**Video Article**

# A Suction Blister Protocol to Study Human T-cell Recall Responses *In Vivo*

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## Abstract

Cutaneous antigen-recall models allow for studies of human memory responses *in vivo*. When combined with skin suction blister (SB) induction, this model offers accessibility to rare populations of antigen-specific T-cells representative of the cellular memory response as well as the cytokine microenvironment *in situ*.

This report describes the practical procedure of a cutaneous recall, an SB induction, and a harvest of antigen-specific T-cells. To exemplify the method, the tuberculin skin test is used for antigenic recall in individuals who, prior to this study, underwent a Bacillus Calmette-Guérin vaccination against an infection with *Mycobacterium tuberculosis.* Finally, examples of multiplex and flow cytometric analyses of SB specimens are provided, illustrating high fractions of antigen-specific polyfunctional CD4+ T-cells available by this sampling method compared with cells isolated from the blood.

The method described here is safe and minimally invasive, provides a unique opportunity to study both innate and adaptive immune responses *in vivo*, and may be beneficial to a broad community of researchers working with cell-mediated immunity and human memory responses, in the context of vaccine development.

## Video Link

The video component of this article can be found at https://www.jove.com/video/57554/

## Introduction

A skin SB is an artificially induced blister, which allows for the harvest of cells and fluid directly from the skin. The technique of raising SBs by vacuum is a well-known tool within the field of dermatology used for the study of skin immunology in health and disease[1,2,3,4,5,6,7,8]. This report demonstrates how the SB technique combined with cutaneous antigenic recall (SB cutaneous recall method) can provide direct insights into adaptive immune responses *in vivo*.

The principle behind the SB induction is simple: a light vacuum is applied to a small area of the skin. The negative pressure will eventually force the epidermis to separate from the dermis, creating a local blister filled with fluid and cells[1,2,9]. The blister fluid can be harvested by a fine needle aspiration and the content can be used for further study *in vitro*.

In recent years, there has been an increasing interest in the SB method for the study of *in vivo* immune responses other than diseases restricted to the skin[10,11]. The study of the adaptive immunity in humans is limited by the fact that the cells and cytokines of interest are sampled from peripheral blood because an invasive sampling of the lymph node or gastrointestinal mucosal tissue may be unacceptable and unethical. An example is the study of long-lived human memory T-cell responses after vaccination[12]. In such trials, the sampling of relevant T-cells can be a great challenge, because the relevant population of cells that mediate immunity resides in the lymphoid tissue, and only a very limited number of specific T-cells circulate in the peripheral blood.

The SB technique offers a unique opportunity to the study of memory T-cells and other specific cell populations. Following the cutaneous inoculation of antigen, T-cells specific for the antigen are recruited from their lymphoid hideaways to the skin and can easily be sampled from the SB. The methodology and research applications of this cutaneous recall model were described by Akbar *et al.* in 2013[2]. A commonly used antigen for skin recall is the commercially available purified protein derivative (PPD) of mycobacteria used to perform a tuberculin skin test (TST)[13], where PPD is injected into the dermal layer of the skin. In individuals with an existing immunological memory towards PPD [*e.g.*, individuals with an *Mycobacterium tuberculosis* (*Mtb*) infection or a prior Bacillus Calmette-Guérin (BCG) vaccination], the antigen deposition results in a recall response with migration to the skin and an *in vivo* clonal expansion of PPD-specific CD4+ T-cells[2,11,14,15]. As a result, high fractions of PPD-specific polyfunctional CD4+ T-cells accumulate in the skin ready for SB sampling. T-cells collected by this method have proven

to be robust and are sufficiently abundant to be characterized by a range of immunoassays and by a long-term *in vitro* culture[15]. Thus, the cutaneous recall model and the SB induction may prove a valuable method to study *in vivo* T-cell responses by *ex vivo* analyses, and increased knowledge of this approach may benefit researchers with interests in cellular immunology and vaccinology.

