Electron microscopy analysis of FcRγ localization after its capture by T cells by trogocytosis[1]

Stéphanie Balor[*,†], Marc Daëron[§,¶], Pierre Bruhns[§,¶] and Denis Hudrisier[*,†]

[*] Université de Toulouse; UPS; IPBS; F-31077 Toulouse, France

[†] CNRS; IPBS (Institut de Pharmacologie et de Biologie Structurale); 205 route de Narbonne, F-31077 Toulouse, France

[§]Institut Pasteur, Département d'Immunologie, Unité d'Allergologie Moléculaire et Cellulaire, Paris, France;

[¶]INSERM, U760, Paris, France;

Author for correspondence: Denis Hudrisier IPBS, CNRS UMR5089, 205 route de Narbonne, 31077 Toulouse. Phone: (+33) 561175910; Fax: (+33) 561175971; email: denis.hudrisier@ipbs.fr,

Running head: Electron microscopy of FcRγ captured by T cells

Keywords: T cell – trogocytosis – Fc Receptors – Cell activation

[1] This work was supported by the Region Midi-Pyrénées and the Agence Nationale pour la Recherche.

Abstract

T cells acquire various proteins from their cellular partners by the process of trogocytosis. We recently demonstrated that the FcγRIIIA receptor and its associated FcRγ are captured by T cells during their co-culture with FcγR-expressing target cells upon both antigen- or antibody-mediated stimulation. Interestingly, we found that FcR captured by T cells could bind ligands but did not transmit detectable intracellular signals or signaling-depending functions upon ligand binding suggesting their improper integration in the recipient T cell membrane. In this study, we provide morphological data in support of this hypothesis. Indeed, we show that the FcRγ-subunit, which we used as a fusion to GFP, was clearly present at the plasma membrane of donor cells, but was detected within structures that were in close contact of, but apparently not integrated in, the plasma membrane of recipient T cells.

Introduction

Trogocytosis refers to the process whereby most hematopoietic cells capture fragments of the plasma membrane of the cells with which they interact. This is particularly the case for T cells, which perform trogocytosis upon recognition of their cognate antigen on antigen-presenting cells (APC). Consequently, T cells harbor on their surface not only the antigen but also a series of molecules that they do not synthesize themselves. Many different roles have been proposed for trogocytosis in T cell biology but their physiological relevance and underlying mechanisms have not been demonstrated (1-4). Most of the functions of trogocytosis identified so far require that molecules captured by T cells interact with their ligand on the same or different cells without the need for intracellular signalling events. For instance, capture by T cells of the antigen and of co-stimulatory molecules followed by interaction with the TCR and CD28 on other T cells results in T cell-T cell interactions leading to activation, tolerance or exhaustion (4). The transfer of several receptors by trogocytosis has been reported on T cells (5-7), B cells (8) or NK cells for instance (9-12) but it is unclear whether they could insert properly in the T cell membrane and transmit intracellular signals similar to those elicited in the presenting cell, naturally expressing these molecules. We recently showed that Fc receptors (FcR) as well as the associated FcRγ subunit could be detected at high levels on murine and human T cells after their intercellular transfer from FcγR-expressing APC (13). Capture of FcγR occurred during co-culture of T cells with FcγR-expressing APC both upon antibody (Ab)- or antigen-mediated T cell stimulation. Once captured by T cells, FcγR were displayed in a conformation compatible with physiological function and conferred upon T cells the ability to bind immune complexes, as well as to provision B cells with this source of antigen. However, we could not detect downstream signals or signaling-dependent functions following triggering of FcγR captured by T cells. In addition, biochemical studies suggested the improper integration of FcγR in the recipient T

cell membrane (13). However, we had no morphological evidence supporting this notion. In this study, we analyzed the location of FcγR on APC and on T cells having captured these receptors by trogocytosis.

Material and Methods

Cell lines and mice. The HEK 293 cell line transfectant expressing FcγRIIIA + FcRγ^{GFP} were used as target cells. The CD8$^+$ T cells specific for OVA peptide 257-264 (SIINFEKL) presented by the H-2K^b MHC class I molecules were obtained from the OT-I TCR-transgenic mice (Charles River, LeGenest, France) (14). Total splenocytes from OT-I mice were cultured in the presence of 0.1 μM of the appropriate antigenic peptide and used in trogocytosis or functional assays 4 to 6 days after stimulation.

Reagents, antibodies and molecular biology. Fluorescently labelled monoclonal antibodies (mAb) against mouse CD8α (53.6.7.2) and FcγRIIB/FcγRIIIA (2.4G2) and streptavidin were from Becton-Dickinson/Pharmingen (Le-Pont-de-Claix, France). Unlabeled mAb to H-2K^b (Y3) was purified from culture supernatant of the corresponding hybridoma. Ab to GFP were from Abcam (Paris, France).

Trogocytosis. HEK expressing a flagged form of FcγRIIIA (15) + FcRγ^{GFP} cells (13) were incubated in T175 culture flasks (25-30x10^{6}cells/flask) with T cells (10x10^{6}cells/flask in 10 ml final volume) for 1 hour at 37°C. T cells were previously incubated or not with 5 μg/ml of the anti-H-2K^b mAbs for 30 minutes at 4°C. T cells were then analyzed by flow cytometry on a FACSCalibur or LSRII (Becton Dickinson) using anti-CD8 or anti-CD4 mAb. Staining with anti-FcγR and detection of GFP fluorescence was used to detect trogocytosis.

Immuno-electron microscopy. Cells were fixed for 1 hour with 4% formaldehyde (EMS, Delta-Microscopies, France) in buffer A (0.1 M cacodylate buffer, pH 7.2, containing 0.1 M sucrose, 5mM $CaCl_2$ and 5 mM $MgCl_2$) at room temperature, washed 3 times in the same

buffer and treated with 50 mM ammonium chloride for 30 minutes. Cells were then concentrated in 2% Agar in buffer A. After Agar concentration, cells were infused in high concentrations of sucrose before being frozen in liquid nitrogen. Frozen samples were then substituted in 0.5% uranyl acetate in methanol at -90°C in a cryosubstitution apparatus (AFS, Leica Microsystems). Then infiltrations and embedding.in Lowicryl HM20 were performed at -45°C and the Lowicryl resin was polymerised with UV light.

