Asian Land Mammal Age (ALMA), traditionally viewed as late Paleocene. The first Asian primates and artiodactyls show up in the succeeding Bumbanian ALMA, widely regarded as early Eocene.

However, all hypotheses about how the Gashatan and Bumbanian ALMAs relate to the P/E boundary are based purely on fossils. Paleontologists have been forced to estimate the ages of Gashatan and Bumbanian fossils on the basis of the fossils themselves, and no consensus has emerged on how to do this (*3*, *7*–*8*). In the absence of independent geochronological evidence, it is difficult to test the possibility that Asia was a Paleocene "Garden of Eden" for many groups of modern mammals (*9*).

Bowen *et al.* (*1*) take a crucial first step toward untying this Gordian knot, thereby illuminating the processes that underlie the biotic turnover at the P/E boundary. Their solution is based on a geochemical anomaly that provides a datum for P/E boundary strata worldwide, much like the well-known iridium anomaly at the Cretaceous/Tertiary boundary. Marine and terrestrial strata near the P/E boundary have revealed a consistent negative spike in the $^{13}C/^{12}C$ ratio, known as the carbon isotope excursion (CIE). The CIE is attributed to large-scale "melting" of oceanic methane hydrates, which would have released massive volumes of methane and its oxidation product CO_2 into Earth's atmosphere (*10*). Hence, the CIE is indicative of intense global warming at the P/E boundary.

In the Bighorn Basin, the incoming wave of Eocene mammals shows up about 10,000 years after the peak of the CIE (*11*). Bowen *et al.* (*1*) now document the CIE in Asia, in an area in China's Hunan Province that is less densely fossiliferous than the Bighorn Basin. Their results show that the Bumbanian ALMA is at least as old as the CIE. The hyaenodontid and perissodactyl specimens from the underlying Gashatan ALMA must therefore be Paleocene in age, older than any similar fossils from North America and Europe.

The new geochronological data of Bowen *et al.* (*1*) strengthen Asia's claim as the birthplace of numerous modern groups of mammals, including our own order, the Primates (see figure) (*7*, *9*). They also point toward global warming as the driving force behind the most profound biotic reorganization of the Age of Mammals. Further field work is needed to clarify whether the biogeographic dominance of Asian mammals at the P/E boundary extends to other organisms and across a longer time interval. Such efforts will go a long way toward integrating the diverse fields of climate change, phylogenetics, geochronology, and biogeography.

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PERSPECTIVES: IMMUNOLOGY

A Perfect Mismatch

Klas Kärre

Patients with leukemia or certain genetic diseases can be treated by transplantation of bone marrow or hematopoietic stem cells. The patient's own bone marrow (and leukemia cells residing in the marrow) are destroyed by irradiation or cytotoxic drugs and are replaced with marrow or stem cells from a donor (allogeneic transplantation). As in organ transplantation, there is a risk that the immune system of the recipient will reject the transplant, termed a host-versus-graft (HvG) reaction. However, because the donor graft gives rise to a new immune system in the recipient, hematopoietic cell transplantation also involves the risk of a tissue-damaging reaction in the opposite direction, called graft-versus-host (GvH) disease. GvH can also be favorable for the patient, as it may help to eradicate residual leukemia cells (the graft-versus-leukemia or GvL effect). T cells of the immune system, which recognize differences between individuals in HLA molecules of the major histocompatibility complex (MHC), are crucial mediators of these reactions.

In a model example of preclinical-to-clinical translational research, Ruggeri *et al.* (*1*), reporting on page 2097 of this issue, demonstrate that another cell of the immune system can influence the outcome of hematopoietic cell transplantation in a surprising and favorable way. So-called natural killer (NK) cells in the donor graft can prevent leukemia relapse in leukemic recipients, and at the same time seem to prevent the destructive complications of GvH and HvG.

One normally tries to avoid complications during hematopoietic cell transplantation by matching the HLA tissue type of donor and recipient at as many loci as possible. The chance that a sibling carries the same HLA type as the patient (that is, has inherited the same combination of HLA genes from the mother and father) is 25%. In many cases there is no familial match, nor can a matching unrelated donor be easily found. In such situations, it is possible to transplant tissue from a family member for whom only one of the HLA-carrying chromosomes is matched with that of the recipient (haploidentical transplantation). Such a partial match is provided by either parent of the patient, and by 50% of the siblings. T cells do not react to the self HLA proteins but do recognize the presence of nonself HLA. In the haploidentical match, there will thus be strong reactivities against the nonmatched HLA molecules, and therefore the graft must be exhaustively depleted of T cells. T cell depletion dramatically reduces GvH reactions, but at the price of a higher incidence of leukemia relapse because the GvL effect is reduced.

