CD5 sweetens lymphocyte responses

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The cell walls of pathogenic and commensal bacteria and fungi contain a variety of carbohydrate-containing moieties, not produced by animals. The presence of these lipopolysaccharides (LPSs), lipoteichoic acids (LTAs), peptidoglycans (PGNs), and zymosans in host tissues thus alerts the host to invasive bacterial or fungal infections. Several families of pattern recognition receptors (PRRs) expressed by epithelial and phagocytic cells contribute to recognition of microbial cell wall components. These PRRs include toll-like receptors (TLRs), nod-like receptors (NLRs), and scavenger receptors. Cell surface TLRs and cytosolic NLRs respond to microbial components by eliciting production of cytokines and chemokines that activate or amplify host inflammatory and immune responses. In contrast, at least some members of the scavenger-receptor cysteine-rich (SRCR) superfamily contribute more to the resolution of inflammation. In this issue of PNAS, a lymphocyte-expressed SRCR, CD5, is shown to bind the fungal polysaccharide β-glucan (1). Together with a recent study on CD6 (2), this work shows that SRCRs expressed on the lymphocyte cell surface are PRRs that detect diverse microbe-derived carbohydrates. Moreover, previous data implicating CD5 in negative regulation of lymphocyte antigen receptor signaling suggest that ligation of these PRRs with microbial components may profoundly influence lymphocyte activation and autoimmunity (Fig. 1).

CD5 is a 67-kDa membrane-spanning glycoprotein comprised of 3 extracellular SRCR repeats, a transmembrane region, and a cytoplasmic tail with conserved threonine and tyrosine residues important for signal transduction. In this issue of PNAS, Vera *et al.* (1) show that a naturally occurring soluble isoform of CD5 (rshCD5) binds to *Schizosaccharomyces pombe*, *Candida albicans*, and *Cryptococcus neoformans* fungal cells with an affinity estimated in the low nM range. The rshCD5 also bound to zymosan and binding was competed by β-1,3-glucan but not mannan or the bacterial carbohydrate-containing moieties PGN, LPS, or LTA. Furthermore, FITC-zymosan bound to a $CD5^{neg}$ and $CD6^{neg}$ Jurkat T cell isolate (2G5) only when these cells were transfected to express membrane-associated CD5. Thus, despite similarity to CD6, a homologous SRCR protein shown to bind PGN, LPS, and LTA (2), CD5 appears to be a β-glucan-specific PRR. Presumably, subtle differences in the architecture of the SRCR domains between CD5 and CD6 are responsible for the respective ligand preferences.

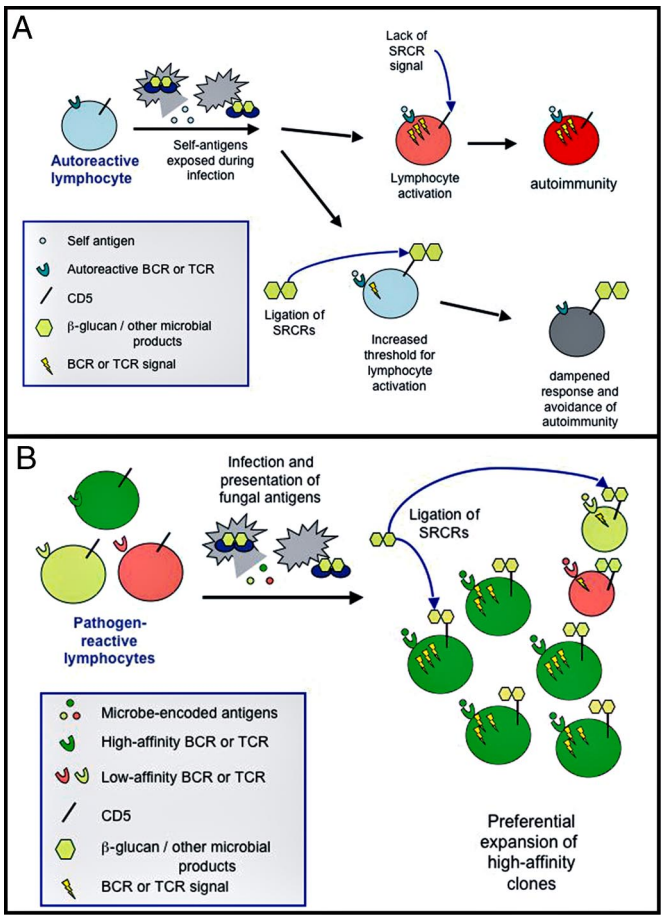

Fig. 1. Potential immunological consequences of pathogen-associated molecular pattern recognition by lymphocyte SRCRs. (*A*) Preventing autoimmunity. Infection exposes normally cryptic self-ligands that stimulate anergic lymphocytes or lymphocytes with low affinity for self antigens. In the presence of microbial products that signal through CD5 or other SRCRs, the threshold for TCR or BCR signaling is increased and activation of autoreactive lymphocytes is dampened. (*B*) Tuning of the antimicrobial T cell response. Infection results in presentation of numerous microbial antigens and activation of lymphocytes with multiple distinct specificities. Lymphocytes with the highest reactivity to microbial antigens receive stronger antigen receptor signals and overcome negative signals from CD5 or other SRCRs to block by competition lymphocytes with more weakly reactive BCRs or TCRs.