Then ultra-thin sections were performed on a Leica Ultracut microtome and were mounted on Formvar-carbon-coated 200 mesh nickel grids. After 15 minutes on a drop of PBS (pH 7.6), the sections were quenched in 0.05 M glycine in PBS and incubated in blocking solution (PBS+5%BSA) for 30 minutes (all from Aurion, Delta-Microscopies, France) followed by anti-GFP primary Abs (Abcam) diluted in PBS-1% BSA for 2 hours at room temperature. The sections were washed for 30 minutes with PBS containing 1% BSA and incubated for 1 hour with goat anti-rabbit IgGs conjugated to 10 nm colloidal gold particles (BioValley, Marne-La-Vallée, France) in PBS containing 1% BSA. The grids were then washed for 20 minutes in PBS, fixed for 5 minutes with 1% glutaraldehyde in PBS and rinsed in distilled water. Finally, thin sections were stained with 1% uranyl acetate and lead citrate and examined at 80 kV on a Jeol 1200-EX electron microscope at the IFR 109 electron microscopy facility.

Results

OT-I CD8$^+$ T cells acquire murine FcRγ^{GFP} upon co-culture with FcγRIIIA/ FcRγ^{GFP} -expressing HEK cells. We recently showed that FcγRIIB/IIIA as well as the FcRγ subunit could be captured by trogocytosis triggered in T cells by mAb such as the anti-H-2K^b mAb or by antigen (13). In order to obtain a large number of T cells having acquired FcγR during trogocytosis we tested if FcγR capture could be detected in trogocytosis experiments performed in large T175 culture flasks. As shown in Fig. 1, we found that, in these experimental conditions as well, OT-I CD8$^+$ T cells captured FcγRIIIA from HEK cells transfected with cDNA encoding murine FcγRIIIA plus the FcRγ^{GFP} subunit (also called FcεRIγ or γ) when co-cultured in the presence of the anti-H-2K^b mAb. Capture of FcγRIIIA was detected using anti-FcγRIIB/IIIA mAb (Fig. 1A) and capture of the FcRγ^{GFP} subunit (Fig. 1B) was determined using GFP fluorescence. This material was then used in electron microscopy experiments in order to visualize the localization of these molecules on donor APC and on recipient T cells. Note that most HEK cells remained adhered when T cells were harvested explaining why so few cells appear in the lower panels. Furthermore, using LSR-II flow cytometer, we found it very difficult to have both T cells and HEK cells in the same FSC/SSC dot plots. As our study was focused on T cells, most HEK cells present in the sample were not present in the SSC/FSC gating we used, centered on T cells. Therefore, the cells appearing in the lower panels are not only very few and, in addition, they are not representative in terms of the fluorescent signals recorded on the whole HEK cell population.

Donor HEK cells and recipient OT-I T cells can be easily distinguished based on morphology. As we wanted to analyze the localization of FcR on a two-partner cell culture, we first determined if staining with specific Abs was necessary to distinguish OT-I T cells

from HEK cells or if morphological parameters were sufficient. As shown in two examples provided in Fig. 2, donor HEK cells and recipient T cells can unambiguously be identified based on morphological features. First, HEK cells are much larger (size) than OT-I T cells (size). Second the cytoplasm of HEK cells appears as very light as compared to that of OT-I T cells, including much larger size and weaker electronic density of the cytoplasm for HEK. Therefore one can easily analyze either OT-I T cells or HEK cells without performing cell-specific staining with Ab.

FcRγ^{GFP} captured by T cells exhibit different localizations as compared to FcγR endogenously expressed by target cells. Next we examined the localization of FcRγ by immuno-electron microscopy in both donor cells (expressing this molecule endogenously) and recipient T cells (expressing it after capture). We did not manage to perform these experiments using the 2.4G2 mAb specific for FcγRIIB/IIIA. However, a previous work having validated the use of polyclonal rabbit anti-GFP Ab in electron microscopy experiments (16), we analyzed the localization of FcRγ^{GFP}, using this antibody when cells were detected using gold-labeled anti-rabbit antiserum. Figure 3 shows a collection of images showing that FcRγ^{GFP} was clearly present at the plasma membrane as well as in intracellular compartments of donor HEK-FcγRIIIA-FcRγ^{GFP} transfectants. In particular it was present in structures resembling microvilli. However, in T cells having captured FcRγ^{GFP}, we detected the molecule within structures of heterogeneous size that were in close contact but apparently not integrated in the T cell membrane (Fig. 4). When looking at the area of contact between HEK transfectants and T cells, FcRγ^{GFP} are clearly detectable in the plasma membrane of HEK cells, while in T cells FcRγ^{GFP} was present in vesicular or tubular structures closely apposed to but not inserted into the T cell membrane (Fig. 5, 6 and 7). Altogether our results are suggestive of an improper integration of FcRγ^{GFP} in the plasma membrane of T cells.

Discussion

Although the transfer of proteins between immune cells has been anecdotically reported for years, the widespread nature of this phenomenon and its potential biological significance has only been described recently. Indeed, protein transfer, and in particular trogocytosis, has been proposed to play a role in fundamental processes such as the initiation and regulation of immune responses (1, 3). Thus, it is critical to identify molecules that can be captured by immune cells, to understand the molecular basis of the transfer and most importantly, to define the function of captured molecules on recipient cells.