The rationale for the Ruggeri *et al.* study in leukemia patients and mice was that NK cells should also influence hematopoietic cell transplantation in a manner determined by HLA, albeit by different rules. It is well established that NK cells can be activated by sensing the absence of self MHC on other cells (missing self recognition) (*2*). They express killer cell inhibitory receptors (KIRs) that recognize self HLA molecules on target cells (*3*, *4*). The receptors then transmit an inhibitory signal that cancels a program for cytotoxic action previously triggered by contact with the target cell. In the absence of the inhibitory signal, cytokine secretion and killing of the target cell proceeds by default. Furthermore, NK cells distinguish groups of HLA molecules rather than single allelic variants. This meant that Ruggeri *et al.* could classify their haploidenti-

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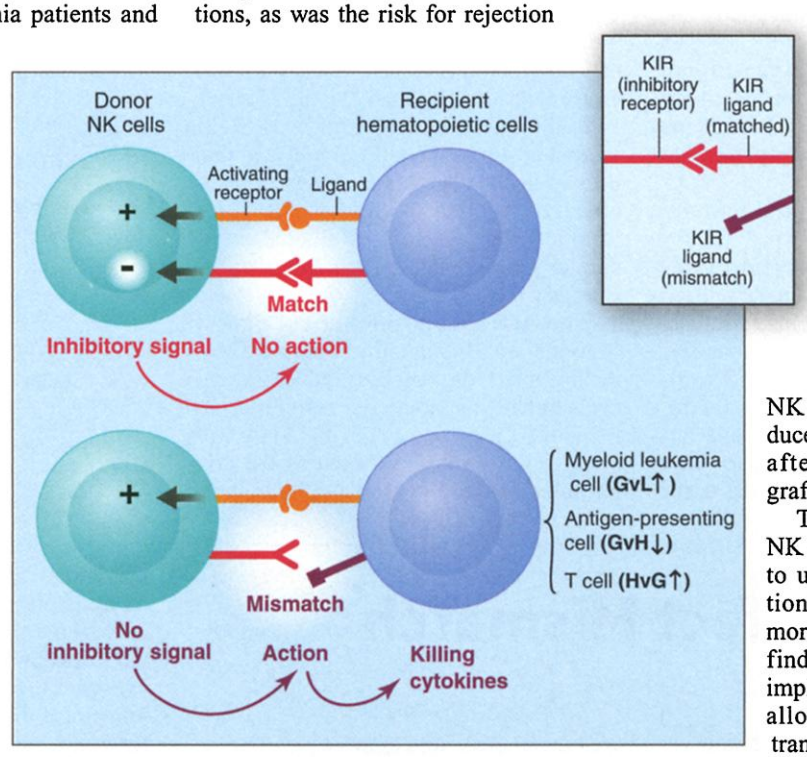
cal transplants into at least two categories on the basis of HLA typing: (i) donor-recipient combinations where NK cells in the graft would sense missing HLA molecules in the host and thus would be mismatched and alloreactive in the GvH direction, and (ii) matched combinations where this would not occur. Ruggeri and colleagues wanted to find out if alloreactive NK cells in the hematopoietic cell graft could affect the outcome of transplantation in acute leukemia patients and in mouse transplant models. Previous research had established that MHC class I molecules control rejection of bone marrow grafts by host NK cells in mice (*5*). But what about the reactivities in the opposite direction, GvH and GvL, and what about the corresponding reactions in humans in a clinical situation?

Ruggeri *et al.* found that a mismatch in appropriate HLA groups—NK KIR ligands, to be more precise—between host and donor tissue did matter. The most impressive influence, associated with a mismatch in the GvH direction, was a dramatically reduced risk of relapse in acute myeloid leukemia (AML) patients. Ruggeri *et al.* interpret this finding as involvement of graft NK cells in a GvL effect. Even if there was no direct evidence for a role for NK cells, several observations support their interpretation. First, the relevant HLA mismatches correlated strongly with the ability to generate donor alloreactive NK cell clones in vitro that killed hematopoietic cells from the recipient. Second, these alloreactive NK cell clones could also kill leukemia cells from AML patients. In acute lymphatic leukemia patients, NK cells could not kill the malignant cells, and in this situation the mismatch in the GvH direction was not associated with reduced risk of relapse. Third, transfer of human NK cells to mice could eradicate previously transplanted AML cells as long as there was an appropriate HLA (KIR ligand) mismatch. Previous work in mice (not involving a KIR ligand mismatch) also supports the notion that NK cells can mediate a GvL in the absence of a GvH effect (*6*).

