Journal of Immunological Methods 467 (2019) 12–18

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

A novel method to efficiently isolate medullary thymic epithelial cells from murine thymi based on UEA-1 MicroBeads

Dana Dohr[1], Robby Engelmann[*,1], Brigitte Müller-Hilke

AG Clinical Immunology, Institute of Immunology, Rostock University Medical Center, Rostock, Germany.

ARTICLE INFO

Keywords:
Medullary thymic epithelial cells
Isolation
UEA-1 MicroBeads
CD45-pluriBeads

ABSTRACT

Objective: The central mechanism for establishing a self-tolerant and functional T cell repertoire includes the promiscuous expression of otherwise tissue-restricted proteins by medullary thymic epithelial cells (TEC). We here demonstrate a novel and highly efficient method for isolating this rare key cell type.
Methods: We combined the enrichment of medullary TEC via UEA-1 MicroBeads with the subsequent depletion of residual $CD45^+$ hematopoietic cells via specific size exclusion and compared our results to the standard Percoll enrichment method and isolation procedure via flow cytometric cell sorting.
Results: The addition of 2 µl UEA-1 MicroBeads per 10^8 thymus cells turned out best for optimal enrichment (an average of 22% purity compared to 1.2% for Percoll) and yield (an average of 1.73×10^5 medullary TEC per thymus compared to 5.16×10^4 for Percoll). After depletion of residual $CD45^+$ cells, our method not only reached a purity of 75.5% but also turned out less stressful for the cells as compared to flow cytometric cell sorting.
Conclusion: We here provide a fast and versatile procedure for enriching medullary TEC that yields higher purity and recovery rates than the standard Percoll enrichment method Our enrichment procedure in combination with $CD45^+$ depletion via specific size exclusion is comparable to the current gold standard flow cytometric cell sorting method.
Significance statement: We developed a fast and versatile procedure to isolate a high number medullary TEC to investigate the biochemical processes of medullary TEC in more depths.

1. Introduction

The thymus is an epithelial-mesenchymal meshwork surrounded by a capsule of connective tissue (Anderson and Takahama, 2012; Kurd and Robey, 2016). The thymus is essential to establish a functional T cell compartment. T cell progenitors migrate from the bone marrow into the thymus and come into contact with thymic epithelial cells (TEC). TEC structure the thymus anatomically and functionally into a cortical and a medullary area in order to support thymocyte development into mature naive T cells (Anderson and Takahama, 2012; Kurd and Robey, 2016). Furthermore, the developing thymocytes induce the differentiation of the cortical and medullary TEC. This bidirectional communication between TEC and thymocytes is called thymic crosstalk which rules thymic function (Abramson and Anderson, 2017). The differentiated medullary TEC are then induced to express otherwise tissue restricted antigens (TRA) against which the developing thymocytes are selected (Derbinsk et al., 2001).

It is a well-accepted notion that the thymic crosstalk and the expression of TRAs are central mechanisms to establish a self-tolerant and functional T cell repertoire (Anderson and Takahama, 2012). However, questions such as how the terminal differentiation of medullary TEC to the involucrin-positive state is orchestrated, how the AIRE-independent fraction of TRAs is regulated and how AIRE is capable of promote TRA expression on the molecular basis are still open. Thus, methods to enrich and isolate the medullary TEC out of the thymic meshwork in a gentle and efficient way are highly warranted.

Current standard protocols for the enrichment of TEC make use of Percoll density centrifugation (Stoeckle et al., 2013) or specific complex digestion methods (Gray et al., 2008; Jain and Gray, 2014; Seach et al., 2012) followed by a depletion of the $CD45^+$ cells based on magnetic sorting. Many protocols include stressful techniques like flow cytometry cell sorting (Seach et al., 2012; Stoeckle et al., 2013). However, the

Abbreviations: TEC, thymic epithelial cells; TRA, tissue restricted antigen; UEA, Ulex Europaeus Agglutinin; RB, AutoMACS running buffer

* Corresponding author at: Schillingallee 70, 18057 Rostock, Germany.
E-mail address: robby.engelmann@med.uni-rostock.de (R. Engelmann).

[1] These authors contributed equally.

https://doi.org/10.1016/j.jim.2019.02.001
Received 7 September 2018; Received in revised form 1 February 2019; Accepted 4 February 2019
Available online 05 February 2019
0022-1759/

Table 1
Staining reagents for flow cytometry.

Staining reagent	Target cell	Final concentration
Anti-CD45:PerCP (Biolegend, Koblenz, Germany, #103130)	Hematopoietic cells	2 µg/ml
Anti-EpCAM:BV421 (Biolegend, Koblenz, Germany, #118225)	TEC	2 µg/ml
Anti-Ly51:PE-Cy7 (Biolegend, Koblenz, Germany, #108314)	Cortical TEC	2 µg/ml
UEA1:FITC (Sigma Aldrich, St. Louis, MO, USA, #L90061MG)	Medullary TEC	20 µg/ml
Anti-CD80:PE (Biolegend, Koblenz, Germany, #104708)	Mature medullary TEC	2 µg/ml
Zombie Red Fixable Viability Dye (BioLegend, Koblenz, Germany, # 423109)	Dead cells	1:100

recovery and viability of the isolated TEC is still to be improved and did not meet our needs. Therefore, we developed a novel method to isolate medullary TEC. It is based on the magnetic enrichment of Ulex Europaeus Agglutinin (UEA)-1-binding medullary TEC followed by the depletion of $CD45^+$ cells by size exclusion. The enrichment via UEA-1 MicroBeads improved the purity and the recovery of medullary TEC compared to the standard Percoll method. Furthermore, when followed by the depletion of $CD45^+$ cells via cell straining it reaches the purity of flow cytometry cell sorting but applies less stress to the cells.

