps and avoids unnecessary cell loss
- Available for many different surface markers of cells from various species

Cell labeled with MicroBeads

### Indirect magnetic cell labeling

- For virtually any cell type from virtually any species
- If no direct MicroBeads for the cell type of interest are available
- Labeling of the cells with a primary antibody of choice followed by magnetic labeling with Anti-Fluorochrome , Anti-Biotin, Streptavidin, or Anti-Immunoglobulin MicroBeads
- For virtually any monoclonal or polyclonal primary antibody
- Method of choice for magnetic separation according to dimly expressed antigens

Cell labeled with Anti-Fluorochrome MicroBeads

Cell labeled with Streptavidin MicroBeads

Cell labeled with Anti-Biotin MicroBeads

Cell labeled with Anti-Immunoglobulin MicroBeads

MACS MicroBeads
Streptavidin MicroBeads
Biotinylated antibody
Fluorochrome-conjugated antibody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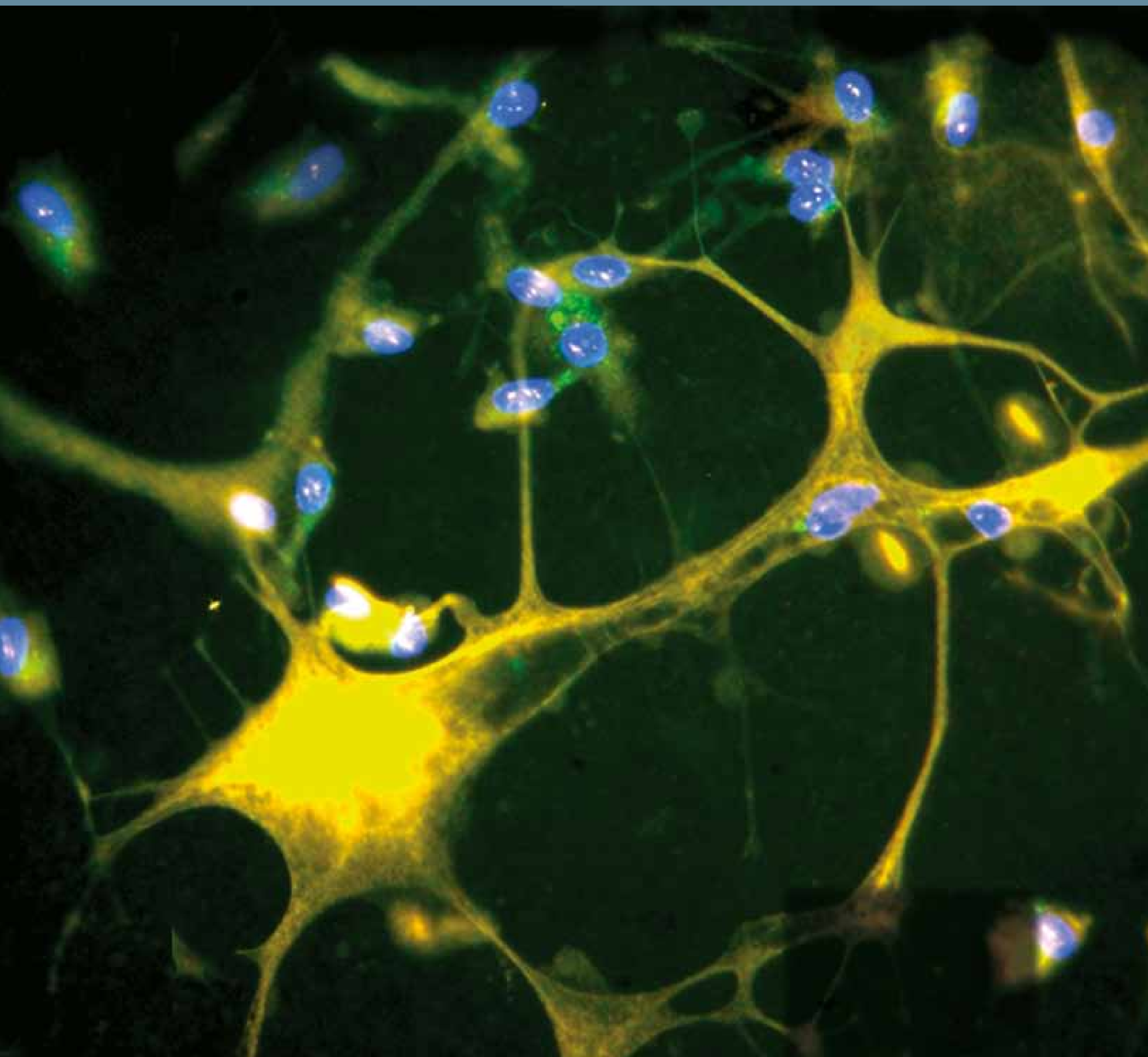
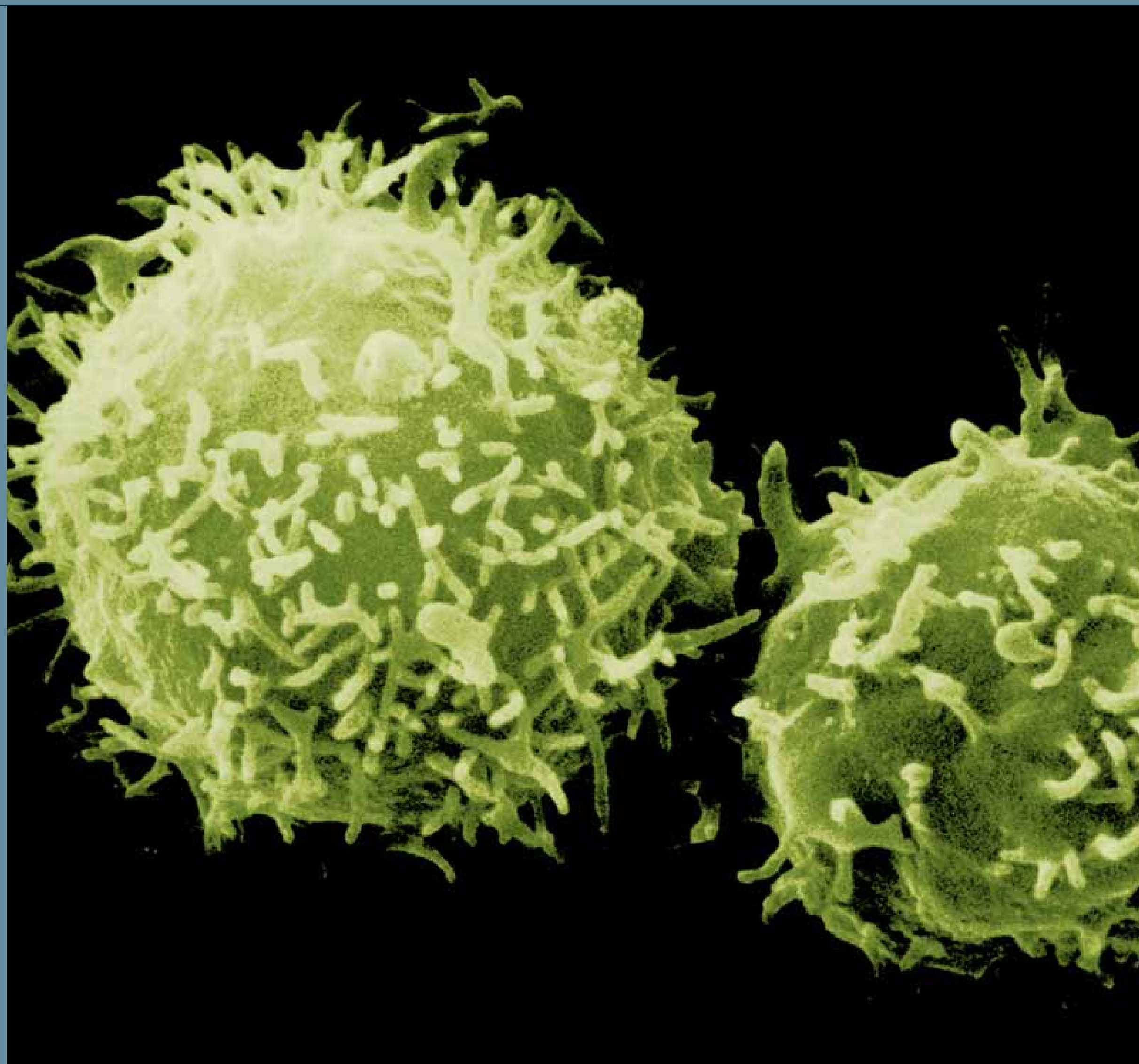
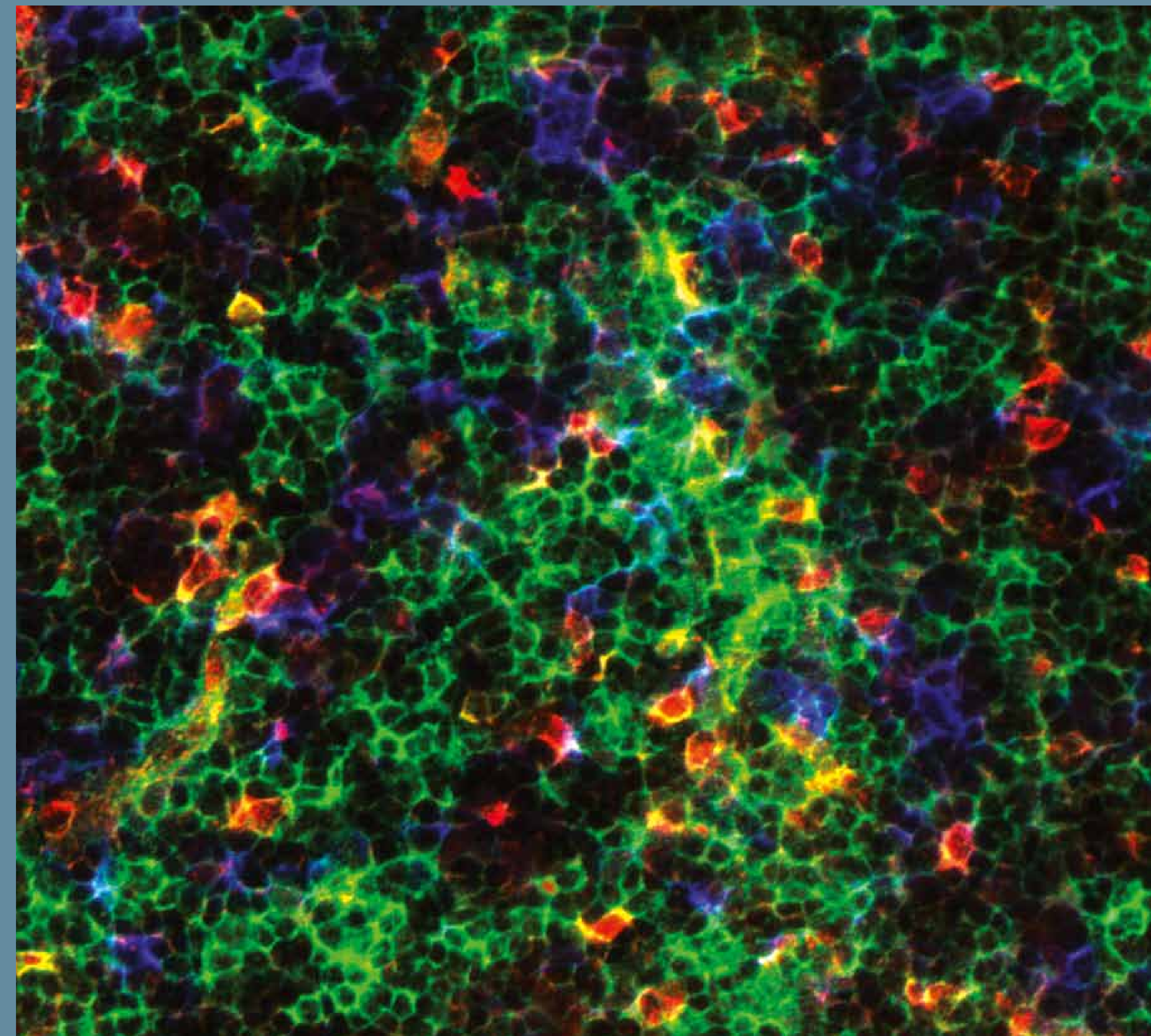
# MACS® Image Gallery



