mechanism that restores liver mass after necrotic or apoptotic injury has occurred. The link between intracellular signals resulting in mitogenic and antiapoptotic effects of these agents remains to be completely dissected.


Selectin and selectin ligand binding: a bittersweet attraction

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Inhibition of leukocyte migration into target organs has long been an attractive, though challenging, basis for anti-inflammatory strategies. However, to date, the manipulation of leukocyte rolling along blood vessels has not yielded successful new therapies. An important study (see the related article beginning on page 1008) may now open new avenues in this exciting field of anti-inflammatory therapies by introducing a putative inhibitor of poly-N-acetyllactosamine biosynthesis that affects selectin ligand activity and shows efficacy in a rodent skin inflammation model.


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Nonstandard abbreviations used: sialyl Lewis X (sLeX); cutaneous lymphocyte-associated antigen (CLA); s14, 4-galactosyltransferase (GalT); s2,3-sialyltransferase (ST3GalII); s1,3-fucosyltransferase (FucT); P-selectin glycoprotein ligand-1 (PSGL-1); contact hypersensitivity (CHS); peracetylated-4-fluorinated-α-glucosamine (4-F-GlNAc).

Tissue-specific localization of T cells is a requirement for immune surveillance in the skin and in addition plays a pivotal role in the pathogenesis of numerous inflammatory skin disorders. Indeed, the evidence that T cells are crucial factors in mediating psoriasis, allergic contact dermatitis, atopic dermatitis, and cutaneous T cell lymphomas is so strong that these diseases are now considered as T cell–mediated dermatoses (1). Consequently, insight into mechanisms of T cell recruitment to the skin (and other target organs) may lead to novel anti-inflammatory therapies, and the subject is therefore of particular interest.

Selectin and selectin ligand interactions mediate leukocyte rolling along the endothelium

The multistep cascade of T cell migration has been well described (Figure 1), and the molecular basis for T cell skin homing has been reviewed recently (2). The first steps of T cell localization to all tissues include leukocyte tethering and rolling along the vessel wall, which is mediated primarily by interactions between selectin and selectin ligand (3). A number of studies have demonstrated the pivotal role of E- and P-selectin for leukocyte rolling as well as their overlapping and mutually compensating functions (4). Therefore, it was not surprising that a neutralizing antibody solely against E-selectin was found to be without beneficial effects in a recent clinical trial (5). Probably for these reasons, the development of a potent, but E-selectin–specific low-molecular weight antagonist called ESA-2 (6) was stopped. The lesson learned from these findings was that potent and clinically active selectin antagonists have to interfere with at least two of the three selectins (E, P, and L) in order to show in vivo efficacy. Some such antagonists have been reported recently (7).
Synthesis of sialyl Lewis X is essential for selectin ligand binding
The tetrasaccharide sialyl Lewis X (sLeX), which is closely related to cutaneous lymphocyte-associated antigen (CLA), binds to all three selectins and appears to be an interesting drug target. Its expression by T cells requires the action of multiple glycosyltransferases such as core 2 β1,6-N-acetylglucosaminyltransferase-I, N-acetylglucosamine-6-O-sulfotransferase, β1,4-galactosyltransferases (GalTs), the α2,3-sialyltransferase (ST3GalIV), and α1,3-fucosyltransferases IV and VII (FucTIV and FucTVII). ST3GalIV, FucTIV, and FucTVII are responsible for synthesizing terminal sialofucosylations that serve as E-selectin ligands. Mice deficient in FucTVII are characterized by a marked reduction of E-, P-, and L-selectin ligand activity and impaired leukocyte extravasation into inflamed tissue, indicating a major role for FucTVII in the generation of selectin ligands. In mice deficient for FucTVII and FucTIV, leukocyte rolling is completely absent, and contact hypersensitivity (CHS), which is substantially reduced in FucTVII-deficient mice, is virtually absent (10, 11). Discovery of low–molecular weight antagonists of FucTVII, which plays the dominant role for selectin ligand production, is ongoing but has not yet reached clinical development (12, 13). Inhibition of FucTVII might be challenging, since FucTVII antagonists have to penetrate the cell and Golgi membrane to reach their target. More important is the fact that in humans, FucTIV may synthesize active selectin ligands in the absence of FucTVII, a compensatory mechanism that was observed in one patient carrying a homozygous missense mutation in the FucTVII gene (14).