This report provides the first stepwise guide on how to induce human skin suction blisters in PPD-injected skin. The cutaneous antigen recall model is demonstrated using the TST in BCG-vaccinated volunteers. Finally, the relevant *ex vivo* analysis of the cells and cytokines isolated by SB is exemplified. Phenotypical and functional characteristics of PPD-specific T-cells obtained by the SB method are thoroughly described elsewhere[2,10,11,16,17,18,19]. This report aims to discuss the practical and immunological aspects of the methodology to ease the application of this technique by other research groups.

## Protocol

All methods described below, including the use of human volunteers, have been approved by the Danish Committee on Health Research Ethics (H-15002988) and the Danish Data Protection Agency (jr.nr. 2015-57-0102). PPD must be a certified product approved for human use and administered within the correct dosage provided by the manufacturer. Any deviation in dosage or administration may require additional ethical approvals and volunteers must give informed consent.

# 1. Tuberculin Skin Test

1. Obtain oral and written informed consent from the volunteer. Prior to the injection, ensure that the volunteer understands and accepts the procedure including the possible adverse effects.
   NOTE: Inclusion criteria specific to this protocol are age > 18 years and a documented BCG vaccination. Inclusion criteria may vary depending on the research question (*i.e.*, an additional inclusion criterion could be evidence of positive TST).
2. Use a ready-made solution of PPD for human use [20 tuberculin units (TU)/mL]. Disinfect the rubber cap of a vial using an alcohol swab.
3. Prepare the injection site
   1. Locate the injection site on the ventral side of the volunteer's forearm.
   2. Place the arm palm-up on a plain surface and choose an area on the upper third of the forearm, approximately 5 - 10 cm from the elbow joint. Stay clear of scars, veins, or damaged skin.
   3. Disinfect the area using an alcoholic swab.

4. Prepare the PPD-solution
   1. Use a 1 mL sterile syringe with a 27 - 30G/short (*e.g.*, 12 mm) fixed needle.
   2. Gently mix and aspirate 0.1 mL of the PPD solution. Make sure that there are no visible bubbles.

5. Intradermal PPD injection
   NOTE: This step describes how to perform a single PPD deposition, but multiple depositions of PPD in the same arm is possible. However, this may require specific ethical approval.
   1. Stretch the skin and point the needle at a 5 - 15° angle to the skin with the bevel facing upwards.
   2. Insert the tip of the needle into the dermal layer of the skin and make sure the tip is almost visible through the epidermis.
   3. Slowly inject 0.1 mL of PPD solution.
      NOTE: If administered correctly, a pale papule of 6 - 10 mm will appear immediately. The papule disappears after approximately 10 min.
   4. When making two or more depositions, aim for a maximum separation (5 - 10 cm) of the injection sites while keeping in a good distance from the elbow and wrist area.
      NOTE: This prevents a potential confluence of the skin test response and allows the uninterrupted positioning of two suction chambers.

# 2. Evaluation of the Skin Reaction - Day 3

1. After 48 - 72 h, note the size of the skin reaction. Measure the induration (the swelling) and **not** the erythema (the redness) of the reaction.
2. Palpate the hard swelling using fingers and then mark the edges of the induration using a ballpoint pen. Use a ruler to measure the induration diameter and note the result in mm.
   NOTE: A reading of the induration size may guide the choice of orifice diameter for the suction chamber.

# 3. Suction Blister Induction - Day 7

1. Assemble the suction device unit.
   Note: Suction device units are vacuum pumps with attached chambers for suction blister induction. The device used in this demonstration is custom made, but suction device units are also commercially available. The pump needs to be adjustable within the range of -20 to -40 kPa and provide a steady and reliable negative pressure. Regional ethical approval should be obtained prior to conducting experiments using this method.
   1. First, prepare the suction chamber by assembling the bottom orifice plate and the window plate with the chamber and the connecting hose. Attach the chamber-connecting hose tightly to the vacuum pump.
      Note: The chambers should include a bottom plate with a hole for the blister induction, which should be suitable for contact with the human skin, and a top plate with a window, allowing for the visual monitoring of the blister.

