Single Lymphocyte Analysis with a Microwell Array Chip

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Abstract

Following genomics and proteomics, cytomics, a novel method of looking at life, has emerged for analyzing large populations of cells on a single-cell basis with multiple parameters in a quantitative manner. We have developed a highly integrated live-cell microarray system for analyzing the cellular responses of individual cells using a microwell array chip that has 234,000 microwells each of which is just large enough to fit a single cell. Compared with flow cytometry and microscope-based methods, our system can analyze the history of the cellular responses of a large number of cells. We have successfully applied the system to analyze human antigen-specific B-cells and produced human monoclonal antibodies (MoAb) against hepatitis B virus surface antigen. We have also constructed a mouse system to assess hepatitis B virus-neutralization activity and have demonstrated the neutralization activity of our antibodies. Our technology should expand the horizons of cell analysis as well as enable generation of human MoAb for antibody-based therapeutics and diagnosis for infectious diseases such as hepatitis viruses.

Key terms

lymphocyte; microwell array chip; monoclonal antibody; hepatitis B virus; intracellular calcium

Cytomics is a novel perspective from which to look at life and to study the “cytome,” analyzing large populations of cells on a single-cell basis with multiple parameters in a quantitative and observer-independent manner (1). To meet the discipline of cytomics, sensitive fluorescence detection devices and sophisticated image analysis procedures have been developed, including tools based on flow cytometry or those based on microscopy (2). Flow cytometers have enabled us to analyze fluorescent signals of large numbers of cells flowing through sheath fluid, but they cannot track the history of the fluorescent signals from each cell of interest. In contrast, tools based on microscopes have enabled us to analyze signals of individual cells at various time points. However, we cannot analyze a large number of cells with a microscope, especially when cells are nonadherent.

B-cells, a major cell population in immune systems, produce antibodies that specifically recognize antigens, such as infectious microbes, neutralize their infectivity, and eliminate them by various immune effector mechanisms. B-cells express mono-specific antibodies on the cell surface as antigen receptors that recognize extra-cellular antigens. It has been estimated that an individual human contains on the order of 10^9 clones of B-cells with distinct specificities (3). It has also been reported that the frequencies of antigen-specific B-cells are quite diverse, which may be due to the form of immunogen. For example, more than 10% of B-cells produce...
the specific antibodies to rabies (4), but only 0.01% of B-cells produce antibodies to hepatitis B virus surface (HBs) antigen (HBs-Ag) after vaccination (5). To analyze the response of individual B-cells to an antigen, it is necessary to analyze a large number of cells at the level of a single cell by cytomtics.

To meet the demand for this type of analysis, we have developed an analysis system using a microwell array chip that has a large number of microwells whose size and shape just fit a single cell (6). By applying an individual cell to each microwell, an array of live cells was prepared, and the cellular responses of individual cells, such as alteration of intracellular Ca\(^{2+}\) concentration, were monitored using a fluorescent scanner that was modified to scan cells. Because the position of each cell was fixed, we could repeatedly analyze the cellular response of the same cell. Consequently, a cell microarray system, a combination of a microwell array chip and a cell scanner, enabled us to analyze the time course of the cellular responses of a large number of cells at the single-cell level. Recently, Deutsch et al. (7) and Biran and Walt (8) reported live cell arrays for analyzing a large number of cells at the single-cell level. These cell arrays were chiefly designed for analyzing cells, and the retrieval of objective cells from wells without disturbing surrounding cells might be difficult. In contrast, our cell microarray system was aimed at not only analyzing cells but also retrieving objective cells from an array.

Hepatitis B virus (HBV) infection is one of the world’s major health problems, especially in East Asia. It causes self-limiting acute hepatitis, but the infection may often become chronic, causing hepatic cirrhosis and hepatic cell cancer. Vaccines based on recombinant DNA technology have been developed and applied for the protective immunization of humans. Such vaccination, however, does not always provoke a sufficient and rapid antibody response, and HB immunoglobulins (HB Ig) have been employed in combination with HB vaccination (9). HB Ig injection, however, poses some serious problems such as contamination by unknown infectious agents and lack of a continuous supply in hospitals. Human monoclonal antibodies (MoAb) represent an alternative to HB Ig therapy.

