half of which were sexual and half effectively asexual. (In each case, the mutation started with 20 copies per population to avoid early extinctions by drift, which would be uninteresting because they should occur equally under both treatments.) They then monitored the mutation's trajectory over 10 generations. Consistent with theory, the beneficial mutation spread at similar average rates for several generations under the two treatments. In later generations, however, the beneficial mutation continued to increase in the sexual populations, but not in those that were asexual. Although the beneficial mutations gave a transient fitness boost to the backgrounds in which they landed, the resulting clones were not usually the eventual winners. (It would be nice to see most beneficial mutations go extinct in the asexual populations, as theory predicts they eventually should, but that would require the experiment to continue for many more generations.) Rice and Chippindale also showed that the initial fitness variation was sufficiently large, relative to the mutation's selective advantage, that this outcome was expected according to the theory. These experiments strongly support the hypothesis that sex is advantageous because it accelerates adaptive evolution—not by combining multiple beneficial mutations, but by freeing individual ones from the baggage of mutations at other genetic loci.

So now we know why sex evolved, right? Not so fast. As seductive as these experiments are, the big question is hardly answered, for two reasons. First, understanding the evolution of sex is really two different questions: the origin of sex, and its long-term maintenance in the face of its costs. As Rice and Chippindale conclude, their experiments are about the "persistence over geological time" of sexual recombination. Second, most competing hypotheses for the evolution of sex are not mutually exclusive, especially if one is interested in why sex is maintained (*10*). There may have been one particular advantage that got sex going, but once it evolved, several forces might make it difficult to return to an asexual state (*11*). Sex may bring beneficial mutations together, purge the genome of deleterious mutations, and—as now shown—allow beneficial mutations to fly free of the baggage of deleterious mutations. The experiments of Rice and Chippindale notwithstanding, evolutionary geneticists will continue to be very interested in sex.

References

1. A. Weismann, *Nature* **36**, 607 (1887).
2. W. R. Rice, A. K. Chippindale, *Science* **294**, 555 (2001).
3. H. J. Muller, *Am. Nat.* **66**, 118 (1932).
4. J. A. De Visser, C. W. Zeyl, P. J. Gerrish, J. L. Blanchard, R. E. Lenski, *Science* **283**, 404 (1999).
5. J. Maynard Smith, *Evolution of Sex* (Cambridge Univ. Press, Cambridge, 1978).
6. S. P. Otto, Y. Michalakis, *Trends Ecol. Evol.* **13**, 145 (1998).
7. A. S. Kondrashov, *Nature* **336**, 435 (1988).
8. J. R. Peck, *Genetics* **137**, 597 (1994).
9. R. L. Malmberg, *Genetics* **86**, 607 (1977).
10. S. A. West, C. M. Lively, A. F. Read, *J. Evol. Biol.* **12**, 1003 (1999).
11. R. E. Lenski, *J. Evol. Biol.* **12**, 1034 (1999).

PERSPECTIVES: IMMUNOLOGY

Stress, NK Receptors, and Immune Surveillance

Drew M. Pardoll

Ever since the immune surveillance hypothesis proposed that the immune system could naturally recognize and eliminate tumors, investigators have been seeking cells that could serve such a purpose. Natural killer (NK) cells of the innate immune system that do not express the T cell receptor (TCR), rather than T cells of the adaptive immune system that do express the TCR, seemed to fit the bill. However, activating receptors expressed by NK cells and their associated ligands have only recently been identified, finally opening the door to the molecular dissection of NK cell involvement in immune surveillance. Now, papers by Girardi *et al.* (*1*) on page 605 of this issue and Diefenbach *et al.* (*2*) in *Nature* identify not only NK cells but also T cells expressing the γδ or αβ TCR as the prime movers in immune surveillance and natural antitumor immunity. The missing link turns out to be the NK cell receptor NKG2d, which is expressed by γδ and αβ T cells as well as by NK cells. These findings unite innate and adaptive immunity within the arena of cancer immunology.

