HLA in sequence

By Lauren Martz, Staff Writer

A group of Australian researchers and an FDA-led team have independently identified a mechanism behind human leukocyte antigen allele-mediated autoimmune reactions to certain drugs.^{1,2} The findings could expand the use of human leukocyte antigen genotyping in clinical trials, patient care and drug design to improve therapeutic outcomes and safety.

Meanwhile, a team from **Stanford University** has published a new technology that could improve the accuracy and reduce the cost of human leukocyte antigen (HLA) genotyping.³ Improved HLA genotyping technology could help facilitate patient screening for known drug-HLA interactions and could help identify new interactions (*see* Box 1, “Human leukocyte antigen genotyping”).

HLAs are proteins that present pathological antigens to T cells to induce an adaptive immune response. The *HLA* genes that encode the proteins are highly polymorphic, with most of the diversity originating at the cleft where HLA interacts with and binds antigens. The binding region controls the selection of pathological or self antigens that the HLA molecules present to T lymphocytes.

Autoimmune adverse drug reactions—such as abacavir hypersensitivity syndrome in patients expressing the major histocompatibility complex class I B 5701 (HLA-B 5701) allele and Stevens-Johnson syndrome in carbamazepine-treated patients expressing HLA-B 1502—have been associated with the expression of specific *HLA* alleles, although the underlying mechanisms were murky.

GlaxoSmithKline plc markets Ziagen abacavir to treat HIV/AIDS. **Novartis AG** markets Tegretol carbamazepine to treat epilepsy.

In a paper published in *Nature*, an Australian team led by Anthony Purcell, Jamie Rossjohn and James McCluskey showed that the two drugs bound the antigen-binding sites of specific HLA alleles and altered the repertoire of antigen proteins that were bound. The result was the presentation of self antigens to T cells, which in turn induced an autoimmune reaction.

Purcell is associate professor and senior research fellow in the

Box 1. Human leukocyte antigen genotyping.

A **Stanford University** team has developed a new human leukocyte antigen (HLA) sequencing method that could be more cost effective and accurate than existing technologies to extend the applications of HLA genotyping, which has previously focused on matching bone marrow donors and recipients.

HLA polymorphisms are clinically relevant because specific alleles have been associated with various autoimmune diseases including multiple sclerosis (MS), celiac disease, rheumatoid arthritis (RA) and type 1 diabetes.^{4–7} Additionally, matching HLA genotypes is essential for host acceptance of bone marrow transplants.⁸

Despite the clear importance of knowing a patient's HLA genotypes, the actual sequencing is expensive and time consuming. Most methods exclusively sequence the antigen-binding cleft because it is the most variable, but this can lead to undetected sequence variation in other regions of the *HLA* genes.

In a paper published in the *Proceedings of the National Academy of*

Sciences, Stanford's Michael Mindrinos and colleagues developed a high throughput HLA sequencing method that maps most of the *HLA* genome to reduce or eliminate ambiguity.

The method also identified previously undetected HLA alleles that could be implicated in disease susceptibility or could further improve matching of patient and donor transplant compatibility.

Mindrinos is associate director of the **Stanford Genome Technology Center**. The paper also included researchers from the university and the **Howard Hughes Medical Institute**.

The team developed primers to sequence the *major histocompatibility complex class I A (HLA-A)*, *HLA-B*, *HLA-C* and *major histocompatibility complex class II DR β1 (HLA-DRB1)* genes.

Sequencing the amplified DNA matched 99% of reference DNA samples from 40 cell lines with known HLA genotypes. In 59 clinical samples, sequencing determined HLA genotype and identified 3 new HLA alleles—2 previously

undetected short insertions and 1 single base pair deletion. Genotyping of a few samples can be done within five days.

Mindrinos said the benefits of his sequencing technology are “higher resolution, lower cost and higher throughput. This technology has reduced the cost of HLA typing and improved its accuracy such that it can soon be part of a standard patient profile, equivalent to a blood type.” He told *SciBX* that the method is less expensive because it can analyze more than 2,000 samples per instrument run, which is over 1,000 samples more than competing high throughput sequencing technology.