We have applied our cell microarray system to detect human B-cells that respond to HBs-Ag from the peripheral blood of HBs-Ag-vaccinated volunteers, and obtained HBs-Ag-specific MoAb that neutralized HBV to prevent its infection of human hepatocytes. Our technology may contribute to the production of human MoAb not only for HBV but also for other various human health-threatening infectious agents, such as AIDS and SARS as well as microbes that might be used for bioterrorism.

**Materials and Methods**

**Microwell Array Chip**

A microwell array chip was manufactured using micromachining (microelectromechanical system) techniques (10) (Supplementary Fig. 1). Briefly, a thin film of silicon dioxide was grown on a silicon surface by thermal oxidation (11), a photoresist was coated on the thin film, and microwell patterns were transferred from a mask via photolithography using a Karl Suss MA6 Mask Aligner (SUSS MicroTec AG, Garching, Germany). Then, the exposed oxidized silicon surface was etched by silicon deep reactive ion etching (12) to form microwells using buffered hydrofluoric acid as a solvent of silicon dioxide. Then, a fluorocarbon polymer was formed on the photoresist and the sidewall of a microwell using enhanced chemical vapor deposition (13). Finally, the photoresist was removed and the fluorocarbon polymer was lifted off the surface of the silicon chip.

**Preparation of Lymphocytes for Intracellular Calcium Analysis**

To analyze the efficiency of the cell microarray system, we used splenocytes of MD4 transgenic mice (C57BL/6-Tg(Igh-MD44Ccg/J from The Jackson Laboratory, Bar Harbor, ME), of which the transgene encodes mouse antibody (HyHEL10) for hen egg lysozyme (HEL) (14). The splenocytes were prepared and loaded with 0.1 μM CellTracker orange (Ex, 535 nm; Em, 585 nm; Invitrogen, Carlsbad, CA) and 0.1 μM Fluo-4 (Ca\(^{2+}\)-dependent fluorophore; Ex, 473 nm; Em, 532 nm; Invitrogen), as previously described (6). The procedure for the transgenic mice experiments was approved by the Committee for Recombinant DNA Experiments (#19-9) and Animal Experiments at the University of Toyama (# 2006-Med-33). The mice were examined to determine whether they were transgenic by staining peripheral blood lymphocytes with biotinylated HEL and PE-conjugate of streptavidin. The mice with HEL-specific Ab-expressing B-cells were used for the experiments.

For preparation of human B-cells, peripheral blood lymphocytes were isolated from healthy donors according to the standard Ficoll–Hypaque method (Lymphoprep; IBL, Takasaki, Japan). B-cells were purified using MACS (Miltenyi Biotech K.K., Tokyo, Japan), and loaded with CellTracker orange and Fluo-4.

**Cell Microarray Analysis and Antibody Preparation**

Cell microarray analysis was performed as previously described (6). Briefly, cells were loaded onto a microwell array
chip and the Fluo-4 fluorescence of the individual cells before stimulation was measured by scanning the chip with a cell scanner (CRBIO IIe-FITC, Hitachi Software, Tokyo, Japan) that was modified from a DNA chip scanner (CRBIO IIe) by changing a 635-nm laser to a 473-nm laser and whose minimum resolution was improved to 2.5 μm. The cells were then stimulated with antigen at room temperature in air by exchanging the buffer on the chip with buffer containing antigen, and the cellular Flu-4 fluorescence was measured with the scanner after stimulation. The Flu-4 fluorescence intensities of the individual cells before and after antigen-stimulation were plotted in a scatter diagram with TIC-Chip Analysis software (Hitachi Software). Cells whose fluorescence increased more than fivefold were retrieved as antigen-activated B-cells from each well with a micromanipulator (TransferMan NK2, Eppendorf, Hamburg, Germany). To prepare an antibody from a retrieved B-cell, antibody cDNA was amplified with single-cell RT-PCR, inserted into an expression vector, and transfected into 293T cells to obtain a supernatant containing the antibody as previously described (6) (see supplementary Fig. 2).

