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LOX-1 Unlocks Human Plasma Cell Potential

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Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is best known for promoting atherosclerosis. In this issue of *Immunity*, Joo et al. (2014) find that dendritic cells triggered through LOX-1 can directly support plasmablast production via the production of the cytokines APRIL and BAFF.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is one of the seven members of the "Dectin-1 cluster" located within the natural killer gene (NKG) complex on human chromosome 12 (mouse chromosome 6) (Huysamen and Brown, 2009). While the other members of this cluster of C-type lectin-like receptors, which includes MICL (CLEC12A), DNGR1 (CLEC9A), and Dectin-1 (CLEC7A), are primarily expressed on myeloid cells, LOX-1 (CLEC8A) is expressed on endothelial and smooth muscle cells, platelets, fibroblasts, and B cells, as well as macrophages and dendritic cells (DCs) (Huysamen and Brown, 2009; Joo et al., 2014). LOX-1 is also differentiated from the other members of Dectin-1 cluster by the fact that it is one of the many different types of scavenger receptors that are capable of binding to oxidized low-density lipoproteins (oxLDL), as well as a range of other endogenous and exogenous ligands. The recognition of oxLDL by LOX-1 expressed on vascular endothelial cells appears to play a significant role in the development of atherosclerosis (Mehta et al., 2007). However, the expression of LOX-1 on macrophages and DCs coupled with its ability to bind a range of ligands including gram-negative and gram-positive bacteria, have suggested that it might also play a role as an immunomodulatory pattern-recognition receptor similar to the other members of the Dectin-1 cluster. Indeed, as with adjuvant strategies involving MICL and CLEC9A, targeting of antigen to LOX-1 with antibody conjugates is an effective technique for enhancing antigen-specific immunity (Caminschi and Shortman, 2012).

Targeting of antigen to DCs is typically used to establish strong $CD8^+$ or $CD4^+$ T cell responses, taking advantage of the fact that DCs are efficient at presenting peptide antigens with both class I and class II MHCs and are strong sources of costimulatory ligands. The role of DCs in stimulating humoral immunity is primarily thought to be mediated via the activation of $CD4^+$ cells, such as the strong induction of T follicular helper cells that occurs in response to antigens targeted via CLEC9A (Caminschi and Shortman, 2012). However, there is a significant body of evidence that DCs can also promote antibody responses through direct interactions with B cells. For example, it is well established that DCs can be potent sources of the B cell stimulatory and survival factors BAFF and APRIL and can thus directly induce class switching and plasmablast differentiation (MacLennan and Vinuesa, 2002). In addition, DCs have been shown to harbor intact antigen on their cell surface (Qi et al., 2006), indicating that they have the capacity to stimulate B cells through both the B cell receptor (BCR) and accessory receptors. Nevertheless, the contribution of direct activation of B cells and plasmablasts by DCs during in vivo humoral responses is unclear and requires further elucidation of how DCs might coordinate the signals they deliver to B-lineage cells.

In this issue of *Immunity*, Joo et al., (2014) examined the possibility that triggering of LOX-1 on immune cells might play a role directly stimulating antibody production by B cells. As a first step, a murine monoclonal antibody directed against human LOX-1 was generated. Staining of human PBMCs with this antibody revealed cell surface expression of LOX-1 on DCs, monocytes, and B cells, but not on T cells. To examine LOX-1 function on human DCs, the authors derived DCs from interleukin-4 (IL-4) + GM-CSF cultured $CD14^+$ blood monocytes and then incubated them with the anti-LOX-1 mAb. In contrast to DCs incubated with control antibody, those stimulated via LOX-1 were found to be strong inducers of the proliferation and plasmablast differentiation of human blood $CD19^+$ B cells. Secretion of high levels of immunoglobulin M (IgM), IgG, and IgA antibodies was observed in cultures with LOX-1-stimulated DCs, as was the induction of Ig class switching. Plasmablasts generated in these cultures showed the upregulation of CCR10 and downregulation of CXCR5 expression typically associated with plasmablast differentiation, as well as increased chemotaxis to the CCR10 ligands CCL27 and CCL28. Although B cells themselves did express LOX-1, direct stimulation of B cells with the LOX-1 mAb did not trigger appreciable B cell proliferation or differentiation. It did, however, lead to increased expression of CCR7 and CCL19-mediated chemotaxis by B cells, suggesting that direct stimulation of B cells by LOX-1 ligands might modify their migration in vivo.

