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CUTTING EDGE

Cutting Edge: Membrane Nanotubes Connect Immune Cells[1,2]

Björn Önfelt, Shlomo Nedvetzki, Kumiko Yanagi, and Daniel M. Davis[3]

We present evidence that nanotubular highways, or membrane nanotubes, facilitate a novel mechanism for intercellular communication in the immune system. Nanotubes were seen to connect multiple cells together and were readily formed between a variety of cell types, including human peripheral blood NK cells, macrophages, and EBV-transformed B cells. Nanotubes could be created upon disassembly of the immunological synapse, as cells move apart. Thus, nanotubular networks could be assembled from transient immunological synapses. Nanotubes were seen to contain GFP-tagged cell surface class I MHC protein expressed in one of the connected cells. Moreover, GPI-conjugated to GFP originating from one cell was transferred onto the surface of another at the connection with a nanotube. Thus, nanotubes can traffic cell surface proteins between immune cells over many tens of microns. Determining whether there are physiological functions for nanotubes is an intriguing new goal for cellular immunology. The Journal of Immunology, 2004, 173: 1511– 1513.

The concept that immune surveillance is sometimes facilitated by the assembly of an immunological synapse triggered a wave of research in imaging immune cell interactions and triggered much discussion on the similarity of intercellular communication controlling disparate biological processes (for example, reviewed in Refs. 1–5). In this study, analogous to very recent observations in neural cells, we report another unexpected mechanism for intercellular communication between immune cells. Rustom et al. (6) recently demonstrated that cultured PC12 rat neural cells or kidney cells could be connected via membrane nanotubes, perhaps related to cytonemes in the *Drosophila* wing imaginal disc (7). Applying single photon-excitation resonance scanning confocal microscopy to image immune cell interactions in vitro, we observed such nanotubes between live immune cells.

Materials and Methods

Cells and tissue culture

721.221 transfectants and peripheral blood NK cells were prepared and imaged as described previously (8, 9). Peripheral blood macrophages were prepared according to standard procedures. Briefly, PBMCs were incubated for 2 h in a plastic flask that was treated with 2% gelatin (Sigma-Aldrich, St. Louis, MO). Nonadherent cells were removed and the remaining adherent cells were incubated for 24 h in X-vivo medium (Cambrex Bio Science Walkersville, Walkersville, MD) with 1% autologous serum before being washed with cold PBS. Cells were checked for CD14 expression by flow cytometry and cultured in X-vivo medium for 7–12 days before use. J774 cells were grown in DMEM with supplements, were harvested, and imaged in the chamber before adhering and spreading. For staining with the lipid probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate (DiD)[4] (Molecular Probes, Eugene, OR), 10^6 721.221 cells were washed in PBS, incubated in 1 ml of PBS with 1 μM DiD for 40 min at 37°C, and washed again. Stained cells were left to rest for 24 h, washed again, and coincubated with 221/GPI-GFP for 24 h before imaging. DiD was kept as 1 mM stock solutions in DMSO at room temperature.

Live cell imaging

Imaging was performed with an inverted resonance scanning confocal microscope (TCS SP2 RS; Leica Microsystems, Deerfield, IL). Before imaging, cells were washed with PBS or phenol-red-free medium and resuspended for imaging in full culture medium (without phenol red). All images were obtained with cells kept at 37°C and 5% CO_2.

Results and Discussion

Nanotubular highways (also referred to as tunneling nanotubes (6, 10) that create supracellular structures (11)), or long "membrane tethers", were readily seen between transfectants of the EBV-transformed human B cell line 721.221 (12) (Fig. 1*A*), human macrophages prepared from peripheral blood (Fig. 1*B*), murine macrophage J774 cells (Fig. 1*C*), and connecting human peripheral blood NK cells to 721.221 cells (Fig. 2*C*). Fluorescence microscopy revealed that the nanotubes contained GPI conjugated to GFP constitutively expressed in 721.221 transfectants (Fig. 1*A*), indicating that immune cell nanotubes are derived in part from cell surface membrane. Intriguingly, we found that nanotubes were able to connect multiple cells simultaneously, thereby establishing complex communication networks between immune cells. This is reminiscent of model nanotubular networks previously seen to connect liposomes (13, 14). Fig. 1*C* shows a nanotubule network spanning over 80 μm connecting three murine macrophage cells. This three-way nanotube is constructed such that the junction is positioned to minimize the length of tube needed to connect these three cells (analysis not shown). One mechanism by which this could be achieved would be that the nanotubes are constructed with fluid membrane that can flow easily between the nanotubes and

