# Isolation and Characterization of Single Anti-U1A-specific B Cells from Autoimmune Patients

RUUD M. T. DE WILDT,[a] FRANK H. J. VAN DEN HOOGEN,[b]
WALTHER J. VAN VENROOIJ,[a] AND RENÉ M. A. HOET[a,c]

[a]*Department of Biochemistry*
*University of Nijmegen*
[b]*Department of Rheumatology*
*University Hospital Nijmegen*
*6500 HB Nijmegen, the Netherlands*

Patients with systemic lupus erythematosus (SLE) or SLE-overlap syndromes often produce autoantibodies directed to U1RNA-binding proteins.[1] Previously, we isolated and characterized autoantigen-binding Ab fragments directed to the U1RNA-associated A protein (U1A) from several variable (V) gene combinatorial phage libraries.[2] Although these libraries have proven to be very successful, heavy ($V_H$) and light ($V_L$) chains are randomly combined during construction of such libraries. Because we were interested in the utilization of the original $V_H/V_L$ pairings of the encoding autoantibodies, we developed a single cell culture system for B cells using mouse thymoma EL-4 B5 cells.[3] In combination with a preselection via recombinant antigen and single cell sorting using fluorescence-activated cell sorting (FACS), we were able to enrich for autoantigen-specific B cells. $V_H$ and $V_L$ genes originating from single U1A-specific B cells were cloned in a phage display vector for expression of single-chain variable fragments (scFv).

Human mononuclear cells were isolated from SLE patients and selected against U1A-coated plates or biotinylated U1A coated to streptavidin-coupled superparamagnetic microbeads (MACS). Adhering cells were collected from the plates via trypsin treatment or were directly used in the case of U1A-bound MACS (FIGURE 1). The selected lymphocytes were plated as single CD19/CD20-positive cells using FACS with an automatic cell deposit unit. Single selected B cells were seeded on 96-well plates containing 20,000 irradiated EL-4 B5 cells and 10% supernatant of phorbol myristate acetate (PMA)– and phytohemagglutinin (PHA)–activated human T cells (FIGURE 2). Cultures were grown for 10–11 days and tested in ELISA for antigen (Ag)–specific antibodies and total Ig production. Cell cultures that were positive in the Ag-specific ELISA screening were used for RNA isolation. An RT reaction was performed with an oligo-dT primer and the cDNA was used in a first PCR with $V_H$ family–specific primers or a mixture of V-kappa or V-lambda primers (in the case of the light chains) as in Marks *et al.*[4] These first PCR products were used in a second

[c]To whom all correspondence should be addressed.

PCR with nested joining-region primers to introduce the appropriate restriction sites for cloning into pHENIX.[2]

Typical percentages of Ig-positive cells determined by ELISA after 10–11 days of culturing on EL-4 B5 cells in the presence of 10% human T cell supernatant varied between 50% and 70%. The frequency of Ag-specific B cell clones varied between 1% and 2.5% as a percentage of Ig-positive wells. Assuming that the frequency of Ag-specific cells in the periphery varies between $10^{-5}$ and $10^{-4}$, this would indicate an enrichment factor of 100–1000. Distributions of IgG, IgM, and IgG/IgM double-