The author is in the Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA. E-mail: dmpardol@jhmi.edu

The first NK receptors to be clearly defined bound both classical and nonclassical MHC (major histocompatibility complex) class I molecules and blocked the killing of target cells by NK cells (*3*). This inhibition was associated with ITIM domains (immunoreceptor tyrosine-based inhibitory motifs) in the cytoplasmic tails of these NK receptors. ITIMs provide docking sites for phosphatases that oppose the activity of tyrosine kinases, enzymes that are essential for NK cell activation. However, certain members of the NK receptor family—CD16, KIR2DS, Ly49D/H, and CD94/NKG2c—do not contain ITIMs. Instead, they are associated with the adapter molecule DAP12, which has an ITAM domain (immunoreceptor tyrosine-based activating motif) capable of activating NK cells (*4*). Thus, the view is emerging that the activity of NK cells is balanced by opposing activating and inhibitory signals. But, other than the down-regulation of MHC class I molecules, it has been unclear which other molecules are likely to tip the balance in favor of NK cell activation. The discovery of ligands for the NKG2d receptor (*5–7*) has shed new light on NK cell activation as well as on T cell–dependent adaptive immunity.

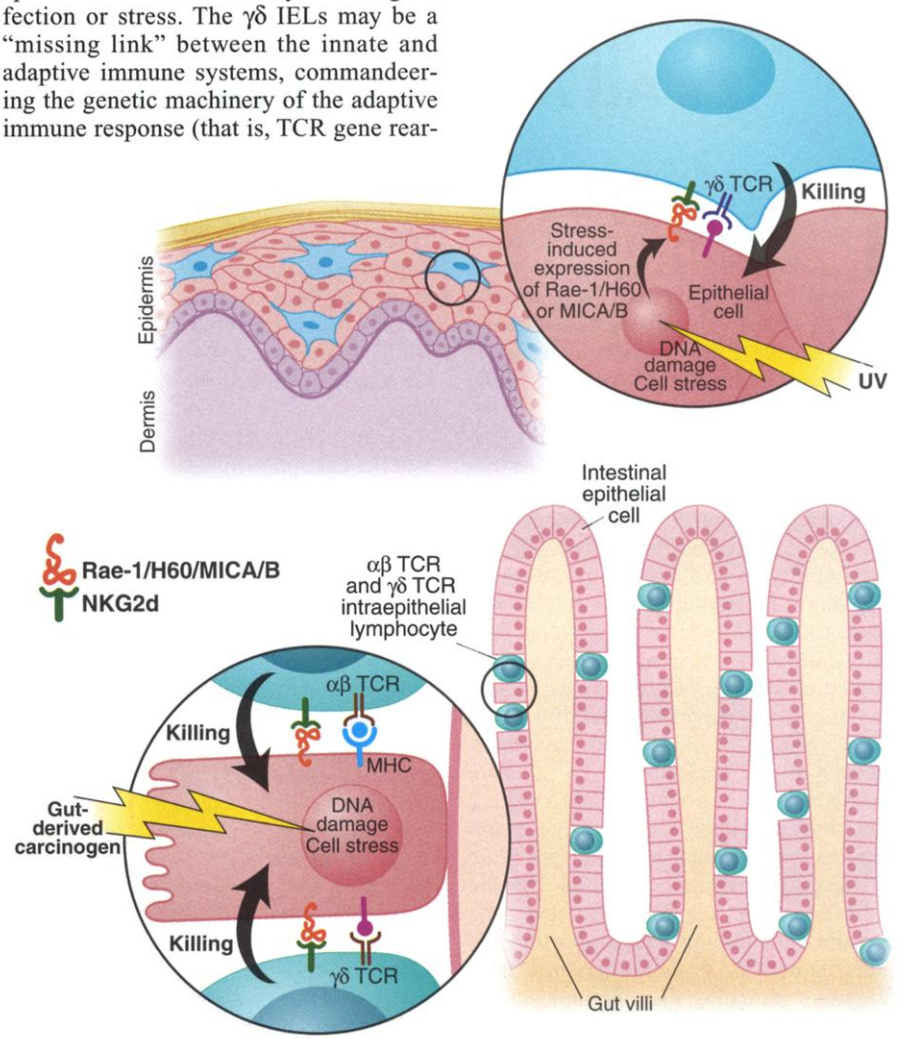
Encoded within the NK receptor gene complex, NKG2d differs from other known NK receptors in three ways. First, it is associated with the adapter molecule DAP10, which contains neither conventional ITIMs nor ITAMs. Instead, DAP10 contains a YXXM motif in its cytoplasmic tail that binds to phosphatidylinositol (PI) 3-kinase after phosphorylation of its tyrosine residues. Second, NKG2d is expressed by all NK cells as well as by $CD8^+$ αβ and γδ T cells, suggesting that it is important in both innate and adaptive immunity. Third, at least some of the ligands for NKG2d can be induced by environmental stresses and are expressed on the surface of many tumor cells. The two best characterized NKG2d ligands are MICA and MICB in human cells, which are nonclassical MHC molecules whose expression is induced by classic stress stimuli such as heat shock (see the figure). MICA and MICB do not have known murine orthologs, but mouse NKG2d does bind to many products of the retinoic acid inducible gene family *Rae-1α–ε* as well as to the product of the *H60* gene. Although expression of *Rae-1* and *H60* genes apparently is not induced by heat shock, they are up-regulated in at least one example of genotoxic stress (application of carcinogens to mouse skin) (*1*). In common with MICA and MICB, Rae-1 and H60 appear to be up-regulated in a number of tumors, leading Girardi *et al.* and Diefenbach *et al.* to directly evaluate Rae-1 and H60 involvement in immune recognition and tumor surveillance in mice.

