Video Article

T Cells Capture Bacteria by Transinfection from Dendritic Cells

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Abstract

Recently, we have shown, contrary to what is described, that CD4⁺ T cells, the paradigm of adaptive immune cells, capture bacteria from infected dendritic cells (DCs) by a process called transinfection. Here, we describe the analysis of the transinfection process, which occurs during the course of antigen presentation. This process was unveiled by using CD4⁺ T cells from transgenic OTII mice, which bear a T cell receptor (TCR) specific for a peptide of ovalbumin (OVAp), which therefore can form stable immune complexes with infected dendritic cells loaded with this specific OVAp. The dynamics of green fluorescent protein (GFP)-expressing bacteria during DC-T cell transmission can be monitored by live-cell imaging and the quantification of bacterial transinfection can be performed by flow cytometry. In addition, transinfection can be quantified by a more sensitive method based in the use of gentamicin, a non-permeable aminoglycoside antibiotic killing extracellular bacteria but not intracellular ones. This classical method has been used previously in microbiology to study the efficiency of bacterial infections. We hereby explain the protocol of the complete process, from the isolation of the primary cells to the quantification of transinfection.

Video Link

The video component of this article can be found at <http://www.jove.com/video/52976/>

Introduction

When a pathogen infects its host, there is usually an activation of the innate and adaptive immune responses, necessary for bacterial clearance. Innate immunity is the first line of defense that prevents most infections. Innate immunity distinguish in a precise way elements that are conserved among broad groups of microorganisms (pathogen-associated molecular patterns, PAMPS)¹. The mechanisms of innate immunity include physical barriers such as skin, chemicals barriers (antimicrobial peptides, lysozyme) and the innate leukocytes, which include the phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells². These cells identify and eliminate pathogens, either by attacking them through contact or via phagocytosis, which includes pathogen engulfing and killing. This system does not allow lifelong defense, in contrast to adaptive immunity, which confer immunological memory against pathogens. The adaptive immune system is the second line of defense and is able to recognize and react to specific antigens of multiple microbial and non-microbial substances³. The main components of the adaptive immune system are the lymphocytes, which include B and T cells. B cells are involved in the humoral response, secreting antibodies against pathogens or exogenous proteins. However, T cells represent the cell-mediated immunity, modulating the immune response with cytokines secretion or killing pathogen-infected cells⁴.

Antigen presenting cells (APCs) including dendritic cells or macrophages, constituents of the innate immune system, can recognize phagocytose pathogens and process bacterial components into antigens, which are presented at the cell surface by the Major Histocompatibility Complex (MHC)⁵⁻⁷. After APCs have phagocytized pathogens, they usually migrate to the draining lymph nodes, where they interact with T cells. T lymphocytes can recognize specific peptide-MHC complexes by their T cell receptors. The immune synapse (IS) occurs in the interface between an antigen-loaded APC and a lymphocyte during antigen presentation^{8,9}. Some bacteria can survive phagocytosis and disseminate systematically within APCs. In this view, infected APCs serve as bacterial reservoirs or "Trojan horses" that facilitate bacterial spread¹⁰. The intimate contact between APCs and lymphocytes that take place during the course of IS formation also function as a platform for exchange of part of membranes, genetic material and exosomes and can be hijacked for some viruses to infect T cells; this process is called transinfection¹¹⁻¹³.

Some pathogenic bacteria (*Listeria monocytogenes*, *Salmonella enterica* and *Shigella flexneri*) are able to invade T lymphocytes *in vivo* and modify their behaviour¹⁴⁻¹⁶. We have recently described that T lymphocytes are also able to capture bacteria by transinfection from previously infected dendritic cells (DCs) during the course of antigen presentation¹⁶. T cell bacterial capture by transinfection exceedingly more effective (1,000-4,000x) than direct infections. T cells capture pathogen and non-pathogen bacteria indicating that transinfection is a process driven by T cells. Strikingly, transinfected T (tiT) cells rapidly killed the captured bacteria and did so more efficiently than professional phagocytes¹⁶. These results, which break a dogma of immunology, show that the cells of adaptive immunity can perform functions that were supposedly exclusive of the innate immunity. In addition, we showed that tiT cells secrete large amounts of pro-inflammatory cytokines and protect from bacterial infections *in vivo*.