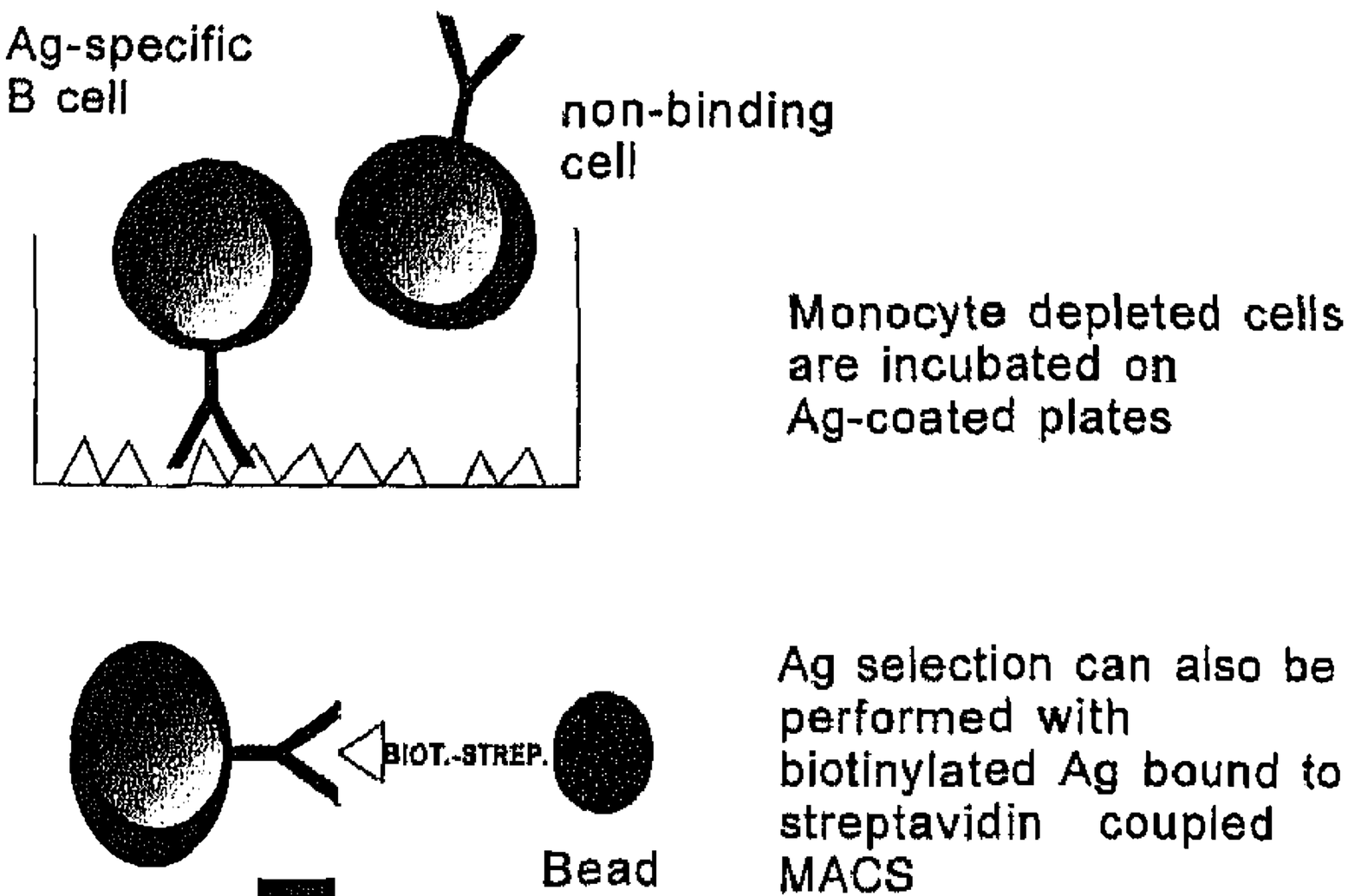


**FIGURE 1.** Antigen selection on peripheral blood mononuclear cells. Human mononuclear cells were isolated from autoimmune patients (SLE) and used to select on U1A-coated plates or biotinylated U1A coated to streptavidin-coupled MACS. Selected cells were collected from the plates via trypsin treatment or were directly used in the case of U1A-bound MACS. Magnetically sorted cells (MACS) can be processed using FACS.

positive isotypes in Ig-producing single B cell cultures were 3:3:1. After culture in the EL-4 B5 system, the B cells obtained a plasmablast-like phenotype expressing $CD38^{HIGH}$ and $SYNDECAN\text{-}1^{MOD.}$ (plasma cell marker). PCR products using cDNA from a single B cell clone amplified with separate $V_H$ family–specific primers and constant region primers (IgG- or IgM-specific) gave rise to only one $V_H$ product, which indicates clonality. In the case of the light chains, a product with either V-kappa or V-lambda primers was obtained. Five IgM and 2 IgG anti-U1A-specific clones have been isolated. So far, we have sequenced only one clone with V regions derived from