Given these strong indications of a GvL effect, one might also expect a less favorable side of the coin in the same donor-recipient combinations: NK cell–mediated destructive GvH reactivity against other host tissues. Surprisingly, the risk for generalized GvH was if anything reduced in these combinations, as was the risk for rejection (HvG). The interpretation of this finding is that NK cells predominantly attack the hematopoietic cells of the host. This means that NK cells would spare epithelial cells of host tissues, which are targets in GvH disease. NK cells would, however, attack bone marrow–derived antigen-presenting cells, thus preventing these cells from presenting host antigens to graft T cells, the step that initiates GvH. The NK cells would also attack host T cells, thereby preventing them from rejecting the graft.



The beneficial effect of natural killer (NK) cells on the outcome of partly HLA-mismatched (haploidentical) hematopoietic cell transplantation. **(Top)** When donor and recipient tissues are matched for KIR ligands—which are subgroups of HLA-A, -B, and -C alleles—NK cells can sense the appropriate HLA molecules and are inhibited from further action. **(Bottom)** In the case of a KIR ligand mismatch, the KIRs of NK cells do not engage ligand and the action initially elicited by activating receptors proceeds. NK cell attack of leukemia cells may explain the graft-versus-leukemia (GvL) effect, whereas NK cell attack of host antigen-presenting cells may explain why there is less of a graft-versus-host (GvH) reaction against nonhematopoietic tissues of the host. Attack of host T cells by NK cells may explain how NK cells prevent rejection of the graft by the host and suggests that the patient may benefit from receiving donor NK cells as part of a pretransplant conditioning regimen. NK cells express several types of activating and inhibitory receptors, and so there may be other processes that affect GvL, GvH, and HvG. T cells can also affect these reactions.

The Ruggeri *et al.* group went back to their mouse colony and obtained evidence to support their conclusions, using the mouse counterpart of KIR, inhibitory receptors of the Ly49 family (*7*). They even took the concept one step further: If NK cells indeed reduce the risk for rejection and GvH by attacking host immune cells, it should be possible to use NK cells as a supplement in the pretransplant "conditioning" of leukemia patients. The authors were able to show in their mice that, provided NK cells had a relevant inhibitory receptor and came from a mouse strain with a relevant mismatch in H-2 (the mouse counterpart of HLA), then pretransplant transfer of donor NK cells to recipient mice reduced the risk of GvH and HvG after transplant of the donor graft.

The pretransplant transfer of NK cells allowed Ruggeri *et al.* to use a less aggressive conditioning treatment and to retain more T cells in the graft. These findings have important clinical implications because they could allow safer hematopoietic cell transplantation, even in patients who would not be able to tolerate the usual conditioning regimen. The authors also show that transfer of alloreactive NK cells after transplantation could help to establish the bone marrow graft. These findings may have a major impact on new clinical procedures, such as donor lymphocyte infusions and "mini-transplants" where transfer of blood lymphocytes without conditioning is being used to treat malignancies (*8*).

The Ruggeri *et al.* study reveals several ways in which NK cells can be exploited in transplantation and immunotherapy. One seemingly paradoxical consequence of the new work is that tomorrow's typing of donor hematopoietic cells or lymphocytes may involve a deliberate search for the "perfect mismatch" at certain HLA loci—that is, for the mismatch that drives favorable NK cell–mediated effects. Further research must center on proving the beneficial effects of NK cells in transplants with ap-

propriate host-recipient HLA mismatches. An important issue is to understand why the NK cells mediate GvL rather than GvH. Is this a reflection of their primary engagement with bone marrow–derived cells, or simply with any blood-borne cells? Are other malignant cells (such as blood-borne metastatic tumor cells) also targeted by NK cells, as evidence from numerous studies in mice would suggest, and if so, which molecules are involved? How can the receptor repertoire of NK cells eventually be adapted to the HLA phenotype of the host? A possible answer to the last question is provided by Wang *et al.*'s work (*9*), also reported in this issue on page 2094. These authors have investigated the signaling pathway associated with mouse Ly49 inhibitory receptors that is known to involve the Src homology 2–containing inositol phosphatase SHIP. They show that this phosphatase is important for shaping the NK cell receptor repertoire during development, possibly by controlling the survival of different NK cell subsets.

We look forward to seeing how these new findings translate to the clinical setting, where hopefully NK cells will improve the outcome of hematopoietic cell transplants for patients with leukemia and other malignant diseases.

