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ANTIVIRAL IL-33-STUMULATED GROUP 2 INNATE LYMPHOID CELLS (CD-90 AND CD-117) FROM MOUSE LUNGS

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ABSTRACT

This article represents not only a significant scientific value, but also a great teaching and instruction value for researchers and motivated students to improve the skill spectrum in flow cytometry. This article provides step-by- step procedure description and results based on in vivo studies on murine lung tissue, cell of interest isolation with dual protocols for flow cytometry method as well as IL-33 impact in dynamics. The newly described innate lymphoid cells of group 2 (ILC2) play an important role in type 2 immune reactions, epithelial repair in mucosal tissue and metabolic homeostasis. ILC2 releases large amounts of type 2 cytokines such as interleukin 4 (IL-4), IL-5 and IL-13, which stimulate type 2 immunity, such as protection against worms. However, without strict regulation, the level of ILC2 can cause undesirable type 2 immune pathologies, including allergic inflammation of the respiratory tract, hypersensitivity of the respiratory tract and atopic dermatitis. Viral infections of the respiratory tract, which are typical triggers of type 1 immune reactions, often lead to type 2 pulmonary immune pathologies, such as asthma and its exacerbations. Interestingly, pulmonary virus infections induce the release of IL-33 with subsequent induction of ILC2-mediated type 2 pulmonary immunopathology, which is independent of the adaptive immune system.

Keywords: Innate immunity, Cytokines, Interleukins, IL-33, CD-90, CD117, ILC2

INTRODUCTION

Type 2 immunity is important for the immune defense against worm infections, but can also be caused by infections with respiratory viruses such as respiratory syncytial virus, flu virus or rhinoviruses [1, 2]. This is surprising, since viral infections of the respiratory tract are powerful inducers of innate and adaptive immune reactions of type 1. It is important to note that viral respiratory infections are the main cause of pathologies mediated by type 2 immunity, including bronchial asthma and its exacerbations [3]. Type 2 immune reactions are characterized by the release of characteristic type 2 cytokines, such as IL-4, IL-5 and IL-13, secreted by two main cell populations: the adaptive CD4+ T helpers of type 2 (Th2) and their innate analogues, recently discovered by the congenital cells of group 2. lymphoid cells (ILC2) [4, 5]. ILC2 is in a stable state on the surfaces of the mucous membranes, including the lungs and intestines [6-8], as well as the skin and bone marrow, and multiplies rapidly when activated [9]. ILC2 belongs to a group of innate lymphoid cells, which consists of natural killer cells, lymphoid tissue inducer cells (LTI) and a group of innate lymphoid cells [1-3]. All ILC groups depend on the transcription factor Id2, as well as on the entire gamma chain of the IL-2 receptor. In addition, the transcription factors GATA3 and RORα are required for the development and function of ILC2 [10]. The induction of ILC2 depends on IL-25, IL-33 and stromal thymus lymphopoietin (TSLP), which can be released by cells of hematopoietic and non- matopoietic origin. Two main subgroups of ILC2 have been identified recently, the natural (n) ILC2 as well as the inflammatory (i) ILC2 [11]: nILC2 are elicited by IL-33, whereas iILC2 are induced by IL-25. nILC2 and iILC2 are thought to be mainly distinguished by their cytokine receptor expression pattern, as nILC2 express ST2 (IL-33R).

MATERIALS AND METHODS OF RESEARCH

1. C57BL/6 wild-type (WT) dark brown mice

Animals should be kept in an SPF environment

2. All the experiments should be completed in accordance with legislation outlined in the regulations and standard guidelines of each research facility

3. Syringes: 5 mL, 10 mL.

4. Needles: 23G, 18G 1½.

5. Petri dishes (60 mm x 15 mm).

6. Sharp scissors or razor blades

8. 70 μm cell strainers

9. Gentle MACS Dissociator

Solutions

1. Phosphate-buffered saline (PBS) without calcium and magnesium.

2. Enzyme-free digestion buffer (wash buffer): RPMI1640, 5% fetal bovine serum (FBS).

3. Digestion buffer A: RPMI1640, 5% FBS, 0.5 mg/mL Collagenase Type IV, 0.1 mg/mL DNase I.

4. Digestion buffer B: RPMI1640, 5% FBS, 0.5 mg/mL Liberase TM, 0.1 mg/mL DNase I.

5. Digestion buffer C: RPMI1640, 5% FBS, 0.2 mg/mL Collagenase P, 0.8 mg/mL Dispase II, 0.1 mg/mL DNaseI.

6. Digestion buffer D: Miltenyi Biotec, Lung Dissociation Kit, Cat. No. 130095927.

7. Blocking buffer: PBS, 2% FBS, 2.4G2 hybridoma supernatant (to block Fc receptors CD16 and CD32).

8. FACS buffer: PBS, 2% FBS.

9. Fixation buffer: 4% paraformaldehyde (PFA) or Foxp3 staining kit (eBioscience, Cat. No. 5523) if nuclear transcription factor stain is desired

