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A Supercode for Inflammation

Siegelman and colleagues demonstrate unexpected synergism of CD44 and VLA-4 during lymphocyte extravasation. This is the first time that molecules mediating rolling and firm adhesion have been shown to associate biochemically leading to direct functional cooperation.

Three digit telephone "area codes" are a useful metaphor for immune cell homing, helping organize the numerous receptor-ligand pairs that mediate three discrete steps of tethering by lectin-glycoprotein interactions, activation by G protein-coupled receptors, and firm adhesion by integrins that are needed to initiate extravasation (Springer, 1994). The area code metaphor has worked because the individual "digits" have been seen as independent, and therefore, all three-way combinations of selectin-glycoprotein, GPCR-attractant, and integrin-ligand pairs would be expected to work similarly for mediating attachment and extravasation. A study in this volume of *Immunity* from Siegelman and colleagues demonstrates for the first time that specific pairwise combinations can be much better than others due to the molecular organization of the receptors on the cell surface, creating a supercode (Nandi et al., 2004 [this issue of *Immunity*]).

The study focuses on the tethering molecule CD44, which was originally linked to lymphocyte homing by Butcher and colleagues (Jalkanen et al., 1987). CD44 is induced on effector and memory T lymphocytes and metastatic tumors and has a C type lectin-like N-terminal domain that binds the proteoglycan hyaluronic acid on the surface of activated endothelial cells (Aruffo et al., 1990). CD44 can act in a "selectin-like" manner, mediating rapid but transient attachment of cells that are rapidly flowing over the endothelial surface (DeGrendele et al., 1996). Earlier work from Siegelman's group had suggested a special relationship between CD44 and very late activation antigen-4 (VLA-4), an integrin that binds vascular cell adhesion molecule-1 (VCAM-1) also expressed on activated endothelial cells, but there had been no explanation for this apparent partnership from which other integrins, like lymphocyte function associated-1 (LFA-1), were excluded (Siegelman et al., 2000). In the current work they have discovered that CD44 can be coimmunoprecipitated with VLA-4 but not LFA-1 and that the association between CD44 and VLA-4 requires the cytoplasmic domain of CD44. Furthermore, CD44 lacking the cytoplasmic domain is unable to synergize with VLA-4 to produce firm adhesion both in vitro and in vivo. These data suggest that the combination of CD44-hyaluroinic acid and VLA-4-VCAM-1 acts as a supercode for entry to effector T lymphocytes into sites of inflammation.

The current result may require reevaluation of earlier results suggesting that VLA-4 can directly mediate tethering from flow, a property normally restricted to selectin-glycoprotein interactions and functionally similar interactions like CD44 with proteoglycan (Alon et al., 1995). It is well established that the low-affinity form of LFA-1 can alter the dynamics of rolling adhesion through transient interactions with ICAMs (Kunkel et al., 1996; Salas et al., 2002), but VLA-4 was the only integrin claimed to be efficient at the initial tethering step. In retrospect, it is now possible that many experiments that demonstrated VLA-4-mediated tethering to cellular substrates, particularly activated endothelial cells, may have been based on VLA-4's close functional association with CD44 and the expression of proteoglycan ligands for CD44 on endothelial cells along with VCAM-1. However, experiments with purified VCAM-1 by Alon et al. argue for the capacity of direct VLA-4-VCAM-1 interactions in tethering from flow (Alon et al., 1995). Regardless, it will be important to test anti-CD44 antibodies in these assays since ligands for CD44 could contaminate human serum albumen preparations used for blocking or even be directly carried by highly glycosylated VCAM-1 molecules, allowing a cryptic contribution of CD44 to the integrin-mediated tethering.

The physical basis for this special synergism between CD44 and VLA-4 may be that firm adhesion sites can be directly nucleated from the transient tethering sites when VLA-4 is preconcentrated with CD44. The specific molecules that link the cytoplasmic domain of CD44 to VLA-4 were not identified specifically, but a number of candidates were coprecipitated with CD44 and VLA-4. The study does not address the GPCR-mediated activation step, which was replaced by phorbol ester-stimulated activation in the in vitro experiments. The in vivo studies demonstrate that either a triggering step is not needed by this unique combination, or it is provided by endogenous signals, perhaps specific GPCR-ligand interactions, at inflamed sites in vivo. If it is correct that the advantage of this configuration stems from the ability to directly mature tethering sites into firm adhesion sites then it would also make sense to locate the activation mechanism in the same complex. It will be interesting to see if this prediction is correct or if it is sufficient to preconfigure the tethering and firm adhesion steps to achieve this synergism. The results have implications for both effector T cell entry into inflamed sites and tumor metastasis where coexpression of CD44 and VLA-4 (an integrin that is expressed on nonhematopoietic cells also) may be reexamined with great interest (Gilcrease et al., 1996).

