Back to the fold: T cell recognition of HFE, a MHC class Ib molecule that regulates iron metabolism

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Hereditary hemochromatosis (HH), a disease resulting from iron overload, is the most common genetic disorder in the United States, afflicting more than a million Americans. Untreated, HH frequently leads to a middle-aged demise due to the failure of an iron-damaged liver, heart, or pancreas. Those whose ancestors hail from the regions of Europe where the Vikings left their genetic imprint (1) have a 1 in 10 chance of harboring the recessive mutation that is the most common cause of HH. HH was one of the first diseases linked to the major histocompatibility complex (MHC) (2). Inasmuch as the MHC typically encodes proteins like class I molecules that function to present peptides derived from intracellular pathogens to CD8 T cells (T_{CD8+}), it would seem an unlikely locus for an iron metabolism gene. But nature typically works in mysterious ways, and this observation and a number of additional clues eventually led HH sleuths to HFE (3, 4), a gene product that is but one of a number of MHC “class Ib” molecules, molecules highly similar genetically and structurally to class I molecules but with myriad divergent functions. HH results from misfolding of HFE induced by a point mutation that replaces the normal cysteine residue at position 282 with a tyrosine. Despite clear genetic evidence that HFE causes HH in humans, and in mice with targeted deletions in HFE (5), it is unclear exactly how HFE influences iron metabolism (6). Although attention has focused on the known interaction of HFE with the transferrin receptor (7) and possible interactions with other iron-binding proteins, other lines of evidence point to contribution of the cellular immune system to HH (8). In this issue of PNAS, Rohrlisch *et al.* (9) bring HFE back to the immune fold by clearly demonstrating that HFE can be recognized by T cells and that this interaction influences the normal T cell repertoire.

MHC class I molecules consist of a heavy chain with three extracellular domains and a noncovalently bound light chain [β_2 -microglobulin (β_2m)]. X-ray crystallography reveals that the α -1 and α -2 domains of the class I heavy chain form two α -helices and an antiparallel β -sheet that create a relatively snug

groove well suited for binding small molecules. In “classical” class I molecules the groove is typically filled shortly after its synthesis with an 8- to 11-residue peptide derived by proteasomal degradation of whatever gene products (be they of viral or cell origin) are being translated at the time. Classical class I molecules carry these peptides to the cell surface where they are inspected by T_{CD8+} , which are constantly on the prowl to detect cells bearing viral or abnormal cellular (e.g., tumor) peptides. T_{CD8+} bear on their surface a clonally distributed antigen-specific receptor [the T cell receptor (TCR)] that interacts with class I–peptide complexes and delivers a signal that activates the T_{CD8+}

Rohrlisch *et al.* searched for cytotoxic T lymphocyte recognition of HFE.

to release molecules that either kill target cells directly [giving rise to the name cytotoxic T lymphocytes (CTLs)] or modify target cell function in a more subtle manner (e.g., by inducing an antiviral state through release of IFN- γ).

Classical class I molecules (also known as class Ia molecules) are part of an extended family with many relatives that are collectively known as nonclassical, or class Ib, molecules. Some of these, such as HLA-E in humans and Qa-1 in mice, function to regulate natural killer (NK) cells by presenting short peptides just like their classical cousins. Others, such as CD1, bind cellular and bacterial lipids and liposaccharides and present them to T cells. In HFE and other class Ib molecules, such as the mouse thymic leukemia (TL) protein, the closer apposition of the α -helices narrows the groove to an extent that appears to preclude antigen binding (10).

Undaunted by the apparent inadequacies of the HFE-ligand binding groove in antigen presentation, Rohrlisch *et al.* (9) systematically searched for CTL recognition of HFE. Initially, they immunized transgenic mice lacking classical

class I molecules with β_2m -deficient mouse cells expressing human HFE (hHFE) molecule covalently tethered to β_2m . These mice are better able to respond to hHFE than normal mice because the tolerance mechanisms that delete self-mouse HFE (mHFE)-reactive CTLs are not operative in the absence of β_2m , which is required for HFE cell surface expression. Some of the immunized mice developed CTLs that killed cells expressing either HFE- β_2m single-chain molecules or normal noncovalent HFE- β_2m heterodimers. Crucially, CTL activity was blocked by the addition of anti-HFE antibody, directly implicating CTL recognition of hHFE. The authors then used HFE-knockout mice to demonstrate that CTLs could also be induced to mHFE. Recognition of mHFE occurred independent of CD8, which is used by classical CTLs to focus the TCR onto class Ia molecules, and by TL as a ligand on T cells.