Here we present the different protocols used to study the bacterial transinfection process in a mouse model. This model is based on the use of CD4⁺ T cells from transgenic OTII mice, which bear a TCR specific for peptide 323-339 of OVA (OVAp) in the context of I-Ab¹⁷ that interact specifically with bacterial-infected bone marrow-derived DCs (BMDCs)^{18,19} loaded with OVAp, forming stable immune synapses.

T cell transinfection can be visualized and tracked using fluorescence microscopy. Additionally, flow cytometry can be used for detecting infected cells by taking advantage of the fluorescence emitted by bacteria expressing green fluorescent protein (GFP)^{16,20}. Moreover, T cell transinfection can be quantified by a more sensitive approach, the gentamicin survival assay that allows measurement of a large number of events. Gentamicin is an antibiotic that cannot penetrate eukaryotic cells. Therefore, using this antibiotic allows differentiation of intracellular bacteria that survived the antibiotic addition from extracellular ones that were killed²¹.

Protocol

Note: Experimental procedures were approved by the Committee for Research Ethics of the Universidad Autónoma de Madrid and conducted under the supervision of the Universidad Autónoma de Madrid Head of Animal Welfare and Health in accordance with Spanish and European guidelines. Mice were bred in specific pathogen free (SPF) housing and they were euthanized by trained and qualified personnel using carbon dioxide (CO₂) inhalation method.

1. Mouse Bone Marrow-derived DCs Differentiation and Infection

Note: **Figure 1** summarizes this first step. All procedures should be carried out in the hood from this point on, using only sterile media, instruments, pipette tips and culture dishes.

1. Mouse Bone Marrow-derived DCs Differentiation
 1. Dissect tibias and femurs from one C57/BL6 mouse²² and transfer into a plastic dish with 10 ml of RPMI medium.
 2. Cut each epiphysis off, with sterile scissors and expose bone marrow, which has a characteristic bright red color.
 3. Infuse the inside of the bone with 10 ml of phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) and 5 mM ethylenediaminetetraacetic acid (EDTA) (PBS/BSA/EDTA buffer) using a sterile syringe on a sterile Petri dish.
 4. Collect the cell suspension and centrifuge in a tube at 600 x g in a refrigerated centrifuge (4 °C).
 5. Resuspend the cells in 1 ml of Ammonium-Chloride-Potassium (ACK) lysing buffer (0.15 M NH₄Cl, 10 mM KHCO₃ and 0.1 mM EDTA) and incubate for 30 sec at RT in order to eliminate red blood cells.
 6. Add 10 ml of RPMI 1640 medium with 10% fetal bovine serum (FBS) and filter through a cell strainer (70 µm) to remove bone fragments and cell clumps before centrifuging at the same speed (600 xg) because clumps were eliminated by filtering.
 7. Count the cells and adjust to 5 x 10⁵ cells/ml with RPMI 10% FBS (containing 50 µM B-mercaptoethanol, 1 mM sodium pyruvate) and add 20 ng/ml recombinant murine granulocyte-macrophage colony-stimulating factor (rm-GM-CSF) as a final concentration.
 8. Add this suspension to a sterile, microbiological quality, 15 cm Petri dish, and culture in a CO₂ incubator (37 °C, 5% CO₂).
 9. Every three days, spin down both non-adherent cells and detached cells with 5 mM EDTA in PBS and resuspend at 5 x 10⁵ cells/ml of cell density, with fresh medium containing 20 ng/ml rm-GM-CSF.
2. Maturation of DCs and Antigen Loading
 1. At day 9, resuspend the cells at 5 x 10⁵ cells/ml of density in medium containing 20 ng/ml rm-GM-CSF. Then add 20 ng/ml of lipopolysaccharide (LPS) to allow high MHC-II expression on cells and incubate in a non-tissue culture-treated sterile 15 cm Petri dish for 24 hr.
 2. After one day of LPS-stimulation, stain some DC cells with antibodies against mouse CD11c, MHCII and Gr-1 to check DCs differentiation by flow cytometry. An example of flow cytometry analysis of DCs is shown in **Figure 3A** and **3B**.
 1. Incubate DCs (5 x 10⁵ cells/sample) with an anti-mouse-CD16/CD32 monoclonal antibody at 2.5 µg/ml of final concentration in 50 µl PBS/BSA/EDTA buffer per sample. Afterwards, add 50 µl of the antibodies against MHCII (I-A/I-E), CD11c and Gr-1 conjugated with different fluorochromes at 1:100, 1:200 and 1:500 in PBS/BSA/EDTA buffer respectively.
 2. Finally, wash DCs with PBS/BSA/EDTA buffer and analyze by flow cytometry according to manufacturer's protocol.
Note: If the cells are well differentiated, cells are ready for antigen loading.
 3. Wash DCs with RPMI 10% FBS (without antibiotics) and incubate them with 10 µg/ml OTII OVAp (OVA 323-339; I S Q A V H A H A E I N E A G R) on a plastic tube in 1 ml of RPMI 10% FBS medium per 5 x 10⁶ DCs for minimum 1 hr at 37 °C. As a negative control, leave DCs without incubating with OVAp.
3. Infection of DCs
 1. Infect DCs at a multiplicity of infection (MOI) of 10 bacteria (10 bacteria per DC) for 1 hr in a CO₂ incubator (37 °C, 5% CO₂).
 2. Wash DC cells 3x in PBS and 1x in RPMI 10% FBS and centrifuge at 450 x g in order to wash most of extracellular bacteria. Finally, resuspend cells in RPMI 10% FBS medium (without antibiotics) at 20 x 10⁶ cells/ml.

