

EDITORIAL

Clinical and Experimental Allergy

Allergology on a chip

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The miniaturization of the biochemical laboratory has been increasing in recent years, and several examples have already been applied to the field of allergology.

The *chip* comes from the field of electronics, where it can be described as a miniaturized electronic circuit (consisting mainly of semiconductor devices, as well as passive components) that has been manufactured in the surface of a thin substrate of semiconductor material (Wikipedia). In biochemistry, the *microarray* was first used to multiply binding reactions such as DNA/RNA hybridizations. One example of DNA microarray (chip) involves genome-wide screening for single-nucleotide polymorphisms (SNPs), to relate the genotype to various diseases. Such SNP chips can investigate millions of SNPs simultaneously from a subject. Many linkage studies have already appeared in the field of allergy (reviewed in [1]).

Total gene expression of a cell culture or a whole tissue is another popular application of microarray technology. Total gene expression may be investigated by reverse translating mRNA to cDNA and testing this against microarrays of probes selective to each individual transcript that can be expressed in the cell (~50 000 different probes). Such studies have led to an increased understanding of which genes are transcribed during activation and differentiation of different cells in tissues (reviewed and discussed in [2]). Inherent problems in such studies are to obtain sufficiently pure cells (such as e.g. epithelial cells or eosinophils in asthma studies) to draw valid conclusions about an up-regulated gene in a specific cell type. Just small numbers of polluting cells may cause severely skewed results. To overcome this problem, investigators have looked at cell lines such as the mast cell lines or bone marrow-derived mast cells. In the T cell area, cultured T cells have been stimulated with mitogens,

superantigens or allergens [3]. This may, however, create other problems, because cell lines may not be very representative of *in vivo* cells and even the source of stem cells seems to influence the results [4]. Moreover, in stimulated cell cultures, there is often bystander stimulation, causing non-allergen-specific cells to be stimulated.

While the RNA/DNA chemistry is relatively homogeneous, protein–protein interactions are much more variable, and chips for protein analysis have developed less rapidly.

The dream of knowing all IgE specificities in a patient has come much closer to fulfilment with the advent of the allergen chip for IgE measurement. This was first carried out with full allergen extracts [5], and later in a pioneering study 78 recombinant and 16 purified single-allergen molecules were spotted on a chip, which was then used for detection of IgE binding in human sera [6]. The chip has later been found to correlate reasonably well with conventional IgE-detection methods [7]. Later works have elucidated the fine specificity of IgE by coupling overlapping peptides covering all linear epitopes of major peanut allergens to a peptide microarray [8, 9].

A word of caution may be warranted for the possible diagnostic use of allergen chips. Patients become sensitized to allergens from biological sources, not to extracts, recombinant proteins or peptides. While major allergens have been defined and expressed as recombinant proteins for many important allergen sources, some patients may still react only to minor allergens. Moreover, an allergen that may be characterized as minor or even non-relevant in one geographical region may be the major and most important allergen in another region, as has been suggested for the apple allergens Mal d 1 (Bet v 1 homologue) and Mal d 3 (LTP) in Spain vs. Central and Northern Europe [10]. Thus we have to be careful not to restrict our diagnostic window to a narrow selection of allergens. Fortunately, handling microarrays with few allergens is as demanding as handling microarrays with thousands of allergens, so large selection of allergen panels can routinely be screened for using chip technology. The only

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reason for making arrays with selection of allergens is the price.

Apart from the allergen-IgE binding, many other protein-protein interactions can be and have been studied by the microarray technology. The generation of such large amount of data led to systems biology, examples of which are the map of the pathways of the 'integrin adhesome' [11].

In this volume of *Clinical Experimental Allergy* a new and interesting development is reported. Lin *et al.* [12] have used a chip with spotted allergen extracts or single proteins to investigate basophils and basophil-like cell lines. The authors not only observed binding of the IgE-sensitized basophils to the spotted allergens but were also able to perform a basophil activation test using the CD63 as an activation marker. Interestingly, only very high concentrations of allergens or anti-IgE could bind or activate the cells, and clearly the dynamics of solid-phase-coated allergens are different from allergens in solution as is normally used for basophil testing. An issue that may rely not only on the technique presented by Lin *et al.* [12] but also on the IgE-binding microarrays is the biological activity of the spotted allergens. It is a cumbersome but important work to control for this aspect, because absorption to the chip solid-phase, drying and subsequent exposure to atmospheric air may severely damage the conformation of the allergenic molecules. In its present form, the basophil chip assay makes use of highly purified basophils or of sensitized cell lines, but one could speculate that a combined use of specific basophil markers such as histamine [13] and activation markers may make the tedious purification step unnecessary in the future. As discussed by the authors, more studies are needed to describe the sensitivity and specificity of this assay, and also the possibility to determine the basophil sensitivity by performing titrations [14, 15] should be further investigated.

The combination of biology and microelectronics has potential within more areas, however. Culture of cells in the tightly controlled microenvironment of a temperature- and pH-controlled chip has been demonstrated [16], and this opens functional studies. Several studies of rolling and adhesion of leucocytes have been performed, and recently methods for detection of lymphocyte subsets have been devised [17]. Also the possibility to culture not only single-cell suspensions, but whole tissues, have attracted attention and a recent volume of the journal 'Lab on a Chip' has been devoted to this aspect. In an editorial of this volume the editors make the important remark that the development in some instances have been technology rather than market driven. It is an important task for the allergological scientific community to formulate the clinically and scientifically relevant problems that should drive the exploitation of these fascinating new tools.

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