Previously, we showed that T cells efficiently acquired FcγR from APC both during antibody- or antigen-mediated stimulation and displayed them in a correct orientation and topology on their surface (13). After capture of FcγR, T cells could bind FcγR ligands very efficiently, while no detectable biochemical or functional response resulted from this binding. Furthermore, we found indications that uncoupling between ligand binding and functional response upon stimulation of FcγR acquired by T cells could be due to a different behavior of FcγR captured by T cells as compared with receptors endogenously expressed by donor cells that was probably resulting from the incomplete integration of captured FcγR into the T cell membrane (13). Note that, even if they were not capable of transmitting intercellular signals, captured receptors could nevertheless confer on recipient cells new functions that solely require their ability to bind ligands (13). In the current study, we provide morphological data supporting this conclusion by showing that molecules of the FcR complex do not appear to be properly integrated in the membrane of recipient T cells while it is the case in donor, presenting cells.

Our data support a model whereby captured FcR molecules could be present in structures attached, but not integrated, to the membrane of the recipient T cells. This would

explain why, being well orientated FcR, they can still bind extracellular ligands but do not propagate signals inside the T cell (13). Our observations also explain why materials captured by trogocytosis can be, at least partly, removed after treatment with low pH solution (9, 11, 13). Very few morphological analyses of the process of trogocytosis by electron microscopy are available (9, 16, 17, 18). We believe that the images we obtained on the localization of the $FcR\gamma^{GFP}$ subunit resemble those published earlier where captured molecules were found within membranous structures loosely attached to the plasma membrane of NK cells (9) or T cells (17). In another study, heterogeneous materials (compatible with the involvement of both exosomes and shed membranes) were also found to be apposed but not integrated in the recipient T cells membrane (18). However, as this was analyzed at late time points (24 hours) of the co-culture, it is unclear whether this transfer is related to trogocytosis or not (18). It is also unclear if the data provided by Pardigon and Yewdell regarding the capture of TL by CD8αα T cells support a model whereby TL would be properly integrated or not in the recipient T cell membrane (16). However their data clearly indicate that the molecules captured are present within structures much larger than exosomes and not diffuse in the T cell membrane (16). Fluorescent microscopy analyses performed in various experimental systems also revealed that molecules, once captured, do not freely diffuse in the recipient cell membrane but are retained in discrete domains (9, 10, 16, 19-22). Limited information is available regarding the ability of receptors captured by trogocytosis to transmit intracellular signals in recipient cells and to confer on these cells novel functions depending on signaling. For instance, NK cells have been shown to capture receptors for Epstein-Barr virus (CD21)(12) or for poliovirus (CD155) (23). However, this did not apparently sensitize NK cells to virus infection as shown in the first study in which this possibility was examined (12). However the interpretation of these data is complicated by the fact that the absence of detectable infection in these cells could be the result of more complex events, such as the

inability of the virus to replicate within these cells, or reduction of the viral load to undetectable levels due to the production of anti-viral molecules by the NK cell. The group of Parish in Canberra reported that, unlike the endogenously expressed BCRs, those captured by B cells do not transmit intracellular calcium fluxes upon stimulation (8). This is very much in line with our report on FcR (13). Furthermore Patel *et al* have found that MHC class II molecules captured by T cells do not sensitize these cells to complement-mediated killing in the presence of anti-MHC class II Ab, while donor cells were lyzed by the same treatment (17). These data are in favor of a model whereby molecules acquired by trogocytosis could be present in membrane structures adsorbed or apposed to recipient cell membrane but not fusing with those latter. However a recent publication by the group of Parish now reports that T cells having captured TCR from other T cells could kill target cells expressing the antigen recognized by the acquired TCR (6). Although the integration of the acquired TCR and its signaling properties have not been investigated in the recipient T cell themselves, these results suggest that acquired TCR could be functional after capture and thus could be well integrated. More complex scenarios could however account for this observation as improperly-integrated, captured TCR could be co-down-modulated with properly-integrated, endogenously expressed TCR to induce signaling (24, 25). Note that the study by Chaudhri *et al* (6) differs from many others in that activated T cell do give materials (TCR) to neighboring cells rather than acquiring molecules from those (see (2) and references therein). Thus this study appears to be more in line with the observation that activated T cells release their TCR in an exosome-associated form (26). Whatever these differences, it remains to be determined why related processes of intercellular transfer of molecules in a membrane-bound form could result in integration or not of the captured molecules in the recipient cell membrane, depending on the nature of the recipient cell.

Apart from purely methodological explanations, we perceive that there are several possible mechanistic explanations to understand the differences between the apparent discrepancies resulting from the studies discussed above. First, it is possible that the detection level of the function examined by Parish *et al* is so low that even a very low number of acquired TCR that integrate properly is sufficient to confer function on recipient T cells (6). Indeed it is well known that CTL cytotoxicity is a very sensitive function (27, 28) and that as few as 500-1000 TCR could be sufficient to activate T cells (29). However, although direct comparison is not possible, other very sensitive functions, *i.e.* complement-mediated killing and antibody-dependent cell cytotoxicity, have also been used both in the study by Patel *et al* (17) and in our study (13), but remained undetectable after triggering of acquired receptors. Another possibility is that membrane structures conveying the receptor have different fusogenic properties. For instance it is still unknown if, during trogocytosis, intercellular transfer of molecules is mediated by exosomes, microparticules, nanotubes, membrane bridges or patches of torn membranes (1). Possibly, depending on the mechanisms involved, this could result in, no, little or even full integration of the acquired molecule. In the absence of molecular tools to selectively block these different processes, it is still impossible to answer this question. If some donor cells are more prone than others to give membrane materials using one of these mechanisms, and if recipient cells have differential abilities to integrate these structures, one can predict that the nature of the cell partners present in the coculture is a critical parameter.

Note that evidence for the transfer of receptors remaining functional in tumor cell lines could be due to different mechanisms, and that tumor cells and not normal cells were used (30). More examples reporting on the functionality of acquired receptors will be helpful to understand the consequences of trogocytosis in T cell biology and further advance the two

major questions concerning the mechanisms of trogocytosis, *e.g*. the molecular basis of protein transfer and the functional properties of captured molecules.

Acknowledgments

We thank Etienne Joly and members of the I^3 club at the IPBS for stimulating discussions. This work benefited of the assistance of the electron microscopy facility of the IFR 109 (TRI platform).