ELISA for Detection of Anti-HBs Antibody

Maxisorp 96-well plates (Nunc, Roskilde, Denmark) were coated with 50 μl/well of 10 μg/ml HBs-Ag (Kaketsuken, Kumamoto, Japan) in phosphate buffered saline (PBS) and then blocked with 3% casein in PBS. After washing, cell culture supernatant containing the antibody was added to the plates and incubated for 15 min at room temperature. The binding of human antibody to the coated antigen was detected using alkaline phosphatase-labeled anti-human immunoglobulins and p-nitrophenylphosphate. The optical absorbance was measured at 414 nm with an ELISA reader (Lab system Japan, Tokyo, Japan). To confirm the antigen-specificity of an antibody, a competitive binding assay was performed. Briefly, in the ELISA assay described earlier, 0.4, 2.0, 10 μg/ml soluble HBs-Ag was added together with the culture supernatants that contained the anti-HBs antibody.

Estimation of the Epitope

For the estimation of the anti-HBs antibody epitope, competitive ELISA was performed with mouse anti-HBs MoAb (anti-d, Institute of Immunology, Takasaki, Japan; anti-d, Institute of Immunology, Takasaki, Japan; anti a and anti -r, provided from T. Nakashima, Kaketsuken) whose epitopes were already determined. Briefly, to Maxisorp 96-well plates coated with blocked as described earlier was added 50 μl/well of diluted mouse anti-HBs MoAb as a competitor, followed by 50 μl/well of 20 ng/ml sample antibody. After 15-min incubation at room temperature, binding of the sample antibody was assessed as described earlier.

In Vivo Neutralizing Activity Assay

To examine the HBV neutralization activity of the antibodies, chimeric mice having human hepatocytes were used. The chimeric mice were produced by transplanting human hepatocytes into albumin enhancer/promoter-driven urokinase-type plasminogen activator-transgenic SCID mice (uPA/SCID mice) (15). The transplanted human hepatocytes were shown to be infected with hepatitis C virus and HBV (16,17). Human serum albumin produced from the transplanted human hepatocytes was in the range of 1.3–5.2 mg/ml, indicating that 32–66% of the liver cells were replaced with those of human origin. Serum of a chronic hepatitis B patient was used for the preparation of HBV. The titer of the virus was determined by quantitative PCR of the virus genome as previously described (16). Primers for PCR are listed in supplementary Table 1. For the test of neutralization activity, the patient serum that contained 1.0 × 10⁶ copies of the virus was mixed with 30 μg of either control or anti-HBs antibodies, incubated at room temperature for 30 min, and then intravenously injected into mice. For the estimation of neutralization activity, virus titers in the sera of the infected mice were measured every 2 weeks as described earlier.

RESULTS

A Live Cell Microarray System for Lymphocytes

A general view of the live cell microarray system that we have developed is shown in Figure 1A. The cell suspension is prepared from blood or tissue, and loaded with fluorophore whose intensity alters with cell conditions, such as Ca²⁺ concentration, membrane potential, and pH. Then, the live cells are applied onto the microwell array chip that contains an array of 234,000 wells in which only a single cell can be trapped. Cells are stimulated with or without stimulants such as infectious reagents, and the alteration in fluorescence is monitored with a cell scanner. The signal of each cell is analyzed on a single-cell basis. Because the position of each cell on the chip is fixed, the signal of any one cell can be repeatedly analyzed. Then, individual cells of interest are identified, retrieved, analyzed at the molecular level, and used for protein engineering.