Inhibition of carbohydrate side chain elongation blocks E-selectin ligand expression and cutaneous immune reactions in vivo
In this issue of the JCI, Dimitroff et al. (15) introduce a new strategy for the inhibition of selectin ligand production using the fluorinated analog of N-acetylgalactosamine — peracetylated-4-fluorinated-D-glucosamine (4-F-GlcNAc) — as a glycosylation inhibitor. Terminal sLeX structures are expressed on poly-N-acetyllactosamines found on core 2 O- and N-linked glycans displayed by PSGL-1 (Figure 2). Consequently, compounds interfering with the synthesis of poly-N-acetyllactosamine structures should reduce selectin ligand activity and function. Indeed, Dimitroff and colleagues...
demonstrated previously that 4-F-GlcNAc was directly incorporated into native CLA expressed on human T cells, indicating direct inhibition of poly-N-acetyllactosamine elongation (8). It is thought that after conversion to UDP-4-F-GlcNAc and addition to the poly-N-acetyllactosamine backbone, 4-F-GlcNAc blocks the addition of Gal to its 4-OH, leading to premature chain termination during sLeX generation (8). In the present study, the authors extend their observations by neatly demonstrating blockade of lymphocyte E-selectin ligand expression in vivo and prevention of murine CHS reactions in the presence of 4-F-GlcNAc (15). Most interesting, 4-F-GlcNAc inhibited CHS responses with an extremely high efficacy, which reached or even exceeded that of potent glucocorticosteroids or calcineurin inhibitors. On the other hand, in addition to their importance in cell-cell interaction, carbohydrate side chains on glycoproteins also play a significant role in protein confirmation, transport, and stability. Mice deficient in UDP-Gal:GalNAc galactosyltransferase GalT—the enzyme responsible for elongation of poly-N-acetyllactosaminoglycans—are normal at birth but exhibit growth retardation and reduced life span. It was suggested that GalT, although dispensable during embryonic life, plays critical roles in the regulation of proliferation and differentiation of epithelial cells after birth (16). Currently, it is unclear whether incomplete, temporary inhibition of poly-N-acetyllactosaminoglycan elongation by 4-F-GlcNAc will have similar unwanted effects as observed in GalT-deficient mice. Nevertheless, selective glycosyltransferase inhibitors are expected to be more specific and may cause even fewer side effects. Although this indicates the need for further investigations and suggests that a novel drug is still a long way off, the present investigation has indicated that inhibition of selectin ligand activity can be achieved by antagonizing specific glycosyltransferases with small molecules and leads to anti-inflammatory effects in vivo. Remarkably, 4-F-GlcNAc only inhibited elicitation without modulating the sensitization phase in the CHS model (15)—a phenomenon that was also observed in FucTVII-deficient mice (11). This indicates that these strategies have the potential to be effective not only in preventive approaches but also in therapeutic modalities, which is important for the therapy of established disease. Moreover, the lack of inhibitory effects on the sensitization phase may even be an advantage, since it should allow a triggering of the immune response, which is important for host defense against pathogens.

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Finding NEMO: genetic disorders of NF-κB activation

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The pathways between a receptor and transcriptional activation mediated by NF-κB are complex. The study of human gene mutations that result in dysregulation of these pathways has provided insight into the functions of individual components of the pathway, their interrelations, and the significance of these systems to the organism (see the related article beginning on page 1108).


Inducible activation of gene transcription

NF-κB was recognized as a DNA-binding factor that exists in the cytoplasm of resting cells and that accumulates in the nucleus under appropriate conditions (1). The ability of NF-κB to shuttle between the cytoplasm and nucleus in a tightly regulated manner led to the exploration of a complex series of events leading to activation-induced gene expression, and to the discovery of factors that prevent the transit of NF-κB into the nucleus (2). Receptor-mediated NF-κB activation of gene transcription and its stringent control are fundamental to cell development, survival, and function. In this issue of the JCI, Courtois and colleagues report a novel human mutation in a protein that negatively regulates NF-κB activation (3). The resultant mutant dominantly inhibits the activation of NF-κB (see below) and gives rise to a clinical syndrome of ectodermal dysplasia (ED) and susceptibility to infection.

NF-κB activity is imparted by a protein dimer selected from five mammalian homologues: p50, p52, p65 (RelA), Rel, and RelB (p50 and p52 are derived from larger precursors, p105 and p100, respectively). The majority of dimers formed by these individual NF-κB members are capable of activating transcription by binding to κB sites in DNA. The dimerization of these molecules occurs through a conserved N-terminal Rel homology domain (RHD). Importantly, the RHD also serves as the binding site for one of several inhibitors of NF-κB (IκBs).

An IκB can physically interfere with NF-κB dimerization or block nuclear localization sequences within the NF-κB member. The family of molecules possessing these activities consists of at least seven members: IκBα, IκBβ, IκBε, IκBγ, Bcl-3, and inhibitory domains of the p105 and p100 precursor proteins. The cytoplasmic association of an IκB and a NF-κB member is controlled by the phosphorylation of the IκB, which leads to its ubiquitination and proteosomal degradation (Figure 1). The release of an NF-κB protein from IκB allows it to participate in dimer formation, translocate to the nucleus, and activate transcription. The phosphorylation of IκB, therefore, is a critical regulatory step in NF-κB function.

NF-κB activation

Phosphorylation of IκB is mediated by an IκB kinase (IKK), a large, multisubunit signaling complex (signalosome) capable of binding IκB as well as other upstream regulators. The classical IKK signalosome consists of two catalytic subunits, IKKα and IKKβ, and a regu-