Recombinant antibody microarrays—a viable option?

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Protein arrays (or chips) are of considerable interest to biologists as tools for studying protein expression on a global scale. They are constructed by spotting many hundreds of capture molecules onto a solid surface, which can then be used to interrogate a particular sample for protein content. Many of the first protein arrays used antibodies as capture molecules[1–3], because antibodies display a breathtaking specificity of action (even distinguishing between functional groups on a molecule) as a result of the exquisitely designed structure of their binding sites. Several recent studies performed on protein microarrays, however, have raised serious doubts about the usefulness of antibodies in such formats because of concerns as to whether antibodies actually are sufficiently specific[3–7]. One explanation for this view might be that investigators involved in this new field are perhaps not immunologists; another, more likely, explanation is that they do not appreciate that antibodies have to be selected for the properties that they are expected to display in a particular application. Consequently, if the application is antibody microarrays, then the antibodies have to be stable and functional in the microarray format.

One means of addressing this problem is to design a recombinant antibody library on the basis of a single framework, such as one based on single-chain Fv (scFv) fragments[8]. As all scFvs in such a library share the same molecular scaffold, the most stable framework[9] can be adopted, resulting in very similar on-the-chip behavior by scFvs with different specificities.

To illustrate this point, we have created in our laboratory an scFv array with specificities against nine different cytokines. When these are allowed to interact with crude extracts of human dendritic cells containing >10,000 different proteins, scFvs directed against interleukin (IL)-1α, IL-8, IL-16, IL-18, and monocyte chemotactic protein (MCP)-4 do not cross-react with any other proteins from the crude cell lysate (Fig. 1A, B).

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Furthermore, it is evident that the differential intensity of spots shows a specific upregulation of four of the cytokines derived from the activated cells (Fig. 1C). The conclusion is that recombinant antibody fragments are well suited for the array format, but only if selected for that application and not simply taken off the shelf.

With specificity issues put aside, the next challenge for antibody arrays will be the problem of scaling up so that global analysis of up to 10,000 proteins can be achieved. This presents several logistic problems. First, the probes used for a global approach would also have to be based on phage-display libraries containing several billion scFv members, for the mere reason that producing monoclonal antibodies against several thousand antigens would be too cumbersome. The selection of thousands of recombinant scFvs from a phage library is no small task, however, and even specialized companies are today limited to a few thousand scFvs per year[10] (this also assumes that purified proteins from a proteome are available). It is worth noting that, to date, no proteome is available in the form of individually purified proteins.

One possible solution could be to spot enough scFvs from a library onto a chip surface to obtain a representative selection of the available specificities. The question is how large such an array would likely have to be. If we spot all members of a ten-billion-member phage library of scFvs, we would obviously have all available specificities. If we initially performed a pre-selection of such a library, to increase the quality of the members, perhaps 100,000–1,000,000 spots would be enough to serve as a representative selection. In such a case, the term 'specific' would not be relevant, and instead this high-density scFv array would be analyzed on the basis of pattern recognition. In the case of differential proteome analysis, for example, patterns that differ between healthy and diseased tissue could be identified. Using this as a starting point, smaller and more focused arrays could be developed, based on perhaps fewer than 500 antibodies.

Clearly, no fundamental conceptual barriers should prevent the application of antibody microarrays in global protein analysis. What we currently lack are the new formats and nanoengineering approaches that will allow analysis on a scale that will match our ambitions.

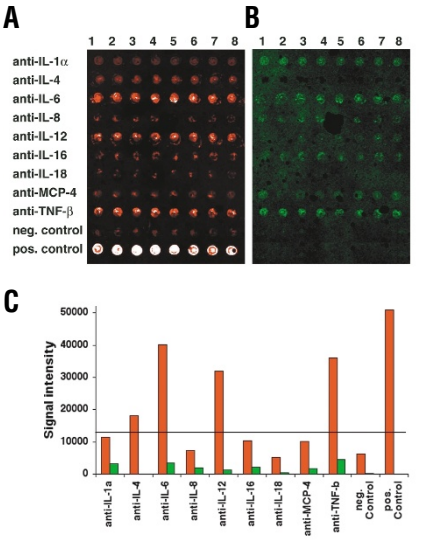

Figure 1. Comparative proteome analysis of activated (Cy5-labeled) versus non-activated (Cy3-labeled) cell lysates from monocyte-derived human dendritic cells (DCs) using antibody microarrays. Nine recombinant human scFv antibody fragments selected from the library[8] that were specific for various cytokines, one negative control (nonspecific scFv), and one positive control (scFv specific for cholera toxin subunit B (CT)) were arrayed in eight replicates, respectively. Cy5-labeled CT was spiked into the positive control. The two differentially labeled proteomes were mixed 1:1 and incubated and analyzed on the same scFv microarray. (A) Cy5-scanned image of the microarray incubated with crude cell lysate. (B) Cy3-scanned image of the microarray incubated with crude cell lysate. (C) Mean signal intensities for each probe (Cy5 values in red, Cy3 values in green). Four cytokines, IL-4, IL-6, IL-12, and tumor necrosis factor (TNF)-β, were upregulated in the activated proteome—that is, they provided a signal at least twice the intensity of the signal for the negative control (cutoff is represented by the solid line). Similar results were obtained when the samples were 'criss-cross' labeled (that is, when the activated proteome was labeled with Cy3 and *vice versa*).

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