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# Intercellular transfer of Ras
## Implications for immunity

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In recent years, it has become evident that lymphocyte are particularly adapted to form short-lived intercellular connections with the cells they scan. Such connections allow the transfer of membrane-anchored proteins, plasma membrane (PM) fragments, and even microRNAs.[1-5] This "unorthodox" intercellular information transfer occurs through various incompletely defined mechanisms. For example, during the formation of an immunological synapse, lymphocytes snatch PM fragments (also termed trogocytosis) and cell surface proteins from antigen-presenting cells (APCs).[6] Additionally, lymphocytes can form intercellular networks through actin-supported, long-range PM extensions, termed tunneling nanotubes (TNTs), which have been shown to facilitate the intercellular transfer of calcium-mediated signals, vesicles, and even viruses.[7]

A few years ago, our early studies in this evolving field demonstrated for the first time that inner PM-anchored proteins, such as Ras family members, could transfer from antigen-presenting cells (APCs) to lymphocytes.[1] This transfer was absolutely depended on cell–cell contact and on the undisturbed function of the actin cytoskeleton. Combining GFP with the C-terminal 9 amino acids of H-Ras, comprising all the membrane-targeting signals, was sufficient to induce the intercellular transfer of GFP. Importantly, we also discovered that when the constitutively active oncogenic form of H-Ras, the H-RasG12V mutant, was acquired by T or NK cells from APCs, during co-culturing, it was functional in the adopting lymphocytes. Namely, we observed enhanced Ras-dependent Raf/MEK/ERK activation and other stimulatory effects, associated with typical RasG12V signaling, in the acquiring lymphocytes. These seminal findings revealed a hitherto unknown mechanism of immune surveillance that enables lymphocytes to sense mutated "deleterious" inner PM-associated proteins within the cells they scan and respond accordingly.[1] Subsequently, a study by Hudrisier and colleagues[3] also showed that H-Ras and K-Ras transfer very efficiently from APCs to lymphocytes. Interestingly, the researchers concluded that intracellular proteins with PM-targeting signals, such as the CAAX motif, display a higher capacity for intercellular transfer than external leaflet-associated proteins.

With respect to the cellular processes that participate and/or regulate trogocytosis and the intercellular transfer of H-Ras, our findings indeed imply that they are somewhat similar but not completely equivalent. Unlike classical trogocytosis, highly specific TCR/peptide-MHC interactions are not required for efficient H-Ras transfer.[1,5] Thus, we hypothesize that the initial diffuse cell–cell contacts that form among lymphocytes and the cells they scan—independent of cognate antigen recognition—provide enough contact regions to promote this transfer. However, the formation of a mature immunological synapse that depends on high avidity specific TCR/peptide-MHC interactions is required for significant typical trogocytosis (PM snatching) to occur.

In a following study, we introduced a novel mass spectrometry-based approach designed to detect the array of proteins synthesized by B cells and acquired by NK lymphocytes during short-term co-culturing. Our novel approach took advantage of the stable-isotope labeling of amino acids in cell culture (SILAC) method. Thus, we labeled B cells (protein donor) with "heavy" amino acids and left the NK cells (protein recipient) unlabeled, such that the labeled proteins acquired by NK cells during co-culture could be identified by their mass shift. We found that, indeed, a large number of proteins (n~170) transfer among B and NK lymphocytes in an actin–cytoskeleton-dependent manner, including the following inner PM-associated proteins: K-Ras, Ral-A, Arf4, Lck, and Rab10.[4]

Our more recent studies, designed to characterize the fine details of Ras intercellular transfer, revealed that TNTs forming between antigen-presenting B cells and T cells, during co-culturing, can also serve as an important conduit for this transfer. In these studies we employed advanced techniques, such as cell trapping by optical tweezers and live-cell imaging by 4D spinning disk confocal microscopy, to first show that close-ended TNTs commonly form after B/T-cell conjugates separate. Next, we determined by fluorescence recovery after photobleaching that GFP-tagged H-Ras (GFP-H-Ras) molecules could shuttle by lateral diffusion in the PM extensions forming the TNTs, and then accumulated within a discrete region at the junction between the B cell-derived TNTs and the T-cell body. Importantly, the GFP-H-Ras molecules continually transferred in discrete quanta from the end-region of the TNT to the T-cell surface and moved away from the junction region.[8]

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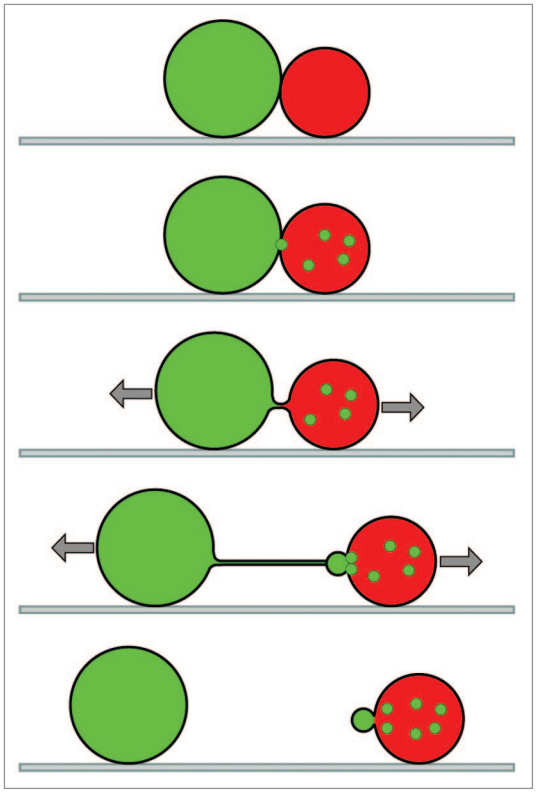

**Figure 1.** Schematic representation of the process of H-Ras transfer from APCs (721.221 B cells) genetically engineered to express GFP-tagged H-Ras (green) to T cells (red). Initially, GFP-H-Ras patches (illustrated as small green spheres) are snatched by T cells during cell–cell conjugate formation. Thereafter, when the 2 cells separate and move in opposite directions (see arrows), GFP-H-Ras enriched (green) TNTs are formed and facilitate the gradual transfer of additional GFP-H-Ras patches to Jurkat T cells.

In conclusion, the experimental data generated by others[3] and us[1,4,5,8] in recent years lead us to propose that the cell-to-cell transfer of selected inner PM-anchored signaling proteins, primarily of the Ras super-family, between the host cells and lymphocytes, by a variety of cellular mechanisms, may be more prevalent than previously recognized. This notion may eventually lead to a paradigm shift in classical theories of cellular immunology. Although our work sheds new light on the topic of intercellular communication in the context of the immune response, it probably just reveals the "tip of the iceberg". (**Fig. 1**)

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