2. Isolation of CD4⁺ T Lymphocytes from OTII Transgenic Mice

Note: **Figure 1** summarizes this second step. Lymph nodes should be used instead of spleen to isolate CD4⁺ T cells, because the proportion of CD4⁺ lymphocytes in lymph nodes (~50%) is larger than in spleen (~25%) and therefore the purification would be more effective.

1. Make Single-cell Suspension of Lymph Nodes
 1. Remove inguinal, axillary, brachial, cervical and mesenteric lymph nodes²³ from OTII transgenic mice and transfer into a plastic dish with 10 ml of RPMI 10% FBS medium.

2. Grind lymph nodes under a sterile hood using two frosted microscope slides. Place lymph nodes on a frosted side of one microscope slide, and rub with frosted side of the second slide until organs have been ground.
 3. Wash single cell suspension in PBS/BSA/EDTA buffer.
 4. Filter the cells through a cell strainer (70 μ m) to remove connective tissue and wash with PBS/BSA/EDTA buffer.
 5. Resuspend the cells in 1 ml of ACK lysis buffer and incubate for 1 min at RT in order to eliminate red blood cells.
2. Isolation of CD4⁺ T Cells
1. Wash again as in step 2.1.3 and count cells to resuspend them at 100×10^6 cells/ml in PBS/BSA/EDTA buffer. Add biotinylated antibodies against CD8, IgM, B220, CD19, MHC class II (I-Ab), CD11b, CD11c and DX5 at 1:250 for 30 min on ice for later negative selection of CD4⁺ T cells.
 2. Wash cells in PBS/BSA/EDTA buffer and incubate the cells (100×10^6 cells/ml) with streptavidin microbeads at the concentration recommended in manufacturer's protocol for 15 min on ice.
 3. Wash cells in PBS/BSA/EDTA buffer and filter them through a cell strainer (30 μ m) before CD4⁺ T cell isolation.
 4. Isolate CD4⁺ T cells by negative selection using a magnetic cell separation machine according to manufacturer's protocol.
 5. Count the cells and adjust to 4×10^6 cells/ml with RPMI 10% FBS medium (without antibiotics).
 6. Fix and keep on ice isolated CD4⁺ T cells (3×10^5 cells) to check the purity by flow cytometer as described²⁴. An example of CD4⁺ T cells purification is shown in **Figure 3C**.