References

1. Davis, D. M. 2007. Intercellular transfer of cell-surface proteins is common and can affect many stages of an immune response. *Nat Rev Immunol* 7:238-243.
2. Hudrisier, D., and P. Bongrand. 2002. Intercellular transfer of antigen-presenting cell determinants onto T cells: molecular mechanisms and biological significance. *Faseb J* 16:477-486.
3. Joly, E., and D. Hudrisier. 2003. What is trogocytosis and what is its purpose. *Nat. Immunol.* 4:815-815.
4. Wetzel, S., and D. Parker. 2006. MHC Transfer from APC to T Cells Following Antigen Recognition. *Crit. Rev. Immunol.* 26:1-21.
5. Patel, D. M., P. Y. Arnold, G. A. White, J. P. Nardella, and M. D. Mannie. 1999. Class II MHC/peptide complexes are released from APC and are acquired by T cell responders during specific antigen recognition. *J. Immunol.* 163:5201-5210.
6. Chaudhri, G., B. J. Quah, Y. Wang, A. H. Tan, J. Zhou, G. Karupiah, and C. R. Parish. 2009. T cell receptor sharing by cytotoxic T lymphocytes facilitates efficient virus control. *Proc Natl Acad Sci U S A.*
7. Bourbié-Vaudaine, S., N. Blanchard, C. Hivroz, and P.-H. Roméo. 2006. Dendritic cells can turn CD4+ T lymphocytes into vascular endothelial growth factor-carrying cells by intercellular neuropilin-1 transfer. *J. Immunol.* 177:1460-1469.
8. Quah, B. J., V. P. Barlow, V. McPhun, K. I. Matthaei, M. D. Hulett, and C. R. Parish. 2008. Bystander B cells rapidly acquire antigen receptors from activated B cells by membrane transfer. *Proc Natl Acad Sci U S A* 105:4259-4264.
9. Williams, G. S., L. M. Collinson, J. Brzostek, P. Eissmann, C. R. Almeida, F. E. McCann, D. Burshtyn, and D. M. Davis. 2007. Membranous structures transfer cell surface proteins across NK cell immune synapses. *Traffic* 8:1190-1204.
10. Roda-Navarro, P., M. Mittelbrunn, M. Ortega, D. Howie, C. Terhorst, F. Sanchez-Madrid, and E. Fernandez-Ruiz. 2004. Dynamic redistribution of the activating 2B4/SAP complex at the cytotoxic NK cell immune synapse. *J Immunol* 173:3640-3646.
11. Vanherberghen, B., K. Andersson, L. M. Carlin, E. N. Nolte-'t Hoen, G. S. Williams, P. Hoglund, and D. M. Davis. 2004. Human and murine inhibitory natural killer cell receptors transfer from natural killer cells to target cells. *Proc Natl Acad Sci U S A* 101:16873-16878.
12. Tabiasco, J., A. Vercellone, F. Meggetto, D. Hudrisier, P. Brousset, and J. J. Fournie. 2003. Acquisition of viral receptor by NK cells through immunological synapse. *J. Immunol.* 170:5993-5998.
13. Hudrisier, D., B. Clemenceau, S. Balor, S. Daubeuf, E. Magdeleine, M. Daeron, P. Bruhns, and H. Vié. 2009. Ligand binding but undetected functional response of FcR after their capture by T cells via trogocytosis. *J. Immunol.* in press.
14. Hogquist, K. A., S. C. Jameson, W. R. Heath, J. L. Howard, M. J. Bevan, and F. R. Carbone. 1994. T-Cell Receptor Antagonist Peptides Induce Positive Selection. *Cell* 76:17-27.
15. Bruhns, P., B. Iannascoli, P. England, D. A. Mancardi, N. Fernandez, S. Jorieux, and M. Daeron. 2008. Specificity and affinity of human Fc{gamma} receptors and their polymorphic variants for human IgG subclasses. *Blood.*
16. Pardigon, N., K. Takeda, B. Saunier, F. Hornung, J. Gibbs, A. Weisberg, N. Contractor, B. Kelsall, J. Bennink, and J. Yewdell. 2006. CD8-Mediated

Intraepithelial Lymphocyte Snatching of Thymic Leukemia MHC Class Ib Molecules In Vitro and In Vivo. *J. Immunol.* 177:1590-1598.
17. Patel, D., R. Dudek, and M. Mannie. 2001. Intercellular exchange of class II MHC complexes: ultrastructural localization and functional presentation of adsorbed I-A/peptide complexes. *Cell. Immunol.* 214:21-34.
18. Buschow, S. I., E. N. Nolte-'t Hoen, G. van Niel, M. S. Pols, T. ten Broeke, M. Lauwen, F. Ossendorp, C. J. Melief, G. Raposo, R. Wubbolts, M. H. Wauben, and W. Stoorvogel. 2009. MHC II in dendritic cells is targeted to lysosomes or T cell-induced exosomes via distinct multivesicular body pathways. *Traffic* 10:1528-1542.
19. McCann, F., P. Eissmann, B. Onfelt, R. Leung, and D. Davis. 2007. The activating NKG2D ligand MHC class I-related chain A transfers from target cells to NK cells in a manner that allows functional consequences. *J. Immunol.* 178:3418-3426.
20. Tomaru, U., Y. Yamano, M. Nagai, D. Maric, P. T. Kaumaya, W. Biddison, and S. Jacobson. 2003. Detection of virus-specific T cells and CD8(+) T-cell epitopes by acquisition of peptide-HLA-GFP complexes: analysis of T-cell phenotype and function in chronic viral infections. *Nat Med* 9:469-476.
21. Wetzel, S., T. McKeithan, and D. Parker. 2005. Peptide-Specific Intercellular Transfer of MHC Class II to CD4 T Cells Directly from the Immunological Synapse upon Cellular Dissociation. *J. Immunol.* 174:80-89.
22. Sabzevari, H., J. kantor, A. Jaigirdar, Y. Tagaya, M. Naramura, J. Hodge, J. Bernon, and J. Schlom. 2001. Acquisition of CD80 (B7-1) by T cells. *J. Immunol.* 166:2505-2513.
23. Fuchs, A., M. Cella, E. Giurisato, A. S. Shaw, and M. Colonna. 2004. Cutting edge: CD96 (tactile) promotes NK cell-target cell adhesion by interacting with the poliovirus receptor (CD155). *J Immunol* 172:3994-3998.
24. San Jose, E., A. Borroto, F. Niedergang, A. Alcover, and B. Alarcon. 2000. Triggering the TCR complex causes the downregulation of nonengaged receptors by a signal transduction-dependent mechanism. *Immunity* 12:161-170.
25. Valitutti, S., S. Muller, M. Cella, E. Padovan, and A. Lanzavecchia. 1995. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. *Nature* 375:148-151.
26. Blanchard, N., D. Lankar, F. Faure, A. Regnault, C. Dumont, G. Raposo, and C. Hivroz. 2002. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J Immunol* 168:3235-3241.
27. Sykulev, Y., M. Joo, I. Vturina, T. Tsomides, and H. Eisen. 1996. Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. *Immunity* 4:565-571.
28. Valitutti, S., S. Muller, M. Dessing, and A. Lanzavecchia. 1996. Different responses are elicited in cytotoxic T lymphocytes by different levels of T cell receptor occupancy. *J. Exp. Med.* 183:1917-1921.
29. Schodin, B. A., T. J. Tsomides, and D. M. Kranz. 1996. Correlation between the number of T cell receptors required for T cell activation and TCR-ligand affinity. *Immunity* 5:137-146.
30. Levchenko, A., B. Mehta, X. Niu, G. Kang, L. Villafania, D. Way, D. Polycarpe, M. Sadekain, and S. Larson. 2005. Intercellular transfer of P-glycoprotein mediates acquired multidrug resistance in tumor cells. *Proc. Natl. Acad. Sci. USA* 102:1933-1938.

