RESEARCH HIGHLIGHT

Harnessing receptor clustering in lipid rafts to tailor the inhibitory effects of monoclonal antibodies to specific cell types

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Certain cell signaling pharmaceutical targets have the potential to provide substantial clinical benefit when inhibited on some cell types yet elicit unwanted collateral damage when impeded on others. Thus, the appropriate therapeutic strategy for this situation would be to block preferentially receptor activation on the desired cell set. Taking advantage of the clustering within lipid rafts of toll-like receptor 4 (TLR4) and Fc gamma receptors (FcγR) during TLR4 activation, we have identified a mechanism that allows an antibody to block more favorably signaling on leukocytes, cells that underlie acute and chronic inflammatory processes. The anti-TLR4 monoclonal antibody (mAb), Hu 15C1, co-engages TLR4 and FcγRs to enhance its inhibitory potency via an avidity effect on FcγR-bearing cells. This novel mechanism of action allows the mAb to block efficiently TLR4 activation on FcγR-bearing inflammatory cells, while limiting the duration of effect on cells lacking FcγRs. As receptor clustering in lipid rafts is a common phenomenon, this mechanism could be exploited to anchor similar receptor-targeting mAbs or formats bearing an antibody Fc domain to desired cell types.

Keywords: Toll-like receptor 4; monoclonal antibody; Fc gamma receptors; receptor clustering; lipid rafts; inflammation

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Targeted therapies continue to represent an evolving class of medicines with many examples of success [1,2]. However, adding to the complexity of targeted medicine, it is now appreciated that a particular target may lead to diseases when aberrantly activated on certain cell types yet play a homeostatic role when 'properly' used by others. Thus, global intervention of such a target may result in the malfunctioning of homeostatic processes, causing unwanted side effects.

TLR4 was first discovered as the receptor for lipopolysaccharides (LPS) of Gram-negative bacteria [3,4]. TLR4 has subsequently been established as a major player in the activation of the innate immunity by sensing danger signals from both pathogens and damaged tissue [5]. Furthermore, animal models of human disease have demonstrated that dysregulated activation of TLR4 plays an underlying role in many inflammatory processes and autoimmune diseases [6,7]. However, as TLR4 is involved

in homeostasis and broadly expressed across many cell types, complete neutralization of TLR4 signaling may be unnecessary to effectively control disease. For example, in a murine model of human graft versus host disease (GvHD), TLR4 deficiency only on the donor hematopoietic cells was protective [8]. In contrast, TLR4 deficiency on the host stromal cells was found to be detrimental [9]. Similarly, in acute kidney injury, damage-associated pattern molecules (DAMPs; ligands of TLR4) promoted impairment of organ function through TLR4 activation on inflammatory cells, while DAMP-mediated TLR4 activation on tubular cells after injury promoted kidney regeneration [6]. Thus, because of this differential therapeutic outcome observed in preclinical studies for a role of TLR4 on hematopoietic versus stromal cells, a TLR4 blocker that preferentially distinguishes between the two types of cells could be the more effective reagent for the patients.

Recently, we have described a mechanism of action for an anti-TLR4 mAb, Hu 15C1, that allows selective, long lasting inhibition of TLR4 activation on leukocytes [10]. The feature distinguishing leukocytes from stromal cells, relevant for this particular effect, is the expression of Fc gamma receptors (FcγRs)[11]. FcγRI is constitutively located in lipid rafts, while FcγRIIA migrates into lipid rafts post cell activation with a TLR4 ligand. In addition, subsequent to this stimulus, TLR4 migrates into lipid rafts. Our studies have revealed that colocalization of TLR4 and FcγRs in lipid rafts brings the receptors into close proximity, such that the introduction of Hu 15C1 harnessed this clustering, simultaneously engaging TLR4 and FcγRs via the Fv and Fc antibody domains, respectively. The biomechanical consequence was an increase in antibody binding avidity, prolonging receptor engagement and ultimately, providing an enhanced inhibitory potency. Our experiments comparing the inhibitory capacity of Hu 15C1 using macrophages versus endothelial cells revealed that the antibody can be up to 100-fold more potent when FcγRs are available on the cell surface [10].

Our studies have gone on to evaluate the relevance of this co-engagement *in vivo* by using an anti-mouse TLR4 mAb that has the capacity to co-engage FcγRs versus a variant of the mAb that is null for FcγR binding. Using a murine model of acute lung inflammation, indeed, the inflammatory process induced by LPS is better controlled with an anti-mouse TLR4 mAb that has the capacity to co-engage FcγRs versus the FcγR null variant [10]. As our experiments with pharmacological reagents demonstrate a similar conclusion to those of the *in vivo* genetic studies, it is intriguing to hypothesize that in certain disease settings, allowing stromal cells, required for homeostasis, to have some extent of TLR4 signaling while much more potently blocking TLR4 activation on leukocytes may be the right balance for clinical intervention. Exploring additional preclinical models will help to illustrate in which human diseases this concept will be relevant.

On myeloid cells, the expression levels of FcγRs are much higher than that of TLR4 (unpublished observations). The substantially higher density of FcγRs on the plasma membrane provides more anchoring sites for the mAb than those for TLR4. The FcγRs further concentrate within lipid micro-domains upon TLR4 activation. Such anchoring to FcγRs potentially renders the mAb available for TLR4 binding on the cell surface even after clearance from the circulation, thus prolonging the inhibitory effect on FcγRs-bearing cells. This is further suggested by an experiment in which the free mAb was removed prior to LPS stimulation. We demonstrated that the capacity to engage FcγRs prolonged the inhibitory duration of the mAb after its removal from the culture medium [10], thus, another feature that could be exploited by mAbs targeting other cell surface proteins.

In addition to FcγRs, TLR4 also clusters with other receptors, including CD11b and the chemokine receptor, CXCR4 [12]. The biological relevance of the clustering of these receptors with TLR4 is unclear. Similar to other well studied receptors, such as the T cell or B cell receptors, clustering may represent a mechanism for enhancing or repressing specific responses via TLR4. Thus, depending on what other proteins are expressed, TLR4 clustering could represent a platform for cell type-specific intervention of receptor function. As we appreciate better the biological relationship of receptors within lipid rafts, cleverer exploitation of this proximal positioning may lead to safer and more powerful therapeutic intervention.

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