So what is it about LOX-1 stimulated DCs that allows them to support B cell proliferation and differentiation? Stimulation of DCs via LOX-1 was found by Joo et al. to trigger the production of the tumor necrosis factor (TNF) superfamily ligands BAFF and APRIL (Joo et al., 2014). Blocking studies verified that the stimulation of B cells by LOX-1 stimulated DCs occurred primarily via the actions of these ligands, APRIL being particularly important for the production of IgM and IgA antibodies and BAFF for the production of IgG. When a series of alternative anti-LOX-1 mAbs were tested for their ability to trigger APRIL and BAFF production by DCs, they were all found to be inferior to the clone (8B4) produced by the authors. Moreover, mAbs directed against a range of other DC-expressed pattern-recognition receptors (DC-SIGN, Dectin-1, DCIR, DEC205) triggered little or no BAFF and/or APRIL

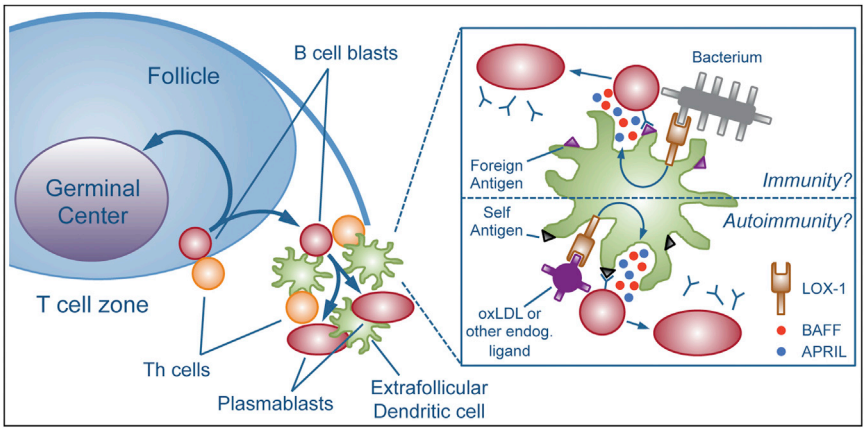

Figure 1. Potential Scenarios for LOX-1-Mediated Augmentation of Humoral Immunity by Extrafollicular Dendritic Cells

(Left) Antigen-activated B cell blasts are expanded in either a T-dependent or T-independent manner near the follicular-T cell zone boundary. T helper (Th) cells can drive the differentiation of germinal center B cells, whereas both T-independent and T-dependent responses can drive migration of B cell blasts to the DC-rich extrafollicular regions of the secondary lymphoid tissues. Here, B cell blasts can differentiate into either switched or unswitched plasmablasts, potentially influenced by signals from extrafollicular Th cells, as well as DCs.

(Right) Extrafollicular B cells could receive stimulatory signals from LOX-1-activated DCs in the form of BAFF and/or APRIL in either protective or autoimmune antibody responses. Thus, LOX-1 on DCs can be triggered by structures on foreign bacteria and, potentially in conjunction with foreign antigen presented on the DC surface, drive B cell proliferation and differentiation. On the other hand, LOX-1 might be triggered by endogenous ligands, such as oxLDLs, and self-reactive B cells therefore triggered with resulting autoantibody production.

production by the DCs. This raised the question of whether the rare ability of the 8B4 anti-LOX-1 clone to activate the B cell stimulatory activity of DCs was simply a quirk of this mAb clone or did in fact reflect a physiologically relevant activity of LOX-1? To answer this question, Joo et al. utilized one of the natural ligands of LOX-1, oxLDL, to trigger DCs. DCs incubated with oxLDL were found to produce BAFF and APRIL and to have B cell stimulatory properties that were indistinguishable from those stimulated with 8B4.