To prepare a live cell microwell array chip for lymphocytes, we manufactured a microwell array chip as shown in supplementary Figure 1. The microwell array chip was fabricated on a silicon substrate by using a micromachining technique (10). A scanning electron micrograph of the chip is shown in Figure 1B. To smoothly apply single cells to and retrieve them from the microwells, we made the surface of the microwell array chip hydrophilic by growing silicon dioxide using a thermal oxidation process (11), and the bottom and the sidewall hydrophobic by coating with a thin fluorocarbon film. To prepare the live lymphocyte array, the lymphocytes were separated, loaded with fluorescent dye, and applied to the microwell array chip. The chip was covered with a cover glass to prevent drying. The array of the live lymphocytes was examined under a fluorescence microscope (Fig. 1C). To raise the array rate and to efficiently hold the single lymphocytes in the microwells, the sizes (diameter and depth) as well as shapes of the microwells were optimized (Fig. 1D and data not shown). When the depth of the microwells is less than 10 μm, the lymphocytes in the microwells were easily moved out during a washing step. As a result, cylindrical microwells with a diameter of 10 μm and a depth of more than 12 μm were most suitable for raising the array rate and efficiently holding the...
lymphocytes. The relationship between the spacing of the microwells and number of arrayed cells was also investigated. When the spaces between the microwells increased, the number of arrayed cells decreased (data not shown). Based on these data, a microwell array chip with wells at a pitch of 20 μm, a diameter of 10 μm, and a depth of 15 μm were used in the following experiment.

Detection of Antigen-Stimulated Activated B-Cells on the Chip, and Comparison of Efficiency with a Flow Cytometry System