3. T Cell Transinfection Measurement by Gentamicin Protection Assay

Note: **Figure 2** summarizes this third step of the protocol.

1. T cell Transinfection
 1. Incubate isolated CD4⁺ T cells with infected DC cells (1:1) (the DCs were infected for 1 hr and washed to eliminate free bacteria) for 30 min to allow immune synapse formation in a CO₂ incubator (37 °C, 5% CO₂).
 2. Add 0.5 ml of isolated CD4⁺ T cells (2×10^6 cells) and 0.1 ml of infected DCs (2×10^6 cells) on a 24-well culture plate. As negative control, infect directly 0.5 ml of CD4⁺ T cells at a MOI of 10 bacteria for 30 min.
 3. Include additional controls separating infected DCs from T cells using a polycarbonate transwell barrier (with 3 μ m of pore size), impeding DC-T physical contact. Add 0.1 ml of DCs inside the transwell and 0.5 ml of CD4⁺ T cells into the lower compartment of the well (24-well plate).
 4. Finally, complete with RPMI medium until there is 0.6 ml in each well to make equal volumes in all samples.
 5. After 30 min of immune synapse formation, add 100 μ g/ml of gentamicin and incubate for 1 hr before collecting cells in a CO₂ incubator (37°C, 5% CO₂).
2. Re-isolation of CD4⁺ T Cells
 1. Collect non-adherent cells and resuspend them in PBS/BSA/EDTA buffer. Most of DCs remain attached to the plastic culture plate. Vortex tubes gently to ensure cell disaggregation. Keep 500 μ l of cell supernatant as control to show that gentamicin has worked well.
 2. Re-isolate CD4⁺ T cells from samples that contain DCs and T cells together as in step 2.2, although most of the DCs should have attached on the plate. Keep the control samples on ice. Take into account only experiments with less than 2% contamination. An example of CD4⁺ T cells purification after transinfection is shown in **Figure 3D**.
3. Lyse T Cells and Seed Them on Bacteria Medium-agar Plates.
 1. Wash CD4⁺ T cells twice with PBS.
 2. Count the cells and resuspend them at 2×10^6 cells/ml in PBS.
 3. Add 500 μ l of 0.1% Triton X-100 in 500 μ l of T cells to lyse them at 1×10^6 cells/ml, releasing the intracellular bacteria out of the T cells.
 4. Make 3 serial decimal dilutions of lysed cells and seed 50 μ l of each dilution on bacteria medium agar plates. Divide plates into 4 portions and seed the different dilutions of lysed cells in each part. Representative results are shown in **Figure 4**.
 1. As a control, also plate the stored cell supernatant of the conjugates on agar, to ensure that gentamicin has worked well. Also plate infected DCs as an additional control. In case low DCs contamination occurs (<2%), subtract the colony forming units (CFUs) corresponding to low DC contamination.

4. Quantification of T Cell Transinfection by Flow Cytometry

Note: **Figure 2** summarizes this step.

1. T Cell Transinfection
 1. Infect BMDCs as in step 1.3, but use GFP expressing bacteria in this case.
 2. Stain CD4⁺ T cells (isolated from lymph nodes in step 2.2) with a cell tracker chloromethyl aminocoumarin (CMAC) for 30 min at 37 °C according to manufacturer's protocol.
 3. Wash labelled CD4⁺ T cells twice with RPMI 10% FBS (without antibiotics).
 4. Incubate infected DCs (after 1 hr incubation with bacteria) with labelled CD4⁺ T cells for 30 min to allow immune synapse formation at 37 °C including all the controls explaining in step 3.1.1.
2. Staining of Infected CD4⁺ T Cells
 1. After 30 min of DC-T immune synapse formation, collect cells and wash them in PBS/BSA/EDTA.
 2. After two washes with PBS/BSA/EDTA buffer, separate aggregated cells by gently vortexing the tubes.
 3. Fix the samples in 0.2 ml/tube of PBS containing 3% PFA for 15 min at RT. Then wash them in PBS.