It appears that any one of the three SRCR repeats present in the CD5 extracellular domain can bind β-glucans, which suggests that polymeric β-glucans could induce cross-linking of cell surface CD5 and intracellular signaling. Consistent with this notion, rshCD5 containing all three SRCR domains aggregated FITC-labeled fungal cells (1). Two additional sets of experiments confirmed that zymosan induces CD5-dependent intracellular signaling cascades. First, sustained phosphorylation of the MAPKs Erk1/2 and MEK was observed in CD5-expressing Jurkat 2G5 cells within 5 min of zymosan treatment. Zymosan-dependent MAPK phosphorylation was not observed in parental 2G5 Jurkats nor in 2G5 Jurkats transfected to express a truncated CD5 lacking its

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cytoplasmic tail. Second, expression of full-length CD5 in the normally $CD5^{neg}$ HEK 293 cell line rendered these cells responsive to zymosan as measured by IL-8 secretion. TLR2-expressing HEK 293 cells are also responsive to zymosan because TLR2 binds fungal cell wall mannans. However, zymosan induced increased IL-8 production in TLR2-expressing HEK cells transfected to also express full-length CD5. Addition of soluble rshCD5 also blocked IL-8 production due to CD5 or TLR2 alone, but at the dose used only partially blocked IL-8 produced by cells expressing both receptors. These data thus clearly indicate that zymosan binds and presumably cross-links cell surface CD5 to activate intracellular signaling cascades.

At this point the impact of β-glucan detection by CD5 on signaling pathways in primary T cells remains to be determined. However, data from previous studies indicate that CD5 normally exerts negative effects on lymphocyte responsiveness to lymphocyte antigen receptor [B cell antigen receptor (BCR) or T cell antigen receptor (TCR)] ligation (3–5). CD5 is present at high levels on the surface of T cells and at somewhat reduced levels on the surface of the "B-1a" subset of B cells. B-1a B cells populate the mouse peritoneal cavity and pleura, comprise a BCR repertoire that is less diverse than that of conventional B2 B cells, and are highly skewed toward recognition of common bacterial and self-antigens (3). At the surface of these lymphocytes, CD5 has been shown to colocalize with the TCR or BCR and to be present in immunological synapses (5–7). These patterns of localization are clearly consistent with potential influences of CD5 on the duration or nature of B or T cell activation. Indeed, CD5 ligation by endogenous protein ligands such as CD72 (8) dampens TCR signaling by recruiting SHP-1 to an ITIM motif in the CD5 cytoplasmic tail (9). In addition, CD5 is reported to enhance production of the immune-suppressive cytokine IL-10 (10). In this context, the results of Vera *et al.* (1) suggest a role for β-glucan detection in the dampening of autoimmune lymphocyte responses and/or the shaping of the lymphocyte repertoire responding to fungal infections (Fig. 1). CD6 or other SCRC family members may serve similar roles in the context of bacterial or viral infections. As cited by Vera *et al.*, there is also evidence that CD5 is upregulated on anergic B cells, regulatory T cells, endothelia, macrophages, and dendritic cells (DCs). Perhaps this upregulation contributes to IL-10 production seen by certain DC populations treated with zymosan (11).

Soluble CD5 can attenuate responsiveness to systemic fungal challenge.

How does CD5 detect fungal pathogens during infection? Studies with the C-type lectin dectin-1 may be illuminating in this regard. Like CD5, dectin-1 binds with high affinity to β-glucans. However, dectin-1 is largely expressed on macrophages, monocytes, neutrophils, and most DC subsets, where it signals via an ITAM motif, Src, and Syk to induce phagocytosis, production of oxidative radicals, and secretion of cytokines (12). The ability of dectin-1 to bind fungal cells depends on the exposure of β-glucans at the fungal cell surface. These sugars comprise up to 50% of the fungal cell wall but are largely hidden beneath a layer of mannans and proteins on certain forms of pathogenic fungi. This masking of β-glucans renders *Candida albicans* largely inaccessible to dectin-1 and impairs detection of intact yeast (13). Presumably CD5 is also unable to detect such intact fungi. However, dectin-1 can detect β-glucan exposure at sites of yeast budding and cell division scars (13). Alternatively, it was recently reported that treatment with echinocandin antifungals increases exposure of β-glucans on the fungal pathogen *Aspergillus fumigatus* and thus triggering of dectin-1 (14). By analogy to bacteria, where antibiotics and bacterial enzymes release cell wall fragments that modulate host immune responses and influence lymphocyte activation (15), it seems likely that β-glucans may also be released from growing or dying fungal cells to trigger CD5.

Fungal infections and liberation or exposure of fungal cell wall carbohydrates activates dectin-1, TLR2, and perhaps other proinflammatory PRRs. Consequently, high systemic levels of these fungal products cause a systemic septic shock-like disease. Given the high binding affinity of CD5 for β-glucans, Vera *et al.* (1) also investigated whether administration of rshCD5 might have therapeutic use as a sink for fungal cell wall components in an in vivo mouse model of zymosan-induced sepsis. Mice administered systemically with a high dose (500 mg/kg) of zymosan had high serum levels of the inflammatory cytokines IL-6 and IL-1β, leukocytosis, increased myloperoxidase (MPO) activity in the liver, organ damage, and death. Treatments with rshCD5 at 1 h before zymosan treatment suppressed cytokine and MPO activity, reduced leukocytosis, and significantly improved survival of the treated animals. Treatment with rshCD5 as late as 3 h after zymosan administration also showed protective effects, whereas rshCD6 had no effect. These data indicate that soluble CD5 can attenuate responsiveness to systemic fungal challenge. However, whether the endogenous soluble CD5 ever reaches sufficiently high serum levels to exert similar effects is not clear. Nonetheless, these findings and the potential roles of CD5 in negative regulation of T and B cell responses should sweeten interest in CD5 and other lymphocyte-expressed SRCRs.

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