We first assessed the efficiency of the cell microarray system using mouse B-cells prepared from MD4 mice that carry a transgene encoding antibody (HyHEL10) to HEL (14). It has been reported that when MD4 B-cells, which express HyHEL10 antibody on their cell surface, are stimulated with HEL, the cells are activated and their intracellular Ca^{2+} concentrations increase (18). We prepared MD4 B-cells, loaded them with a fluorescent Ca^{2+} indicator, Fluo-4 (19), and arrayed them on the chip. The fluorescence of the cells was monitored with a cell scanner that was equipped with a 473-nm laser to activate and monitor the fluorescence of Fluo-4. Before stimulation, the fluorescence of each cell was measured and analyzed with the cell scanner at the single-cell level (left panel of Fig. 2A). Then, MD4 splenocytes were stimulated with HEL and their fluorescence was monitored with the cell scanner (right panel of Fig. 2A). The cell scanner scans the total cell area between 30 and 90 s after the stimulation. Because the address of each cell on the chip is fixed, we could compare the fluorescence intensity of each cell before and after stimulation by using analysis software. As shown in Figure 2B, dots corresponding to cells whose fluorescence was unchanged after the stimulation were located on the "y = x" line. When B-cells were activated with HEL, the intracellular Ca^{2+} level was increased, and the fluorescence intensity was augmented by a factor of about five. Dots corresponding to such activated B-cells shifted upward and were discriminated from those of the unstimulated cells. If cells with a twofold increase in Fluo-4 fluorescence were considered as positive cells, 49% of the cells became positive. If cells with a fourfold increase in fluorescence were considered as positive cells, 17% of the cells were positive. If cells with a twofold increase in fluorescence were considered as positive cells, 17% of the cells became positive. The earlier results demonstrate that a combination of a microwell array chip and a cell scanner (cell microarray system) can monitor the activation of about 200,000 individual cells by monitoring the alteration of intracellular Ca^{2+} concentration. We then compared the efficiency of the cell microarray system for detection of activated MD4 B-cells with that of a flow cytometry system (Figs. 2B and 2C). Splenocytes prepared from MD4 transgenic mice or normal mice were prepared and loaded with Fluo-4. These cells were mixed...
**Figure 2.** Analysis of B-cells with the cell microarray system. (A) Scanned image of intracellular Ca\(^{2+}\) signals of B-cells arrayed on a micro-well array chip before and after stimulation. MD4 B-cells were loaded with Fluo-4, stimulated with HEL on the chip, and scanned with a cell scanner before (left) and after (right) stimulation. (B) Scattered plots of Fluo-4-intensities of MD4 B-cells before and after HEL-stimulation. Various percentages (100%, 10%, 1%, 0.1%, and 0%) of MD4 splenocytes were prepared by mixing with normal splenocytes, loaded with Fluo-4, arrayed on a chip, and stimulated with HEL (10 \(\mu\)g/ml). The cells were scanned with a cell scanner and Fluo-4 fluorescence signals before (x-axis) and after (y-axis) stimulation were calculated and plotted on scatter diagrams. Each spot corresponds to one cell. Cells whose fluorescence was not altered correspond to dots on the blue line (y = x). Dots of the activated cells shifted upward. Cells with more than a fourfold increase in Fluo-4 fluorescence intensity were considered as responding cells and enclosed with a box. (C) The same MD4 B-cell preparation was analyzed with a flow cytometer. Fluorescence histograms of lymphocytes before (light gray) and after (dark gray) stimulation are shown. The percentages of fluorescence positive cells after stimulation, before stimulation, and their differences are shown from top to bottom in each panel. (D) Comparison of cell microarray system and flow cytometry for detecting activated cells. The percentages of HEL-activated MD4 B-cells detected with either the cell microarray system (black column) or flow cytometry (open column) were calculated as in Figures 2B or 2C. The results represent the average of three independent experiments, and error bars represent the standard deviation.
with various ratios such as 100%, 10%, 1%, and 0.1% MD4 spleen cells. Cell preparation was applied on the microwell array chip and stimulated with HEL, and the fluorescence of each B-cell before and after stimulation with HEL was measured with the cell scanner (Fig. 2B). To compare the detection efficiency with flow cytometry, the spleen cell mixture was stimulated with HEL and the alteration of fluorescence intensity was also measured with a flow cytometer (Fig. 2C). Figure 2D shows the percentages of positively detected cells with the cell microarray system or flow cytometry. We selected cells with a more than fourfold increase in Fluo-4 fluorescence after stimulation with antigen as antigen-activated B-cells in the cell microarray analysis. For the flow cytometry analysis, we calculated the positive cells by subtracting the percentage of positive cells before stimulation from the percentage of positive cells after stimulation. As noted, we observed the cell population with high Fluo-4 fluorescence intensity before stimulation, which was detected not only in the cell microarray system but also in flow cytometry (Figs. 2A–2C). The cell microarray system could distinguish the false positive cells and antigen-stimulated cells whose Fluo-4 fluorescence signals increased after antigen stimulation because each cell address was fixed on the chip and the fluorescence levels of the same cell before and after stimulation could be compared. In contrast, flow cytometry could not make the same distinction because it could not repeatedly monitor the signals of specific cells. Therefore, the detection of antigen-stimulated B-cells of low frequency with flow cytometry was hampered by the false positive cells, but was not hampered with the cell microarray system (Fig. 2D). In the case of the microwell array chip, the percentage of positively detected cells was linearly decreased with the dilution of MD4 spleen cells and about 0.2% of the positive cells were still detected in 0.1% of the MD4 spleen cells. The background of the positive cells was around 0.08% (Figs. 2B and 2D). In contrast, an analysis of less than 1% of the MD4 spleen cells with the flow cytometry system could not show the difference because of the false positive cells (Figs. 2C and 2D).

**Application to the Detection of HBV-Specific B-Cells and Generation of MoAb with Neutralizing Activity of HBV Infection**