2. Protocol

2.1. Material

2.1.1. Tools

Two small forceps with a straight and serrated tip, 12-well-plate (Greiner Bio-One GmbH, Kremsmünster, Austria, Cat. #665180), three to four glass Pasteur pipets (230 mm, w/o absorbent cotton filter, Carl Roth GmbH, Karlsruhe, Deutschland, Cat. # 4522.1) with self-made decreasing opening size, 70 µm cell strainer (Greiner Bio-One GmbH, Kremsmünster, Austria, Cat. #542070), 50 ml conical tube (Greiner Bio-One GmbH, Kremsmünster, Austria, Cat. #210270) were used for the digestion of the thymus.

LS MACS columns (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, Cat. #130-042-401) and a quadroMACS magnet (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, Cat. # 130-090-976) were used for the magnetical enrichment procedure.

For cell analyses and sorts we use a BD FACS Aria IIIu machine (BD, Heidelberg, Germany) with FACS Diva Software (version 8.0.2).

2.1.2. Buffers, solutions and kits

Cell culture medium

RPMI 1640 Medium, (GlutaMAX™ Supplement, Thermo Fisher Scientific, Waltham, MA, USA, SKU # 61870-010).

AutoMACS running buffer (RB)

Phosphat-buffered saline (PBS) with bovine serum albumin (BSA) and 0.09% azide (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, Cat. # 130-091-221).

Fc Block

Solution to block the Fc part on cell surface to prevent unspecific bindings (TruStain fcX™ (anti-mouse CD16/32) Antibody, BioLegend, Koblenz, Germany, Cat. #101319).

MicroBeads

UEA-1 MicroBeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, Cat. # 130-098-770) for the magnetically enrichment of medullary TEC.

S-pluriBead Mini Reagent Kit

Contains CD45 S-pluriBead anti-ms (pluriSelect Life Science, Leipzig, Germany, SKU #70-50010-11).

Staining buffer

Phosphat-buffered saline (PBS) with 0.5% bovine serum albumin (BSA) and 0.1% acide.

2.1.3. Enzyme solutions

Dispase (Stock con. 5 kU/ml, BD Biosciences, Heidelberg, Germany, Cat. #354235), Collagenase B (Stock con. 40 mg/ml, Roche, Rotkreuz, Switzerland, Cat. #11088807001) and DNase I (Stock con. 8 kU/ml, Roche, Rotkreuz, Switzerland, Cat #04716728001).

2.1.4. Flow cytometry staining panel

See Table 1.

2.2. Procedure

2.2.1. Prearrangements

1. Prepare four glass Pasteur pipettes with decreasing opening size: cut the outlet ends with a glass cutter at different lengths to create the intended opening sizes and smooth the ends by heating over a Bunsen burner
2. Pre-cool all buffers and solutions and hold them on ice for the whole procedure
3. Thaw the enzyme solutions and bring them to room temperature

2.2.2. Tissue preparation and digestion

1. Sacrifice a mice by CO_2 asphyxiation followed by cervical dislocation
2. Remove the thymus and put it into ice cold cell culture medium till subsequent processing
3. Put the thymus in a well of a 12-well-plate, containing 500 µl medium
4. Mince the tissue with forceps into approx. 2 mm pieces
5. For enzymatic digestion add 160 µl Dispase, 2.65 µl Collagenase B and 6.35 µl DNase I and incubate at 37 °C for a total of 20–30 min
6. For additional mechanical dissociation gently pipette up and down every 10 min using the glass Pasteur pipettes with a decreasing opening diameter until the suspension becomes cloudy and free of large fragments
7. Pass the cell suspension through a 70 µm cell strainer on a 50 ml conical tube and flush remaining cells from the strainer with 10 ml RB

2.2.3. Enrichment of the medullary TEC by magnetic cell sorting

1. Count cells in a Neubauer counting chamber after staining with trypan blue (to analyze the cell composition prior to the enrichment we kept back 1×10^6 cells to stain for flow cytometric analysis)