Diefenbach *et al.* (*2*) and Cerwenka *et al.* (*8*) found that forcing mouse tumors that do not naturally express Rae-1β and H60 to express these ligands resulted in NK-dependent immune rejection of the tumors in vivo. Analysis of additional tumors revealed that $CD8^+$ αβ T cells were sometimes also involved in tumor rejection (*2*). Mice that rejected Rae-1β– or H60-positive tumors were immune to rechallenge with the same tumors, even when they no longer expressed either NKG2d ligand. Immunity to rechallenge depended solely on $CD8^+$ αβ T cells, whose ability to kill tumor cell targets in vitro was greatly enhanced if the tumors expressed Rae-1β or H60. These findings show that NKG2d is directly involved in the activation of NK cells, and that it also acts as a costimulatory receptor (signal 2) for the αβ TCR (signal 1), helping to enhance T cell activation. Consistent with this notion, NKG2d bound to MICA delivers a costimulatory signal that substitutes for the costimulatory molecule CD28 on human $CD8^+$ αβ T cells (*9*). The fact that both NKG2d and CD28 (but not TCR) activate PI 3-kinase further implies that NKG2d is a costimulatory receptor.

If NKG2d ligands mediate NK cell– and T cell–dependent tumor rejection, why then do tumors that naturally express these ligands grow in the host to begin with? Presumably, those tumor cells that do survive are able to balance the expression of activating versus inhibitory ligands such that immunologic tolerance dominates sufficiently to protect them. Consistent with this idea, the amount of Rae-1 or H60 in the experimental tumors of Diefenbach *et al.* was much higher than in tumors that naturally express these ligands. That naturally progressing tumors manipulate their expression of inhibitory and activating ligands in favor of immune tolerance reveals a new strategy for cancer immunotherapy. Selective up-regulation of NKG2d ligands on tumors (either by in vivo gene transfer or with drugs that up-regulate endogenous expression) may enable the balance to be shifted from immune tolerance back to NK cell and T cell activation. Given that $CD8^+$ T cells primed by Rae-1–transduced tumors could reject a subsequent challenge with Rae-1–negative tumor cells, induction of NKG2d ligands on only a subset of tumor cells might be sufficient to achieve antitumor immunity.

Apart from manipulating the immune response to tumors, NKG2d may be crucial for immune detection of precancerous epithelial cells in the skin and intestine, according to Girardi *et al.* (*1*) and others (*10*). Both skin and gut contain unique resident T cells termed intraepithelial lymphocytes (IELs). In the mouse, the majority of epidermal IELs and roughly half of intestinal IELs are γδ T cells (the other half of the intestinal IEL population are $CD8^+$ αβ T cells). The γδ TCR is normally expressed by <2% of T cells in blood and secondary lymphoid tissues. Human intestinal IELs (and possibly IELs in human skin) are enriched in γδ T cells (*11*). Remarkably, both epidermal and intestinal IELs display unique and extremely nonpolymorphic V region repertoires, suggesting specificity for a limited set of antigens selectively expressed in their respective epithelial compartments (*12, 13*). Although not yet fully characterized, these antigens may be self antigens that are expressed by resident epithelial cells or keratinocytes during infection or stress. The γδ IELs may be a "missing link" between the innate and adaptive immune systems, commandeering the genetic machinery of the adaptive immune response (that is, TCR gene rearrangements) to generate receptors specific for molecular patterns common to stress and infection. Girardi *et al.* (*1*) and others (*10*) find that both epidermal and intestinal γδ IELs express NKG2d and become activated when NKG2d binds to Rae-1, H60, or MICA/B.