Legend to Figures.

Figure 1. Both FcγRIII and FcεRIγ^{GFP} are captured by OT-I during trogocytosis triggered by antibodies. HEK cells transfected with a vector encoding murine flagged FcγRIIIA plus FcRγ^{GFP} were incubated with OT-I T cells coated (bottom panels) or not (top panels) with the anti-H-2K^{b} mAb. Cells were analyzed by flow cytometry using anti-CD8 mAb and a biotinylated mAb against FcγRII/III followed by fluorescent streptavidin and by analyzing the GFP fluorescence. Note that HEK cells that were mock-transfected do not allow triggering of trogocytosis by OT-I T cells (not shown).

Figure 2. HEK-FcγRIII/FcRγ^{GFP} and OT-I T cells can easily been distinguished on a morphological basis by electron microscopy. HEK cells transfected with FcγRIII and FcRγGFP and OT-I T cells were processed for electron microscopy analysis then analyzed at a low magnification.

Figure 3. Localization of endogenously expressed FcRγ^{GFP} in HEK-FcγRIII/FcRγ^{GFP} cells. HEK cells transfected with FcγRIIIA and FcRγ^{GFP} were processed for electron microscopy analysis after staining with rabbit anti-GFP antiserum followed by anti-rabbit IgG coupled to gold particles. Arrowheads indicate representative structures showing immunogold labeling in HEK cells.

Figure 4. Localization of acquired FcRγ^{GFP} in OT-I T cells. OT-I T cells were incubated with HEK cells transfected with FcγRIIIA and FcRγ^{GFP} for 1-hour in the presence of the anti-H-2K^{b} mAb in order to trigger trogocytosis. Cells were then processed for electron microscopy analysis after staining with rabbit anti-GFP antiserum followed by anti-rabbit IgG

coupled to gold particles. Arrowheads indicate representative structures showing immunogold labeling in OT-I T cells.

Figure 5, 6 and 7. Localization of acquired FcRγ^{GFP} at the area of contact between OT-I T cells and HEK transfectant. OT-I T cells were incubated with HEK cells transfected with FcγRIIIA and FcRγ^{GFP} for 1-hour in the presence of the anti-H-2K^{b} mAb in order to trigger trogocytosis. Cells were then processed for electron microscopy analysis after staining with rabbit anti-GFP antiserum followed by anti-rabbit IgG coupled to gold particles. The images are focused on the area of contact between T cells and HEK transfectants (right panels) that are presented at a lower magnification (left part of the images).