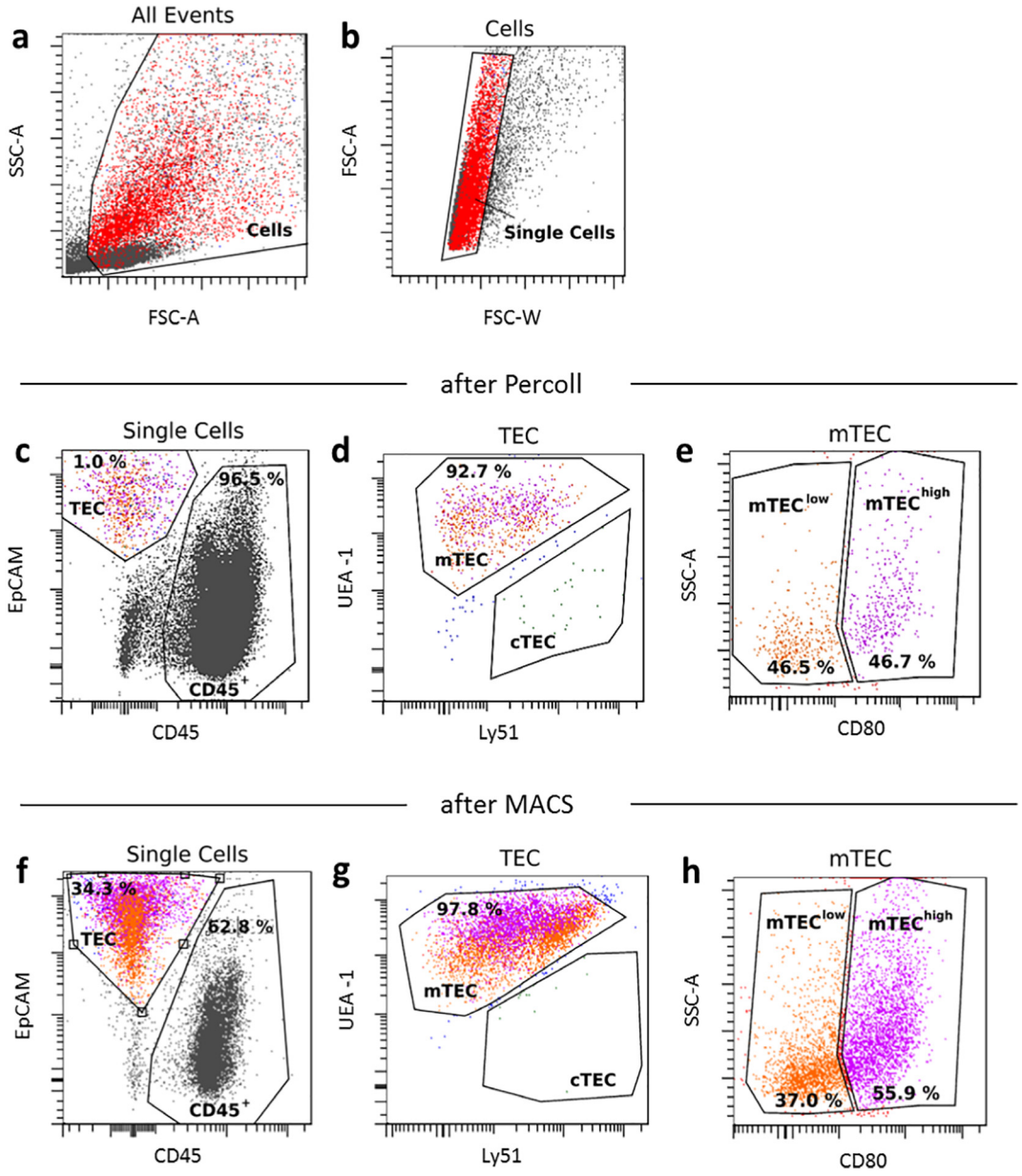

Fig. 1. Identification of different TEC populations after enrichment with UEA-1 MicroBeads (MACS) and Percoll via flow cytometry. A representative flow cytometry experiment is shown. (a) Gate for all cells in the sample. (b) Gate to exclude doublets. (c), (d), (e) analysis after the enrichment with Percoll. (f), (g), (h) analysis after the enrichment with MACS. (c), (f) Gate to discriminate TEC ($CD45^-EpCAM^+$) and hematopoietic cells ($CD45^+EpCAM^-$). (d), (g) Gate for medullary (mTEC) ($UEA\text{-}1^+Ly\text{-}51^-$) and cortical thymic epithelial cells (cTEC) ($UEA\text{-}1^-Ly\text{-}51^+$). (e), (h) Gate to further separate medullary TEC into mature medullary TEC (mTEChigh) and immature medullary TEC (mTEClow) according to their surface expression of CD80.

2. Spin down for 7 min, 300 g, 4 °C
3. Resuspend the cell pellet in 98 µl RB per 1×10^8 cells
4. Add 2 µl UEA-1 MicroBeads per 1×10^8 cells and incubate 10 min at 4 °C
5. Add 300 µl RB per 1×10^8 cells for washing the cells and centrifuge for 7 min, 300 *g*, 4 °C.
6. Resuspend the cells in 500 µl RB per 1×10^8 cells
7. Follow the MACS protocol for LS MACS columns: Insert a LS MACS column into the quadroMACS magnet and equilibrate with 2 ml RB. Afterwards load the sample, wash three times with 2 ml RB, release the column from the magnet and put it onto a 15 ml conical tube.
8. Elute the cells enriched for medullary TEC with 5 ml RB.

