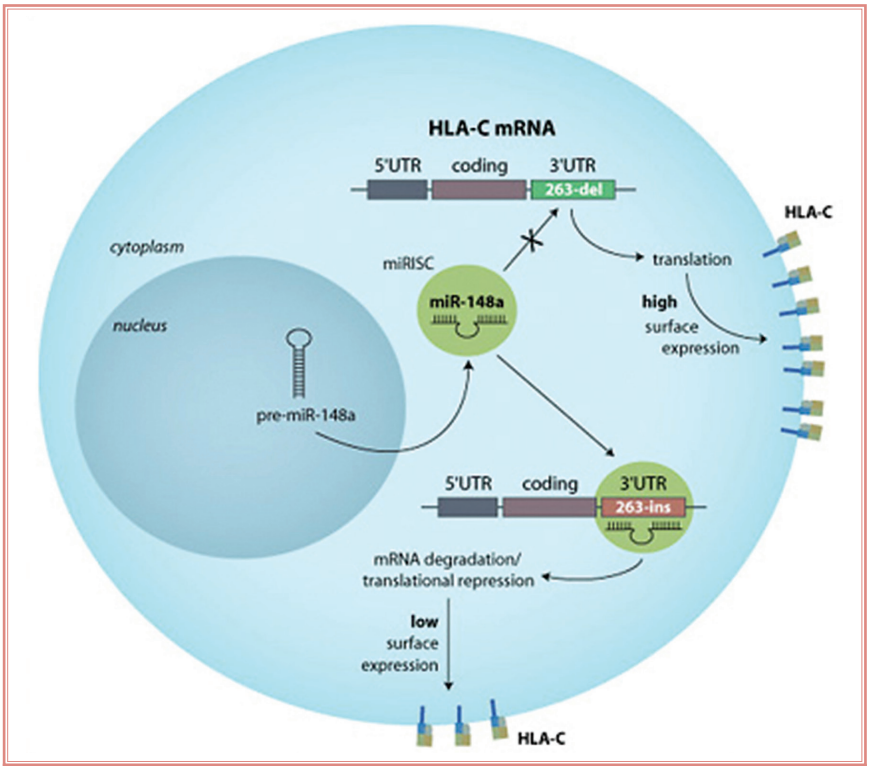
Seeing the Unexpected

Mary Carrington, Ph.D., Senior Investigator in CCR's Laboratory of Experimental Immunology and Director of the SAIC Basic Science Program at the Frederick National Laboratory for Cancer Research, has a talent for seeing unexpected molecular interactions, and for interpreting their implications. While studying the genes that code for human leukocyte antigens (HLAs)—the molecules that distinguish "self" vs. "nonself" on human cells, tissues, and organs—and the role they play in a person's susceptibility to HIV infection, she and her colleagues made a novel discovery. They found that tiny variants called single nucleotide polymorphisms, located within untranslated regions of the HLA-C gene—where microRNAs like to bind—can actually change the amount of "self" molecules produced and displayed.

She and two Postdoctoral Fellows, Smita Kulkarni, Ph.D., who works in her lab, and Ram Savan, Ph.D., who worked with Howard Young, Ph.D., a colleague in the Laboratory of Experimental Immunology, realized immediately that this unexpected post-transcriptional interaction between microRNA and a particular *HLA* variant could be a powerful modulator that regulates *HLA* expression and, in turn, influences host immune responses in human disease, whether triggered by HIV infections, autoimmune diseases, or cancer.

Another Unexpected Interaction

The immune system is a complex network. In many cases, the actions of various components are intertwined, and the effects of one component may be greatly affected by its relationship with a different component. In their study of the host immune responses and diseases, Carrington and her collaborators have made another important and unexpected contribution. They have carefully documented how *HLA* and a completely separate gene cluster, the killer cell immunoglobulin-like receptor (*KIR*) genes, conspire to operate in functionally relevant combinations for good or ill. Working



(*Figure:* M. Carrington, CCR)

Carrington and colleagues have found evidence that a single nucleotide polymorphism (-35 SNP) marks the presence of a variant in the HLA-C microRNA 148a binding site that directly determines whether or not there is an increase in expression of HLA-C.