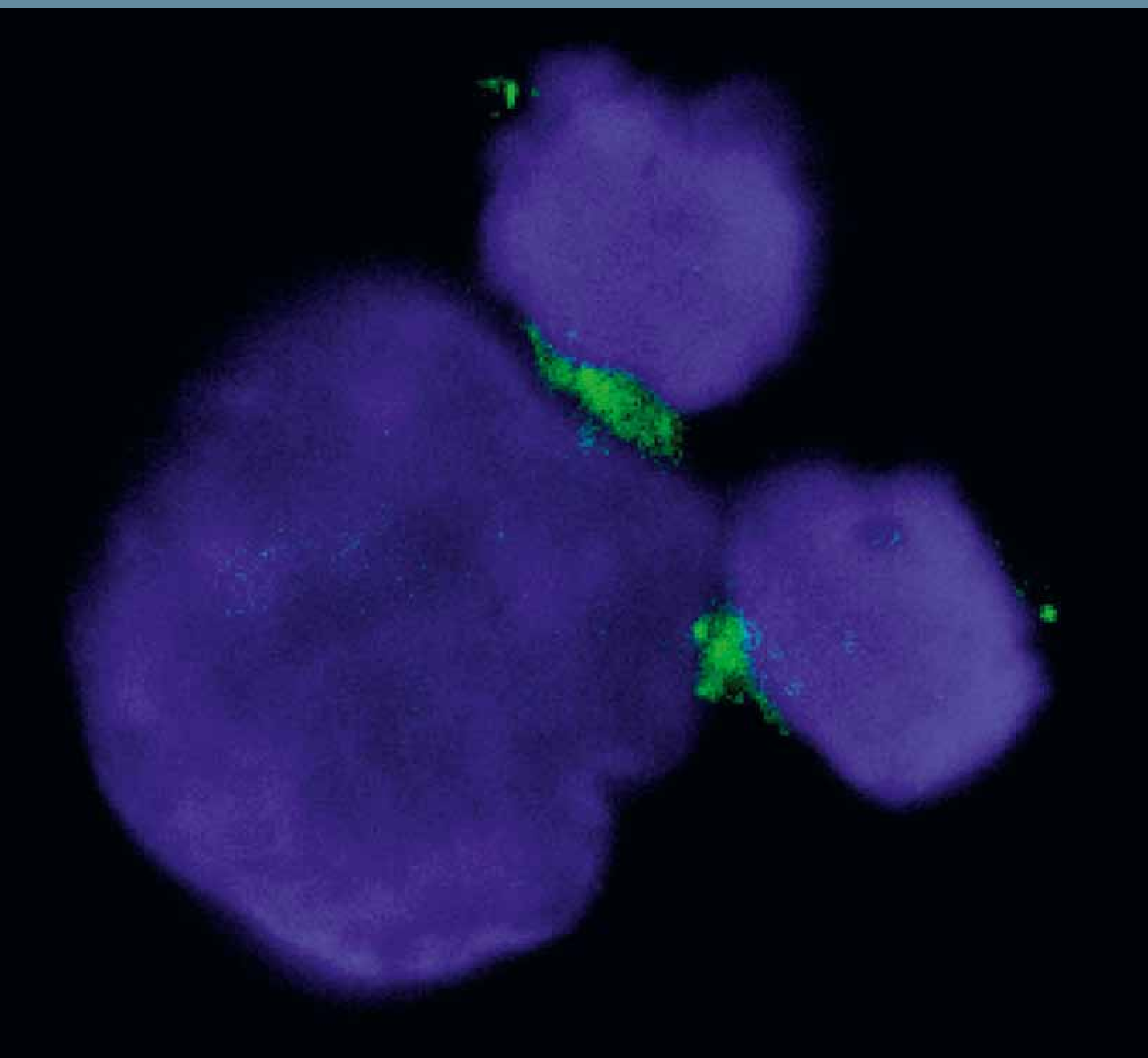
Cells from mobilized PBMCs were magnetically isolated according to the expression of CD133. The cells were cultivated for 3–5 weeks and differentiated into oligodendrocyte-like cells. The cells were stained for GFAP (FITC), MBP (Cy3), and DAPI (nuclei). (Courtesy of Selim Kuçi, Tübingen, Germany.)