According to Mindrinos, applications of the technology include matching transplantations, evaluating responses in clinical trials of therapeutics and vaccines, and conducting disease association studies. His group is now working to extend the HLA genotyping method to cover the four other *HLA* genes.

He said Stanford University is filing a patent application. The technology is unavailable for licensing. —LM

Department of Biochemistry and Molecular Biology at **The University of Melbourne**. Rossjohn is senior lecturer in the Department of Biochemistry and Molecular Biology at **Monash University**. McCluskey is deputy vice-chancellor of research at the University of Melbourne.

The paper also included researchers from the **Australian Centre for Vaccine Development at the Queensland Institute of Medical Research** and the **Cardiff University School of Medicine**.

In cultured antigen-presenting cells, abacavir altered the HLA-B 5701 binding peptide repertoire by about 25%, whereas abacavir treatment did not alter the peptide repertoire in cells expressing closely related HLA alleles.

In isolated T cell lines from healthy HLA-B 5701 donors, culture with abacavir plus peptides that bound HLA-B 5701 only in the presence of the drug induced T cell activation, whereas abacavir alone did not activate T cells.

Crystallization studies of abacavir with the HLA alleles and self peptides showed the drug noncovalently bound and altered the shape of the peptide-binding site.

In a separate paper published in May in *AIDS*, an FDA-led team came to a similar conclusion about abacavir's mechanism of HLA-related drug autoimmunity. The group found that abacavir noncovalently bound HLA-B 5701 to alter the antigen-binding site and allow the presentation of self antigens.

To determine whether the phenomenon occurred in other HLA-drug interactions, the Australian team replicated the studies with HLA-B 1502 alleles and carbamazepine. Indeed, the drug bound noncovalently to the antigen-binding cleft and altered the HLA peptide repertoire by about 15%.

Purcell told *SciBX*, "We are currently investigating other drug hypersensitivity reactions that are strongly associated with different HLA haplotypes. The potential of other small molecules to also modulate immune responses via a similar mechanism is a focus of ongoing research."

Michael Norcross, lead author on the *AIDS* paper and lead research investigator at the FDA's Center for Drug Evaluation and Research, told *SciBX* that next steps for the team include developing assays that cover all of the HLA types in the general human population and studying the impact of other drugs on those HLAs to see if any induce changes in the binding sites, the self peptides recognized and the T cell response.

Norcross cautioned that a drug's interaction with HLA molecules does not tell the entire story because not all people with HLA alleles linked to drug reactions actually have the reactions. Thus, future research is required to identify the other factors that determine whether a patient will develop an adverse reaction.

"HLA genotyping technology will allow us to identify patients with certain types of HLA alleles that may be susceptible to drug interactions and will allow us to screen patient populations. This could help guide clinical trial design and patient selection for particular drugs," said Norcross. *AIDS* patients are currently screened for HLA-B 5701 prior to treatment.

He added, "If we see drugs in development or even approved

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**—Michael Norcross,
Food and Drug Administration**

that change an HLA molecule's structural characteristics, we should further characterize the HLA interactions as a first step. What we do with the known risk of reaction will depend on the frequency and severity of the reactions that occur."

The good news, he said, is it should be possible to identify potential drug-HLA interactions very early in the drug development process. "All that we need to do is look at these molecules in assays to see whether they interact with any forms of HLA," he said. "It would also be very valuable to look for interactions between drug metabolites and HLA molecules."

Purcell said it may be possible to modify existing drugs that carry a risk of severe

autoimmune reactions to prevent their binding to HLA molecules.

"The interaction of abacavir with HLA-B 5701 is very specific, and we predict even small changes to the drug may prevent binding to and shifts in the peptide repertoire of this HLA molecule," he said.

The findings by Purcell's team have not been patented and are not available for licensing. Patent and licensing status for the FDA's study is undisclosed.

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COMPANIES AND INSTITUTIONS MENTIONED

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