10. Red blood cell lysis buffer (e.g., Sigma-Aldrich Cat. No. R7757)

11. Viability stain (e.g., Fixable Live/Dead staining kit, Life Technologies, Cat. No. L34957)

Antibodies for flowcytometry

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1. Lineage antibodies: TCRβ (clone H57-597), TCRγδ (clone eBioGL3), CD11b (clone M1/70), CD11c (clone N418), B220 (clone RA3-6B2), CD3ε (clone 145-2C11), Ter119 (clone Ter119), NK1.1 (clone PK136) to exclude lineage positive cells)

2. CD45 (clone 104) to specifically detect hematopoietic cells

3. KLRG1 (clone 2F1) and Thy1/CD90 (clone 53-2.1) to detect and enrich for ILC2.

4. Sca-1 (clone E13-161.7), c-kit (clone 2B8), ICOS (clone C398.4A), CD69 (clone H1.2F3), CD25 (clone PC61.5), **ST2** (clone RMST2-2 or DJ8;) and GATA3 (clone TWAJ) to phenotypically characterize ILC2

5. BD Canto II Flow Cytometer.

6. Flow Jo software

METHODS

The lungs refer to the lower respiratory tract and are located in the chest along with the heart and thymus. The lung of the mouse consists of five lobes. The following protocol describes the preparation of a suspension of individual cells from the lungs of a mouse. If more than one mouse needs to be treated, the lungs should be kept on ice in a solution of RPMI 1640 + 5% FBS. All solutions used for this preparation should be cold; however, before adding enzymes, buffer solutions are brought to room temperature for digestion. All buffers with enzymes should be freshly prepared. Injection of the cytokine IL-33 (Figure 1).

Figure 1. Injection of the cytokine IL-33

Injection of cytokine IL-33 induces an innate type 2 immune response, including ILC2, in a dose-dependent manner. As a control, PBS and 10, 50 or 100 ng IL-33 were administered for three consecutive days, and ILC2 levels in the lungs were analyzed with flow cytometry 24 hours after the last treatment. Two to five mice were used in each group and a representative result was displayed for each group.

Preparation of a single cell suspension from mouse lungs (Figure 2). Subject the mouse to euthanasia, open the abdominal cavity and carefully separate the chest membrane from the rib cage. Cut the rib cage from the left and right sides and either cut the rib cage from the top or cut the rib cage in half.

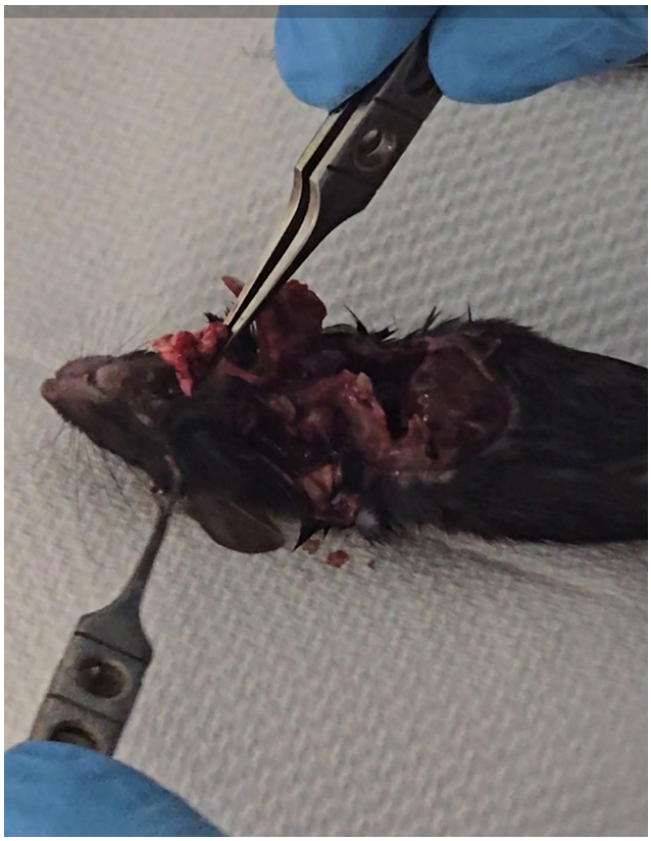

Figure 2. Preparation of a single cell suspension from mouse lungs

Cut through the blood vessels under the lungs. Perform pulmonary perfusion by inserting 1 10 ml of cold PBS ice into the right ventricle using a 10 ml syringe and a 23g needle 1. After perfusion, the lungs should turn white. Remove the heart and thymus, cut out the lungs and place them in 2 ml of washing solution. Remove the trachea, median lymph nodes and all additional tissues taken so that only the lungs (five lobes) are left.