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[4] Abbreviation used in this paper: DiD, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate
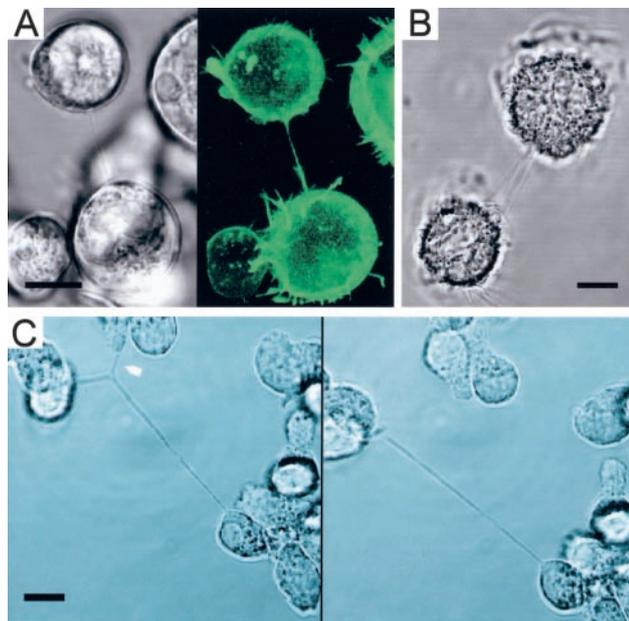

FIGURE 1. Membrane nanotubes connect immune cells. *A,* Nanotubular highways form between EBV-transformed human B cells (721.221). Corresponding fluorescence image shows that nanotubes contain the lipid GPI conjugated to GFP. *B,* Nanotube between primary human macrophages. *C,* Membrane nanotubes can establish communication networks between cells. Shown are three murine macrophage cells (J774), 30 min after incubation in the microscope chamber and before they started to spread, connected together by a nanotubular network. One tube, denoted by the white arrow, breaks after some time, leaving only a nanotubular connection between two cells (*right panel*). Scale bars, 10 μm.

FIGURE 2. Nanotubes can form upon disassembly of the immunological synapse and can facilitate directed trafficking of membrane components. *A,* Time-lapse imaging of J774 cells shows bulges, highlighted by white arrows, moving along the nanotube. Analysis of 15 images showed that for a period of 160 s, two bulges moved with a constant speed of 0.16 μm/s. *B,* Membrane bound GPI-GFP originating from a 221/GPI-GFP cell (green) transferred to a 221/DiD (red) at the nanotubular connection. Shown are several optical slices covering the midsection of the two cells where the connecting nanotube is. *C,* Nanotubes can form after intercellular contact. Disassembly of an immunological synapse between a peripheral blood NK cell (smaller cell) and a 721.221 cell results in a nanotube connecting the cells. *D,* Time-lapse imaging of 721.221 cells forming a transient contact demonstrates that a connecting nanotube can form as cells move apart. Scale bars, 10 μm.

the cell surface. This would also explain why the nanotube contracts between two remaining cells after connection to the third cell breaks (Fig. 1*C, right panel*).

