Cytolytic responses: cadherins put out the fire

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Cytotoxic lymphocytes, such as natural killer (NK) cells and CD8$^+$ T cells, provide an essential defense against intracellular pathogens and tumors. During target cell recognition, these cells receive both activating and inhibitory signals. The cell must evaluate these opposing signals and determine the appropriate response: activation or inhibition. Classically, inhibitory signals are mediated by receptors that recognize MHC class I molecules (1). But recent studies, including one in this issue, suggest that MHC class I–independent inhibitory signals can also result in inhibition of cytotoxic cells.

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Activation of cytotoxic lymphocytes is tightly regulated

NK cells and CD8$^+$ T cells provide defense against virus-infected and transformed cells by releasing perforin and granzymes from cytolytic granules and by secreting the cytokine interferon (IFN)-γ. T cell receptor (TCR) engagement is essential for the activation of CD8$^+$ T cells, although costimulatory receptors also contribute. NK cell activation can be triggered by the ligation of various activating receptors, each of which recognizes a different cell surface molecule on the target cell (2). The activation threshold of these cytolytic cells is set by the integration of inhibitory signals that counteract the activation signals (2). Inhibitory receptors recognize molecules that are expressed on normal cells as a means of protecting healthy cells from attack by NK and CD8$^+$ T cells. The prototypic inhibitory receptors recognize MHC class I molecules and include members of the Ly49 and PIR receptor families in mice, and the KIR and LILR receptor families in humans (2). These receptors contain tyrosine-based inhibitory motifs (ITIMs) in their intracellular domains, which recruit the protein tyrosine phosphatases SHP1 and SHP2. SHP1 and SHP2 dephosphorylate (and thus deactivate) critical signaling molecules, thereby blocking the activation of effector functions. NK cells and CD8$^+$ T cells also express other inhibitory receptors, including CEACAM1, LAIR1, and KLRG1, whose ligands are now being characterized. In this issue, Ito et al. (p. 289) identify the cell adhesion molecule E-cadherin as the ligand that engages KLRG1 in mice and men (3).

KLRG1 inhibits effector cell responses

KLRG1 is a transmembrane receptor belonging to the lectin-like superfamily, which mediates inhibition via a cytoplasmic ITIM motif. KLRG1 is preferentially expressed on cells that provide rapid effector functions in peripheral tissues, including subsets of NK cells and effector–memory CD8$^+$ T cells (4–9). Because these T cells have experienced their cognate antigen and acquired effector functions in the past, they secrete perforin, granzymes, and IFN-γ more promptly upon reexposure to that antigen. KLRG1 is rapidly upregulated on NK cells and CD8$^+$ and CD4$^+$ T cells in mice during infection with murine cytomegalovirus and *Toxoplasma gondii*, and in humans, during chronic infection with human cytomegalovirus, Epstein-Barr virus, and human immunodeficiency virus 1 (5, 6, 10–13). KLRG1 is also expressed on rat mast cells (4, 14).

The inhibitory function of KLRG1, its predominant expression on effector cells, and its inducible expression during infections, collectively suggest that KLRG1 might raise the activation threshold of NK and T cells, thereby down-regulating effector responses in the periphery and preventing immunopathology.

KLRG1 binds to cadherins on epithelial cells

Until now, the KLRG1 ligand was unknown. Using a recombinant KLRG1 tetramer, which is capable of detecting the ligand on epithelial cell lines, and expression cloning, Ito et al. now identify E-cadherin as the KLRG1 ligand (3). A similar finding was independently reported by Gründemann et al. (15).

Cadherins comprise a family of transmembrane glycoproteins that mediate Ca^{2+}-dependent cell–cell adhesion (16). They contribute to tissue morphogenesis during embryonic development and to the maintenance of tissue type and architecture. The extracellular region of cadherins contains tandem repeats that mediate Ca^{2+}-dependent homotypic interactions, and the cytoplasmic domain links them to the actin cytoskeleton by binding to β-catenin. β-catenin recruits α-catenin, which in turn binds directly to actin and to several actin-binding proteins, including α-actinin, vinculin, and formin. This cadherin–catenin–actin complex is essential for the formation and maintenance of the cell–cell adhesion complex known as the adherens junction. The cadherin family includes type I cadherins (epithelial [E]-, neuronal [N]-, placental [P], retinal [R]-, and muscle [M]-cadherins) and type II cadherins (vascular endothelial [VE]-cadherin). E-cadherin is found at epithelial cell junctions and on Langerhans cells (LCs), a unique population of dendritic cells (DCs) found in the epidermis. Other cadherins are found in similar structures in other cell types. According to Ito et al., three of the type I cadherins—E-, N-, and R-cadherins—are recognized by KLRG1 (3).

