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# LYMPHOCYTES, RECEPTORS AND AFFINITY CHROMATOGRAPHY

LESLIE HUDSON*
*Department of Experimental Immunobiology, Wellcome Research Laboratories, Beckenham, Kent (Great Britain)*

CONTENTS



## 1. INTRODUCTION

The former apparent simplicity of the immune response has been destroyed by the recognition of a bewildering array of immunologically active cells, differing not only in specificity but also in effector function and lineage. By isolation, characterisation and recombination of the cellular components of this system it has been possible to describe to a limited extent the complex interactions constituting the immune network.

The criteria by which cells can, and have been, fractionated and characterised are numerous. Fortunately, excellent current reviews are available dealing with the isolation and characterisation of immunologically relevant cells[1] and, more particularly, with the applicability of affinity chromatography to immunology[2]. Thus I wish to limit myself here to a consideration of the general approach to cell affinity chromatography and more especially to those techniques that are likely to carry forward the study of the immune response.

## 2. LYMPHOCYTES, RECEPTORS AND AFFINITY CHROMATOGRAPHY

In principle, the techniques available for the immunospecific fractionation of cells rely basically upon the interaction of a more or less well-defined ligand or affinity molecule with a specific receptor on the surface of the cells to be selected. The diversity in technical design is derived from the method by which cells binding to the ligand are removed from the majority of non-reactive cells. As can be seen from Fig. 1, most affinity separations are achieved on a solid-phase immunoadsorbent composed of a support matrix carrying covalently linked affinity molecules. The fluorescence-activated cell sorter, developed by Herzenberg and his colleagues[3], is one notable

* Present address: Immunology Unit, Department of Biochemistry, Jenner Wing, St. George's Hospital Medical School, University of London, Cranmer Terrace, London SW17 0RE, Great Britain.

exception to this general approach. This is clearly the most powerful tool available by which cells binding affinity molecules may be isolated directly. There are, however, sufficient advantages associated with less sophisticated systems of affinity separation to ensure their continued use.

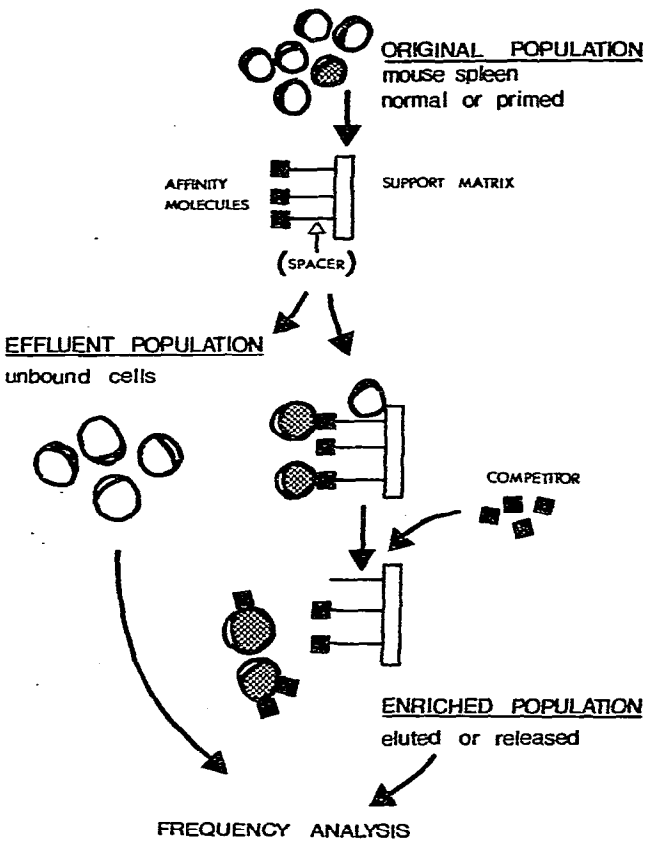


Fig. 1. Flow diagram for the selection of cells by affinity chromatography.