2.2.4. Depletion of the $CD45^+$ cells by pluriSelect

1. Count the cells after elution and spin down as described above
2. Follow the pluriSelect Protocol: Resuspend the cells in 500 µl Wash Buffer and 500 µl Buffer B (included in the Kit) and transfer the suspension into a 2 ml tube. Accordingly, add 40 µl CD45 S-pluriBeads, incubate for 30 min at room temperature on a horizontal roller mixer, attach a S-pluriStrainer on a 50 ml conical tube, equilibrate the strainer with 1 ml Wash Buffer. Afterwards, load the sample and wash with 10 ml Wash Buffer in 2 ml steps. Count the cells in the flow-through and transfer up to 1×10^6 cells into a fresh 1.5 ml tube for flow cytometric quality control.
3. The flow through will contain up to 80% medullary TEC

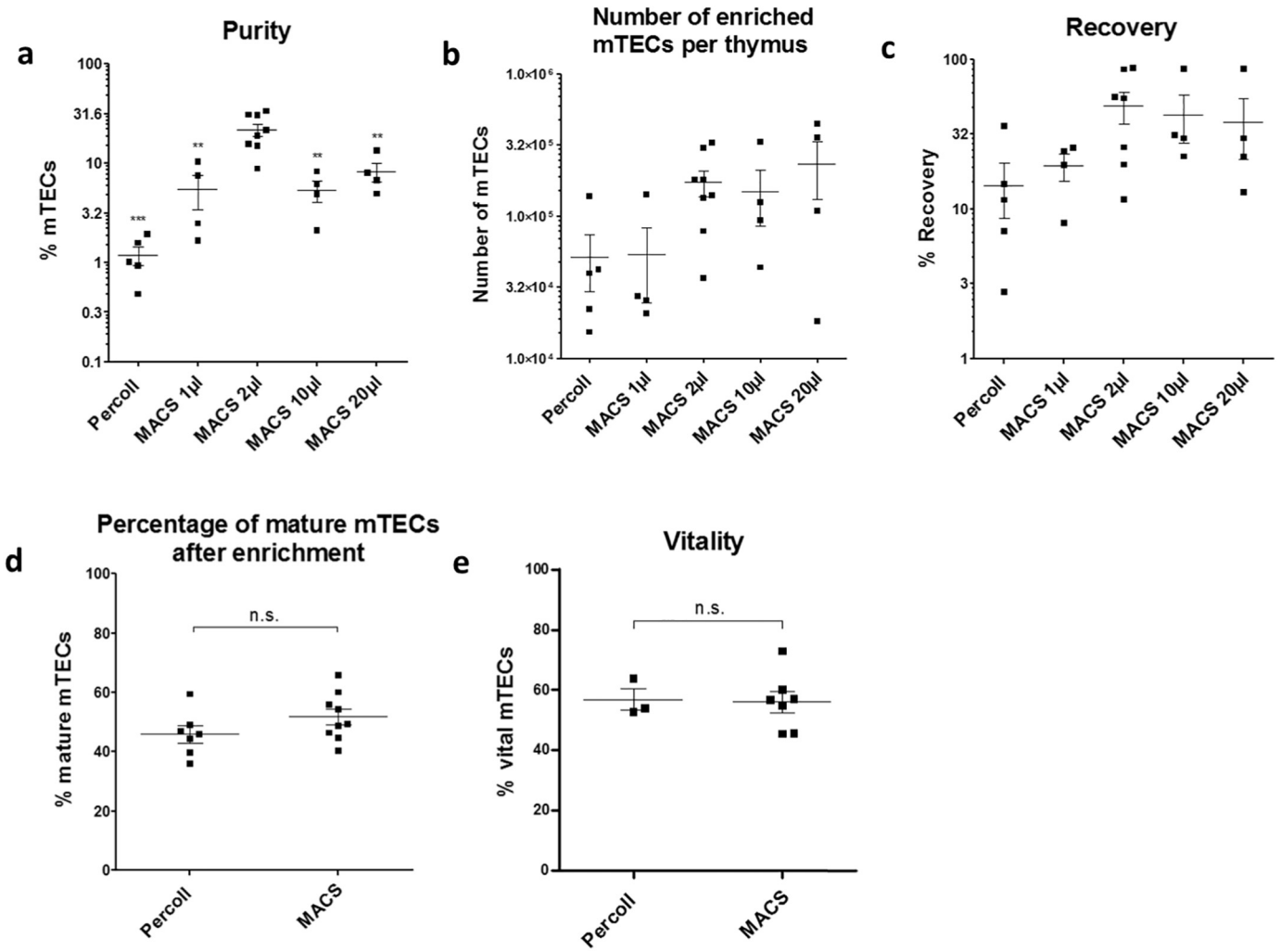

Fig. 2. Purity, number of enriched medullary TEC (mTEC), recovery per thymus and vitality after enrichment with Percoll density gradient centrifugation or MACS using UEA-1 MicroBeads. (a) The percentages of medullary TEC among all single cells, (b) total number of medullary TEC per thymus, (c) the number of medullary TEC after enrichment via Percoll or MACS in relation to the number of medullary TEC prior to the enrichment, (d) the percentage of mature medullary TEC (CD80hi) after enrichment via Percoll or MACS and (e) the percentage of vital medullary TEC after enrichment are shown. Each point represents a biological replicate. Horizontal lines indicate means and vertical lines indicate SEM. *P*-values for (a), (b) and (c) were calculated by One-Way ANOVA followed by Tukey's Multiple Comparison post hoc test. ** $p < .01$ and *** $p < .001$, compared to 2 µl MicroBead- volume. P-values for (d) and (e) were calculated by unpaired *t*-test.