2. Determine the optimal orifice diameter of the orifice plate relative to the size of the skin reaction; the larger the size of the orifice, the larger the blister.
3. Disinfect the orifice plate and the skin of the volunteer using alcohol swabs.
4. Attach the suction chamber to the skin.
    1. Make sure that the volunteer's arm is resting comfortably.
    2. Make sure that the hole in the bottom plate of the suction chamber is situated over the PPD reaction so that the center of the skin reaction is visible.
    3. Secure the chamber loosely using straps: when negative pressure is applied, the chamber will adhere to the skin and maintain its position.

5. Turn the suction device on and adjust the pressure to -20 kPa.
6. After 30 min, increase the negative pressure to -25 kPa.
7. After 60 min, further increase the negative pressure to -30 kPa.
8. Keep the pressure at -30 kPa until a blister is fully formed. Ensure that the negative pressures provided in this protocol are specific to a customized instrument. It may be necessary to adjust the pressure slightly when applying the protocol in other settings.
   NOTE: The blister induction phase ranges from 60 to 180 min (1 - 3 h) with the actual blister formation occurring within the last 30 min. Blistering is often associated with an itchy sensation. The blisters may occur as single or multiple merging blisters. If blister rupture or hemorrhage occurs, it is advised to terminate the blister induction by slowly releasing the pressure.
9. After the blister is fully formed, release the pressure, and carefully remove the suction chamber from the skin without rupturing the blister.
10. Apply a soft dressing on the surrounding skin area and place a hard cap (*e.g.*, from a 50 mL plastic tube) over the blister.
11. Secure the cap with non-allergenic surgical tape followed by a soft stretchy bandage.
12. Instruct the volunteer to avoid excessive physical strain on the blister area for the next 12 - 24 h.

## 4. Harvest of Blister Fluid - Day 8

1. On the 8th day, gently remove the protective dressing and disinfect the blister with skin disinfection spray.
2. Use a 2 mL sterile syringe with a 23G needle to harvest the blister content. Insert the needle into the top/lateral side of the blister roof and slowly aspirate the fluid. Avoid touching the floor of the cavity but make sure to harvest all the fluid.
3. Apply a dressing on the collapsed blister.
4. Transfer the blister fluid to a sterile tube. Note the volume.
5. Spin the blister fluid down at 600 x g using a tabletop centrifuge for 4 min.
6. Transfer the supernatant to another tube and resuspend the cell pellet in 500 μL of cell medium.
   NOTE: The cells are now ready for counting and further analysis. Supernatants can be stored at -20 to -80 °C until use.

## 5. Analyses of Suction Blister Cells and Fluids

NOTE: Providing instructions for the analysis of cells and fluid obtained from skin suction blisters is not within the scope of this protocol. However, in order to provide representative results for this report, intracellular stain flow cytometry and multiplex analyses were performed. The methodology is described in brief below.

1. Flow cytometry
    1. Stimulate suspensions of $1 \times 10^5$ fresh cells isolated from suction blisters from 1 BCG-vaccinated volunteer with 10 μg/mL of PPD and incubate the cells at 37 °C and 5% $CO_2$. Include unstimulated cells for a parallel incubation.
    2. Stimulate suspensions $1 \times 10^6$ PBMCs obtained from 1 BCG-vaccinated volunteer with 10 μg/mL of PPD and incubate the mixture at 37 °C and 5% $CO_2$. Include unstimulated cells for a parallel incubation.
    3. After 2 h, treat all cells with Brefaldin A and monensin and incubate them for 6 h at 37°C.
    4. Stain the cells with a panel of Live/Dead-stain-BV510, anti-CD4-AF780, anti-CD3-BV421, anti-CD8-PerCP-Cy5.5, anti-CCR7-PE, anti-CD45RA-BV786, anti-IFN-γ-AF700, anti-TNF-α-PE-Cy7, and anti-IL-2-FITC.
    5. Include FMO controls in the PBMC panel.
    6. Perform a flow cytometric analysis with a flow cytometer and analyze the results using relevant software.
    7. Gate on the cytokine-producing CD3+CD4+CD8- subsets using the following gating strategy: singlets - > viable CD3+ - > CD4+CD8- - > IFN-γ+, TNF-α+, or IL-2+.
    8. Gate on effector memory cell populations using the following gating strategy: singlets - > viable CD3+ - > CD4+CD8- - > CCR7-CD45RA-.
    9. Calculate individual cytokine profiles using Boolean gating.