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PERSPECTIVES: MICROBIOLOGY

A Tail of Two Specifi-cities

Graham F. Hatfull

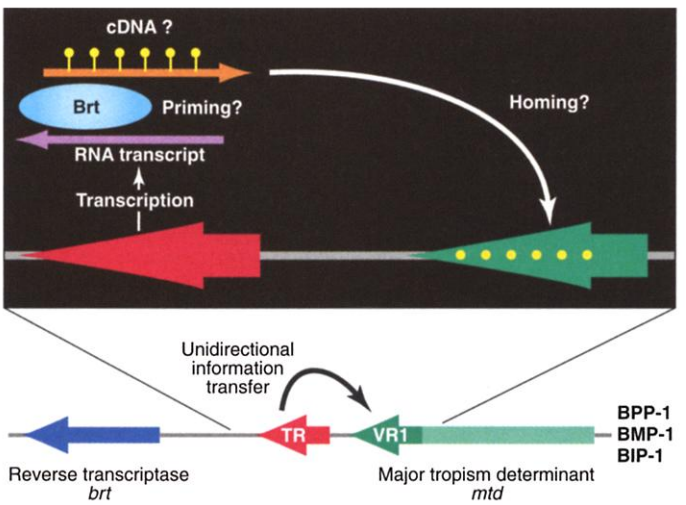
In the world of microbial biowarfare, standing still in the face of a ferocious viral attack is a recipe for disaster. Various subspecies of *Bordetella* bacteria, which cause respiratory illness in mammals including whooping cough in humans, must withstand attack by bacteriophage viruses such as BPP-1. They accomplish this by switching between two distinct phases: One (Bvg^+) expresses virulence genes and colonizes the respiratory tract of hosts, and the other (Bvg^-) is better adapted for growth outside of a host (*1*). Among the genes whose expression is altered on switching between these two phases is *prn*. This gene encodes an adhesion protein, pertactin, which is the receptor for the bacteriophage BPP-1. Thus, BPP-1 infects only the Bvg^+ strain of *Bordetella* that expresses pertactin and not the Bvg^- strain.

But not so fast. On page 2091 of this issue, Liu *et al.* (*2*) show that BPP-1 is not so easily fooled. Remarkably, when faced with this switch in bacterial phenotype, one in a million phage will change tropism to be able to infect the Bvg^- strain. If the bacterium should change back to the Bvg^+ state, then the phage can revert to its original tropism. Because the range of bacterial hosts that a phage infects is typically determined by the nature of its tail fibers, it is not uncommon to find marked variation among phage tail fiber proteins. Some phages use dedicated site-specific recombination systems to switch between alternative tail fiber proteins (*3*). The tropism change in BPP-1 involves a putative tail fiber protein (Mtd), but this change is not dependent on a simple binary switch, as Liu *et al.* show (see the figure) .

Phage BPP-1 normally infects the Bvg^+ but not the Bvg^- strain of *Bordetella*. However, it can assume two additional trophic states: BMP, which infects Bvg^- but not Bvg^+, and BIP, which infects both bacterial strains. The three trophic states of the phage are interconvertible, and switching from one state to another is accompanied by base substitutions in a 134–base pair (bp) segment of the phage genome. This segment, called the variable region (VR1), encodes the carboxyl-terminal 44 amino acids of the *mtd* gene product.

Base substitutions in VR1 are not random but occur predominantly at 22 positions. Most of the base substitutions are in either the first or second positions of codons, and so lead to changes in the amino acid sequence of the Mtd protein. The phage has devised a way to restrict mutations to the 134-bp region, thus avoiding changes that would be deleterious. Just downstream of the *mtd* gene is a nearly identical copy of the 134-bp region, the template repeat (TR), which provides sequence information that is incorporated into the *mtd* gene. Interestingly, all of the 22 highly variable positions in *mtd* have an adenine in the corresponding position in the TR, and there is only one additional adenine in the 134-bp repeat.

How is the sequence information copied from the TR to the *mtd* gene, and why is this a low-fidelity mechanism? Not all parts of the process are yet known, but it is clear that a phage-encoded reverse transcriptase (Brt) is required (see the figure). This enzyme is similar in sequence to the reverse transcriptases of retroviruses and to those encoded by



Tropism changes in bacteriophage. Phages BPP-1, BMP-1, and BIP-1 are closely related trophic variants that differ in their ability to infect Bvg^- and Bvg^+ strains of *Bordetella* bacteria. The end of the *mtd* gene—highly variable among BPP, BMP, and BIP phages—contains a variable region (VR-1) that is about 90% identical to the 134-bp template repeat (TR) located just downstream. Information from the TR is transferred unidirectionally to the VR1 region of *mtd* in a low-fidelity event that is dependent on the phage-encoded reverse transcriptase Brt. The detailed mechanism of information transfer is not known but may involve sequence-dependent misincorporation during Brt-mediated copying of a TR transcript followed by introduction of the new information into VR1. Priming of synthesis of the TR cDNA could be mediated either by an endogenous primer or directly by VR1 after endonucleolytic cleavage.

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