What is the significance of KLRG1–cadherin interactions? Ito et al. show

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that this interaction triggers an inhibitory pathway that down-regulates NK cell cytotoxicity (3). The study by Gründemann et al. found that KLRG1–cadherin interactions predominantly inhibited the expansion of CD8$^+$ T cells and their acquisition of effector functions, but had little effect on NK cell cytotoxicity. This inhibitory effect occurred when CD8$^+$ T cells were primed by nonprofessional antigen-presenting cells (15). Collectively, these studies demonstrate a role for E-cadherin in inhibition of both NK cells and T cells. This may have important implications for tumor immunosurveillance by NK and T cells. For example, E-cadherin expression is frequently down-regulated in certain breast cancers, because of the loss of one allele of the E-cadherin gene *CDH1* in combination with mutation or epigenetic silencing of the remaining allele (17). Cancer cells lacking E-cadherin lose contact inhibition and cell polarity, proliferate more rapidly, and acquire an invasive phenotype, suggesting that the loss of E-cadherin favors tumor growth. However, when NK cells and T cells encounter tumor cells that lack E-cadherin expression, KRLG1 is not engaged. In the absence of KLRG1-mediated inhibitory signals, the activation threshold of the cells is likely to be lower, causing the cells to release cytotoxic granules and IFN-γ to more readily and rapidly eliminate the tumor cells.

In contrast, some tumors may paradoxically exploit KLRG1–cadherin interactions to their advantage. One example is aggressive breast tumors, some of which initially down-regulate E-cadherin to gain motility and metastatic potential, but then reexpress the protein to promote the attachment of metastases and to avoid immune attack (17). In addition, epithelial tumors can switch from expressing E-cadherin to expressing N-cadherin (or other cadherins). N-cadherin endows the tumor with more motility and invasiveness through interactions with ligands on endothelial cells and stromal cells (17). In both cases, tumors escape immunosurveillance by engaging the KLRG1 inhibitory pathway.

Potential cross-talk between KLRG1 and α$_E$β$_7$ integrin

In addition to KLRG1, lymphocytes express another receptor for E-cadherin, the integrin α$_E$β$_7$ (CD103) (18). This integrin is predominantly expressed on lamina propria T cells and intraepithelial lymphocytes (19). Consistent with this expression pattern, α$_E$β$_7$ integrins mediate T cell homing and adhesion to intestinal epithelial cells via interactions with E-cadherin (19). These gut-associated T cells provide immunosurveillance against infected, damaged, or transformed epithelial cells, and participate in tissue repair by secreting wound-healing cytokines. Although KLRG1 is not expressed on intraepithelial lymphocytes (6), it might be up-regulated on some α$_E$β$_7$$^+$ T cells upon infection. NK cell subsets express α$_E$β$_7$ and might coexpress KLRG1 during steady-state or activation. Although not yet tested, coengagement of KLRG1 and α$_E$β$_7$ by E-cadherin on NK cells and T cells might modulate α$_E$β$_7$ function. It has been shown that α$_E$β$_7$ integrin is regulated by inside-outside signals, as α$_E$β$_7$-expressing cells bind more avidly to E-cadherin after the cells are activated with phorbol myristate acetate or anti-CD3 (19). It is thus possible that KLRG1 might transduce signals that inhibit α$_E$β$_7$ integrin activation, thereby reducing its avidity for E-cadherin and its ability to control lymphocyte migration and function. The potential impact of KLRG1–cadherin interactions on mucosal immunity is an attractive theme for future investigations.

KLRG1–cadherin inhibitory interactions: the tip of the iceberg?

The interaction between KLRG1 and cadherins is remarkably similar to other inhibitory interactions between lymphocytes and epithelial cells. Indeed, activated NK cells and T cells recognize another group of cell adhesion molecules, known as human carcinoembryonic antigen (CEA)–related adhesion molecules (CEACAMs). Recognition is mediated by CEACAM1, an inhibitory member of the CEACAM family that is expressed on activated NK cells, T cells, and other leukocytes.