2. Cytokine release demonstrations
    1. For a demonstration of a cytokine release *in vivo*, use a multiplex analysis to quantify the levels of IL-2, IFN-γ, TNF-α, IL-10, and IL-13 in thawed suction blister fluid obtained from 4 volunteers.
    2. For a demonstration of a cytokine release by cells obtained from suction blisters *ex vivo*, use fresh suction blister cells and PBMCs obtained from 1 BCG-vaccinated volunteer.
    3. Infect the PBMC and suction blister cells with 100 colony-forming units (CFU) of *Mtb* H37Rv and incubate them for 4 days.
    4. Use a multiplex analysis to quantify the levels of IL-2, IFN-γ, TNF-α, IL-10, and IL-13 in all culture supernatants.
    5. Adjust the measurements above the assay calculation range to the upper calculation limit.

## Representative Results

Eight healthy adult volunteers (median age: 30 years, range: 26 - 43 years) with a documented previous BCG vaccination (median time from BCG-vaccination: 5.5 years, range: 1 - 30 years) were included. The participants were challenged intradermally with 2 TU of PPD followed by a TST evaluation on day 3. SBs were induced on day 7 and harvested on day 8, and all blisters were raised using suction blister chambers with 10 mm orifice diameters. Seven individuals were given 2 separate PPD inoculations simultaneously in the same arm followed by 2 parallel SB inductions (please refer to the note regarding multiple PPD depositions below step 1.4 in the **Protocol**). Peripheral blood was drawn on day 7 for plasma and a PBMCs isolation by density gradient centrifugation. Plasma and fluid supernatants from SBs were stored at -20 °C. Fresh SB cells (SBCs) were counted using nigrosine stain and microscopy.

The clinical TST responses and SBC yield are presented in **Figure 1**. The median size of the TST indurations was 10.25 mm (range: 0 - 20 mm) and the median cell number per blister was 50,000 (range: 15,000 - 210,000 cells, number of blisters: 15). Two of the volunteers had no clinical response in either of the 2 TSTs and a corresponding low total cell yield of 15,000 cells/blister. The cell yield was associated with the mean clinical response of TST (Spearman's $r = 0.643$, $p = 0.094$).
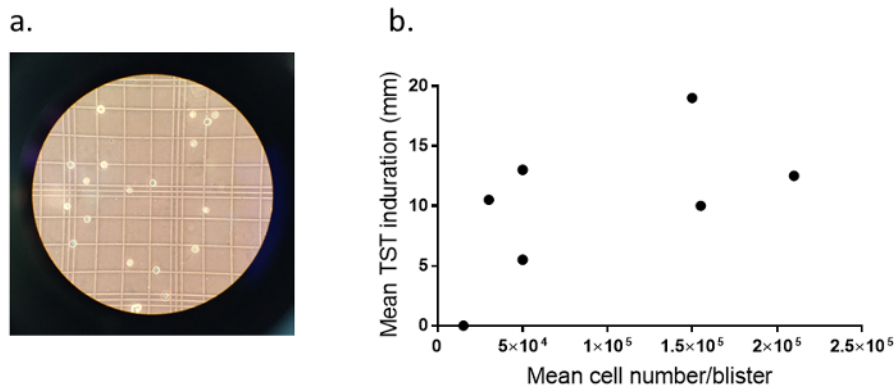


**Figure 1: Suction blister cell yield.** (**a**) This panel shows a representative microscopy of nigrosine-stained cells isolated from SBs raised 7 days post-TST. (**b**) This panel shows relationships between the mean TST induration (mm) and the mean cell yield (*n*/blister) in 8 BCG-vaccinated volunteers (number of blisters = 15). The dots represent individual mean measurements. Please note that 2 dots are overlapping as 2 volunteers both had a TST induration of 0 mm and a cell yield of 15,000. Please click here to view a larger version of this figure.