(*Photo: R. Baer*)

Xiao-jiang Gao, Ph.D., and Mary Carrington, Ph.D.

together, these gene clusters determine how "non-self" invading pathogens or "self" tumors are either tolerated or destroyed by the body's immune system.

KIR molecules, the product of *KIR* genes, are the largest category of receptors expressed on the surface of natural killer (NK) cells. These cells are a key component of the innate immune system, the body's immediate response against foreign invaders and tumor cells. When a foreign cell enters the body, its own HLA class I "self" molecules present a piece of its protein on its surface. The NK cell's KIR receptor binds to it, and sends a signal to the NK cell that activates or inhibits its activity. So by using the invading cell's own HLA "self" markers, KIR molecules help to regulate the NK cell's ability to kill other cells.

Although the *HLA* and *KIR* gene clusters work together, they do not reside together. The *HLA* and *KIR* clusters are located on chromosomes 6p and 19q, respectively, so they are inherited independently. This increases genetic variation, which in turn, creates extensive diversity among HLA class I and KIR molecules. However, to be effective KIR molecules and corresponding specific HLA molecules must be present together to regulate NK cell activity. Therefore, it is not surprising that variation of these gene groups likely influences the risk of a variety of diseases.

> "Over the past several years, we have discovered the breadth and depth of influence exerted by *HLA/KIR* genetic-variant combinations," said Carrington, "but much remains to be discovered, understood, and applied before we can improve the lives of cancer patients and of patients who suffer from infectious diseases."

HLA/KIR Combinations

HLA/KIR variant combinations may have a protective effect. Very strong evidence exists showing that the *HLA/KIR* immune response genes are under natural selection in some regions. "Very different HLA/KIR variant frequencies have been found in particular geographic locations. We believe these frequency differences may be related to the types of diseases endemic to particular regions," said Carrington. "For example, certain HLA/KIR gene forms may be protective against infection with the parasitic protozoan *Plasmodium malariae* in a region where malaria is prevalent, so a higher frequency of

these malaria-protective variants may be found in the population located there. This would probably occur because individuals who inherited the protective variants would live longer and reproduce. Conversely, in other regions of the world, where malaria is not a big problem, there might not be selection pressure for the malaria-protective *HLA/KIR* gene variants."

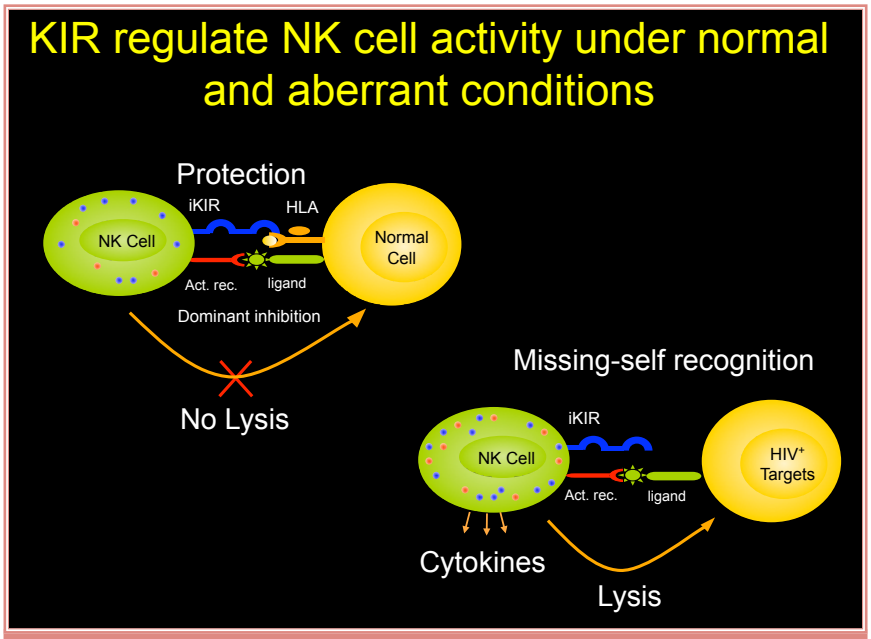
HLA/KIR combinations also play a role in immune surveillance. *HLA* gene variants manufacture products with extensive diversity that serve the immune response well by interacting with an unlimited number of "non-self" fragments. Whether encountering an external pathogen or an enemy from within, such as a cancer cell, the molecules produced by *HLA* variants notify the body's immune system that a response is needed. "*HLA* diversity ensures the survival of our species by providing resistance to a wide breadth of infectious organisms and even the ability to eliminate cancer cells," said Carrington.

