IMMUNOCHEMISTRY

How to find a T cell's 'turn-ons'

A new array-based strategy offers a sensitive, high-throughput tool for identification of T-cell epitopes and monitoring of the cellular immune response to antigen stimulation.

The identification and quantification of T cells specific for a particular antigen is a highly effective means for characterizing a subject's reaction to an immunogenic stimulus, such as from a pathogen or a vaccine. Unfortunately, this can be a daunting process; some effective techniques exist, such as cell proliferation assays, but these can be time-consuming and are ineffective for the conduct of large-scale experiments.

In the body, antigens are presented to the immune system as peptides bound to cell-surface major histocompatibility complex (MHC) proteins. In 2003, Stanford University investigators developed an array-based system that used such immobilized MHC-peptide complexes to bind and isolate T cells that target a specific epitope (Soen *et al.*, 2003), demonstrating new promise for high-throughput T-cell analysis. Now, work by University of Massachusetts Medical School investigator Lawrence Stern and postdoctoral fellow Jennifer Stone takes this technology another step forward, allowing investigators not only to isolate specific T-cell populations, but also to determine the extent and the nature of activation in response to antigen binding (Stone *et al.*, 2005).

Each array spot contains MHC complexed with different peptides, alongside costimulatory antibodies; however, the spots also include antibodies specific for cytokines released by T cells following activation (**Fig. 1**). After binding cells to the array, the results of the experiment are visualized with fluorescent antibodies that also recognize those cytokines. "The actual advance here," says Stern, "is using the capture antibodies together with our MHCs, so that we can not only attract, bind and count the cells, but also look at their functional responses on the microarray." Because the detection process is specific for activation, only functional

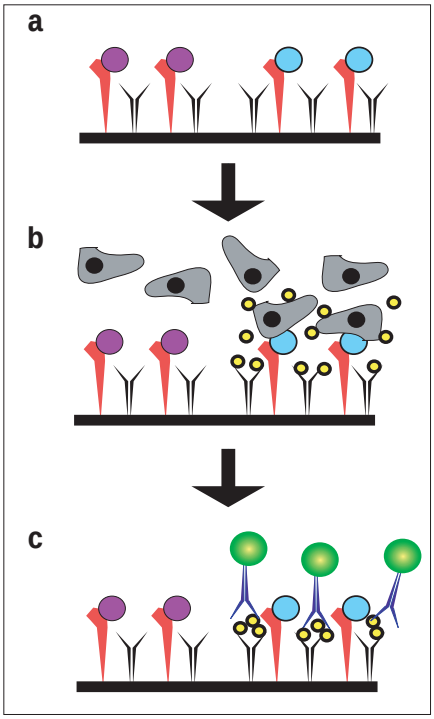

Figure 1 | The Stern group's MHC-peptide array strategy. (**a**) Each spot contains immobilized MHC proteins (red), which present different peptides, and costimulatory antibodies (not shown). (**b**) T cells that recognize a given epitope will bind the array and release cytokines (yellow), which are bound by capture antibodies on the array. (**c**) Fluorescently tagged antibodies (blue) against these same cytokines allow detection.

IMAGING AND VISUALIZATION

INTELLIGENT FLUORESCENCE

A new theory on the role that structure has in the fluorescent properties of fluorescein leads to the intelligent design of improved fluorophores and new fluorescent probes.

Since fluorescein was first described in 1871, it has proven to be a tremendously valuable molecule. Its convenient fluorescent properties are the basis of many fluorescent probes, such as the calcium sensor fluo-3. Unfortunately, it has other properties that make it less than ideal. Now Tetsuo Nagano and his colleagues at the University of Tokyo show that the three-dimensional structure of fluorescein is critical for its fluorescence and they demonstrate how this observation opens the door to the rational design of fluorescent molecules (Urano *et al.*, 2005). This methodology allowed them to devise new fluorescent molecules with considerable advantages over traditional fluorescein.

After years of working with derivatives of fluorescein, Nagano and colleagues hypothesized that the purportedly indispensable carboxylic acid on the benzene ring of fluorescein is not intrinsically involved in fluorescence. Rather its only function is to keep the benzene ring perpendicular to the plane of the fluorophore it is attached to. They tested their hypothesis by replacing this carboxylic group with a methyl group, which produced a highly fluorescent molecule they call a TokyoGreen. Surprisingly, "substitution of the carboxylic acid with a methyl group has not been done in over 100 years since fluorescein was first synthesized," says Nagano.

Their findings allow researchers to predict how the addition or removal of different chemical groups will alter the fluorescence of new derivatives. "This is a great advantage for the development of new probes. Fluorescent probes developed based on our strategy can be tuned to have very weak fluorescence before the reaction of interest and then show remarkable fluorescence increases of up to 2,500 times the initial fluorescence, following the reaction," remarks Nagano.

Using these observations it is much easier for chemists to intelligently replace the carboxylic group with an alternate, group that correctly orients the benzene ring. By adding particular modifying groups, the electron density can be tuned to get the desired fluorescence. This knowledge should simplify

interactions should be observed; furthermore, by simultaneously screening for several different cytokines, Stern's team shows that one can very specifically characterize the nature of T-cell activation.