Girardi and colleagues show that γδ T cells are crucial for immune surveillance against malignant epidermal cells (*1*). Reminiscent of recent immune surveillance studies in RAG- and IFNR-deficient mice (*14*), the incidence of cutaneous malignancies after treatment with a combination of initiator and promoter carcinogens increases in mice lacking δ TCR. Furthermore, Rae-1 and H60 expression is negli-



Fighting on the frontline. Immune surveillance by intraepithelial lymphocytes (IELs) activated by the NKG2d ligands Rae-1 and H60. Two epithelial compartments, skin (top) and intestine (bottom), are prime targets for pathogen invasion and for tumor formation in response to genotoxic stresses such as UV light (skin) or gut-derived carcinogens (gut epithelium). These and other stress stimuli induce expression of the NKG2d ligands Rae-1 and H60 in mice, and MICA and MICB in humans. (**Top**) Skin IELs expressing the γδ TCR are activated by a combination of ligands that engage NKG2d and the γδ TCR (ligand not yet identified). Activated γδ IELs kill cells that express Rae-1, H60, or MICA/B before they become established as tumors. (**Bottom**) In addition to the γδ IELs that recognize the Rae-1, H60, or MICA/B expressed by stressed gut epithelial cells, αβ IELs may recognize peptide–MHC class I complexes on stressed intestinal epithelium.

gible in normal skin but increases dramatically in skin treated with the carcinogen combination, and is even higher at sites where papillomas and carcinomas eventually form (*1*). Killing of Rae-1– and H60-positive skin cells by γδ IELs requires both NKG2d and γδ TCR, suggesting that engagement of NKG2d with its ligand provides the costimulatory signal to the TCR. It remains to be determined whether αβ IELs, which also express NKG2d, behave in a similar way to γδ IELs.

These results suggest that although the ligands for γδ TCR may be constitutively expressed, it is the inducible ligands for NKG2d that are the critical sensors of stress, costimulating IELs with the rapid kinetics characteristic of innate immunity (see the figure). The nature of the stress stimuli that induce the expression of NKG2d ligands has yet to be fully characterized. From the standpoint of tumor surveillance by the immune system, the relevant stressors—UV light in the case of skin, and gut-derived carcinogens in the case of intestinal epithelium—are both likely to be genotoxic (to damage the DNA of cells). Elucidating the stimuli and signals that induce expression of activating ligands for these different IEL populations will shed light on how regional immune networks protect specific epithelial tissues, our body's frontline defense against tumors and pathogens.

References

1. M. Girardi *et al.*, *Science* **294**, 605 (2001); published online 20 September 2001 (10.1126/science.1063916).
2. A. Diefenbach *et al.*, *Nature* **413**, 165 (2001).
3. L. L. Lanier, *Annu. Rev. Immunol.* **16**, 359 (1998).
4. A. B. Bakker *et al.*, *Hum. Immunol.* **61**, 18 (2000).
5. J. Wu *et al.*, *Science* **285**, 730 (1999).
6. A. Cerwenka *et al.*, *Immunity* **12**, 721 (2000).
7. A. Diefenbach *et al.*, *Nature Immunol.* **1**, 119 (2000).
8. A. Cerwenka *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 11521 (2001).
9. V. Groh *et al.*, *Nature Immunol.* **2**, 255 (2001).
10. V. Groh *et al.*, *Science* **279**, 1737 (1998).
11. W. Holtmeier *et al.*, *J. Invest. Dermatol.* **116**, 275 (2001).
12. D. Asarnow *et al.*, *Cell* **55**, 837 (1988).
13. T. Goodman, L. Lefrancois, *Nature* **333**, 855 (1988).
14. V. Shankaran *et al.*, *Nature* **410**, 1107 (2001).