2.2.5. Quality control by flow cytometry

Live/dead staining

1. Wash the cells with 500 µl 1xPBS
2. Resuspend the cells in 100 µl 1xPBS and add 1 µl Zombie Red Fixable Viability Dye
3. Incubate 15 min at room temperature in the dark
4. Wash the cells with 500 µl 1xPBS

Stain for TEC markers

1. Spin down the cells and resuspend the cell pellet in 95 µl Staining buffer and add 1 µl of UEA1:FITC, CD80:PE, CD45:PerCP, EpCAM:BV421 and Ly51:PE-Cy7, respectively (Table 1)
2. Incubate 15 min at room temperature
3. Wash and resuspend the cells with 500 µl FACS buffer
4. Perform the flow cytometric analysis

3. Methods

3.1. Mice

4–6 weeks old, female mice from different mouse lines were used: NMRI (Charles River, Wilmington, MA, USA), Balb/c (Charles River, Wilmington, MA, USA), C57BL/6 (Jackson Laboratory, Bar Harbor, Maine, USA) and SKG (first described by Sakaguchi et al., 2003, provided from Prof. Dr. Alf Hamann, DRFZ in Berlin).

3.2. Density gradient centrifugation of thymus cells

Density gradient centrifugation was performed with Percoll (Sigma-Aldrich). After digestion (as described in section 1.2.2.) up to 1×10^6 cells were kept back for flow cytometry to analyze the cell composition prior to the enrichment. Residual cells were centrifuged and the cell pellet was resuspended in 4 ml dense Percoll ($\rho = 1115$ g/ml) and transferred into a 15 ml tube coated with 1xPBS/2% BSA. The cell suspension was layered with light Percoll ($\rho = 1065$ g/ml) and finally covered with DPBS (Gibco Life Technologies GmbH, USA) containing 2% BSA (Carl Roth GmbH, Deutschland). The density gradient centrifugation was performed at 1450 *g*, 4 °C for 30 min. TEC accumulated