4. Incubate with anti-mouse-CD16/CD32 monoclonal antibody (2.5 µg/ml) in 50 µl/sample of PBS/BSA/EDTA for 15 min on ice to block Fc receptors of dendritic cells.
 5. Add a rabbit anti-bacteria antibody (10 µg/ml as final concentration) in 50 µl/sample of PBS/BSA/EDTA for 30 min on ice to stain extracellular bacteria.
 6. Wash cells with PBS/BSA/EDTA and incubate with an antibody against mouse CD11c conjugated with phycoerythrin (PE) and a secondary antibody against rabbit conjugated with other fluorochrome like Alexa-Fluor 647 at 1:200 in 100 µl/sample of PBS/BSA/EDTA for 30 min on ice.
 7. Wash cells and analyze samples by flow cytometry. Do not forget to include a sample of transinfected T cells using non-fluorescent bacteria as negative control and samples labelled with just one of each fluorochrome used in the experiment to compensate the samples and adjust the flow cytometry settings²⁵. Representative analysis is shown in **Figure 5**.
- Note: If bacteria do not express GFP, to distinguish intracellular and extracellular bacteria, label extracellular bacteria, fix, and permeabilize samples with Triton X-100 at 0.5% in PBS for 5 min. Afterwards stain total bacteria (intracellular + extracellular) with a different fluorochrome.

Representative Results

Herein we described how to perform murine T cell bacterial transinfection from infected bone marrow derived-DCs and how to measure bacterial transinfection via two different approaches: flow cytometry and gentamicin survival assay. **Figure 1** summarizes the procedure to obtain the cells. DCs are generated by incubation of bone marrow cells with GM-CSF for 9 days. Then, DCs are matured with LPS to increase MHCII on its membrane to load them later on with a specific peptide of OVA (OVAp). As a control, some DCs are not loaded with OVAp. Both DCs (OVAp-loaded and non-loaded) are infected at a multiplicity of infection (MOI) of 10 bacteria. On the other hand, CD4⁺ T cells are isolated from lymph nodes of OTII transgenic mice, which recognize the specific OVAp. **Figure 2** depicts the procedure to perform and quantify T cell transinfection including the appropriate negative controls. As described before, some DCs are not loaded with OVAp. In this case, there is an interaction between DCs and T cells, but not immune synapse formation due to the absence of OVAp. An additional control is included, separating DCs and T cells with a polycarbonate transwell barrier (with 3 µm of pore size), which impedes DC-T physical contact. DCs are included inside the transwell; as a result they are not able to pass through this pore size. Another negative control is also incorporated, the direct T cell infection, by incubating T cells directly at a MOI of 10 bacteria. T cell transinfection can be quantified by two approaches: flow cytometry or gentamicin survival assay. To measure by flow cytometry, DCs should have been infected previously with bacteria-GFP and CD4⁺ T cells stain with a cell tracker (T cells-blue). After conjugate formation, the cells should be stained with antibodies against DCs (CD11c-PE). The extracellular bacteria should also be stained in order to quantify the percentage of infected CD4⁺ T cells by flow cytometry later on. To measure transinfection by gentamicin survival assay, cells are incubated with gentamicin for 1 hr to kill extracellular bacteria, then T cells are re-isolated to seed on agar plate. Decimal serial dilutions are made to seed 50 µl of each dilution on agar plate and facilitate the counting of CFUs.

As shown in **Figure 3**, DC differentiation has to be checked by flow cytometry (**Figure 3A** and **3B**). DCs should express CD11c and MHCII (**Figure 3A**) and not Gr-1, which is a marker of neutrophils. As presented in **Figure 3B**, some neutrophils (3.27% of Gr-1⁺, MHC⁺) were presented in the culture, but DCs culture with more than 5% of neutrophils contamination should not be used. CD4⁺ T cells purity should be checked after isolation from lymph nodes (**Figure 3C**) and after separation from DCs conjugates formation (**Figure 3D**). Take into account the experiments with less than 2% of contaminants after conjugates formation.