Published online 20 September 2001;
10.1126/science.1066284
Include this information when citing this paper.

PERSPECTIVES: MOLECULAR ELECTRONICS

It's All About Contacts

K. W. Hipps

The goal of building sophisticated electronic devices from individual molecules has spurred studies of single-molecule rectification (*1*), nanotube-based transistors (*2*), and negative differential resistance from small collections of molecules (*3*). The primary problems facing the molecular electronics designer are measuring and predicting electron transport. Molecular electronics will also require reliable molecular wires to carry signals from one molecular circuit element to another.

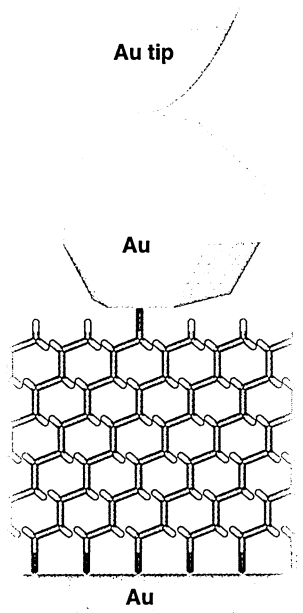
A key requirement in all these studies is the ability to measure the conductivity of a single molecule. To do so, we must connect a macroscopic current source and volt meter to each end of a single molecule. Molecular electronics is thus very much about contacts. Ideally, these contacts should be ohmic so that any nonlinearity in the conductivity of the wire can be correctly attributed and studied. They must also be low in resistance to ensure that the properties measured are those of the molecule and not those of the molecule-contact interface. Moreover, the medium surrounding and supporting the molecule must be several orders of magnitude more insulating than the molecule itself because the contact area of the support with the electrical contacts is often much greater than that between the electrical contacts and the molecule.

To see how hard it can be to determine the conductivity of an individual molecule, consider the case of DNA. Depending on the study, one finds that DNA is an insulator (*4*), semiconductor (*5*), conductor (*6*), or proximity-induced superconductor (*7*).

Dunlap *et al.* (*4*) used a scanning tunneling microscope to image DNA that was held, segment wise, onto a Pt/C-coated surface by patches of Pt/C overcoating. They were able to follow strands of λDNA between and through the coated segments. The uncoated regions had such high resistance that they appeared in negative contrast. The authors estimated a resistivity of 10^5 ohm-cm and concluded that DNA is a good insulator. However, this measurement was more about metal-DNA current injection and short-axis conductivity than about the commonly considered long-axis conductivity through π orbitals in adjacent base pairs.

Porath *et al.* (*5*) studied conduction through a single, 10.4-nm-long, double-stranded DNA oligomer trapped between Pt electrodes. They observed wide band-gap semiconductor-like behavior. An unexpected result was the widening of the gap with increasing temperature. The authors state that the nature of the contact resistance between the metallic electrode and DNA is not well understood and suggest that there is no good metallic contact.

Fink and Schoenenberger (*6*) imaged λDNA ropes assumed to consist of a few double-stranded DNA molecules as they made electrical contact with a sharp tungsten probe. The DNA ropes were suspended across holes in a gold-coated carbon grid and imaged with a low-energy electron point source. A tungsten tip was then used to make contact with a rope and sever it, thereby producing a conduction path from the tip through the rope to the gold-coated grid. The authors estimate a resistivity of 1 milliohm-cm for a single DNA double strand and conclude that DNA is a conductor. The authors clearly have excellent mechanical contact between DNA and tip, but it is not clear that they have good electrical contact. Thus, their measurement is an upper limit for the resistivity. They solve the problem of stray parallel currents by keeping the strands in vacuum during the study.



Molecular solder? Cui *et al.*'s (*13*) experiment allows reliable measurements of molecular conductivity for a range of different molecules.

Very recently, Kasumov *et al.* (*7*) have studied the conductivity of 16-μm-long strands of λDNA combed across a 0.5-μm gap between Re/C electrodes. Their measurements were on small sets of molecules (about 10) rather than a single molecule. The resistance per molecule was less than

The author is in the Department of Chemistry and the Materials Science Program, Washington State University, Pullman, WA 99164, USA. E-mail: hipps@wsu.edu