The findings of Joo et al. indicate that LOX-1 joins the receptors for interferon-α (IFN-α), IFN-γ, and CD40 ligand as DC surface molecules capable of triggering the production of BAFF and/or APRIL by DCs (Joo et al., 2014; Litinskiy et al., 2002). The potential for DCs to support B cell responses when triggered in this way is clear and demonstrable in vitro. Whether DCs impact humoral immunity in this way in vivo and under what circumstances this might occur is more difficult to determine. A link between DCs and plasmablast survival and differentiation in vivo has long been suspected due to the fact that activated B cells undergoing plasmablast differentiation in response to either T-dependent or T-independent antigens migrate extrafollicularly to areas rich in $CD11c^+$ DCs within secondary lymphoid tissues (Figure 1). Similarly, plasmablasts generated in an autoimmune setting colocalize with DCs, and there is some evidence that DCs support autoreactive plasmablasts in vivo under these circumstances (Teichmann et al., 2010). Given that DCs can present intact antigen to B cells (Qi et al., 2006) and that BCR triggering synergizes with the BAFF and APRIL signals delivered by DCs (Litinskiy et al., 2002), one can imagine that DCs might well be a significant driver of plasmablast responses to both foreign and self-antigens. While this might happen in the absence of T cell signals (Litinskiy et al., 2002), extrafollicular T helper cells have also been described that have the potential to collaborate with DCs in supporting plasmablast proliferation and differentiation in response to T-dependent antigen (Chan et al., 2009; Odegard et al., 2008).

How then could triggering of LOX-1 on DCs play a role in in vivo B cell responses? In addition to oxLDL, LOX-1 recognizes a range of other endogenous ligands associated with inflammation (Huysamen and Brown, 2009). Thus, similar to its role on endothelial cells, DC-expressed LOX-1 might act as an inflammatory sensor, in this case facilitating the support of responding B cells through BAFF and APRIL production potentially in conjunction with presentation of intact antigen (Figure 1). Clearly, the LOX-1-mediated triggering of DCs by ligands associated with inflammation has the potential to support B cells in both protective and autoimmune responses (Figure 1). However, the ability of LOX-1 to bind both gram-positive and gram-negative bacteria raises the possibility that DCs could provide a direct support to B cell responses under circumstances where rapid production of antibodies is required to combat replicative pathogens (Figure 1). In the case of T-dependent antigens, LOX-1-activated DCs might collaborate with extrafollicular T helper cells to enhance humoral responses. Ultimately, precise determination of the role of DC-expressed LOX-1 will require sophisticated, cell-specific gene inactivation studies in mice. Nevertheless, the studies of Joo et al. provide a valuable insight into the functional capabilities of DC-expressed LOX-1 in the human context and suggest that it is worth considering as a target molecule in vaccines designed to promote humoral immune responses.

REFERENCES

Caminschi, I., and Shortman, K. (2012). Trends Immunol. *33*, 71–77.

Chan, T.D., Gatto, D., Wood, K., Camidge, T., Basten, A., and Brink, R. (2009). J. Immunol. *183*, 3139–3149.

Huysamen, C., and Brown, G.D. (2009). FEMS Microbiol. Lett. *290*, 121–128.

Joo, H., Li, D., Dullaers, M., Kim, T.-W., Duluc, D., Upchurch, K., Xue, Y., Zurawski, S., Le Grand, R., Liu, Y.-J., et al. (2014). Immunity *41*, this issue, 592–604.

Litinskiy, M.B., Nardelli, B., Hilbert, D.M., He, B., Schaffer, A., Casali, P., and Cerutti, A. (2002). Nat. Immunol. *3*, 822–829.

MacLennan, I., and Vinuesa, C. (2002). Immunity *17*, 235–238.

Mehta, J.L., Sanada, N., Hu, C.P., Chen, J., Dandapat, A., Sugawara, F., Satoh, H., Inoue, K., Kawase, Y., Jishage, K., et al. (2007). Circ. Res. *100*, 1634–1642.

Odegard, J.M., Marks, B.R., DiPlacido, L.D., Poholek, A.C., Kono, D.H., Dong, C., Flavell, R.A., and Craft, J. (2008). J. Exp. Med. *205*, 2873–2886.

Qi, H., Egen, J.G., Huang, A.Y., and Germain, R.N. (2006). Science *312*, 1672–1676.

Teichmann, L.L., Ols, M.L., Kashgarian, M., Reizis, B., Kaplan, D.H., and Shlomchik, M.J. (2010). Immunity *33*, 967–978.