Transfer the lungs to a small Petri dish (60 x 15 mm) (Figure 3) and cut into small pieces with sharp scissors or razor blades. Add 5 ml of the appropriate gap buffer and incubate for 1 hour at 37°C if a gap buffer A, B or C is used. Perform the cleavage using the Miltenyl cleavage kit and the Gentle MACS Dissociator according to the manufacturer's recommendations.

Enter various digestible tissues into a 5 ml syringe (18 g 1½ needles) and distribute the suspension by re-suction to obtain a single-celled suspension. Filter out the cell suspension using a 70 µm thick cell sieve. Rinse a Petri dish with 1 ml of washing solution and also pass it through a cell sieve. If there are still small pieces of fabric left, push them through the cell sieve with the syringe plunger. Rinse the cell filter with additional 5 ml rinsing buffers. Centrifuge the cell suspension at 450 g for 5 minutes and remove the supernatant liquid. Resuspend the cells in 10 ml of FACS buffer. Centrifuge the cell suspension at 450 g for 5 minutes and remove the supernatant liquid. Perform red blood cell lysis (RBC) to remove red blood cells with a buffer for red blood cell lysis according to the manufacturer's recommendations. Rinse the cell suspension once with a FACS buffer to remove all traces of the

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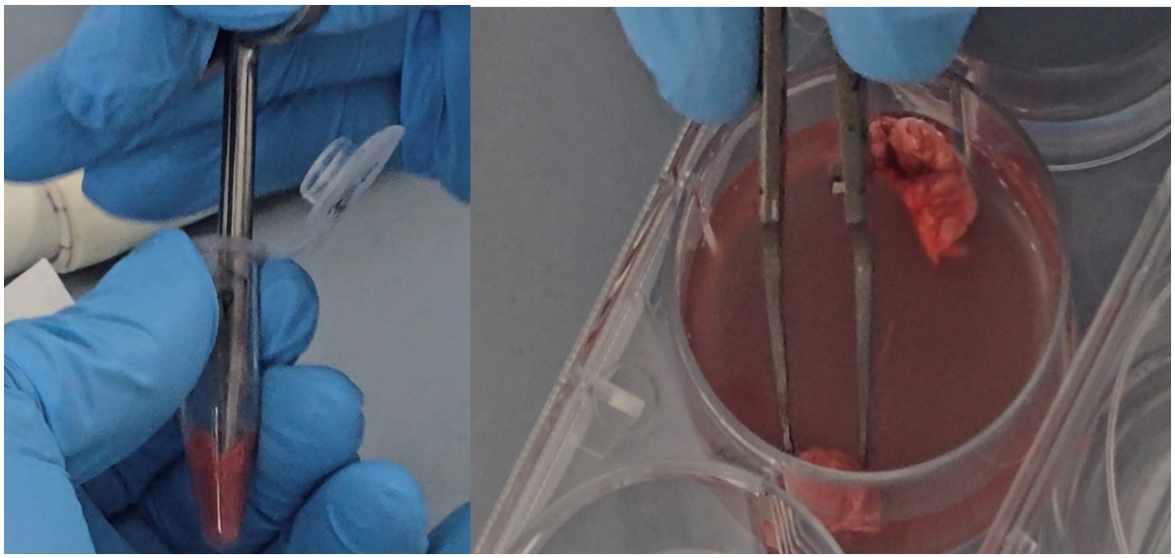