The diversity generated when many different types of *HLA* markers on target cells can coexist in concert with many different KIR molecules on NK cells, increases the effectiveness of the immune surveillance system. As the combinations *HLA/KIR* genetic variants change, their products change, and NK cell activity changes, ranging from strong inhibition to strong activation.

Genetic variation plays another important role, too. KIR molecular diversity also produces inhibitory KIR molecules that allow NK cells to identify normal body cells as "self." This prevents autoimmune attacks on healthy autologous ("self") cells.

HLA-C/KIR Combinations and Cancer

Aware that expression levels of *HLA/KIR* variants can influence host immune responses in both human infection and disease, the Carrington team applied their discoveries to better understand a woman's risk for cervical cancer, which is now known to be caused by infections from one of about 15 strains of HPV, most often HPV16.



(*Figure*: M. Carrington, CCR)

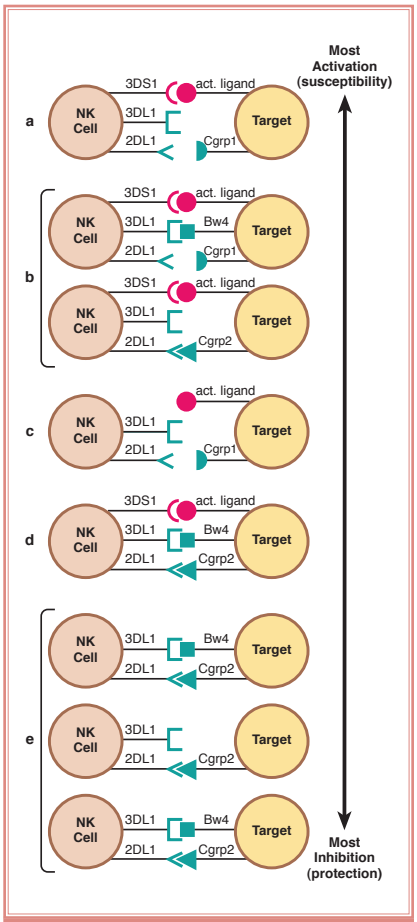
NK cells express KIR on their cell surface that can send either activating or inhibitory signals to NK cells. Inhibitory KIR recognize specific HLA class I molecules on target cells. If these molecules are present, KIR will send a signal to the NK cell not to kill the cell. Some virally infected cells and some tumor cells downregulate HLA class I to escape NK cell recognition. In this instance, the inhibitory KIR will not see its HLA class I counterpart in the normal context and an activating KIR will send a signal to kill the cell because its HLA class I expression is abnormal.

HPV infection is the most common sexually transmitted disease in the nation. While most HPV infections exhibit no symptoms and clear up without treatment, some can persist and lead to cancer. The Carrington team was curious about what constitutes this persistence at the molecular level. Specifically, are certain *HLA/KIR* genotype variants, or genetic variations, involved in a woman's risk of developing cervical cancer?

Carrington and her team have a longstanding collaboration to investigate cervical cancer with Allan Hildesheim, Ph.D., an Investigator in NCI's Division of Cancer Epidemiology and Genetics. In one study, the researchers investigated whether *KIR* genes are involved in the risk of developing cervical neoplasia, a cancer caused by HPV. The two research teams grouped *HLA-B (HLA-B Bw4)* and *HLA-C (HLA-Cw* groups 1 and 2) molecules according to their specificity for certain *KIR* receptors. Having obtained samples from patients in three large cervical cancer studies, the teams compared the presence of the HLA groups in normal and abnormal cervical tissue samples that were classified as intraepithelial neoplasia 3 (CIN3)–also known as stage 0 cervical carcinoma. When they merged data from all studies, Carrington and Hildesheim found that *HLA-Cw* group 2 and *HLA-B Bw*4 groups were significantly associated with a decreased risk of cervical cancer. The presence of both of these alleles, forms of the KIR gene, had a stronger protective effect than the presence of either alone.