Importantly, bulges were seen to traffic along a nanotube coupling together two murine J774 macrophages (Fig. 2*A*). This suggests that nanotubes traffic cargo between immune cells. Indeed, consistent with the observations made by Rustom et al. (6) in PC12 cells, vesicles, stained with DiO (3,3′-dioctadecyloxacarbocyanine perchlorate), derived from one primary macrophage could be seen within a nanotube connecting to another macrophage (data not shown). However, in addition in this study, some GPI-GFP originating from one 721.221 cell (221/GPI-GFP, green) is transferred onto the surface of another 721.221 cell, stained with the fluorescent lipid DiD (221/DiD, red), at the connection with a nanotube (Fig. 2*B*). The nanotube between these cells does contain some red fluorescence near the contact of the nanotube with the 221/DiD cell, demonstrating that lipids from the two cells can mix and the nanotube truly connects these two cells (analysis not shown). This suggests that nanotubes can transport membrane material, including GPI-anchored proteins, along the surface of the tube, in addition to transporting material inside the tube as demonstrated previously (6). Thus, nanotubes may provide a mechanism for the intercellular transfer of cell surface proteins, previously observed following the formation of T cell and NK cell immunological synapses (15–19). Evidence for such an immunological role for nanotubular highways is that HLA-Cw6, tagged at the C terminus with GFP (9), could be seen along the nanotube connecting a peripheral blood NK cell to a transfectant of 721.221 expressing HLA-Cw6-GFP (Fig. 3*A*).
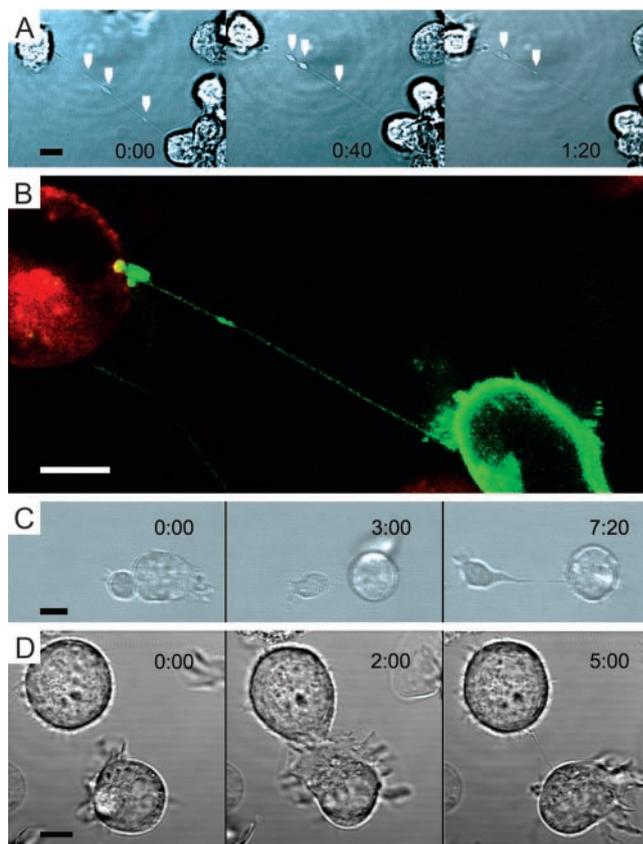
Recent observations suggested that nanotubes could be formed de novo by actin-driven protrusions extending from one cell to another (6). However, in this study, we have observed formation of tubes after cell-cell contact, suggesting an alternative mechanism for establishing nanotubes between cells. An immunological synapse between a NK cell and 721.221 cell (2, 9, 20) was observed to result in a nanotube being formed as cells separated (Fig. 2*C*). Thus, the assembly of nanotubular networks may arise from transient intercellular contacts seen between immune cells in vivo in some situations (21–23). Fig. *2D* shows time-lapse micrographs of 721.221 cells forming a transient contact resulting in a connecting nanotube. Possibly, nanotubular highways formed by this mechanism may originate from "membrane bridges" previously seen between immune cells by electron microscopy (24).