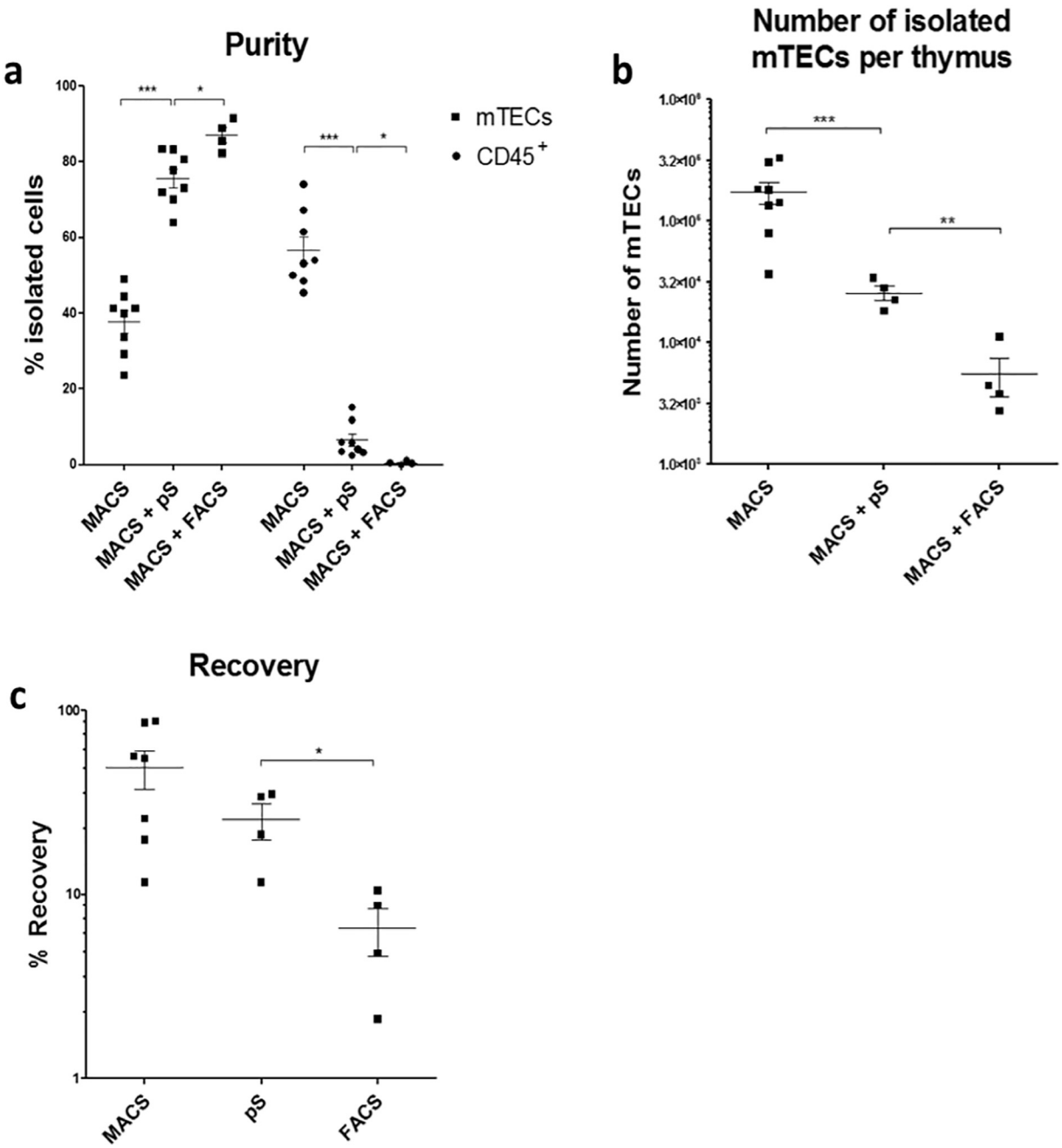

Fig. 3. Purity, number of isolated medullary TEC (mTEC) and recovery after enrichment with MACS followed by pluriSelect (MACS + pS) compared to the enrichment with MACS only (MACS) and MACS followed by FACS (MACS + FACS). (a) The percentage of medullary TEC or CD45$^+$ among all single cells after enrichment, (b) total number of isolated medullary TEC per thymus after enrichment via MACS, MACS followed by pS or MACS followed by FACS and (c) the number of isolated medullary TEC after enrichment via MACS, MACS followed by pS or MACS followed by FACS in relation to the number of medullary TEC prior to the associated enrichment procedure (MACS, pS or FACS) are shown. Horizontal lines indicate means and vertical lines indicates SEM. *P*-values between MACS and MACS + pS were calculated by paired *t*-test and *p*-values between MACS + pS and MACS + FACS were calculated by an unpaired *t*-test. *$p < .05$, **$p < .01$ and ***$p < .001$.

in the interphase between the dense and the light Percoll. Cells were counted and prepared for flow cytometric analysis after transfer into a fresh 15 ml tube.

3.3. Flow cytometry cell sorting

After enrichment via MACS single cell suspension was sorted using a BD FACS Aria IIIu machine using the same antibodies and gating scheme as used for the flow cytometric analyses (Fig. 1). The reanalysis was performed with 123count eBeads Counting Beads (eBioscience, Germany) in order to calculate the absolute number of sorted cells.

3.4. Statistics

Comparisons of multiple groups were performed by Analysis Of Variance (ANOVA) followed by Tukey's Multiple Comparison post-hoc test. Alternatively, paired or unpaired *t*-tests were used to compare the means of two groups. All analyses were performed with GraphPad Prism (version 5.01). Significance levels are indicated by "*" for $p < .05$, "** "for $p < .01$ and "*** "for $p < .001$.