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Proteasomes Get By with Lots of Help from Their Friends

Proteasomes can't do it all. It was previously known that aminopeptidases frequently degrade proteasome-generated peptides. Now it appears that another protease, tripeptidyl peptidase II (TPP II), plays a critical role in cleaving proteasomal produced peptides into shorter peptides that can then be degraded by aminopeptidases.

Nature abounds with nasty, selfish things like viruses and tumor cells bent on their own survival at our expense. Fortunately, for protection our vertebrate ancestors endowed us with $CD8^+$ T cells and MHC class I molecules (and NK cells, etc.). $CD8^+$ T cells roam our nooks and crannies, sifting through countless MHC class I molecules for the faintest peptidic traces of trouble. Unless a cell happens to be a professional antigen-presenting cell, nearly all of the peptides displayed by class I molecules will have been synthesized by the cell's own ribosomes. Virtually all of these peptides are generated from longer polypeptides by the action of proteases. Current evidence suggests that most of peptides are derived from the significant fraction of polypeptides degraded with a half-life of $\sim$10 min following their synthesis (Yewdell et al., 2003). Presumably these proteins are degraded due to some defect that prevents them from attaining a stable conformation in a reasonable time frame.

This year marks the tenth anniversary of the landmark study demonstrating that proteasomes are responsible for the vast majority of protein degradation in cells (including degradation of newly synthesized proteins), and are involved in the generation of most class I peptide ligands (Rock et al., 1994). Class I molecules are finicky about peptide length; each peptide presented has an optimal length for high- affinity binding that ranges from 8 to 11 residues. It was initially thought that proteasomes acted as a "molecular ruler," generating peptides of the proper length for high-affinity class I binding. More recent studies, however, demonstrated that this was the exception rather than the rule(r). Most proteasome-generated peptides require further trimming. It is now clear that aminopeptidases trim proteasome- generated peptides both in the cytosol/nucleus (where proteasomes are located) and in the endoplasmic reticulum (ER), following delivery of peptides by the transporter associated with antigen processing (TAP). ER aminopeptidases appear to play a particularly important role in peptide generation (Serwold et al., 2002; York et al., 2002).

For obscure, but no doubt important, reasons cells are devoid of active carboxypeptidases. It has been generally accepted that proteasomes must liberate the C termini of class I peptide ligands (Shastri et al., 2002). With the findings of Reits et al., published in this issue of *Immunity*, it now appears that a second large cellular protease with endopeptidase activity contributes to the generation of C-terminal cleavages that produce MHC class I binding peptides. Most importantly, the authors provide convincing evidence that this protease, tripeptidyl peptidase II (TPP II), plays an essential role in generating many MHC class I peptide ligands.

The participation of TPP II in antigen presentation was first implied by the findings of Glas et al. (1998), who reported that a large protease (later identified as TPP II by Geier et al. [1999]) could replace the functions of proteasomes in cells adapted to grow in the proteasome inhibitor NLVS. Subsequently, however, it was shown that NLVS-resistant cells remained sensitive to all other proteasome inhibitors tested, raising doubts regarding the functional overlap between TPP II and proteasomes (Princiotta et al., 2001). In any event, Geier et al. demonstrated that TPP II exhibits endopeptidase activity, suggesting the possibility that it contributes to the many independent examples of ongoing antigen presentation in proteasome inhibitor treated cells (Shastri et al., 2002). The proverbial smoking gun was provided by Seifert et al. (2003), in demonstrating that generation of a defined viral peptide required catalytically active TPP II but not proteasomes.

In their present study, Reits et al. place TPP II squarely in the main stream of class I processing pathways. This group has pioneered the (in their own words) "single cell biochemistry" approach to antigen processing by creative application of fluorescent molecules and confocal microscopy. In this installment, they use internally quenched fluorescent peptides microinjected into cells to characterize peptide degradation, which is inferred by increased fluorescence that accompanies cleavage between the fluorescent and quencher groups attached to amino acid side chains. This is an exquisite applica-