Fig. 1

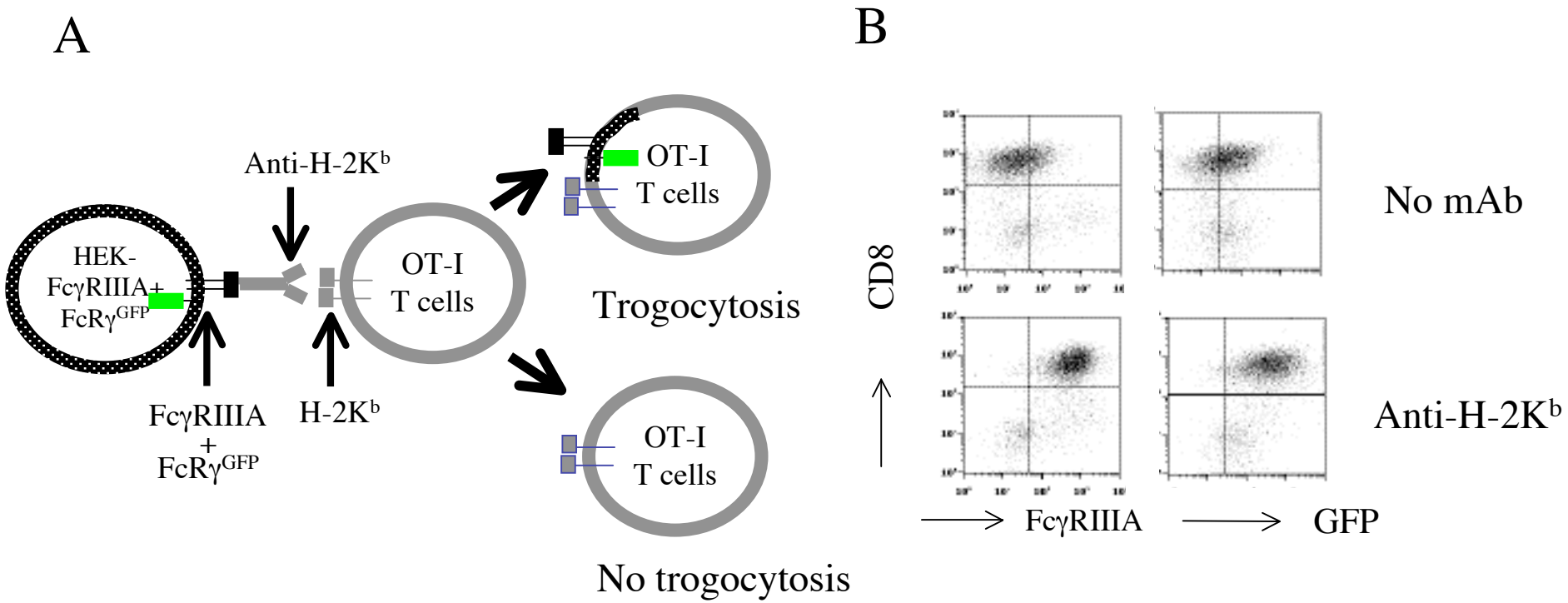
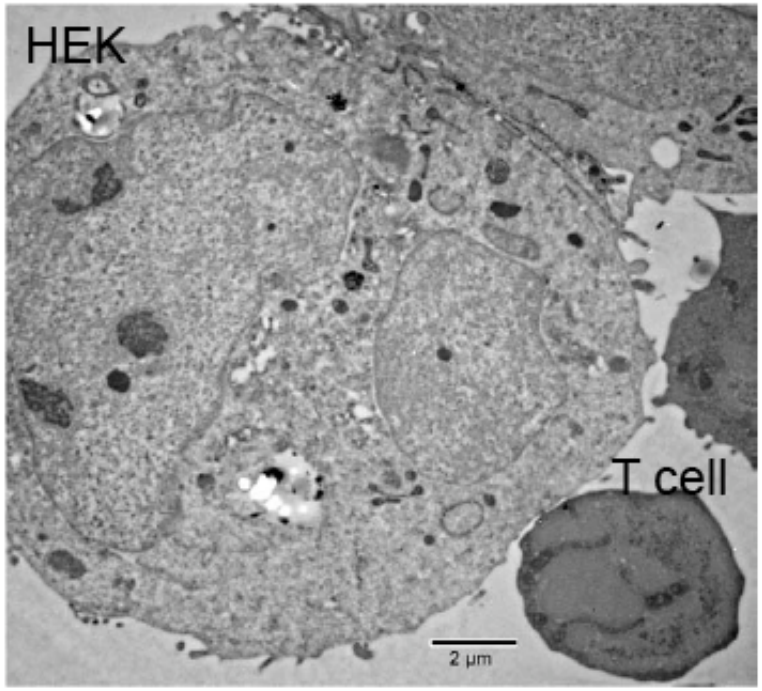