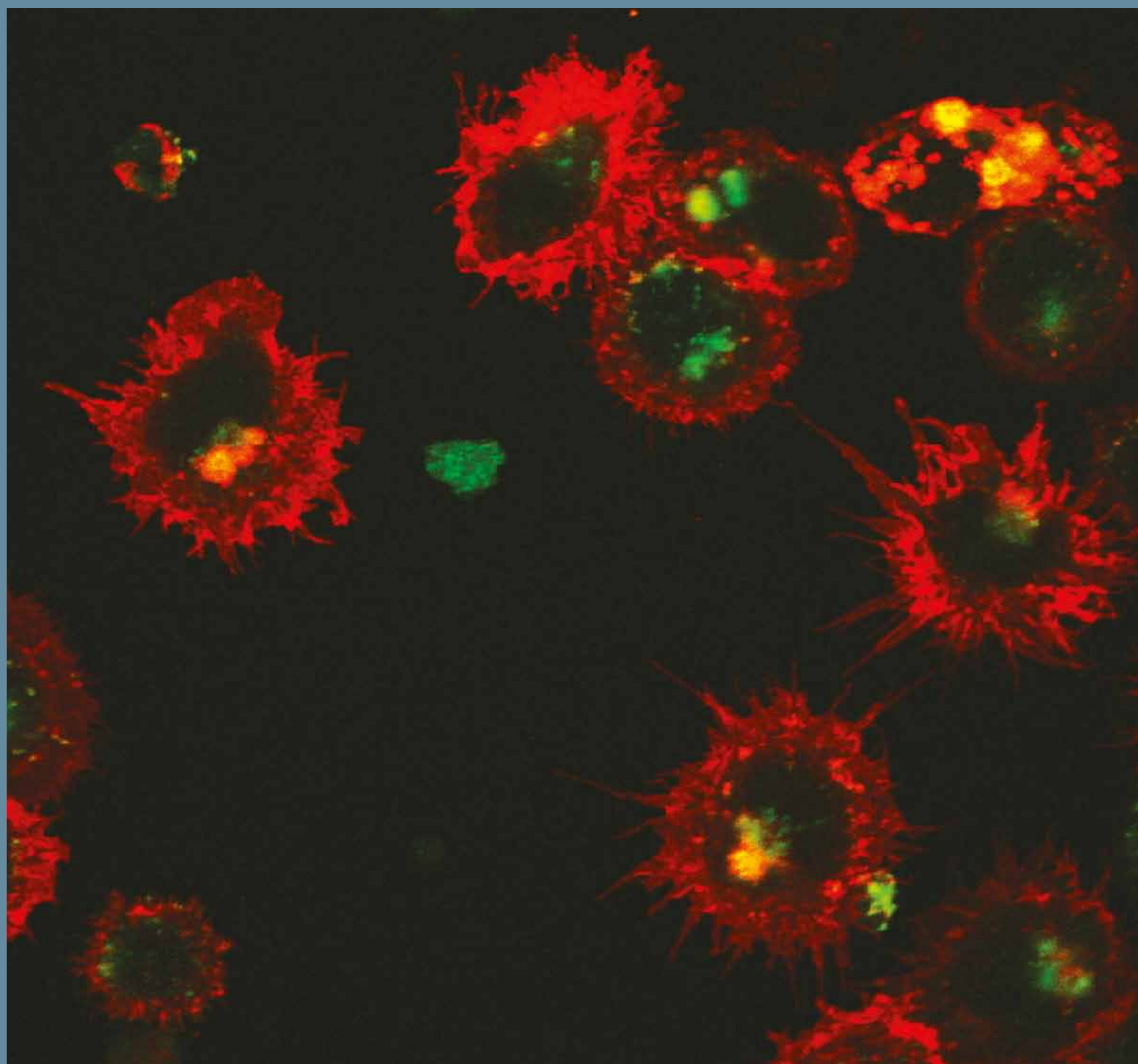
$CD8^+$ T cells were isolated by positive selection using CD8 MicroBeads and analyzed via scanning electron microscopy. (Courtesy of Prof. Groscurth, Zürich, Switzerland.)