Because the efficacy of detecting single cells using the cell microarray system was verified, we tried to apply the system to detect human antigen-specific B-cells and prepare human antigen-specific MoAb. Volunteers were vaccinated with recombinant HBs-Ag according to the vaccination protocol. Seven days after the last vaccination, B-cells were prepared from peripheral blood, loaded with Fluo-4, applied on a microwell array chip, and stimulated with HBs-Ag on the chip. The fluorescence intensity of individual cells before and after stimulation was measured with the cell scanner. As shown in Figure 3A, the fluorescence intensity of the minor cell population increased with the HBs-Ag stimulation. Figure 3B shows the percentage of activated B-cells before and after the boost of HBs-Ag in the volunteers. The HBs-Ag boost almost doubled the percentage of positive cells (Fig. 3B). We then retrieved the positive cells from the microwells using a micro-manipulator and the heavy chain and light chain variable regions of cDNA were augmented from a single B-cell by RT-PCR. The cDNAs were inserted into expression vectors, which were then cotransfected to 293T cells derived from human embryonic kidney (Supplementary Fig. 2). The culture supernatants of the 293T cells were collected and the specific binding activity of the antibody was analyzed with ELISA. Sixty-six IgM and 12 IgG cDNAs were cloned from 377 individual B-cells. In 12 IgG cDNAs, 3 IgG (116, 477, and 478) were found to bind HBs-Ag specifically (Fig. 3C). It has been reported

**Figure 3.** Detection of HBs-Ag-specific B-cells and production of HBs-Ag-specific antibodies with the cell microarray system. (A) Scatter diagram of peripheral blood B-cells before and after stimulation with HBs-Ag (100 μg/ml). (B) Percentage of HBs-Ag-stimulated B-cells in peripheral blood B-cells before and after application of HBs vaccine. Black bars show the average. (C) Antigen specificity of anti-HBs antibody. The antigen specificity of 116, 477, and 478 antibodies were examined with a competitive binding assay. Binding of the antibodies to plate-coated HBs-Ag was examined in the presence of various doses (0.4, 2, 10 μg/ml) of soluble HBs-Ag. Representative data of three to four independent experiments are shown. (D) Determination of epitope of anti-HBs antibodies. Binding of the antibodies to plate-coated HBs-Ag was examined in the presence of competitive MoAb to specific epitopes (a, r, d) on HBs-Ag. *P < 0.01.
that there are three major epitopes, a, d/y, and r/w on HBs-Ag (20). The epitopes recognized by the IgG antibodies were examined by competition with epitope-specific antibodies. As shown in Figure 3D, 116 was found to bind epitope “a” since the “a”-epitope-specific antibody inhibited the binding of 116 to HBs-Ag. Because the other antibodies (477 and 478) were not competed with the antibodies used, we could not determine their epitopes. The affinities of the 116, 477, and 478 antibodies were determined with surface plasmon resonance. It was revealed that their $K_d$ were $1.2 \times 10^{-9}$ M, $5.9 \times 10^{-7}$ M, and $1.4 \times 10^{-7}$ M, respectively (data not shown).

Finally, we tested to see if these antibodies inhibit the infection of HBV in human hepatocytes. We used chimeric mice to which human hepatocytes had been transplanted (16,17) (Fig. 4A). We injected i.v. $1.0 \times 10^6$ copies of HBV into the mice in the presence of control, 116, 477, or 478 antibody. Infection and replication of the virus was determined by detecting the viral genomic DNA in the serum with PCR. Figure 4B shows that the virus DNA was detected 4 weeks later in the chimeric mice injected with the virus together with either the control or 477 antibody. In contrast, the virus DNA was not detected in the serum of the mice that had been injected with the virus together with either the 116 or 478 antibody, demonstrating that these antibodies showed the neutralizing activity.

**DISCUSSION**

We described a live cell microarray chip, which arrays a large number of nonadherent cells, such as lymphocytes, by accommodating them in microwells whose size and shape are optimized to trap only a single cell. By stimulating individual cells on the chip and monitoring cell signals such as intracellular Ca$^{2+}$ levels before and after antigen-stimulation, we could efficiently detect the activation of individual cells. Since the cell scanner repeatedly scans 234,000 cells in about 2 min/scan, it can follow the history of cell signals every 2 min. We verified that this system is more suitable for analyzing the “cytome” (21,22) when compared with microscopy and flow cytometry-based systems, which cannot analyze the history of the cellular responses of large numbers of cells at the level of single cells. Using a novel microwell array chip, we could successfully analyze human B-cells of volunteers who had been vaccinated with HBs-Ag and generate some MoAb that were able to neutralize the HBV infection of human hepatocytes.