Immunoadsorbents with antigens or antibody[4,5], alone or in combination[6], have been used to separate lymphoid cells by adsorption via antigen-binding or other surface receptors or determinants. More recently affinity molecules with a greater chemical definition of binding specificity, for example, lectins or haemagglutinins[7] or histamine[8] have been used, although the immunological basis for ligand specificity is less clear.

The requirements of a suitable support matrix, such as ease of derivatisation, high capacity and minimal direct interaction with the cells to be selected, have led to a general adoption of cross-linked agaroses and dextrans, and to a lesser extent, acrylamide gels. In addition, the beaded presentation of these materials is advantageous because of the flow and surface area characteristics associated with beaded affinity columns[2]. Recently, Eckert *et al.*[9] has developed a beaded support by cross-

linking calf serum with glutaraldehyde. Although not as well defined as the other gels mentioned, it has the advantage of minimal non-specific adsorption of non-activated lymphoid cells.

The magnitude of interaction of cells with the affinity molecules is sufficient to retard their passage through the affinity column, but not sufficient to effect their retention. Capture of the specifically retarded cells requires interaction with the matrix, consequently, in cell affinity chromatography, specificity and capacity are somewhat antithetical.

The effect of moving the affinity molecule away from the environment of the matrix by means of a spacer or extension arm is less dramatic in cell, as opposed to molecular, affinity chromatography because of the relative cellular and molecular dimensions involved. However, spacers are available that can be degraded enzymatically[10], thermally[11] or chemically[12]. Thus the release of adsorbed cells can be effected more efficiently than by earlier techniques requiring the solubilisation of the total support matrix[13].

The release of bound cells by competition with free affinity molecules is obviously advantageous as it introduces specific desorption, as well as specific adsorption into the affinity technique. However, because of the multivalent interactions of the bound cells with the affinity column producing a very low dissociation rate, release by direct competition is not possible. A shear force is required to release the cells from the column in the presence of free competitor, this then stops re-association of the cell with the insoluble affinity molecules. Techniques are available whereby this shear force can be generated hydrodynamically[14], by centrifugation[15], by mixing[16,17] or musically[18].

Within the field of antigen-specific cell fractionation, probably the most widely studied cell population is that derived from mouse spleen containing effector or memory cells elicited by previous immunisation with the antigen to be used for affinity selection. In this case, a strict analysis of the original, enriched and depleted cell populations is possible in terms of the frequency of antibody-forming cell precursors for the antigen under test[11,19].

Much of the present research on fractionated cells is centred on the functions of the purified cells rather than on the techniques of their fractionation. It is now possible to enrich or deplete lymphocyte populations of almost any of their known cellular components. For example, using dinitrophenyl–human serum albumin (DNP–HSA) conjugated to Sephadex G200, it has been possible to fractionate spleen cell populations from mice primed to $DNP_4$ fowl gamma globulin ($DNP_4FGG$)[17]. When cells were prepared seven days after *in vivo* priming, plaque-forming cells secreting anti-DNP antibody (DNP–PFC) could be adsorbed to DNP–HSA Sephadex columns, and then recovered either by elution with DNP–lysine or by dextranase solubilisation of the matrix[13]. Although the DNP–PFC's released by DNP–lys competition were functional and showed a 30-fold enrichment over the original population, those recovered by matrix solubilisation were inactive. This disparity in findings is probably due to effector cell blockade[20]; the monovalent hapten used for elution being less efficient than the multivalent hapten released by enzymatic digestion[13]. Similar columns using DNP–lys as the affinity molecules can be used for the retention of DNP-specific memory cells obtained from the spleens of mice immunised more than six months previously with $DNP_4FGG$. Cells released by solubilisation of the

matrix show a 5–6-fold enrichment when assayed in a Mitchison adoptive transfer system with hapten-carrier conjugates as antigens[21]. However, a greater enrichment (10–100-fold) could be obtained when bound cells were eluted with a stepwise gradient of free DNP–lysine between 0.03 and 3.0 m*M*. Fractions of the eluted cells were assayed *in vivo* for the production of antibody-forming cells after challenge with antigens. It was found that the affinity of the antibody was inversely proportional to the concentration of DNP–lys used to elute the plasma cell precursors. The isoelectric focusing banding pattern of anti-DNP antibody showed a very limited heterogeneity within each fraction, suggesting that a relatively low number of clones had been selected. In our present work, we are attempting to use these purified memory cells in hybridisation studies. It is hoped that the relatively high frequency of antigen-specific, non-activated lymphocytes might enable us to produce hybrids responsive to antigenic stimulation.