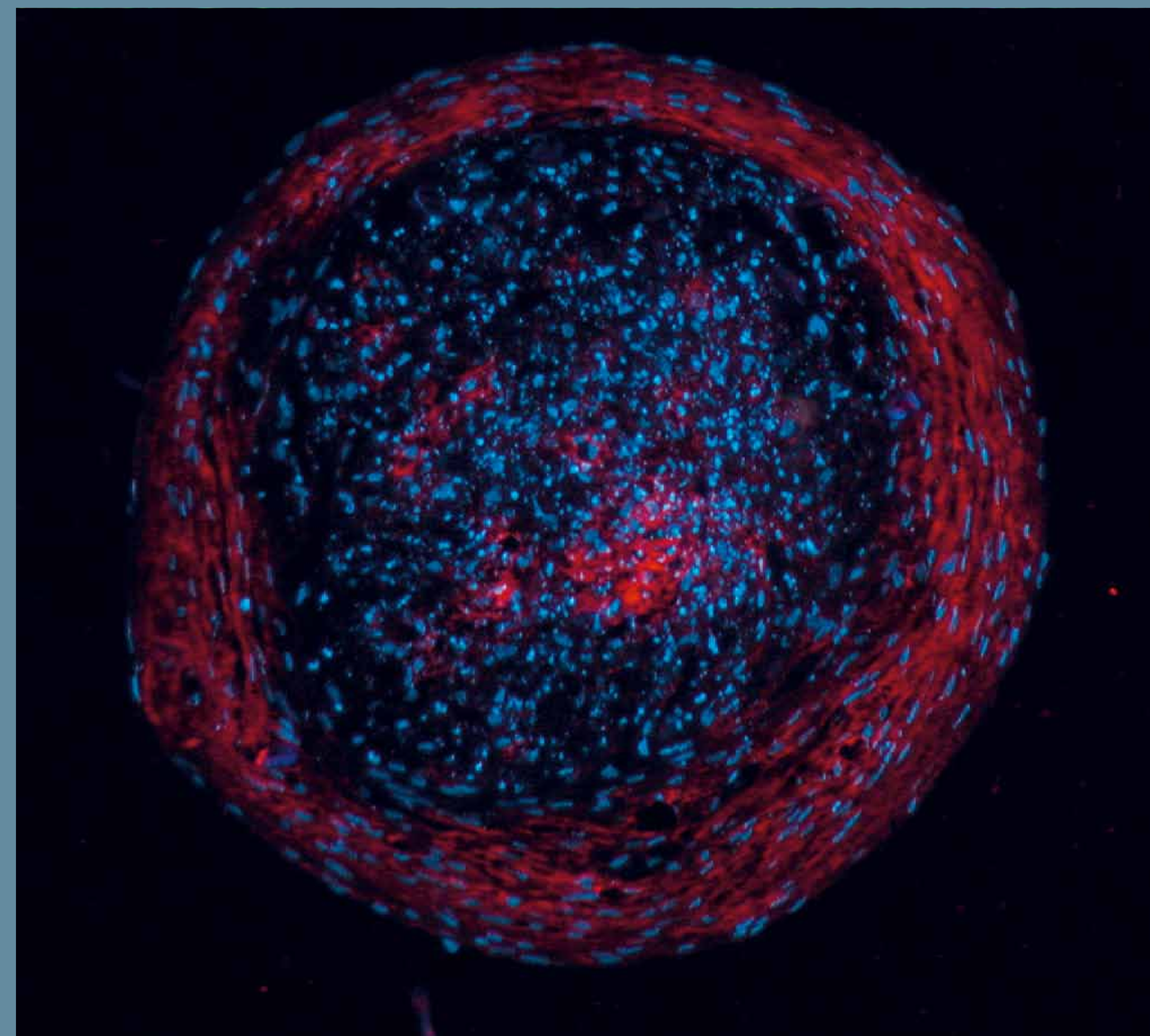
Acetone-fixed cryosections from murine lymph nodes were stained with Anti-mPDCA-1-Biotin, followed by counterstainings with Streptavidin-Cy3 (red), Anti-Ly-6C-FITC (green), and CD11c-APC (blue).
(Courtesy of Drs. L. Ohl and R. Förster, Hannover, Germany.)