CEACAM1 can bind several members of the CEACAM family expressed on epithelial cells, including CEACAM1 itself, and these interactions inhibit NK cell cytotoxicity against epithelial cells (20). Like E-cadherin, the expression of high levels of CEACAM1 on some tumor cells increases their motility and invasiveness (21). However, CEACAM1 expression on tumor cells may also promote tumorigenesis by triggering the CEACAM1 inhibitory pathway on NK and T cells, thus blocking their ability to recognize and eliminate tumor cells. As lymphocytes express multiple orphan inhibitory receptors, it is possible that additional MHC class I–independent inhibitory pathways will be identified, which might help explain the aggressiveness of certain epithelial tumors.

The complexity of cytotoxic lymphocyte–epithelial cell interplay

The overall picture of cytotoxic lymphocyte–epithelial cell interactions is becoming increasingly complicated in view of the recent demonstration that NK cells and T cells can bind cell adhesion molecules through both inhibitory and activating receptors. NK cells and T cells have been shown to recognize adhesion molecules known as nectins and nectin-like proteins (necls). Nectins and necls, like cadherins, mediate cell–cell adhesion through homotypic and heterotypic interactions (22). These proteins form adherens junctions in epithelial tissues and are linked to the actin cytoskeleton through actin-binding proteins such as afadin, DAL-1/4.1B, and human discs large (hDLG) protein (22, 23). NK cells and T cells express the receptor CD226, which recognizes necl-5 and nectin-2, and the receptor CD96, which recognizes necl-5 (24–26). In addition, activated NK cells and T cells up-regulate the receptor CRTAM, which binds to necl-2 (27). Unlike KLRG1, however, the receptors specific for the nectins and necls trigger NK cell and T cell activation, either alone or in association with the β2 integrin LFA-1 (28).

The role of these activating receptor interactions and their impact on tumor

immunosurveillance is not completely understood. Initial studies indicated that lymphocyte recognition of the nectins and necls provides costimulatory signals that trigger NK cell cytotoxicity, as well as T cell proliferation and IFN-γ secretion. Thus, we speculate that nectins and necls are not easily accessible to the immune system in normal tissues, either because these molecules are hidden in interepithelial junctions or are already engaged in homotypic interactions that preclude epithelial–lymphocyte interactions (Fig. 1). However, upon neoplastic disruption of tissue organization, nectin and necls may become accessible to NK and T cells for recognition (Fig. 1). If this is the case, tumors should decrease their expression of nectins and necls to escape immunosurveillance. Consistent with this model, many epithelial tumors—particularly nonsmall cell lung carcinomas—lack necl-2 expression because of the loss of heterozygosity of one *Necl-2* allele and the epigenetic inactivation of the second allele (23). This lack of necl-2 reduces epithelial cell adhesion and promotes invasion, and also allows neoplastic cells to avoid detection by NK cells and T cells. Some tumors, however, express high levels of other members of the nectin/necl family, such as necl-5, which also promotes motility and the metastatic potential of tumor cells (29). Why lymphocytes expressing activating receptors for Necl-5 do not eliminate these tumor cells is unclear. In vivo, tumors may secrete or shed soluble forms of necl-5 that act as decoys to prevent the recognition of necl-5 on their surface. Alternatively, lymphocytes may express yet unknown inhibitory receptors for necl-5.