Regarding the viability of cells on the chip, Fluo-4 fluorescence intensities of about 90% of the B-cells were increased by stimulation with anti-IgM antibodies (data not shown). As about 90% of B-cells express IgM on the cell surface, the result indicates that most of the B-cells were alive on the chip during the assay. The diameter and depth of the microwells were critical in preparing an array of single cells (data not shown and Fig. 1D). When the diameter or the depth was insufficient, array rate was decreased, and when they were too great, fluorescence signals of two to three cells were observed under a fluorescence microscope (data not shown). In this study, well size and shape were optimized to those of lymphocytes, so that we observed single fluorescence signals from each well, as shown in Figure 1C.

Recently, Deutsch et al. reported a live cell array to perform cell-based assays on thousands of individual cells (7).
Microcavities were arrayed in a honeycomb-like structure and could monitor cell responses, such as reactive oxygen species generation, on a single-cell basis (23). Biran and Walt reported an optical imaging fiber-based single live cell array for analyzing yeast or bacterial cells (8). These cell arrays were specifically designed for analyzing cells, and it may be difficult to retrieve objective cells from wells without disturbing the surrounding cells. In our cell microarray, each well was separated so that we could retrieve single objective cells without disturbing the surrounding cells.

Therapeutic MoAb have been developed into beneficial and profitable medical products in molecule-targeted therapeutics (24). To produce fully human MoAb, the bacteriophage display method and the Epstein-Barr virus method have been developed (25,26). The bacteriophage display method requires the preparation of a large-scale bacteriophage library (~10^11 clones) to get an antigen-specific antibody, making the method difficult to perform in ordinary laboratories. With the availability of a cell scanner, microwell array chips, and a micromanipulator, our system is easy to operate and can be performed in any laboratory that is so equipped. Recently, we have developed a prototype of equipment that can automatically perform processes from cell application to cell retrieval, making the system more appropriate as a procedure for producing human MoAb. Concerning the Epstein-Barr virus method, the virus can transform human B-cells to produce human antibodies. Because only a part of the B-cells can be transformed with the virus, the method cannot efficiently screen antigen-specific B-cells. Our system can screen antigen-specific B-cells directly from freshly separated human peripheral blood lymphocytes and produce antigen-specific MoAb.

We showed that the human anti-HBs antibodies that we had produced exhibited neutralization activity to inhibit HBV infection of human hepatocytes. To demonstrate the neutralization activity, we constructed an estimation system using chimeric mice transplanted with human hepatocytes (16). As HBV can infect only fresh human hepatocytes or that of primates, but not that of rodents, examination of neutralization activity has mostly been performed on chimpanzees (27), which is very expensive and might be contrary to the principles of animal welfare. The use of chimeric mice described in this study is simple and not expensive when compared with the conventional method using chimpanzees because mice are small, easy to breed, and much less expensive.

In conclusion, we have demonstrated and described a system for analyzing a large number of cells on a single-cell basis, which can be applied to “cytomics” analysis, and to the production of human MoAb directly from human peripheral blood B-cells. The system might be applicable for analyzing T-cells to detect antigen-specific T-cells and for cloning TCR cDNAs. By changing the chip design, we are able to analyze various kinds of cells such as hybridoma, hepatocytes, and nerve cells as well as lymphocytes. Our technology should expand the horizons of cell analysis as well as the generation of human MoAb for antibody-based therapeutics and diagnosis of hepatitis virus infection.

ACKNOWLEDGMENTS

We thank M. Suzuki, I. Maruyama, H. Nakazato, and Y. Shimizu for their helpful contributions to our discussions, T. Nakashima for providing anti-HBs MoAb, S. Hirota for technical assistance, and K. Hata for secretarial assistance.

LITERATURE CITED