To demonstrate the flow cytometric SB characterization, SBCs were obtained from a 43-year-old volunteer who had been BCG-vaccinated 30 years earlier and had no known exposure to *Mtb*. The SBCs were isolated following the induction of a single blister (induration: 1.4 mm/100,000 SBCs). **Figure 2** shows representative plots of the intracellular cytokine staining of the SBCs *versus* PBMCs.

In this volunteer, the fraction of CD3+CD4+CD8- SBCs increased from 67% in unstimulated cells to > 90% upon a PPD *in vitro* restimulation, whereas the fraction of CD3+CD4+CD8- PBMCs remained constant (~ 51%, **Figure 2**). Over 92% of the *in vitro* PPD-, as well as the unstimulated CD3+CD4+CD8- SBCs, were of the effector memory type (CCR7-CD45RA-, data not shown). The overall fractions of specific CD3+CD4+CD8- cells induced by PPD-stimulation were higher in the SBCs compared to the PBMCs (33.1 *vs*. 0.2%, unstimulated samples subtracted). In the PBMCs, the fractions of polyfunctional PPD-specific CD3+CD4+CD8- cells were all < 0.05%.
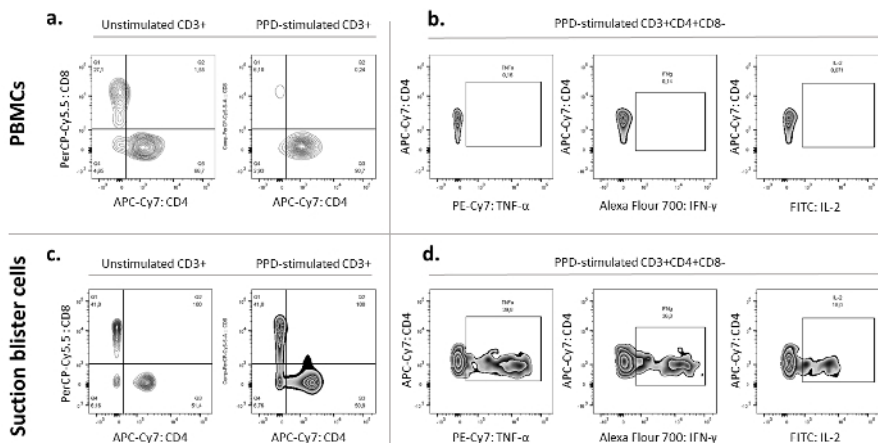


**Figure 2: Representative flow cytometry plots of SBCs *versus* PBMCs.** Panels **a** and **b** show representative density plots of CD8+ and CD4+ populations in (**a**) unstimulated *vs*. PPD-stimulated PBMCs and (**c**) SBCs. Panels **b** and **d** slow plots of intracellular cytokine staining in PPD-stimulated CD3+CD4+CD8- cells for (**b**) PBMCs and (**d**) SBCs. Please click here to view a larger version of this figure.

As expected, CD3+CD4+CD8- SBCs were activated *in vivo*, illustrated by a high proportion of the cells staining for upregulated cytokines (12%), with the predominant cytokines being TNF-α and IFN-γ. However, upon PPD-stimulation, the cytokine secreting cells shifted towards a triple- or double-positive IFN-γ+TNF-α+IL-2+ (17%) and IFN-γ+TNF-α+ (15%) profile (**Figure 3b**, PBMC profiles from the same donor are included for comparison in **Figure 3a**). The cytokine expression profiles for PPD-stimulated effector memory CD4+ T-cell subsets (CD3+CD4+CD8-CCR7-CD45RA-) were comparable to the CD3+CD4+CD8- population presented in Figures 2 and 3 (data not shown).
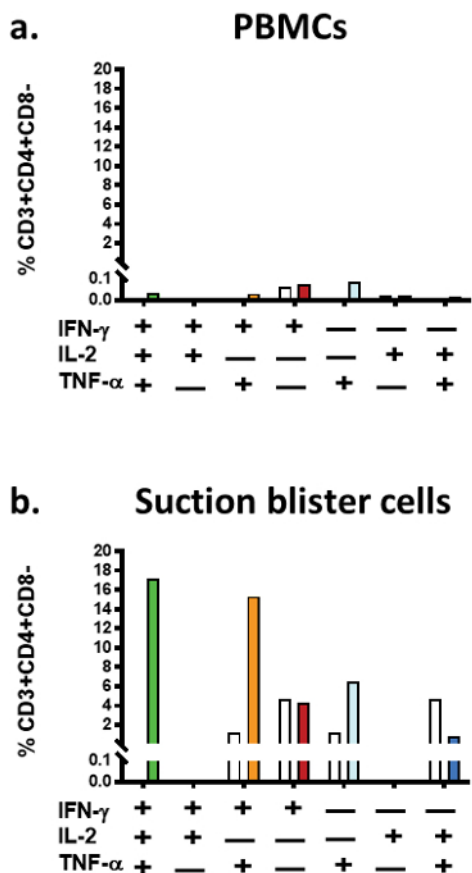