Particularly important is the surprisingly long length of the nanotubes that were seen to connect immune cells. An example of the creation of a particularly long nanotube connecting a peripheral blood NK cell to 721.221, transfected to express GFP-tagged HLA-Cw6 (9), is shown in Fig. 3*A*. This nanotube eventually extends to well over 140 μm, out of the field of view. In
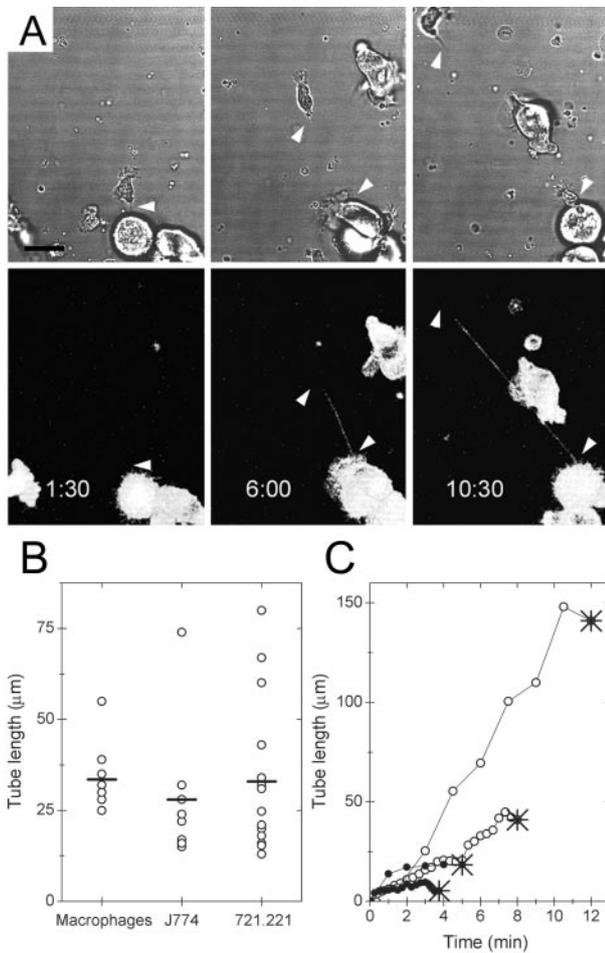
FIGURE 3. Nanotubes connect cells over tens of microns and last many minutes. *A*, Time-lapse imaging of a peripheral blood NK cell (smaller cell) and a 721.221 transfectant, expressing GFP-tagged HLA-C, shows the creation of a particularly long nanotube connecting the cells over 140 μm. *Upper row*, The cells seen by bright field microscopy; *lower row*, several optical slices of the corresponding GFP fluorescence. Arrows mark the contacts of the nanotube with each cell. This nanotube grew with a speed of around 0.2 μm/s and lasted over 15 min. Immunological cell surface proteins such as class I MHC protein can be seen on such a nanotube. Scale bar, 25 μm. *B*, The length of nanotubes between pairs of like cells, i.e., pairs of primary macrophages, J774 cells, or 721.221 cells, was measured. Each point shown represents the length of one tube connecting a particular pair of cells and the average for each cell type is marked with a bar. A wide variation in the length of nanotubes was observed, the average being around 30 μm. *C*, Some nanotubes grew rapidly as cells moved apart, while others remained a particular length for some time before breaking. Plot shows the length of nanotubes increasing over time, calculated from time-lapse images of nanotubes formed after a transient intercellular contact. Time 0 denotes the moment that the contacting cells started to move apart. In each case, a star (∗) denotes the point where the nanotubes break, except for the particularly long nanotube where the star denotes the point where the tube continues out of the frame of view. This long nanotube broke over 15 min after the cells began to move apart. ○, Nanotubes between peripheral blood NK cells and 721.221; ●, nanotubes between pairs of 721.221 cells.

general, between different cell pairs, there was a large range in the length of nanotubes from 10 to well over 50 μm, with no obvious correlation between the length of tether and cell type (Fig. 3*B*). The average length of a nanotube seen in all cell-cell connections was 30 μm. The length of nanotubes sometimes increased rapidly as cells moved apart, and at other times re-

mained static for long periods of time, in all cases generally lasting for the order of minutes before breaking (Fig. 3*C*). For the particularly long nanotube seen in Fig. 3*A*, the tether grew at an approximately constant rate of 0.2 μm/s and lasted for over 15 min (Fig. 3*C*).

Thus, in summary, we present evidence that membrane nanotubes represent a novel mechanism for intercellular communication in immunology. These observations raise many important new questions, such as whether or not such membrane tethers would be possible in tissues or in blood flow. Broadly, determining whether there are physiological functions for membrane nanotubes is an intriguing new goal for immunology and neuroscience alike.