The usefulness of this type of approach for the selection of antigen specific cells has been extended recently by Scott[16]. For example, cells binding polymerised flagellin (POL), were isolated by reacting the cell population with fluorescein(Fl)-conjugated POL. The cells reacting with Fl–POL were then isolated from the non-reactive population by filtration through a column of anti-Fl antibodies coupled to Sepharose. The column bound cells were then released by gentle mixing in the presence of Fl-bovine serum albumin.

Although the majority of procedures based on antigen-specific affinity chromatography have yielded purified B, but not T, lymphocytes, Scott[16] was able to isolate functional T effector cells by binding BALB/c cytotoxic cells to Fl-coupled EL4 lymphoma cells. The "cellular complex" was then isolated on an anti-fluorescein column and recovered as above.

The functional complexity of lymphocyte populations is by no means limited to that derived from the diversity of antigen binding receptors. It has long been known that cells capable of indirect regulation of antibody production have both positive and negative functions. It is difficult to discern the subtle attributes of those systems of affinity chromatography that have been able to isolate not only B cells but also helper T cells (positive regulators[22] and suppressor T cells (negative regulators)[12,23] apparently by direct interaction with antigen. Superficially similar systems have yielded B cells uncontaminated by T cells and therefore devoid of helper or suppressor functions[14,17].

The use of column-bound cells to adsorb unwanted antibodies from antisera[24] has recently been elegantly extended by Sela and Edelman[25]. Cells adsorbed to Con A coupled Sephadex were cross-linked with glutaraldehyde and then used as a cellular immunoadsorbent to isolate anti-carbohydrate antibody from normal sera. The potency of this technique was demonstrated by the definition of antibodies able to precipitate a glycoprotein associated with the developing, but not the growing stage of slime molds.

This technique seems an excellent tool for the analysis of cell–cell interactions. Not only should it be possible to produce antibodies to probe sites important for structure formation and ultimately organogenesis, but immobilised cells might also provide a good substrate for the isolation of receptors responsible for cell–cell recognition. Indeed, a good testing ground might be the isolation of the putative receptor for sheep erythrocytes present on human T cells.

A preliminary insight into the workings of immune lymphocyte populations has been gained by the use of various complex and relatively ill defined antigens, from hapten-carrier conjugates to sheep erythrocytes. For greater insight, more defined systems for cell fractionation will be needed, using molecules of a simplicity on the scale of the mediators of cell reactivity already known in pharmacology and endocrinology. Thus it seems likely that interest might once more cycle back to the basic systems of fractionation, because the degree of definition of cell populations is now insufficient for the increasing precision of biophysical, biochemical and immunochemical techniques. Thus, methods are required for the clonal selection and expansion of monospecific, monofunctional lymphocyte populations obtained by affinity isolation.

## 3. SUMMARY

The ability to isolate, characterise and recombine, in a predetermined manner, immunologically reactive cells is one of the most powerful tools with which to investigate the detailed workings of the immune network. However, the study of cell affinity chromatography centres on the cell rather than on the technique. Hence, none of the systems, with the notable exception of the fluorescence-activated cell sorter, have been subjected to extensive technological innovation. There is an obvious need for a greater characterisation of the criteria by which cells are fractionated to to improve reproducibility. These essentially biophysical techniques could then serve as a basis for defining the cell type isolated. This would remove the need to define the isolated cell only in terms of the pre-existing lymphocyte subjects, which rarely correspond directly.

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