Figure 4 shows an example of T transinfection experiment using *Salmonella*. As illustrated in **Figure 4A**, plates are divided in four portions to seed the serial dilutions in each part. *Salmonella* have grown on LB agar plates and colony-forming units can be counted. As shown in **Figure 4A**, *Salmonella* was captured by T cells from infected DCs and this process was clearly enhanced with OVAp recognition (plates on the left of **Figure 4A** and **Figure 4B**). However, when there was a physical barrier impeding the contact between DCs and T cells, there was virtually no transinfection, as in the case of doing a direct T cell infection (plates on the right of **Figure 4A** and **Figure 4B**).

As shown in **Figure 5**, T cell transinfection can be quantified by flow cytometry using bacteria expressing a bright GFP. DCs and T cells can be differentiated by size and complexity in a forward and side scatter plot, but different markers can be used to well distinguish them. CD4⁺ T cells were labelled with a cell tracker (CMAC) and DCs with an antibody against CD11c. Extracellular bacteria were stained with a rabbit antibody against *Salmonella* and secondary antibody anti-rabbit conjugated with Alexa Fluor 647. Therefore, the percentage of infected CD4⁺ T cells can be quantified because they are labelled with CMAC (not with CD11c-PE) and expressed GFP (bacteria-GFP), but they were not stained with the antibody against extracellular bacteria (Alexa Fluor 647).

Figure 1. Flow chart of DC generation and CD4⁺ T cells isolation. On the left hand side of the figure, the process to differentiate dendritic cells (DCs) from bone marrow cells is detailed. After dissecting femurs and tibias, bone marrow cells are extracted from the interior of the bones. Afterwards, red blood cells (RBC) are lysed and bone marrow cells are cultured with recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) for 9 days. Once they are well differentiated, DCs are incubated with lipopolysaccharide (LPS) to increase Major Histocompatibility Complex II (MHCII) expression. Then, DCs are loaded with a specific ovalbumin peptide (OVAp) and infected with a multiplicity of infection (MOI) of 10 bacteria. Some of them are not loaded with OVAp as a control. On the right hand side of the figure, how to isolate CD4⁺ T cells from OTII transgenic mice is represented. Lymph nodes (LNs) are removed and ground, obtaining a single-cell suspension. Finally, CD4⁺ T cells are isolated by negative selection using a magnetic cell separation machine. [Please click here to view a larger version of this figure.](#)

Figure 2. Flow chart of T cell transinfection procedure. Infected (bacteria are depicted in green) and OVAp loaded-DCs (red cells) are incubated with CD4⁺ T cells (blue cells) to allow immune synapse formation. Infected DCs (but not loaded with OVAp) are also incubated with T cells, which allows interaction between both cells but without immune synapse formation. An additional control is included, separating CD4⁺ T cells and DCs with a polycarbonate transwell barrier (+TW), which impedes DC-T cell contact. Direct T cell infection is also incorporated as negative control. Finally, T cell transinfection can be quantified by two methods, by flow cytometry (left side of the figure), which indicates the number of T cells capturing bacteria, and by gentamicin survival assay (right side of the figure) a very sensitive method (one infecting bacterium can be detected) indicating the bacterial infection rate. [Please click here to view a larger version of this figure.](#)

Figure 3. Flow cytometer analysis of bone marrow derived-DCs and isolated CD4⁺ T cells. Representative dot plots of bone marrow derived-DCs analysed by flow cytometry. (A) DCs expressed MHCII and CD11c (85.8%; upper right quadrant) (B) DCs expressed MHCII but not Gr-1 (85%; lower right quadrant). However, there was a 3.27% of cells that expressed Gr-1 but not MHCII cells (upper left quadrant). These cells were slight contaminated neutrophils. (C and D) Histograms representing the purity of CD4⁺ T cells isolated from lymph nodes (C), and after conjugate formation with dendritic cells (D). [Please click here to view a larger version of this figure.](#)