Finally, exposure of nectins and necls may also play a role in tissue remodeling during injuries and chronic wounds. For instance, recognition of nectin and necls may serve as a mechanism for the removal of damaged cells by NK or T cells, thereby restoring normal tissue architecture. Alternatively, if nectins and necls on epithelial cells are continuously accessible to NK cells and T cells, these epithelial cells may be targeted for killing, hindering the healing process.
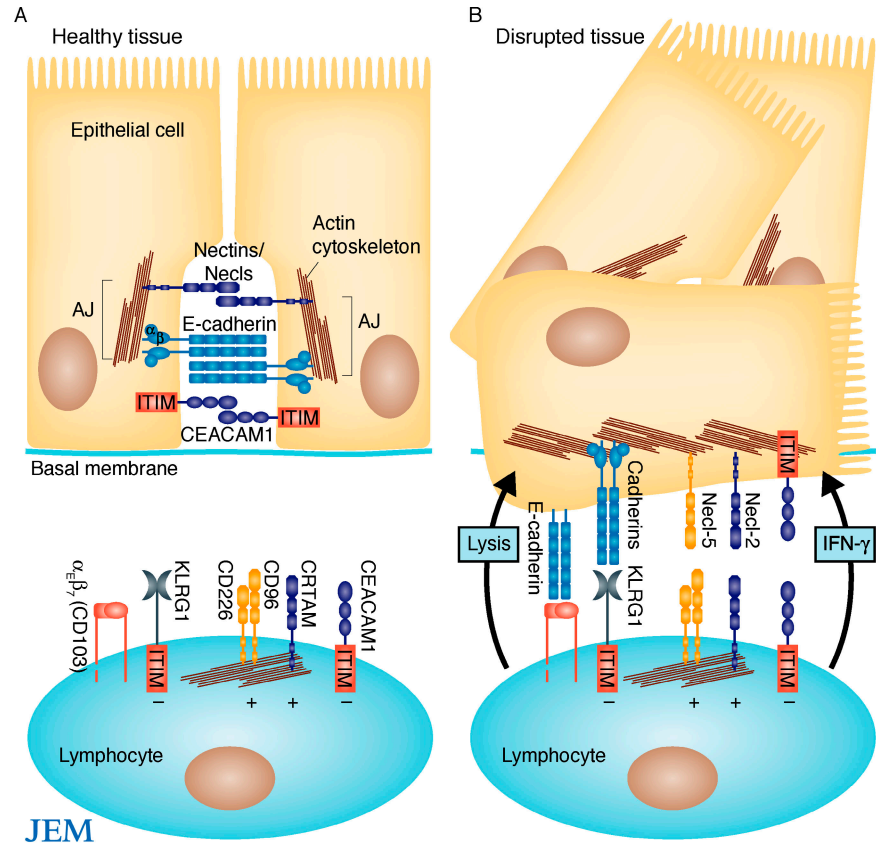

Figure 1. Lymphocytes express multiple receptors that recognize adhesion, which contribute to normal epithelial tissue architecture. (A) E-cadherin and nectins/necls form adherens junctions (AJ) in healthy epithelial tissues. These receptors, as well as CEACAM1, mediate cell–cell interactions via homotypic and/or heterotypic interactions. (B) Loss of cell polarity and tissue architecture (resulting, for example, from the growth of tumor cells) might favor lymphocyte–epithelial cell interactions over epithelial–epithelial interactions. KLRG1 and the integrin $\alpha_E\beta_7$ on NK and T cells bind to E-cadherin (and possibly N- and R-cadherins) on epithelial cells. CEACAM1 mediates homotypic adhesion between lymphocytes and epithelial cells. Both KLRG1 and CEACAM1 contain a cytoplasmic ITIM and deliver inhibitory signals. CRTAM binds necl-2, whereas CD226 and CD96 bind necl-5. CRTAM, CD226, and CD96 transduce positive signals that trigger cytotoxicity and IFN-γ release by NK and T cells.

Concluding remarks

The characterization of KLRG1 as a cadherin receptor, and previous studies showing NK and T cell recognition of CEACAM, nectins, and necls, highlights the capacity of the immune system to detect molecules involved in tissue organization. The challenge now is to understand how all these potential interactions are integrated in physiological settings and how the resulting signals modulate the immune response against tumors that disrupt tissue organization.

The impact of each interaction is most likely determined by several factors. The distribution of lymphocytic receptors on different cell subsets and their expression under steady-state, inflammatory, and neoplastic conditions are crucial issues that need to be investigated further. Another important factor is the accessibility of the epithelial cell ligands for their lymphocytic receptors. Such availability might be inversely proportional to homotypic and heterotypic interactions mediated by cadherins, CEACAMs, nectins, and necls. It is conceivable that a loss of cell polarity and tissue architecture may favor lymphocyte–epithelial cell interactions over epithelial–epithelial interactions (Fig. 1). The hierarchy of these cell–cell contacts will also depend on binding affinities and binding sites. It has been shown, for example, that

the $\alpha_E\beta_7$–binding site on E-cadherin does not overlap the site involved in homophilic E-cadherin interactions (19). Thus, E-cadherin–E-cadherin binding does not preclude $\alpha_E\beta_7$ binding to E-cadherin and thus both signals could be transduced simultaneously. Structural studies are now necessary to define the molecular basis of KLRG1–cadherin binding. Studies investigating the unknown aspects of these receptor–ligand interactions will not only contribute to our understanding of the maintenance of tissue organization and the control of tumor progression, but may also provide novel targets for intervention in tumor therapy.

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