Figure 3. Transferring the lungs into a petri dish

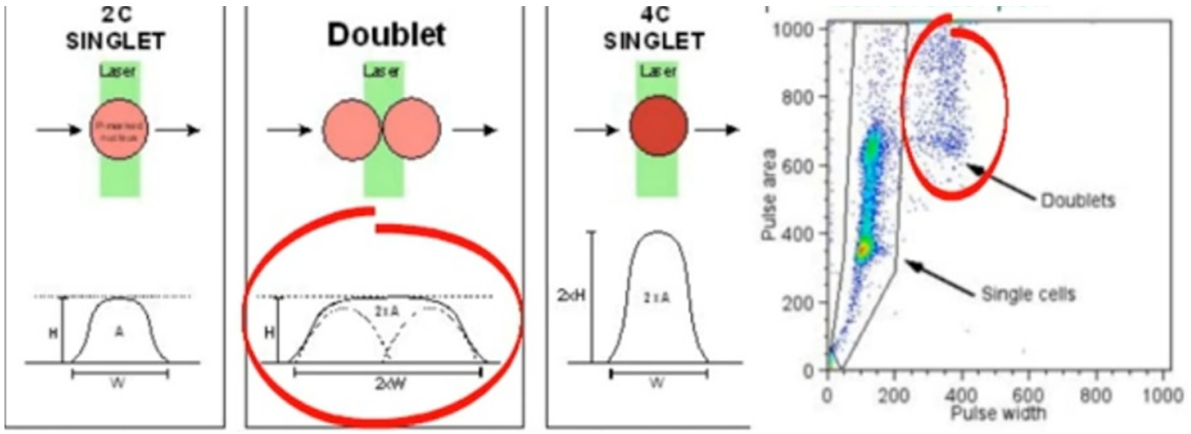

Figure 4. Doublet discrimination in NK

buffer for lysis of red blood cells. Continue the blocking and staining of the cell suspension for flow cytometry analysis.

Staining procedure

Before staining the cells for flowing cytometry, Fc receptors (CD16 and CD32) are blocked to avoid binding of non-specific antibodies, on ice for 15 minutes using the supernatant 2.4G2 hybridoma diluted in the FACS buffer. Centrifuge the cell suspension at 450 g for 5 minutes and remove the supernatant liquid. Add the appropriate antibody mixture diluted in the FACS buffer to the cells, resuspend and incubate on ice for 30 minutes. Add cooled PBS to the cells, centrifuge at 450g for 5 minutes and remove the supernatant liquid. Note that the cells are washed only with PBS, since FBS can suppress the staining of vitality. If viability staining has not been performed, the cells can be washed with a FACS buffer containing FBS. Repeat this: add the PBS cooled on ice to the cells, centrifuge at 450g for 5 minutes and remove the supernatant liquid. Incubate the cells on ice for 30 minutes to make them viable. Add a FACS buffer to the cells, centrifuge the cell suspension at 450 g for 5 minutes and drain the supernatant liquid. Repeat the centrifugation of the cell suspension for 5 minutes at 450 g and remove the supernatant liquid. The cells can be analyzed immediately by flow cytometry or fixed with 4% PFA for 15 minutes at room temperature. If nuclear staining is required, the cells are fixed, permeabilized and stained with the Foxp3 staining kit, as recommended by

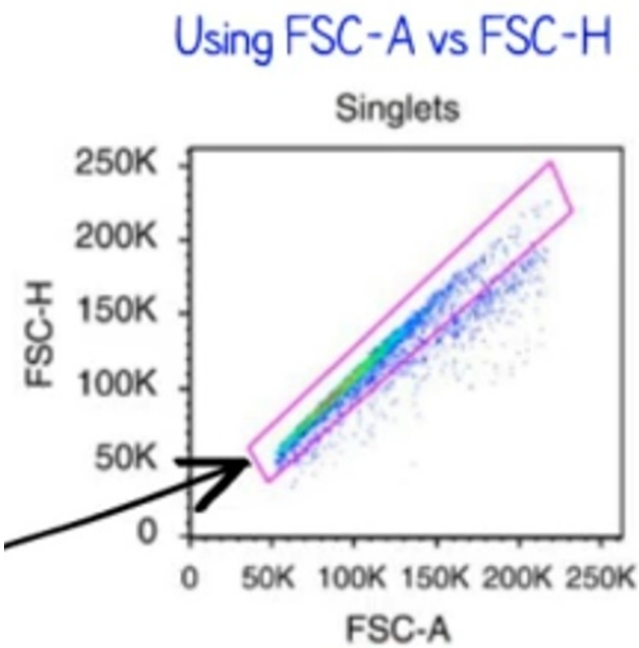

Figure 5. The FSC-A vs FSC-H strategy to exclude doublets. Digital instruments provide an option to equalize A and H for a given parameter. In BD- instruments this option is called ‘area scaling’