"This suggests that these *HLA* class I variants may be exerting influence on cervical cancer based on

(*Image:* M. Carrington, CCR)



Model shows KIR-mediated NK cell types associated with risk of developing cervical neoplasia.

Resistance to cervical neoplasia increases when genotypes are ordered by their ability to confer the most activation (susceptibility) to the most inhibition (protection). The red shapes represent activating receptors and HLA class I molecules; the green shapes represent inhibitory receptors and HLA class I molecules.

their capacity to bind inhibitory KIR molecules, which regulates NK cell activity," said Carrington. By contrast, the presence of an activating KIR receptor, KIR3DS1, was found more frequently in CIN3 tissue samples than occurred in noncancerous cells. So, activating and inhibitory KIR molecules appear to be involved in a woman's risk of developing cervical cancer.

Allelic groups of *HLA* whose gene products bind KIR3DS1 molecules could trigger an immune response, while those produced by *HLA-Cw* group 2 and *HLA-B Bw4* allelic groups could bind to KIR2DL1 and KIR3DL1 molecules, respectively, and avert immune activation. "Therefore, it appears that *KIR*-associated immune activity is linked to cervical pathogenesis," said Carrington.

Aware that only a few HPV infections can persist and lead to cancer, the Carrington team developed a model to explain this observation in molecular terms. They suspected that there is a gradation of influences, ranging from *HLA/KIR* genotype combinations that are most activating to ones that are most inhibitory. Then they discovered examples of just that—inhibitory HLA/KIR molecule pairs that decrease the risk of developing cervical cancer and the presence of the activating receptor KIR3DS1—particularly when not accompanied by *HLA-Cw* group 2 and *HLA-B Bw4* groups—that results in susceptibility to cervical cancer.

Variants Are Complicated

Genetic epidemiological studies show that compound genotypes expected to result in immune-activating phenotypes may be associated with protection against a particular infectious disease, and may also be associated with susceptibility to autoimmune disease. Genotype is the internal inheritable allelic information within a person. Phenotype is the external physical manifestation of all the inheritable allelic information within a person. Overall, Carrington and her collaborators have found a general trend for the association of an overactive KIR component with a favorable outcome in infectious diseases, but with an unfavorable result in autoimmune disease and cancer.

HLA-C/KIR and Cervical Cancer Risk

Continuing their collaboration with Hildesheim, Carrington and her team are studying a large collection of CIN3/cervical cancer samples from the Guanacaste Natural History Study (NHS). NHS is a population-based cohort of 10,000 women from Guanacaste, a rural province of Costa Rica with a high incidence of invasive cervical cancer. Cervical and blood samples were obtained and HPV tests were conducted annually for over seven years.

Having these cervical samples available now permits the Carrington team to expand on their earlier discovery of the interaction between untranslated regions of the *HLA-C* gene and microRNAs in HIV infection. Having discovered that microRNA post-transcriptional activity with *HLA-C* can actually change the amount of "self" molecules, the team can now study *HLA-C* variants and their interactions with KIR genotypes in relation to cervical cancer risk.

"In particular, my colleagues and I are now interested in identifying all the factors that can influence levels of *HLA-C* expression in cervical tissue because we have evidence that there is a decreased risk of cervical neoplasia when strongly inhibitory *HLA-C/KIR* combination genotypes are present," explains Carrington. "Over the past several years, we have discovered the breadth and depth of influence exerted by *HLA/KIR* genetic-variant combinations," said Carrington, "but much remains to be discovered, understood, and applied before we can improve the lives of cancer patients and of patients who suffer from infectious diseases."

To learn more about Dr. Carrington's *research, please visit her* CCR Web *site at http://ccr.cancer.gov/staff/staff.asp?*Name=carrington.