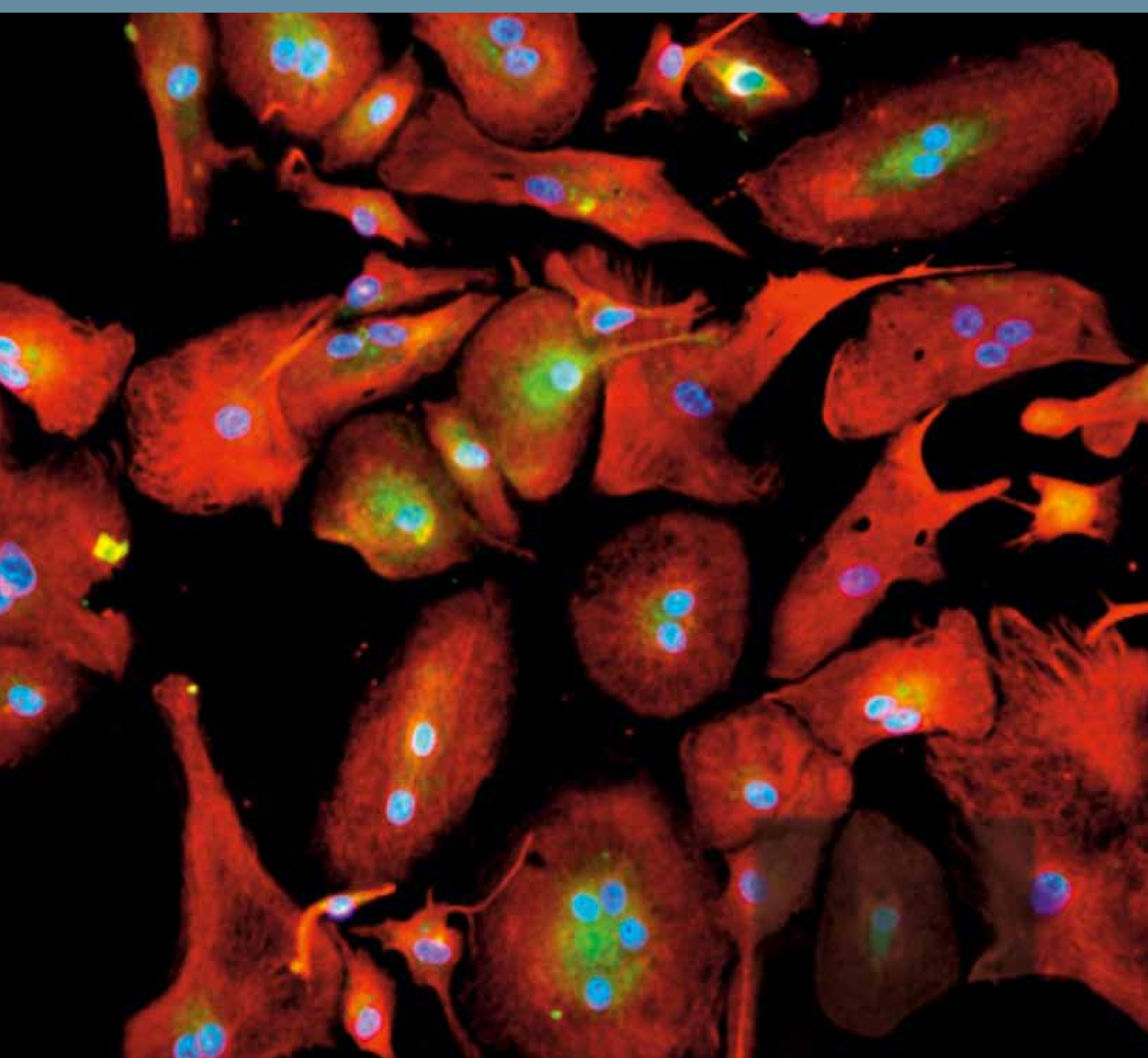
The image shows one AML blast attacked by two NK cells with capping of CD69 (green) at the immune synapse.
(Courtesy of Dr. Mark Lowdell, London, UK.)