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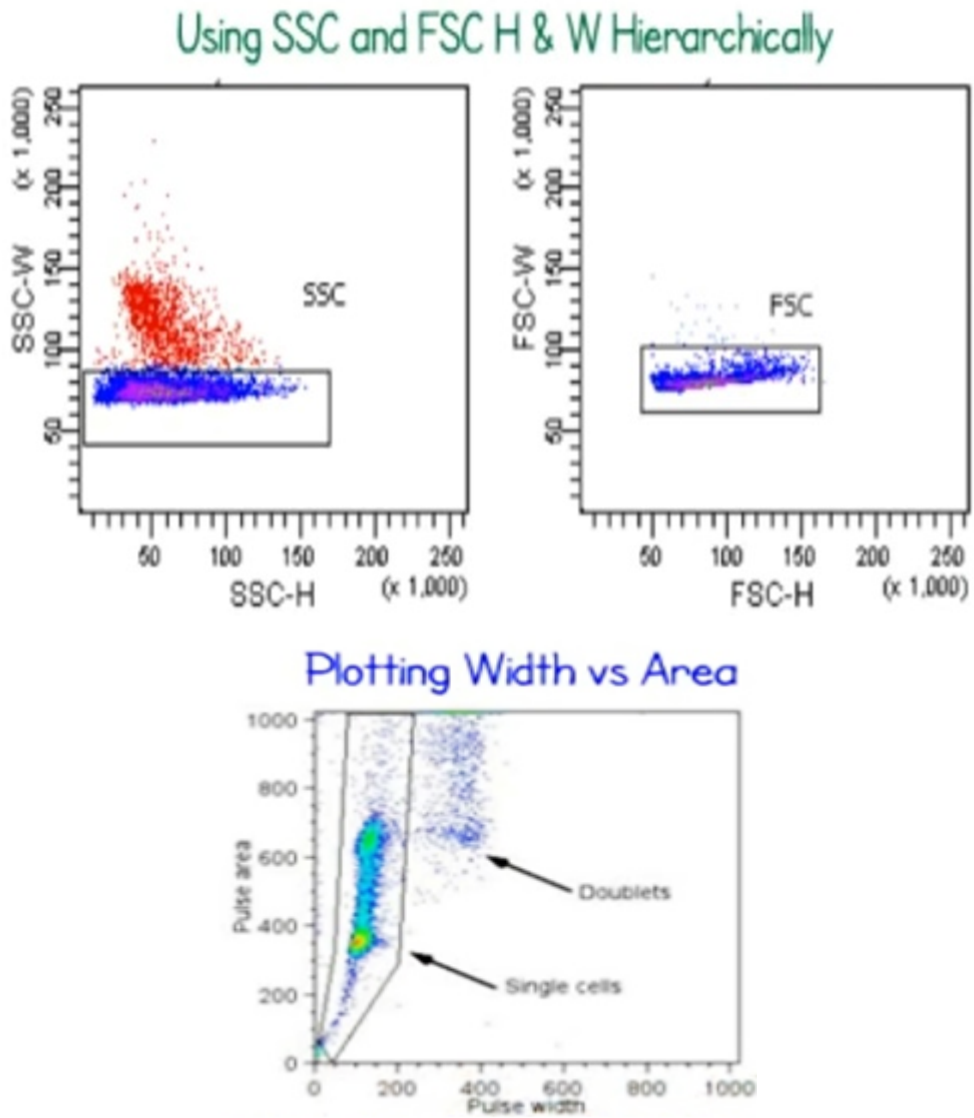

Figure 6. Using SSC and FSC-H and W-Hierarchically

the manufacturer.

Flow cytometry tutorial

Forward scatter (FSC) is the amount of light scattered in the forward direction. FSC is roughly proportional to the cell/particle size.

Side scatter (SSC) is the light scattered at large angles. SSC is proportional to the internal complexity of a cell.

H-Hight -is the intensity of signal, W-Width- is time taken for a cell to pass through the laser beam and also the duration of the signal. A-Area – is a procession and definition of a voltage pulse which is a signal from a cell/particle is detected and serves as marker for a cell or particle.

Doublet discrimination in NK - (natural killer) cells: The disproportions between H, W and A can be used to identify doublets in a laser beam signal. The singlet shows a clear red gradient as a marker in voltage pulse (Figure 4).

When the scaling property is set for the range, cells of a certain size show very similar values to A and H. Therefore, all singlets are grouped diagonally and separated from duplicates and clots (the black arrow in Figure 5). It is important to note that this strategy requires scaling the area with some experimental cells (not clumps or doublets).

Using SSC and FSC-H and W-Hierarchically adjusted method– this strategy is more accurate and recommended since it is not affected by area scaling (H is independent of W). Plotting width vs area – this strategy too does not require area scaling with experimental cells unlike H vs A (Figure 6).

RESULTS

In comparison with other areas of the mucous membrane (for example, the small intestine), the flow cytometric analysis of ILC2 in the lungs is difficult, since ILC2 is very rarely found in the lungs, especially in a stable state, as well as in infectious or inflammatory diseases. The data are collected with the BD Canto II flow cytometer and analyzed with Flow Jo (Figure 7 A, B).

The following definition strategy was used for pulmonary ILC2 (Fig. 1a); At first, only individual cells were selected in the FSC-H and FSC-A graph (Fig. 1a, step 1); secondly, cell residues and dead cells were excluded (Fig. 1a, step 2 and step 3). Pulmonary ILC2 are CD45-positive hematopoietic cells (Fig. 1a, step 4) and negative by origin (Fig. 1a, step 5), but positively stained twice on Thy1 and KLRG1 (Fig. 1a, step 6).