Fig. 2



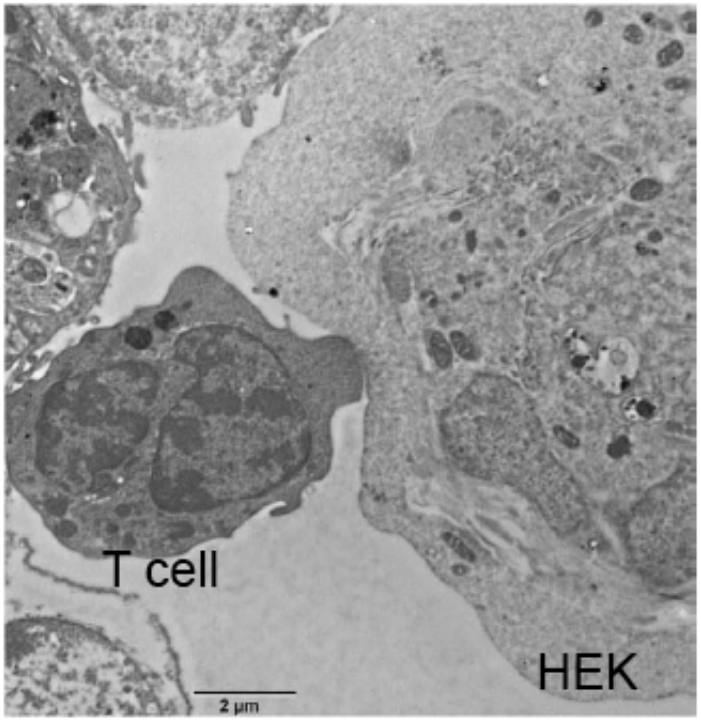


Fig. 3

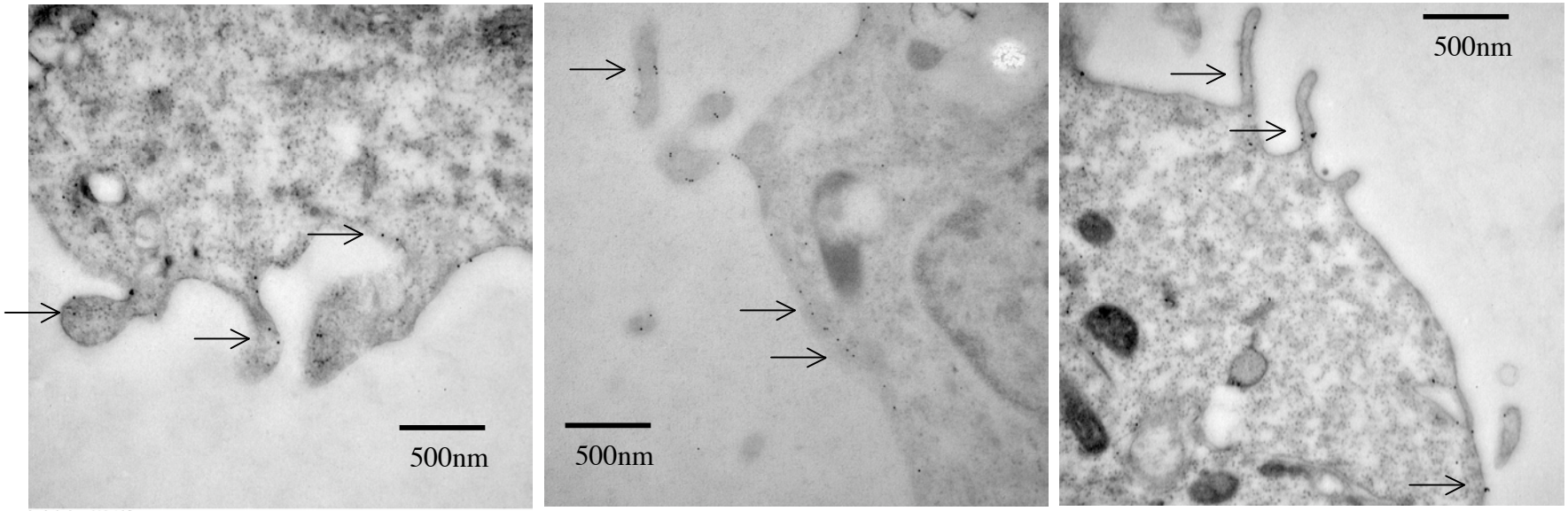


Fig. 4