**Figure 3: Cytokine profiles for CD4+ PPD-stimulated and unstimulated SBCs versus PBMCs.** These panels show individual profiles for cytokine producing (**a**) CD3+CD4+CD8- PBMCs cells and (**b**) CD3+CD4+CD8- SBCs. The bars represent fractions of antigen-specific cytokine profiles in unstimulated cells (white bars) and upon an *in vitro* stimulation with PPD (colored bars).

To explore the SB fluid as a source of information on the cytokine microenvironment at the site of the skin testing, the cytokine levels were measured in fluid harvested from SBs induced 7 days post-TST (**Figure 4a**). The median levels of IFN-γ, TNF-α, and IL-2 were 339 pg/mL, 19 pg/mL, and 1 pg/mL, respectively (*n* = 6). The plasma levels were generally very low. Cells isolated from SBs produced high levels of pro-inflammatory cytokines *ex vivo* (**Figure 4b**). Titrations of the SBCs from 1 volunteer were cultured for 4 days in the presence of virulent *M. tuberculosis* ± autologous PBMCs. The IFN-γ levels were > 30-fold higher in cultures containing SBCs, irrespective of the presence of PBMCs.
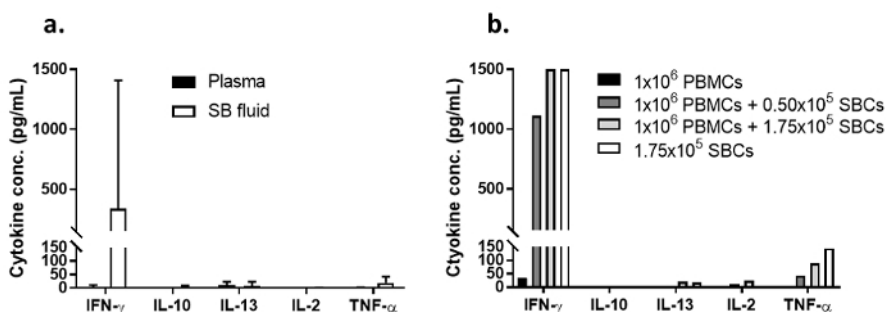


**Figure 4: Cytokine levels in SB fluid, plasma, and *Mtb*-infected culture supernatants.** (**a**) This panel shows cytokine levels in SB fluid supernatants and plasma from 6 BCG-vaccinated volunteers. The bars represent the median cytokine levels; the error bars represent the range. (**b**) This panel shows the cytokine levels in supernatants from 4 parallel 600 µl *ex vivo* 4-day cultures of 1 x 10^6 PBMCs (black bars), 1.75 x 10^5 SBCs (white bars), and 1 x 10^6 PBMCs spiked with either 0.5 or 1.75 x 10^5 SBCs (grey bars) infected with *Mtb*. Please click here to view a larger version of this figure.