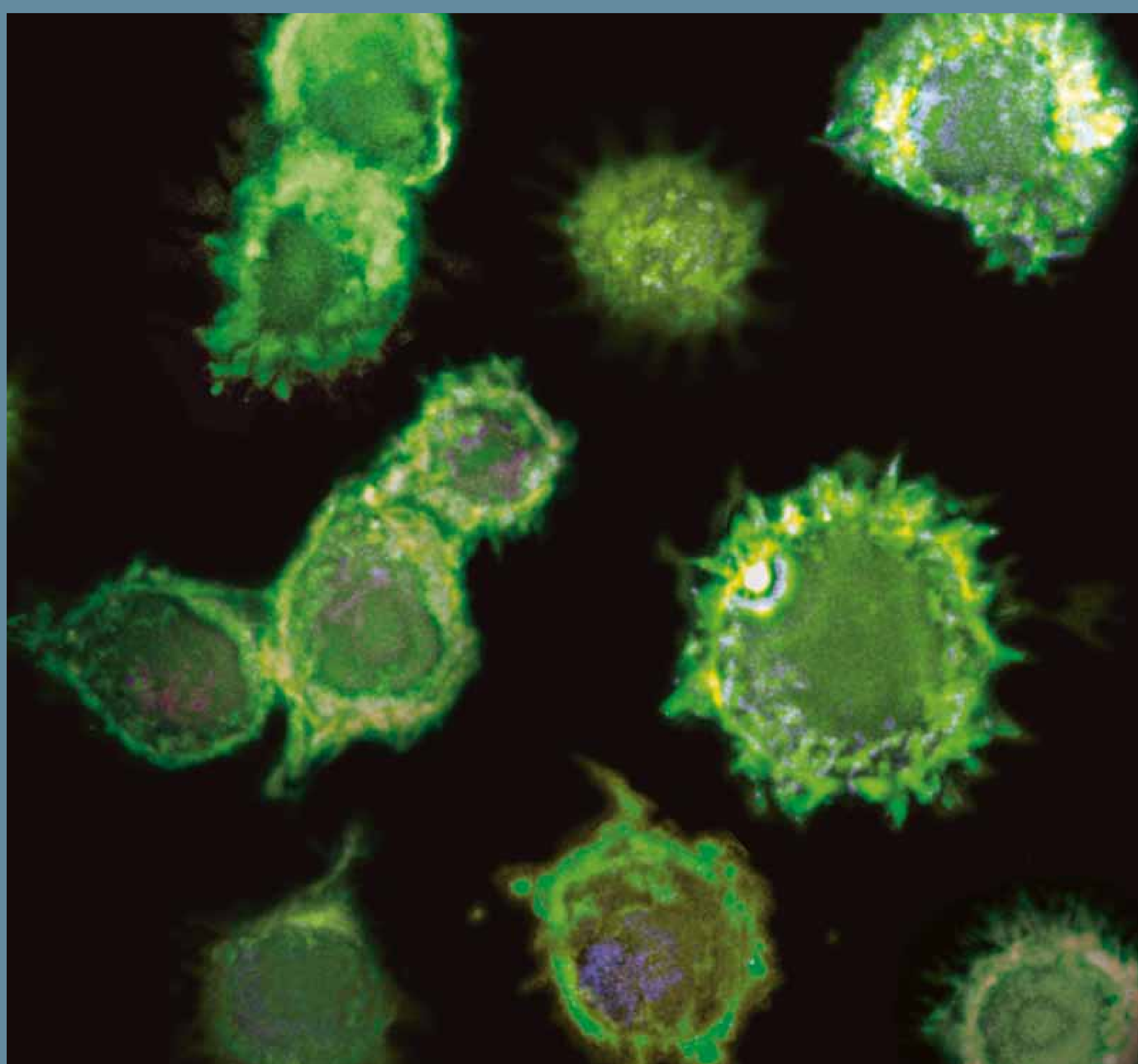
Plasmacytoid dendritic cells were isolated using MACS® Cell Separation and cultured for 48 hours with CpG B and IL-3.
The cells were stained for HLA-DR (red) and CD63 (green).



Chondrocytes were differentiated from $CD271^+$ multipotent mesenchymal stromal cells (MSCs) for 21 days in NH ChondroDiff Medium. After cultivation, paraffine-embedded sections were stained for aggrecan (red) and nuclei (blue).



Cells from mobilized PBMCs were magnetically isolated according to the expression of CD133. The cells were cultivated for 3–5 weeks and differentiated into astrocyte-like cells. The cells were stained for GFAP (Cy3), EPO (FITC), and nuclei (DAPI). (Courtesy of Selim Kuçi, Tübingen, Germany.)



Dendritic cells were generated by culturing isolated $CD133^+$ cells in the presence of Flt-3 ligand, SCF, GM-CSF, TNF-α, and TGF-β for 10 days. Thereafter, $CD1a^+$ cells were isolated using CD1a MicroBeads and stained with CD1a-FITC.

**Would you like to contribute to the MACS® Image Gallery?**

**Please send us your favorite cell images!**

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This could be your image.

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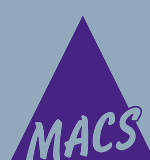


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