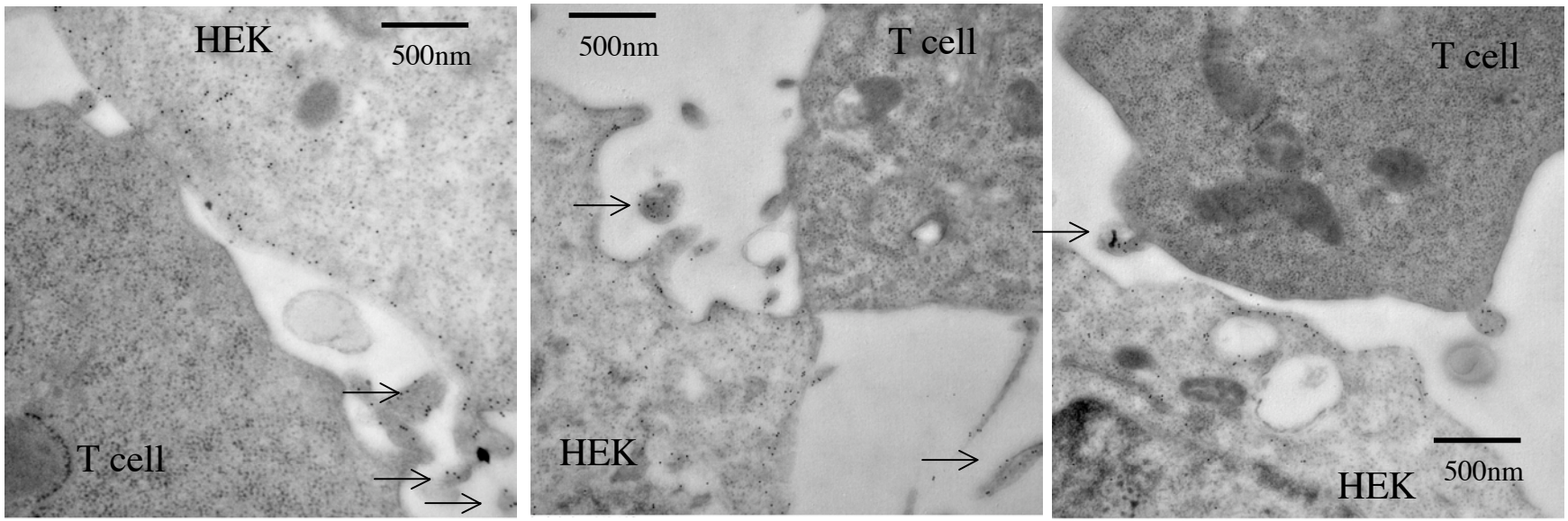


Fig. 5

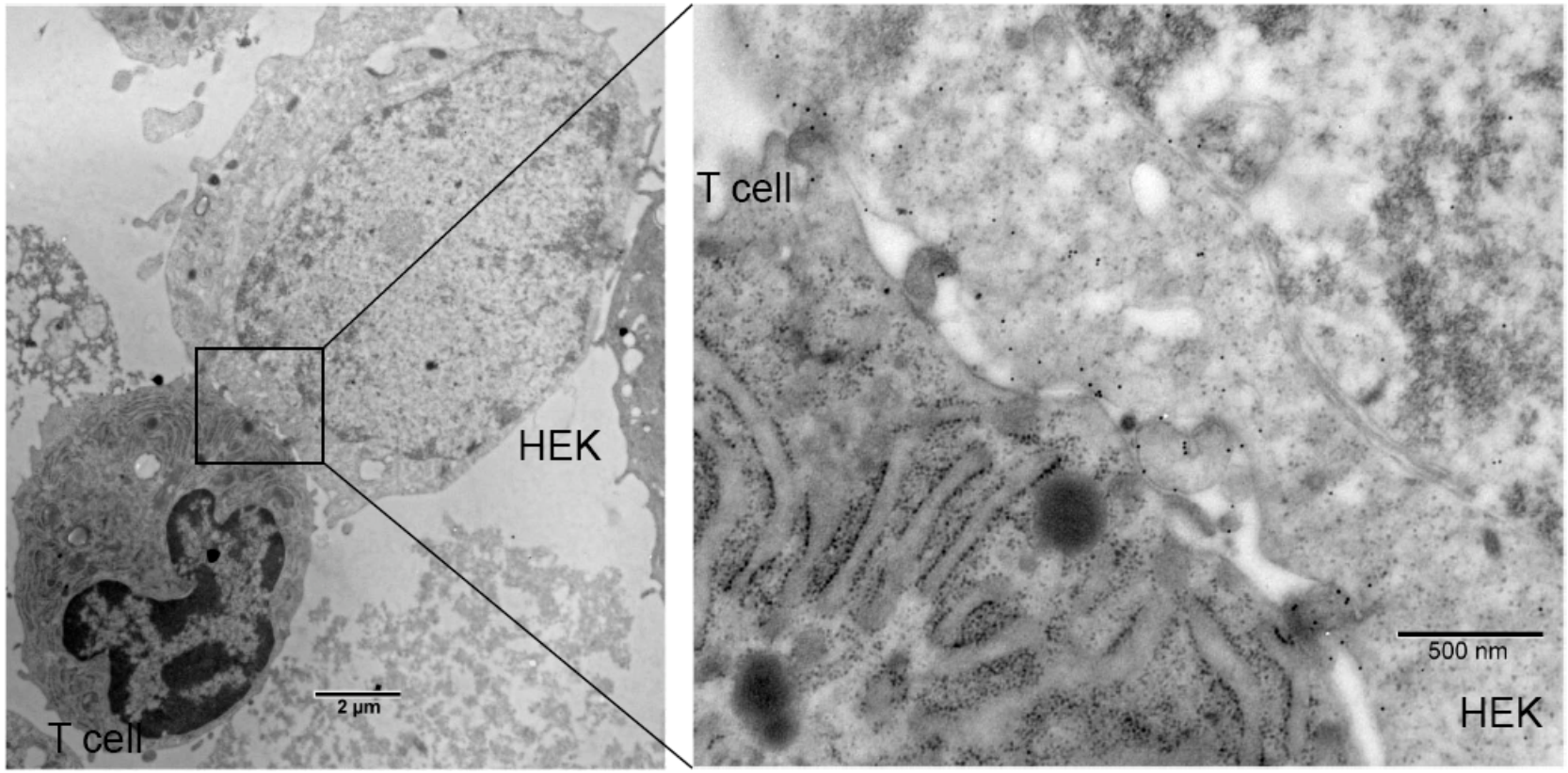


Fig. 6

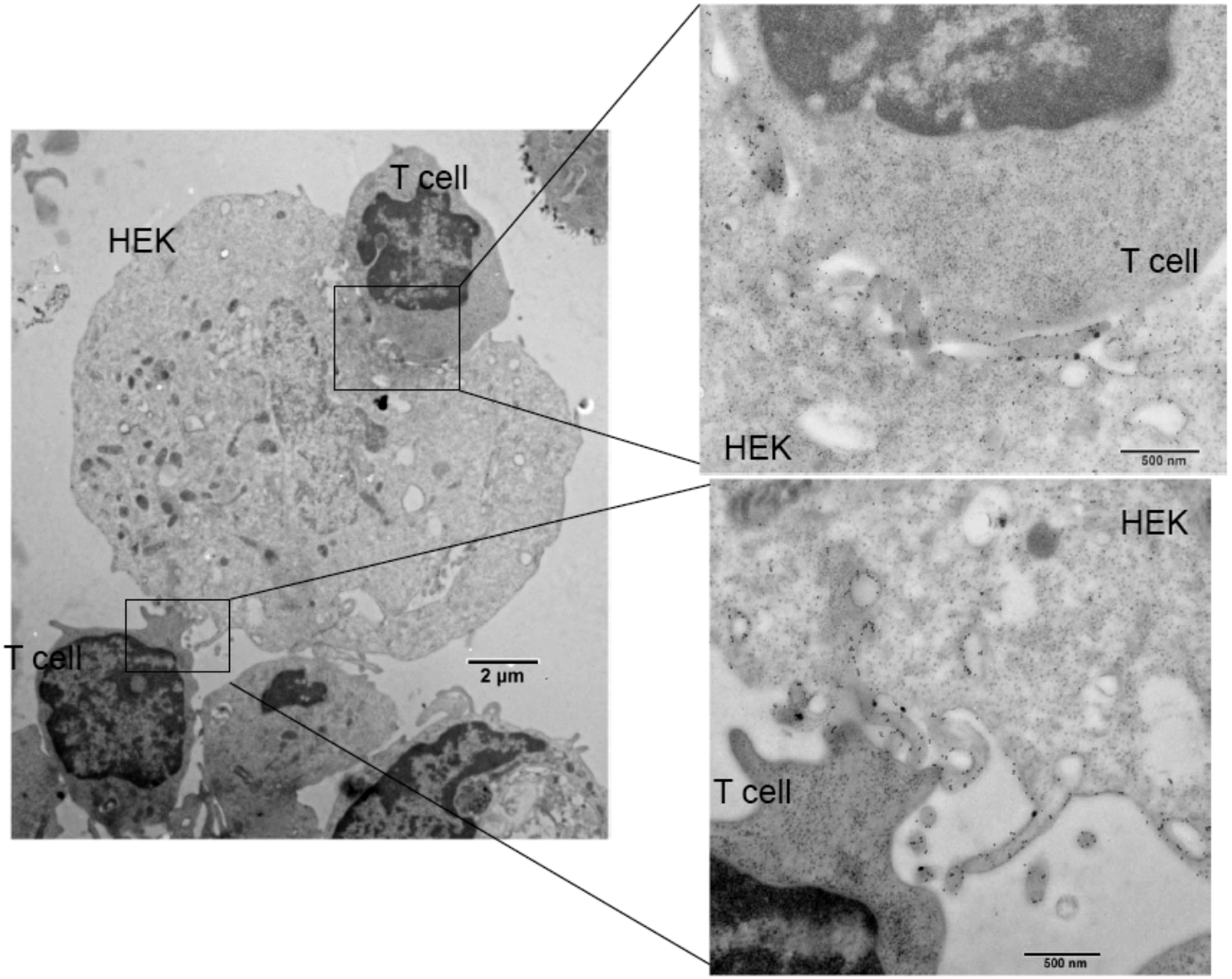


Fig. 7