## Discussion

This manuscript describes a practical procedure for the study of human immune memory responses *in vivo*, using cutaneous antigen recall and cell harvest by suction blister induction. TST was used as an example of intradermal antigen deposition and BCG-vaccine recall. Finally, an example of SB specimen characterization by flow cytometric and multiplex cytokine analysis was provided, demonstrating that roughly a third of the SB cells were antigen-specific polyfunctional T-cells of the effector memory phenotype.

The critical steps of this protocol include the intradermal injection technique, the suction blister induction, and the blister puncture. Firstly, a correct intradermal deposition requires trained personnel. An incorrect deposition can lead to suboptimal results. PPD is generally a well-known and safe cutaneous antigen, but its composition may vary between manufacturers, limiting comparability[13,20]. This report provides instructions for a single intradermal deposition of 2 TU, and optionally two parallel depositions (2 x 2 TU), which may require additional ethical approval. However, other studies have used 10 TU, which increase the likelihood of a strong skin reaction[10,21]. During the SB induction step, small stepwise increases in the negative pressure will reduce the risk of hemorrhage and blister rupture. The chances of contamination with red blood cells or leucocytes from the bloodstream are generally low[2]. The aseptic puncture technique prevents microbial contamination and avoiding contact between the puncture needle and the dermal blister floor reduces impurities of debris or resident skin cells. However, some researchers prefer to harvest SB fluid by applying a rolling pressure to the punctured blister[10]. It may be necessary to puncture the septa within the blister. The SB technique itself is minimally invasive; collapsed SBs heal without scarring and infections are very rare.[2] However, some degree of hypo-pigmentation may occur and SB induction should probably be avoided in people with a history of colloid scarring.

Technical limitations include low total cell yields and consequently limited options for long-term storage. Relationships between the leucocyte yield, clinical TST responses, the blister size, and erythrocyte contamination has been thoroughly described elsewhere[2,10]. In the BCG-recall experiments presented here (**Figure 1**), the median total yield from each blister was 50,000, implying a small-scale experimental set-up using these cells, especially if SBCs are studied alone[15]. However, the specific T-cell population in an SB sample mostly exceeds what is found in PBMC samples in both relative and absolute cell counts. In the example presented in **Figure 2**, the number of triple-positive PPD-specific T-cells in a sample of 100,000 SB cells were more than 2x greater than the number found in 1 x $10^6$ PBMCs from the same donor (data not shown). A visual scoring of the TST reaction is the most common clinical method for the evaluation of *M. tuberculosis* memory. Of note, the underlying adaptive immunological reaction does not peak at the same time as the clinical skin reaction[2,21]. Not all BCG-vaccinated or *Mtb*-exposed individuals will develop strong TST reactions and strategies for classification, and a handling of samples from TST non-responders, as well as a preexisting mycobacterial sensitization in the study population, need to be considered before initiating a recall trial using this method. Also, in both SB sampling and visual scoring, there is a potential for a theoretical bias in individuals with reduced skin responses due to global or skin-related impaired immunity as seen, for instance, in HIV-infection and certain age groups[2,13,18,20]. In addition, an immunological boosting of the TST reaction with repeated testing is well known[20,22]. However, the SB cell phenotypes remain rather constant when TSTs are repeated[10]. This observation supports the role of SB recall in longitudinal studies of cellular immune responses.

For T-cell immunologists, SB recall allows for the harvest of high fractions of antigen-specific cells. However, the timing of SB for a sampling from a PPD-deposition is critical as both the cellular composition and the cytokine microenvironment change over time. In this protocol, blisters were induced 7-day post-challenge and the isolated cells were primarily CD4+ effector memory T-cells including high fractions of cells with a co-expression of IFN-γ, TNF-α, and IL-2. These observations are in line with previous studies describing how T-cell activation, proliferation, and differentiation occurs in PPD-primed skin[2,15,21]. Kinetic SB studies in PPD-sensitized individuals suggest that the very early skin response is unspecific; however, already within the first days following the PPD-challenge, the response becomes dominated by CD4+ T-cells (both central memory and effector memory phenotypes have been described) and, after 3 days, the response is dominated by high fractions of PPD-specific cytokine producing CD4+ T-cells[2,8,10,15,21]. This adaptive cytokine response remains detectable for more than 2 weeks[2,8,10,23]. Day 7 post-TST appears to be the most optimal time point for the collection of cytokine producing memory CD4+ T-cells[2]. However, other time points may, of course, be relevant depending on the antigen and response of interest. Of note, in one study, the adaptive PPD response has been reported to peak a little earlier with a decrease in the pro-inflammatory cytokine secretion already at 5 days post-TST[10].