An organism's TCR repertoire must have the capacity to recognize its set of class Ia molecules bearing peptides from all of the pathogens the host might encounter. This is even more difficult than it might first appear, because the class I genes are the most polymorphic known in vertebrates; for each locus there are typically hundreds to thousands of alleles present in populations. In humans, the TCR repertoire encompasses $\approx 10^{11}$ T cells expressing 10^8 different TCRs. TCRs consist of two noncovalently bound chains (α and β). TCRs are generated via somatic mutation during the rearrangement of families of 50 α -variable (αV) and 20 β -variable (βV) genes with smaller gene segments that are then fused with the constant region of each chain. Unlike class Ia molecules, which can be recognized by T cells expressing any of the V genes, CTL recognizing mouse and human HFE are much less diverse: indeed, all of the hHFE-reactive CTLs studied express the TCR $\alpha V14$ – $\beta V2$ combination. The recognition of HFE by this TCR was elegantly confirmed by transducing a T cell line with the $\alpha V14$ and $\beta V2$ chains and demonstrating that the cell was now activated by cells expressing HFE.

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Rohrlich *et al.* then used this hard-won information in an insightful manner by demonstrating that targeted deletion of *HFE* in mice is associated with a decrease in the number of T cells expressing α V6 TCRs. This finding establishes that mHFE participates in the selection of the TCR repertoire. Although this is not unprecedented (CD1 is also known to shape the TCR repertoire), it is an entirely unexpected finding that suggests a more general role for HFE regulating immune responses.

These remarkable findings raise a number of intriguing questions. First, because the α -helices in HFE are in closer proximity than those in classical class I molecules and lack any cargo in between, what is the basis for TCR cross-reaction between HFE and class Ia molecules? The authors suggest that recognition is based primarily on the interaction of TCR α -chains with HFE, and they propose this as a model for peptide-independent recognition of class Ia molecules by alloreactive T cells, i.e., T cells specific for MHC-mismatched class Ia molecules that participate in organ rejection.

Second, do T cells with HFE-specific TCRs play a role in iron metabolism?

Rohrlich *et al.* suggest that activation of HFE-specific T cells may release cytokines such as IL-6 and TNF- α that induce hepatic synthesis of hepcidin, a polypeptide hormone that decreases tissue availability of iron (11). One of the principal sites of expression of HFE is

HFE participates in the selection of the T cell receptor repertoire.

the small intestinal epithelium, where dietary iron is absorbed. The small intestinal epithelium is also home to large numbers of intraepithelial lymphocytes (IELs), whose functions remain a mystery despite intensive investigation. Could some of these IELs be HFE-specific T cells that participate in regulating iron absorption?

Third, does HFE have other roles to play in HH and cellular immunity? A recent publication (12) demonstrates

that the HFE Cys-282 to Tyr mutation reduces expression of class Ia molecules by interfering with normal loading of peptides in the endoplasmic reticulum, which could alter cellular immune recognition of HFE-expressing cells and contribute to the HH phenotype. More generally, could HH play a completely unexpected role in the biogenesis of class Ia molecules in cell types that express HFE? Does a broader role for HFE in cellular immunity account for the interest taken in HFE by cytomegalovirus, whose US2 protein induces the rapid degradation of HFE (13)?

Although the present clues are tantalizing, much remains to be learned about the extent to which HFE function is truly integrated with that of the cellular immune system. Still, the HFE saga reminds us that nature abhors strict categorization but rather generously exploits multitasking to accomplish the tasks at hand using relatively limited number of gene products.

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