Stern is looking to apply this new technology to study immune responses to viral infection—specifically, vaccinia and dengue—and he sees this as a potentially powerful tool for quickly identifying immunogenic peptides from these pathogens. "My personal feeling is that the technology will be most useful if we can pick up responses in blood," says Stern, "...and the sensitivity appears to be enough to pick up cells at a frequency that you might expect to find them in blood from a person who's had a vaccine or been exposed to an infectious agent." But he adds, "we haven't actually tested peripheral blood samples from individuals [yet]," and this remains a priority for future development.

Nevertheless, this pilot study highlights the method's promise in terms of both efficiency and economy. "The advantage of this technology in general is really that we're parallelizing and miniaturizing existing assays, so they can be done on a larger scale," says Stern. Initial experiments using a nonfluorescent, precipitatable detection substrate also suggest that even facilities without expensive array-scanning equipment, could avail themselves of this high-throughput diagnostic tool. "Potentially... you could put some blood or a blood product on it, process it, and just read the results by eye." In the fight to stay one step ahead of the latest pathogenic threats, this could offer an important edge to clinicians and researchers on the front lines.

Michael Eisenstein

RESEARCH PAPERS

Stone, J.D. *et al.* HLA-restricted epitope identification and detection of functional T-cell responses by using MHC-peptide and costimulatory arrays. *Proc. Natl. Acad. Sci. USA* **102**, 3744–3749 (2005).

Soen, Y. *et al.* Detection and characterization of cellular immune responses using peptide-MHC arrays. *PLoS Biol.* **1**, E65 (2003).

the design of specialized fluorescent probes by removing much of the trial and error that is inherent in present approaches. Furthermore, the use of hydrophobic group, will create a TokyoGreen that is almost freely membrane permeable.

To demonstrate these advantages of TokyoGreens, Nagano and colleagues developed a new membrane permeable fluorescent probe for β-galactosidase that responds faster than a previously described probe. Nagano describes the new situation as follows: "Until now, fluorescent probes were mainly developed by trial and error. Our recent findings [allow] us to convert most chemical reactions into fluorescence off/on switching reactions by using appropriate fluorophores and adjusting the electron densities of electron donor moieties. Hence we can develop many novel fluorescence probes based on this rational design strategy." They now have preliminary results in applying their TokyoGreen strategy to other fluorophores, so it is likely that this research will result in many new and useful fluorescent probes.

Daniel Evanko

RESEARCH PAPERS

Urano, Y. *et al.* Evolution of fluorescein as a platform for finely tunable fluorescence probes. *J. Am. Chem. Soc.* **127**, 4888–4894 (2005).

NEWS IN BRIEF

GENOMICS

Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*

To assemble a more comprehensive dataset of genes involved in the crucial stages of *C. elegans* development, Sönnichsen *et al.* have conducted a massive gene-silencing study, testing a library of double-stranded RNAs targeted against over 19,000 different genes and profiling phenotypes via time-lapse microscopy.

Sönnichsen, B. *et al. Nature* **434**, 462–469 (2005).

STEM CELLS

Low O_2 tensions and the prevention of differentiation of HES cells

Researchers working with human embryonic stem cells (hESCs) typically maintain them under standard culture conditions of 5% CO_2, even though the physiological environment of the mammalian reproductive tract is hypoxic by comparison. Ezashi *et al.* show that culturing hESCs in such hypoxic conditions (3–5% O_2) reduces hESC differentiation without affecting viability.

Ezashi, T. *et al. Proc. Natl. Acad. Sci.* USA **102**, 4783–4788 (2005).

SPECTROSCOPY

A concept for rapid protein-structure determination by solid-state NMR spectroscopy

Recent studies have shown that solid-state NMR can offer an effective approach for determining the structure of proteins that are difficult to crystallize, although this typically requires synthesis of several labeled protein variants. Lange *et al.* present a variation on this technique that allows rapid determination of a three-dimensional structure with a single, isotope-labeled protein sample.

Lange, A. *et al. Angew. Chem. Int. Ed. Engl.* **44**, 2089–2092 (2005).

RNA INTERFERENCE

A systematic analysis of the silencing effects of an active siRNA at all single nucleotide–mismatched target sites

Ongoing research into the process of RNA interference has demonstrated that total complementarity between a small interfering RNA (siRNA) and its target is not an absolute requirement. To identify where mismatches can be functionally tolerated, Du *et al.* systematically mutate each nucleotide in the sequence of two different siRNA-targeted mRNA transcripts and assess silencing efficiency.

Du, Q. *et al. Nucleic Acids Res.* **33**, 1671–1677 (2005).

GENE REGULATION

Temperature-sensitive protein-DNA dimerizers

In previous work, Hauschild *et al.* demonstrated the generation of molecules containing a DNA-binding domain linked to a 'hook' element that interacts with a DNA-binding protein of interest, selectively improving the binding affinity of the target protein. They now show that changes to the linker connecting these domains can confer temperature sensitivity, creating new possibilities for the design and regulation of these artificial transcription factors.

Hauschild, K.E. *et al. Proc. Natl. Acad. Sci. USA* **102**, 5008–5013 (2005).