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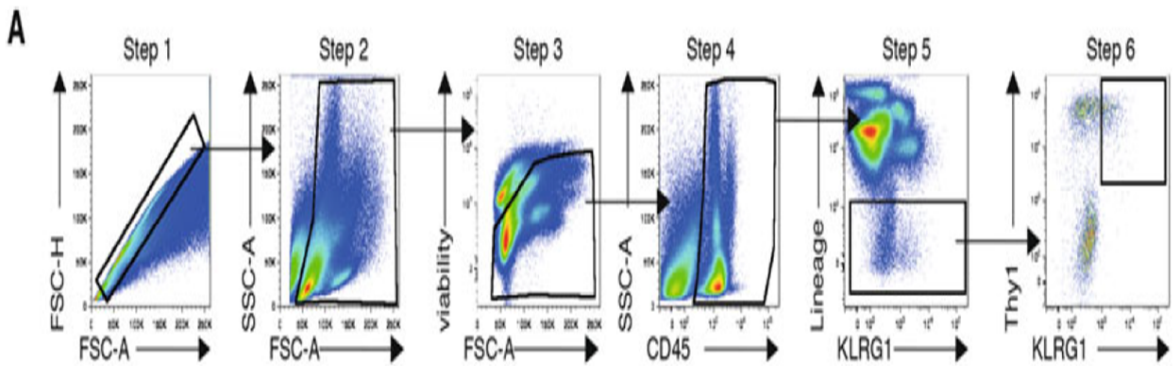


Figure 7 A. Characterization of Group 2 innate lymphoid cells (ILC2) in the mouse lung. Gating strategy for the identification of ILC2

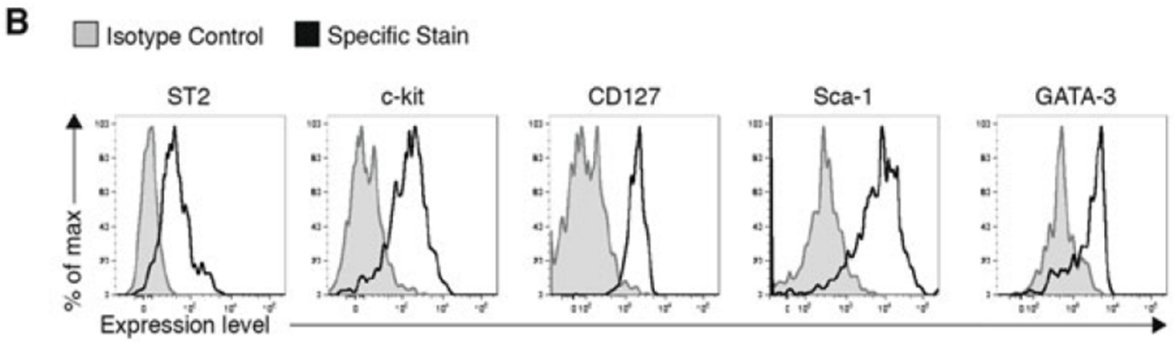
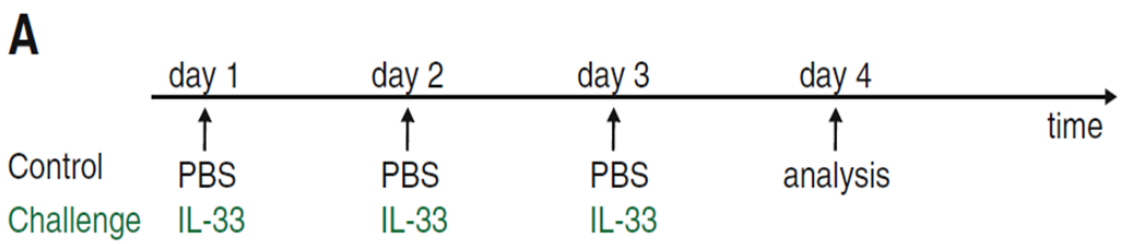


Figure 7 B. Upon gating on single cells and exclusion of dead cells, ILC2 are defined as CD45+Lineage (Lin)-Thy1+KLRG1+ cells expressing (B) ST2 (IL-33R), c-kit, CD127, Sca-1, and GATA-3