Acknowledgments

References

1. Bromley, S. K., W. R. Burack, K. G. Johnson, K. Somersalo, T. N. Sims, C. Sumen, M. M. Davis, A. S. Shaw, P. M. Allen, and M. L. Dustin. 2001. The immunological synapse. *Annu. Rev. Immunol. 19:375.*
2. Davis, D. M. 2002. Assembly of the immunological synapse for T cells and NK cells. *Trends Immunol. 23:356.*
3. Kupfer, A., and H. Kupfer. 2003. Imaging immune cell interactions and functions: SMACs and the immunological synapse. *Semin. Immunol. 15:295.*
4. Huppa, J. B., and M. M. Davis. 2003. T-cell-antigen recognition and the immunological synapse. *Nat. Rev. Immunol. 3:973.*
5. Davis, D. M., and M. L. Dustin. 2004. What is the importance of the immunological synapse? *Trends Immunol. 25:323.*
6. Rustom, A., R. Saffrich, I. Markovic, P. Walther, and H. H. Gerdes. 2004. Nanotubular highways for intercellular organelle transport. *Science 303:1007.*
7. Ramirez-Weber, F. A., and T. B. Kornberg. 1999. Cytonemes: cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell 97:599.*
8. Eleme, K., S. B. Taner, B. Önfelt, L. M. Collinson, F. E. McCann, N. J. Chalupny, D. Cosman, C. Hopkins, A. I. Magee, and D. M. Davis. 2004. Cell surface organization of stress-inducible proteins ULBP and MICA that stimulate human NK cells and T cells via NKG2D. *J. Exp. Med. 199:1005.*
9. Davis, D. M., I. Chiu, M. Fassett, G. B. Cohen, O. Mandelboim, and J. L. Strominger. 1999. The human natural killer cell immune synapse. *Proc. Natl. Acad. Sci. USA 96:15062.*
10. Schuldt, A. 2004. Getting connected. *Nat. Cell Biol. 6:281.*
11. Baluska, F., D. Volkmann, and P. W. Barlow. 2004. Cell bodies in a cage. *Nature 428:371.*
12. Shimizu, Y., and R. DeMars. 1989. Production of human cells expressing individual transferred HLA-A, -B, -C genes using an HLA-A, -B, -C null human cell line. *J. Immunol. 142:3320.*
13. Karlsson, A., R. Karlsson, M. Karlsson, A. S. Cans, A. Stromberg, F. Ryttsen, and O. Orwar. 2001. Networks of nanotubes and containers. *Nature 409:150.*
14. Karlsson, M., K. Sott, M. Davidson, A. S. Cans, P. Linderholm, D. Chiu, and O. Orwar. 2002. Formation of geometrically complex lipid nanotube-vesicle networks of higher-order topologies. *Proc. Natl. Acad. Sci. USA 99:11573.*
15. Huang, J. F., Y. Yang, H. Sepulveda, W. Shi, I. Hwang, P. A. Peterson, M. R. Jackson, J. Sprent, and Z. Cai. 1999. TCR-mediated internalization of peptide-MHC complexes acquired by T cells. *Science 286:952.*
16. Carlin, L. M., K. Eleme, F. E. McCann, and D. M. Davis. 2001. Intercellular transfer and supramolecular organization of human leukocyte antigen C at inhibitory natural killer cell immune synapses. *J. Exp. Med. 194:1507.*
17. 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.*
18. Davis, D. M., T. Igakura, F. E. McCann, L. M. Carlin, K. Andersson, B. Vanherberghen, A. Sjostrom, C. R. Bangham, and P. Hoglund. 2003. The protean immune cell synapse: a supramolecular structure with many functions. *Semin. Immunol. 15:317.*
19. 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.*
20. Vyas, Y. M., H. Maniar, and B. Dupont. 2002. Visualization of signaling pathways and cortical cytoskeleton in cytolytic and noncytolytic natural killer cell immune synapses. *Immunol. Rev. 189:161.*
21. Germain, R. N., and M. K. Jenkins. 2004. In vivo antigen presentation. *Curr. Opin. Immunol. 16:120.*
22. Miller, M. J., S. H. Wei, I. Parker, and M. D. Cahalan. 2002. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. *Science 296:1869.*
23. Stoll, S., J. Delon, T. M. Brotz, and R. N. Germain. 2002. Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science 296:1873.*
24. Stinchcombe, J. C., G. Bossi, S. Booth, and G. M. Griffiths. 2001. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity 15:751.*