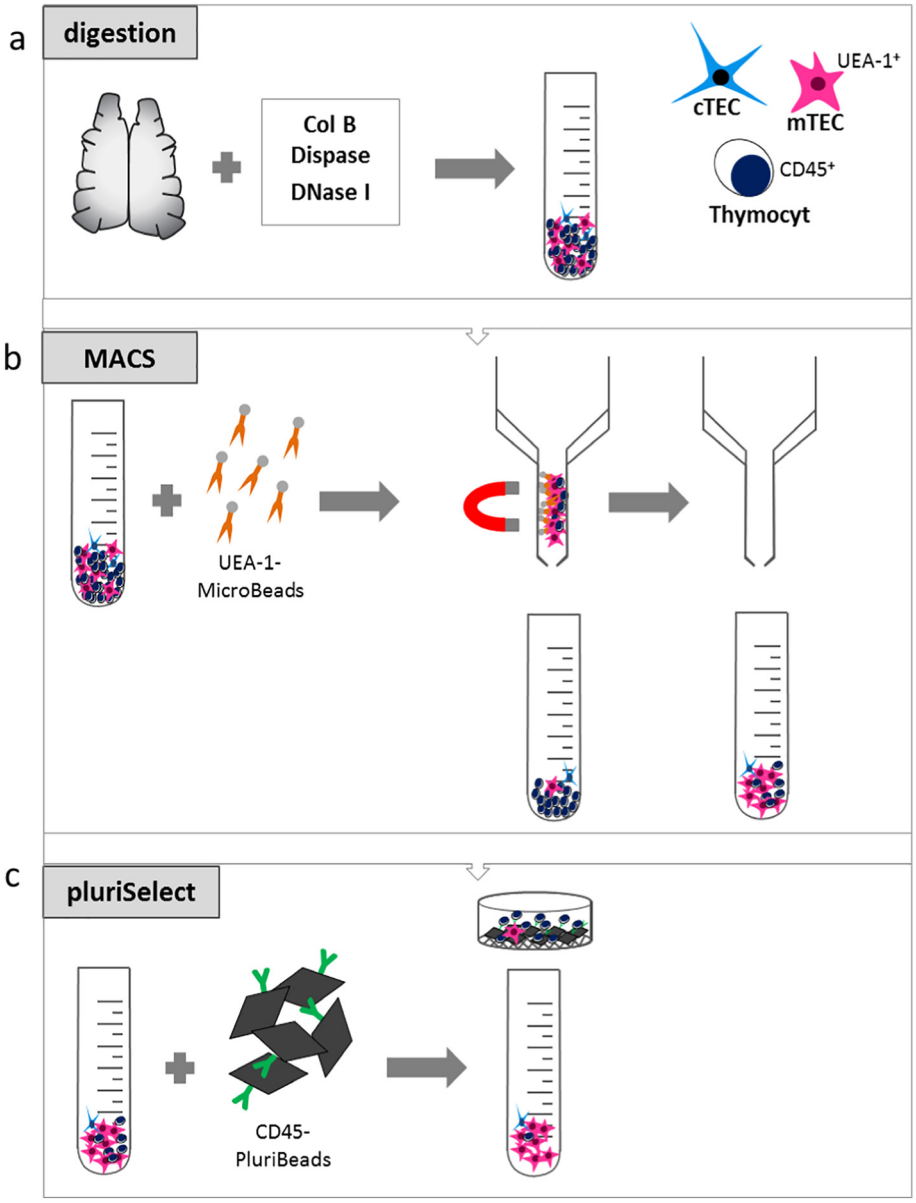

Fig. 4. Workflow of our established method. (a) Digestion of the thymus tissue with Collagenase B, Dispase and DNase I cocktail to create a single cell suspension of medullary TEC, cortical TEC and hematopoietic cells. (b) Enrichment of the medullary TEC by MACS. Medullary TEC are labeled by UEA-1 MicroBeads and are magnetically enriched. (c) Depletion of the remaining hematopoietic cells by pluriSelect. $CD45^+$ cells are labeled by CD45-pluriBeads and are subsequently depleted by size exclusion.

4. Results

4.1. Magnetic enrichment of medullary TEC with UEA-1 MicroBeads is superior to the standard Percoll method

We started out to improve the first enrichment step. Therefore, we used different amounts of UEA-1 MicroBeads to magnetically enrich medullary TEC from digested thymus preparations. In parallel, we performed the standard Percoll density gradient centrifugation protocol. The gating scheme to identify the TEC populations is exemplified in Fig. 1.

The purity of medullary TEC is significantly higher after magnetic enrichment of UEA-1-binding cells as compared to the enrichment with Percoll. Using a MicroBead volume of 2 µl showed an optimal purity of 22% in average, which was 18 times higher compared with the average purity of 1.2% after Percoll (Fig. 2a). Higher volumes of UEA-1 MicroBeads resulted in a significant drop in purity again. These results could be independently reproduced by two researchers in our lab (data not shown).

Furthermore, the number of enriched medullary TEC per thymus and the overall recovery of medullary TEC showed a trend to an improvement using the magnetic isolation of UEA-1-binding cells as compared to Percoll (Fig. 2b and c). The enrichment with Percoll yielded an average of 5.16×10^4 medullary TEC per thymus, whereas using 2 µl MicroBeads resulted in 1.73×10^5 medullary.

TEC per thymus. Similarly, the recovery of medullary TEC (relation between the number of medullary TEC after enrichment to the number of medullary TEC prior to the enrichment) reached 14.4% in average with Percoll and increased threefold to 48.8% after magnetic enrichment of $UEA\text{-}1^+$ cells. Finally, we checked the percentage of mature medullary TEC and viability of the cells after both procedures and could not detect any significant difference (Fig. 2d and e). In summary, the magnetic enrichment yielded higher purity, at minimum a comparable recovery and no difference in both the percentage of mature medullary

TEC and the viability of the cells compared to the widely used Percoll method.

4.2. Subsequent depletion of CD45$^+$ cells via cell straining shows similar purity but improved recovery of medullary TEC compared to flow cytometry cell sorting

After the first enrichment step the cell mixture still contains a high proportion of CD45$^+$ cells (about 60%). Therefore, an additional isolation method is needed to further increase the purity. We compared flow cytometry cell sorting as the gold standard with a cell straining method that remove the vast majority of CD45$^+$ cells (Fig. 3). In this independent experimental series we again enriched medullary TEC using the optimal 2 µl UEA-1 MicroBeads and reached an average purity of 38% as compared to the 22% for the first experimental series.

After depletion of CD45$^+$ cells by cell straining, the purity of medullary TEC increased substantially to an average of 75.5%. As expected, flow cytometry cell sorting reached a significantly higher purity (87%) (Fig. 3a). However, the number of isolated medullary TEC per thymus (Fig. 3b) and the recovery rate proved to be significantly lower after flow cytometry cell sorting compared to the size exclusion method (Fig. 3c).

In summary, the magnetic enrichment of UEA-1-binding cells combined with the subsequent depletion of CD45$^+$ cells via cell straining shows slightly lower purity but an increased yield of viable medullary TEC as compared to the current standard methods.