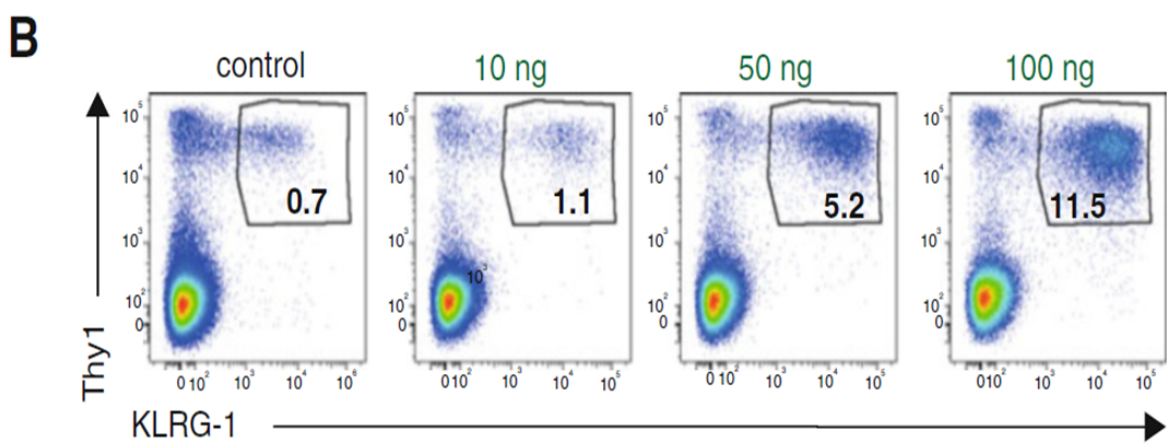


Figure 8. A -Induction of pulmonary Group 2 innate lymphoid cells (ILC2) in vivo. (A) Outline of experimental setup.

B - Intranasal administration of the cytokine IL-33 induces an innate type 2 immune response including ILC2 in a dose-dependent manner.

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Pulmonary ILC2 are further defined by their expression of ST2 (IL-33R), c-kit (CD117), CD127 (IL-7Rα), Sca-1, and GATA3 (Fig. 1b).

PBS as a control, and 10, 50 or 100 ng IL-33 were administered at three consecutive days and levels of pulmonary ILC2 analyzed by Flow Cytometry 24 after the last treatment. Two to five mice have been used per group and a representative result for each group is shown (Figure 8).

CONCLUSION

In our experience, both antibodies (eBioscience, clone RMST2-2; and MD Bioproducts, clone DJ8) stain ST2 very well and are recommended for the characterization of ILC2. Interestingly, clone DJ8 can also be used as a blocking antibody. This should be taken into account when cells are subsequently used for experiments with IL-33 stimulation. Lungs are perfused with ice-cold PBS (10 ml) or erythrocyte lysis is performed before blocking and staining with erythrocyte lysis buffer (for example, Sigma-Aldrich, Cat. No. No. R7757). If more than one lung is processed, 6-well plates can be used instead of small petri dishes. We use four lungs for each group of digestive buffers (digestive buffers A-D) to analyze pulmonary ILC2. To avoid a non-specific binding of staining antibodies, Fc receptors can be blocked with commercially available antibodies. Alternatively, the supernatant from the 2,4G2 hybridoma cell line, which produces antibodies against the Fc receptors CD16 and CD32, can be used. In summary, it can be said that the described protocol can also be used to detect ILC2 in other mouse strains. However, the pedigree cocktail must be carefully adjusted. For example, NK cells do not express NK1.1 in Balb / c mice. Here, NK1.1 is replaced by CD49b (clone DX5) to exclude NK cells.

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CONFLICTS OF INTEREST

There are neither conflicts nor claimed issues.

REFERENCES

1. Hansel TT, Johnston SL. Openshaw PJ Microbes and mucosal immune responses in asthma // Lancet. – 2013. – Vol. 381. – P. 861-873.

2. Wynn TA Type 2 cytokines: mechanisms and therapeutic strategies // Nat Rev Immunol. –205. – Vol. 15. –P. 271-282.

3. Edwards MR, Bartlett NW, Hussell T, Openshaw P, Johnston SL the microbiology of asthma // Nat Rev Microbiol. – 2012. – Vol 10. – P. 459-471

4. Artis D., Spits H. The biology of innate lymphoid cells // Nature. – 2015. –Vol. 517. – P. 293-301.

5. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? // Nat Rev Immunol. – 2010. – Vol. 10. – P. 225-235.

6. Moro K et al. Innate production of T(H)2 cytokines by adipose tissue associated c-kit (+) Sca-1(+) lymphoid cells // Nature. – 2012. – Vol. 463. – P. 540-544.

7. Neill DR et al. Nuocytes represent a new innate effector leukocyte that mediates type- 2 immunity // Nature. – 2010. – Vol. 464. – P. 1367-1370.

8. Price AE et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity // Proc Natl Acad Sci U S A. – 2010. – Vol. 107. – P. 11489-11494.

9. McKenzie AN., Spits H., Eberl G. Innate lymphoid cells in inflammation and immunity // Immunity. – 2014. – Vol. 41. – P. 366-374.

10. Diefenbach A, Colonna M, Koyasu S. Development, differentiation, and diversity of innate lymphoid cells // Immunity. – 2014. – Vol.41. – P. 354-365.