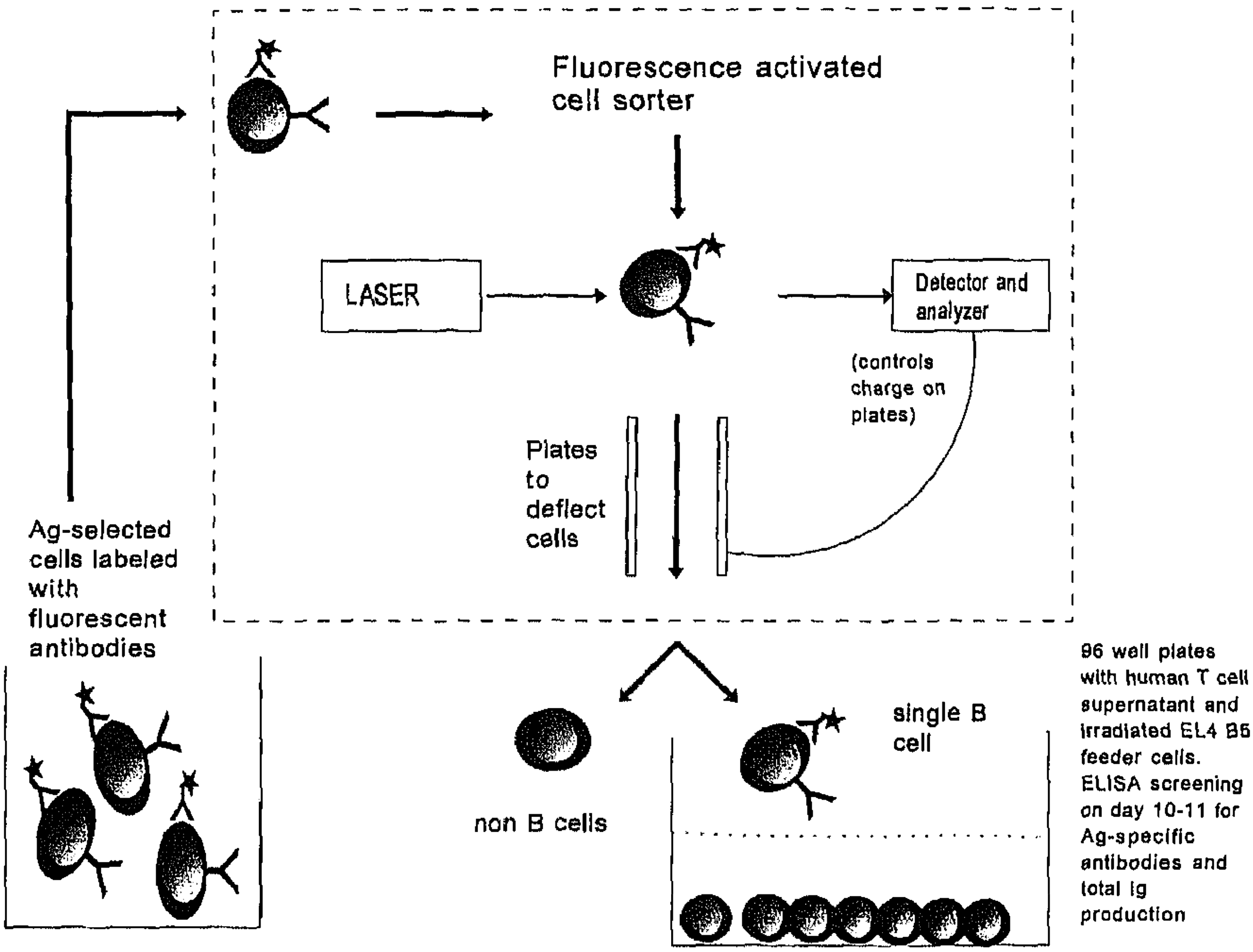


**FIGURE 2.** The selected lymphocytes were plated as single cells using FACS with an automatic cell deposit unit in 96-well plates containing 20,000 irradiated EL-4 B5 cells and 10% supernatant of phorbol myristate acetate (PMA)– and phytohemagglutinin (PHA)–activated human T cells. Cultures were grown for 10–11 days and tested in ELISA for Ag-specific antibodies and total Ig production.

DP-49 ($V_H3$) for the heavy chain and lv318 for the lambda light chain. We have been able to show binding of the cloned V regions to the U1A protein in ELISA. Further studies are currently being performed to isolate functional recombinant antibodies containing original $V_H/V_L$ pairings.

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