Rafts for antigen presentation?

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The partitioning of membrane-associated proteins into discrete microdomains (commonly dubbed "membrane rafts") can profoundly influence their biological activities. Anderson *et al*.[1] have shown that major histocompatibility complex (MHC) class II proteins are constitutively associated with membrane rafts in both mouse and human B cell lines. This association augments antigen presentation, particularly at lower experimental concentrations of antigen. Their proposed mechanism is that multiple antigen–class II complexes that are confined within individual membrane rafts become locally concentrated and thereby stimulate T cell receptors (TCRs) more efficiently. If this were so, it would be necessary for the number of antigen–class II complexes to exceed the number of membrane rafts on the antigen-presenting cell (APC) surface by several-fold. If complexes numerically exceeded rafts by *n*-fold, on average, each raft would have *n* complexes. As *n* falls below unity, the tendency for complexes to aggregate by virtue of their random association with rafts will fall to zero. Based on physical measurements, it can be estimated that the plasma membrane of a cell typically contains several thousand membrane rafts[2,3]. However, T cells can become activated by APCs that bear as few as 200 antigen–class II complexes on their surfaces[1]. Thus, under physiological conditions, *n* may often be less than 1. The mechanism of raft-enhanced antigen presentation proposed by Anderson *et al.* may be, therefore, of limited physiological relevance.

We suggest that at very low concentrations of antigen, the aggregation of class II with membrane rafts can usefully serve to increase the local concentration of antigen–class II complexes only if class II bearing specific antigen is preferentially recruited to a restricted pool of membrane rafts. Such recruitment could be initiated by the binding of cognate TCRs, leading to the preferential partitioning of specific antigen–class II complexes into membrane rafts located within the immunological synapse on the surface of the APC. In the THP-1 cell line, cross-linking antibodies to class II can induce it to enter membrane rafts, from which it is normally excluded in these cells[4]. Class II bearing specific antigen may be similarly induced to enter the membrane rafts of B cells during interactions with T cells, though the constitutive presence of class II within the rafts makes this more difficult to observe[1] (and R. Huby *et al.*, unpublished data).

Perhaps by measuring the contribution that membrane rafts make to the presentation of *in vitro*–loaded antigen by fixed APCs, Anderson *et al.* may be restricting their observations to an effect that occurs only at unphysiologically high amounts of antigen. It will be necessary to assess the contribution that membrane rafts make to the presentation of lower concentrations of antigen, by unfixed APCs, before their biologically relevant, dynamic contribution to the process of antigen recognition can be judged.

1. Anderson, H., Hiltbold, E. & Roche, P. *Nature Immunol.* **1,** 156–162 (2000).
2. Friedrichson, T. & Kurzchalia, T.V. *Nature* **394,** 802–805 (1998).
3. Varma, R. & Mayor, S. *Nature* **394,** 798–801 (1998).
4. Huby, R. D., Dearman, R. J. & Kimber, I. J. *Biol. Chem.* **274,** 22591–22596 (1999).

Response

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Huby *et al*.[1] have offered their interpretation of our recent study[2] in which we showed the importance of membrane lipid rafts in major histocompatibility complex (MHC) class II–restricted T cell activation. They propose that because the plasma membrane contains so many lipid rafts and so few specific peptide–class II complexes are required to activate T cells, it is unlikely that lipid rafts could serve to concentrate the specific class II–peptide complexes that are necessary for T cell activation. However, it is not known yet how many lipid rafts are on the surface of antigen presenting cells (APCs); how many class II molecules are present in each lipid raft; whether antigen-loading onto newly synthesized class II molecules is truly random; or whether the specific peptide complexes required to activate antigen-specific T cells are randomly distributed on the plasma membrane.

Huby *et al*. propose that aggregation of class II with lipid rafts serves to increase the local concentration of class II–peptide complexes only if specific class II–peptide complexes are recruited to and associate with a restricted pool of membrane rafts at (presumably) the immunological synapse. This model predicts that disrupting raft integrity on APCs before chemical fixation would not alter the efficiency of antigen presentation to T cells. However, our paper showed that raft disruption inhibits T cell activation by fixed APCs—a result that does not fit into their model.

Using paraformaldehyde-fixed APCs at low doses of protein antigen, we show the importance of lipid raft microdomains in antigen presentation. As mentioned, the effect of raft-disrupting drugs on live cells is reversible and these drugs can directly inhibit T cell activation. These considerations necessitated chemical fixation of the APCs after the addition of raft-disrupting drugs, thereby preventing reassembly of raft-protein complexes, and removal of these drugs before analysis of T cell activation. Of course proteins are not fixed on the plasma membrane of APCs *in vivo* and they often reorganize on the plasma membrane after ligation. By analogy with the well documented ability of lipid rafts to reorganize on the plasma membrane of T cells, we propose that engagement of specific class II molecules with the TCR on antigen-specific T cells leads to the aggregation of the rafts containing these complexes at the immunological synapse. As there are presumably "irrelevant" and "relevant" class II–peptide complexes in the same lipid raft, one might be able to visualize this directly.

On APCs, a significant fraction of surface class II molecules constitutively resides in lipid raft microdomains. Huby *et al*. originally proposed that class II association with lipid rafts only occurred after ligation of class II molecules with antibodies or, presumably, TCR engagement[3]. It is unlikely that the constitutive association of class II molecules with lipid rafts is a biological accident and therefore not physiologically relevant. In addition to a potential role for the concentration of lipid rafts containing class II–peptide complexes after engagement with the TCR, we propose that the "pre-packaging" of class II in lipid raft microdomains might serve to facilitate even further the concentration effects necessary for T cell activation. We look forward to rigorously testing and revising this model.

1. Huby, R. D., Chowdhury, F. & Lombardi, G. *Nature Immunol.* **2**, 3 (2001).
2. Anderson, H., Hiltbold, E. & Roche, P. *Nature Immunol.* **1**, 156–162 (2000).
3. Huby, R. D., Dearman, R. J. & Kimber, I. J. *Biol. Chem.* **274**, 22591–22596 (1999).