11. Huang Y et al. Multipotential 'inflammatory' type 2 innate IL-25-responsive, lineage-negative KLRG1(hi) cells are lymphoid cells // Nat Immunol. – 2015. – Vol.16. – P. 161-169.

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ТЫШҚАННЫҢ ӨКПЕСІНЕН ВИРУСҚА ҚАРСЫ IL-33-ЫНТАЛАНДЫРЫЛҒАН 2-ТОПТАҒЫ ТУА БІТКЕН ЛИМФОИДТЫ ЖАСУШАЛАР (CD90 ЖӘНЕ CD117)

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ТҮЙІН

Бұл мақала маңызды ғылыми құндылықты ғана емес, сонымен қатар зерттеушілер мен ынталы студенттер үшін ағындық цитометриядағы дағдылар спектрін жақсартуға бағытталған оқыту мен оқытудың үлкен құндылығын біл-діреді. Бұл мақалада процедуралардың кезең-кезеңімен сипаттамасы және ағынды цитометрия әдісі үшін қос хаттама-лары бар қызығушылық тудыратын жасушаларды оқшаулау, сондай-ақ IL-33 динамикасына ӘСЕР ету арқылы өкпенің мурин тінін in vivo зерттеуге негізделген нәтижелер берілген. Жаңадан сипатталған 2-топтағы туа біткен лимфоидты жасушалар (ILC2) 2 типті иммундық реакцияларда, шырышты тіндердің эпителийін қалпына келтіруде және метабо-ликалық гомеостазда маңызды рөл атқарады. ILC2 құрттардан қорғау сияқты 2 типті иммунитетті ынталандыратын интерлейкин 4 (ИЛ-4), ИЛ-5 және ИЛ-13 сияқты 2 типті цитокиндердің көп мөлшерін шығарады. Алайда, ҚАТАҢ рет-теусіз ILC2 деңгейі 2 типті жағымсыз иммундық патологияларды, соның ішінде тыныс алу жолдарының аллергиялық қабынуын, тыныс алу жолдарының жоғары сезімталдығын және атопиялық дерматитті тудыруы мүмкін. 1 типті им-мундық реакциялардың типтік қоздырғыштары болып табылатын тыныс алу жолдарының вирустық инфекциялары көбінесе демікпе және оның өршуі сияқты 2 типті өкпе иммундық патологияларына әкеледі. Бір қызығы, өкпенің ви-рустық инфекциялары IL-33 шығарылуын тудырады, содан кейін АДАПТИВТІ иммундық жүйеге тәуелсіз ILC2-дел-далдық 2 типті өкпе иммунопатологиясын индукциялайды.

Түйінді сөздер: Туа біткен иммунитет, Цитокиндер, Интерлейкиндер, IL-33, CD90, CD117, ILC2.

УДК: 615.371

СТИМУЛИРОВАННЫЕ ПРОТИВОВИРУСНЫМ IL-33 ВРОЖДЕННЫЕ ЛИМФОИДНЫЕ КЛЕТКИ 2-Й ГРУППЫ (CD90 И CD117) ИЗ ЛЕГКИХ МЫШИ

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АБСТРАКТ

Эта статья представляет собой не только значительную научную ценность, но и представляет собой большую учеб-ную ценность для исследователей и мотивированных студентов, желающих улучшить свои навыки в области проточ-ной цитометрии. В этой статье приводится пошаговое описание процедуры и результаты, основанные на исследова-ниях in vivo на мышиной легочной ткани, выделении интересующих клеток с помощью двух протоколов для метода проточной цитометрии, а также влияние IL-33 в динамике. Недавно описанные врожденные лимфоидные клетки 2-й группы (ILC2) играют важную роль в иммунных реакциях 2-го типа, восстановлении эпителия в тканях слизистой оболочки и метаболическом гомеостазе. ILC2 высвобождает большое количество цитокинов 2-го типа, таких как ин-терлейкин 4 (IL-4), IL-5 и IL-13, которые стимулируют иммунитет 2-го типа, например, защиту от глистов. Однако без строгого регулирования уровень ILC2 может вызывать нежелательные иммунные патологии 2-го типа, включая аллергическое воспаление дыхательных путей, гиперчувствительность дыхательных путей и атопический дерматит. Вирусные инфекции дыхательных путей, которые являются типичными триггерами иммунных реакций 1-го типа, ча-сто приводят к легочным иммунным патологиям 2-го типа, таким как астма и ее обострения. Интересно, что легоч-ные вирусные инфекции индуцируют высвобождение IL-33 с последующей индукцией ILC2-опосредованной легоч-ной иммунопатологии 2-го типа, которая не зависит от адаптивной иммунной системы.

Ключевые слова: Врожденный иммунитет, цитокины, интерлейкины, IL-33, CD90, CD117, ILC2