SB fluid contains high levels of both pro-inflammatory cytokines and other proteins shown to be representative of the skin microenvironment[15,24]. Kinetics studies have shown that TNF-α and IFN-γ levels in SB fluid peak after 3 days while IL-2 levels peak after 7 days[2,10], probably reflecting the dominance of adaptive responses at this later stage. Because SB cells have been activated *in vivo*, they exhibit a high spontaneous cytokine release as well as a potential for a specific release upon restimulation (**Figure 3** and **4**).

This report focuses on CD4+ T-cell immunity. As demonstrated in **Figure 2**, the majority of the isolated T-cells were indeed CD4+, while there were almost no CD8+ T-cells in the suction blister fluid. This is in line with other SB PPD-recall studies reporting low proportions and an inferior migratory capacity of CD8+ T-cells compared to CD4+ T cells[2,8,10,23]. A further characterization of the CD8+ contribution would be preferable; however, this is beyond the scope of this report.

T-cells are considered essential for the immune control of *Mtb*; however, it has been difficult to identify a reliable correlate of the protection reflected in the adaptive immune response from the blood[25,26]. This roadblock severely hinders the development of new TB vaccines, as there currently is no alternative to large and very costly efficacy trials[27]. TB vaccine developers determine vaccine immunogenicity by assessing small and transient changes in the vaccine-specific T-cell populations in the blood[28,29]. However, it is questionable whether the small fraction of circulating antigen-specific T-cells found in the blood is relevant (*i.e.*, capable of migrating to the site of an infection and representative of the T-cell-rich microenvironment controlling *Mtb*)[27,30,31].

Based on previous studies and the data presented herein, the SB cutaneous recall model represents an untapped potential for the study of vaccine-specific T-cells. Not only does the model enable a recall of a vaccine response generated decades ago, it also allows the evaluation of the true memory potential in a tissue-specific context[15,18,19,21]. Novel, specific skin tests, which include antigens also comprised of candidate subunit TB vaccines, suggest new opportunities for vaccine evaluation using this model[28,32]. Furthermore, transcriptomic analyses suggest that a cell-mediated immune response generated in PPD-challenged skin is similar to the response found in the *Mtb*-infected lung[18].

While skin punch biopsies also allow for a cell harvest from the skin and provide spatial information, compared with SB sampling, the method is more invasive and requires enzymatic or mechanic processing to prepare single-cell suspensions[33]. Measurements of cytokines and cell markers are comparable between the two methods[2,10].

The suction blister method has already been applied in many areas of medical research besides dermatology, either alone or in combination with a systemic or local skin challenge. Examples include studies of sepsis, Epstein-Barr virus-associated lymphoproliferative disease, diabetic neuropathy, glucocorticoid intake effects, and human trials testing therapeutic antibodies or models for T-cell-targeted therapies[10,34,35,36,37,38].

From a therapeutic point of view, the SB cutaneous recall method offers unique advantages to study the central T-cell memory potential and-from both a biological and technical point of view-the skin seems to provide a relevant sampling compartment[2,15,18,19,21]. In particular, compared to traditional, passive sampling of circulating PBMCs, the SB cutaneous recall method allows for the study of T-cells that have proven the ability to migrate from the lymph node in response to their relevant antigen, and complete the local expansion and differentiation in a tissue-specific *in vivo* context[15,18,19,21].

In conclusion, the model demonstrated here could be relevant for researchers within the field of human adaptive immunity and T-cell targeting agents (*i.e.*, in infectious disease vaccinology or cancer research). The TST model applied in this protocol is, of course, of special relevance in the field of TB vaccinology. However, the basic concept of this model is highly applicable in other fields of research.

## Disclosures

The authors have nothing to disclose.

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