5. Discussion

We established a novel method for the gentle and efficient isolation of medullary TEC (Fig. 4). Our method provides a higher purity and recovery than described for current gold standard protocols. For instance, the protocol of Stoeckle et al., 2013 generates a purity of 0.5% of human TEC after enrichment via Percoll and 10% after depletion of CD45$^+$ cells. The data of Gray et al., 2008 demonstrated purities of about 2% of non-hematopoietic cells after using a special digestion protocol and 32% medullary TEC after depletion of CD45$^+$ cells.

They enrich 2.56×10^5 medullary TEC per thymus and show a recovery of 78%. In summary, the used methods in current protocols enrich TEC to a maximum of 53.6% purity (Jain and Gray, 2014) and a number of medullary TEC of 2.56×10^5 cells per thymus (Gray et al., 2008). Moreover, they obtain these results after the depletion of CD45$^+$ cells. We here reach purities of about 30% and a number of medullary TEC of 3×10^5 cells by using our MACS protocol already prior to the depletion. Furthermore, we only need a very low amount of UEA-1 MicroBeads (0.2 µl per 10^7 cells).

UEA-1 has been shown to be lower expressed on the immature mTEC subset (Danzl et al., 2014). However, we did not observe a significant difference in the proportions of mature CD80hi mTECs among the total mTEC population. This argues for an unbiased isolation in respect to these mTEC subsets. However, we cannot fully exclude the possibility that our method enriches the mature mTEC subset.

Seach et al., 2012 suggested that the usage of Liberase enzyme mixtures is superior in breaking down the meshwork of TEC in comparison to the combination of collagenase/ dispase/ DNAse. However, we did not replicate these results most likely because we include more vigorous mechanic forces to the thymus fragments during digestion by using glass pipettes with decreasing opening sizes. Our data shows that flow cytometry sorting of medullary TEC is the method of choice when a high purity is warranted. However, in terms of recovery of viable cells our method is superior with only slightly decreased purity of 75%. Additionally, we were able to generate cell cultures with cells obtained from our enrichment method (data not shown). Following the instruction of C. Röpke in *Epithelial Cell Culture Protocols* seeded cells developed an adherent and confluent cell culture after seven days. Over the time the number of medullary TEC were increased whereas the CD45$^+$ cells were decreased. In contrast, we do not expect the same possibility to generate a medullary TEC culture after using flow cytometric cell sorting. This method is both stressful and insufficient in quantity (Fig. 3b and c).

The property of murine medullary TEC to bind UEA-1 on its surface is conserved in human medullary TEC. Thus, our established method is likely be able to isolate medullary TEC from human thymi without extensive modifications.

In summary, we now have a novel method in our hands to isolate a high number medullary TEC to investigate the biochemical processes of medullary TEC in more depths.

Funding

The source of funding is an intramural grant (FORUN 01-14). The funding agency had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Abramson, J., Anderson, G., 2017. Thymic epithelial cells. Annu. Rev. Immunol. 35, 85–118.

Anderson, G., Takahama, Y., 2012. Thymic epithelial cells: working class heroes for T cell development and repertoire selection. Trends Immunol. 33, 256–263. https://doi.org/10.1016/j.it.2012.03.005.

Danzl, N.M., Jeong, S., Choi, Y., Alexandropoulos, K., 2014. Identification of novel thymic epithelial cell subsets whose differentiation is regulated by RANKL and Traf6. PLoS One 9, e86129. https://doi.org/10.1371/journal.pone.0086129.

Derbinsk, J., Schulte, A., Kyewski, B., Klein, L., 2001. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat. Immunol. 2, 1032–1039. https://doi.org/10.1038/ni723.

Gray, D.H.D., Fletcher, A.L., Hammett, M., Seach, N., Ueno, T., Young, L.F., Barbuto, J., Boyd, R.L., Chidgey, A.P., 2008. Unbiased analysis, enrichment and purification of thymic stromal cells. J. Immunol. Methods 329, 56–66. https://doi.org/10.1016/j.jim.2007.09.010.

Jain, R., Gray, D.H.D., 2014. Isolation of thymic epithelial cells and analysis by flow cytometry. In: Curr. Protoc. Immunol. John Wiley & Sons, Inc., Hoboken, NJ, USA. https://doi.org/10.1002/0471142735.im0326s107. S. 3.26.1–3.26.15.

Kurd, N., Robey, E.A., 2016. T-cell selection in the thymus: a spatial and temporal perspective. Immunol. Rev. 271, 114–126. https://doi.org/10.1111/imr.12398.

Sakaguchi, S., Takahashi, T., Hata, H., Nomura, T., Sakaguchi, N., 2003. SKG mice, a new genetic model of rheumatoid arthritis. Arthritis Res. Ther. 5, 10. https://doi.org/10.1186/ar811.

Seach, N., Wong, K., Hammett, M., Boyd, R.L., Chidgey, A.P., 2012. Purified enzymes improve isolation and characterization of the adult thymic epithelium. J. Immunol. Methods 385, 23–34. https://doi.org/10.1016/j.jim.2012.07.023.

Stoeckle, C., Rota, I.A., Tolosa, E., Haller, C., Melms, A., Adamopoulou, E., 2013. Isolation of myeloid dendritic cells and epithelial cells from human thymus. J. Vis. Exp., e50951. https://doi.org/10.3791/50951.