Figure 4. T cell transfection quantified by gentamicin survival assay. LB agar plates were divided into 4 portions corresponding to decimal serial dilutions of lysed CD4⁺ T cells. **(A)** Colony forming units of *Salmonella enterica* grew on LB agar plates corresponding to T cell transfection in the presence (+) or absence (-) of OVAp on the surface of infected DC, and in conditions allowing DC/T cell contact (-TW) or in the presence of a physical barrier impeding such contacts (+TW). An empty plate corresponding to direct T cell infection (negative control) is also shown. There was almost no transfection when DC/T cell contact was impeded (+TW). **(B)** Quantification of Colony Forming Units (CFUs) from several experiments showing the rate of bacterial captured by T cells from infected DCs. Direct T cell infection, and conditions impeding DC/T cell contact produce little to no levels of T cell infection. When DC/T cell contacts were permitted, there was T cell transfection, and it was enhanced by antigen recognition (+OVAp). Column bars represent the mean of 3 independent experiments. Error bars indicate the SD. Significant differences are represented by ***, corresponding to $p < 0.0001$. [Please click here to view a larger version of this figure.](#)

Figure 5. T cell transfection quantified by flow cytometry. Representative dot plot showing DCs and T cell conjugates after transfection process. CD4⁺ T cells are small and round as forward and side scatter (FCS - SSC) dot blot shows, and in order to be well differentiated, they were stained with a cell tracker (CMAC). DCs expressing CD11c are not labelled with CMAC and are larger than T CD4⁺ T cells with irregular shape. Infected CD4⁺ T cells containing intracellular bacteria-GFP but negative for extracellular bacteria (6.19%) are shown in the lower right quadrant. [Please click here to view a larger version of this figure.](#)

Discussion

T cells or T lymphocytes are a type of leukocytes that play a central role in cell-mediated immunity and belong to the adaptive immune response²⁶. T cells are refractory to being infected *in vitro* but some reports indicate that they can be infected *in vivo*^{14,15}. The intimate contacts of APC and T cells during immune synapse serve as platforms for exchanging biological material¹³, including some viruses such as HIV¹¹. It was recently shown that contrary to the dogma, T cells, the paradigm of cells of adaptive immunity, are also able to efficiently capture bacteria by transfection from infected DCs^{11,16}. Herein we show the detailed protocols to quantify T cell bacterial transfection *in vitro* from infected BMDCs.

T cell bacterial transfection was enhanced by antigen recognition¹⁶. We showed that by using CD4⁺ T cells isolated from OTII transgenic mice recognizing a specific OVAp, BMDCs loaded with OVAp and mice infected with different bacteria (here data is presented from *Salmonella enterica*). CD4⁺ T cell isolated from OTII mice are incubated with infected DCs for 30 min to allow immune synapse formation. The data

presented here correspond to the bacteria that T cells can capture from DCs in this short period of time. We know that longer exposures of T cells to infected DCs resulted in larger bacterial captures (not shown), but as T cells also destroy the uptaken bacteria very quickly, quantification of bacterial transinfection occurring during longer DC/T cell time contacts proved to be difficult. Gentamicin survival assay is a very sensitive method to quantify T cell transinfection because it is able to detect one single infecting bacterium²¹. After incubation with gentamicin that kill just extracellular bacteria, T cells are isolated and lysed to seed on bacteria medium agar plates. The isolation of T cells after forming conjugates with DCs is a critical step within the protocol. Only experiments with less than 2% of contaminants should be taken into account. DC contamination can be measured by flow cytometry and CFUs corresponding to low DC contamination should be subtracted. Alternatively, CD4⁺ T cells purity can be improved by using cell sorting.

Another approach to quantify T cell transinfection is by flow cytometry, using GFP expressing bacteria and labelling the extracellular bacteria with a compatible fluorophore. One limitation of this approach is that bacteria have to express a GFP that is sufficiently bright for detecting infected cells against the autofluorescence background. Alternatively, bacteria could be stained before infecting DCs with a cell tracker. Another approach would be staining the extracellular bacteria, permeabilizing the T cells, then labelling all the bacteria (intracellular + extracellular) with a different fluorochrome.

Regarding future directions of this protocol, we are trying to set up a protocol to quantify the number of T cells that captured bacteria after longer exposition to infected DCs, by using bacteria decorated with magnetic particles. T cells capturing bacteria would retain the magnetic particles and therefore it should be easy to isolate them by using magnets.

Disclosures

The authors have nothing to disclose.

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