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Hybridoma technologies for antibody production

Hybridoma technology features effective usage of innate functions of both immune cells and cancers, allowing production of hybridoma cells, which continuously generate monoclonal antibodies specific to antigens of interest. For standard generation of hybridoma cells, B lymphocytes must be somatically fused with myeloma cells using various technologies. However, the methods generally do not necessarily result in selective fusion of target B lymphocytes with myeloma cells. To overcome this problem, we have developed a new hybridoma technology that involves preselection of B lymphocytes with target antigens based on immunoglobulin receptors and selective fusion of B cell–myeloma cell complexes with electrical pulses. The advanced methodology, termed B-cell targeting, multitargeting and stereospecific targeting, may be applicable to simultaneous production of monoclonal antibodies, selective production of stereospecific monoclonal antibodies, and also to efficient generation of human monoclonal antibodies for clinical purposes.

KEYWORDS: antigen-based selection ▪ B lymphocyte ▪ biotin–avidin interaction ▪ electrical pulse ▪ hybridoma cell ▪ immunoglobulin receptor ▪ monoclonal antibody ▪ myeloma cell ▪ selective fusion

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Hybridoma technology, originally established by Köhler and Milstein [1], continues to be of critical importance for raising monoclonal antibodies against antigens of interest. The most important point with this approach is the utilization of innate functions of immune cells, maturated in lymphoid organs, through a humoral immune response and of cancers.

The maturation pathway of B lymphocytes is stringently regulated in vertebrates. B lymphocytes that can produce antibodies first arise in bone marrow as hematopoietic stem cells and differentiate into pro-B, pre-B and immature B lymphocytes. These latter have important functions, harboring immunoglobulin receptors on their surfaces that can specifically bind antigens. Before leaving the bone marrow, IgM-type antigen-specific receptors on immature B lymphocytes are utilized to remove those reacting with self-antigens. This process is extremely important to self-antibody attack. Only immature B lymphocytes insensitive to self-antigens are able to differentiate into mature B lymphocytes, expressing both IgM- and IgD-type immunoglobulin receptors, which occurs predominantly in the bone marrow but can also take place in secondary lymphoid organs such as the spleen and lymph nodes [2]. When immune cells encounter foreign antigens, an immune response swiftly begins. First, professional antigen-presenting cells capture the invaded foreign antigens and present their immune information on their cell surfaces in association with MHC class II molecules. This information is then transferred to helper T lymphocytes, which are thereby activated and selectively stimulate antigen-sensitized mature B lymphocytes that harbor the same peptide–MHC complexes on their cell surface as that observed on the antigen-activated professional antigen-presenting cells. Activated B lymphocytes may differentiate further into plasma cells, which synthesize and secrete antibodies of the same antigenic specificity as that of the immunoglobulin receptor on the B lymphocyte. The second major pathway after antigen activation is to become a memory B lymphocyte that is capable of being activated for a secondary, and more rapid, response to the same antigen. The production of memory B lymphocytes is associated with class switching and somatic hypermutation. Repeated stimulation by the same antigen can help increase the production of high-affinity antibodies, a process known as affinity maturation.

With the original hybridoma technology, hemagglutinating virus of Japan (HVJ) and polyethyleneglycol (PEG) were utilized for somatically fusing antigen-sensitized B lymphocytes and myeloma cells to produce hybridoma cells [1,3]. However, this caused

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nonspecific fusion between many kinds of cells without control. There are two critical points for successful production of novel monoclonal antibodies based on hybridoma technology. The first point is immunization to facilitate differentiation of B lymphocytes into more matured forms. The second point is selective fusion of targeted antigen-sensitized B lymphocytes with myeloma cells. If these critical points are achieved, hybridoma technology allows generation of monoclonal antibodies with high affinity and specificity, contributing not only to basic science research, but also to medical analyses and therapeutics on the basis of specific binding between antigens and antibodies.

This article covers the next generation of hybridoma technology, which features antigen-based preselection of B lymphocytes and their selective fusion with myeloma cells using electrical pulses. Feasible future applications of the advanced technology are also discussed.

Conventional hybridoma technology

Hemagglutinating virus of Japan & polyethyleneglycol

The original establishment of hybridoma technology was an outstanding achievement [1]. There are several important points that should be emphasized. First is the use of cancerous cells to confer B lymphocytes with immortality. Somatic fusion of B lymphocytes in spleen cells with cancerous myeloma cells give rise to hybridoma cells, which continuously produce antibodies *in vitro*. The other, is realization of the production of monoclonal antibodies specific to an epitope in an antigen. Since a single B lymphocyte generates an antibody specific to one epitope, monoclonal antibodies generated by hybridoma technology can specifically recognize single epitopes of an antigen. In addition, the monoclonal antibodies yielded by this technology are always homogeneous. Before the advent of hybridoma technology, only polyclonal antibodies from sera of immunized animals were available, which cross-reacted with several epitopes and were often inhomogeneous, depending upon their production batches.

Although the original hybridoma technology was established more than 35 years ago [1], it is still applied because of its simple protocol. After mixing spleen and myeloma cells, cell mixtures can be somatically fused by the addition of fusing reagents such as HVJ and PEG, as shown in Figure 1. HVJ was first utilized for cell fusion, but later, PEG became available. Although the protocol is very simple, there is one serious problem; PEG, as well as HVJ, result in very low efficiency without selectivity owing to the failure to control cell fusion. In effect, more time and procedures are needed for isolating the target hybridoma cells.

Improved hybridoma technology

Pearl-chain formation

To improve the original concept, other hybridoma technologies have subsequently been established. The pearl-chain formation [4], outlined in Figure 2, uses an electric field. Myeloma cells and spleen cells, including immunized B lymphocytes, form a monolayer on electrode surfaces by the application of a nonuniform alternating current field and become fused with application of electric field pulses to produce hybridoma cells. This method certainly increased the fusion efficiency, but it still brought about nonspecific spleen cell–spleen cell and also myeloma cell–myeloma cell fusion. In addition, even when a spleen cell and a myeloma cell were successfully fused to yield a hybridoma cell, it was impossible to control selective fusion of only the desired B lymphocyte with a myeloma cell.

Laser radiation

Another improvement was also achieved with laser radiation [5]. Performed under a microscope, a lymphocyte is transferred by trapping laser so that it comes into contact with a myeloma cell (SP2 cell). The contact surface of the lymphocyte–myeloma cell is then irradiated with a pulse laser beam, as outlined in Figure 3. Such a fusion process operated manually has an obvious advantage because it can precisely control the fusion between a lymphocyte and a myeloma cell. However, it usually takes a long time to manually fuse all cells under a microscope because at least 10^6 cells sensitized B lymphocytes would be expected to exist in a spleen from an immunized mouse. In addition, it is impossible to discern the specifically targeted immunized B lymphocytes for fusion with myeloma cells.

Consequently, while the pearl-chain formation and laser radiation methods have certain advantages over conventional hybridoma technology regarding the frequency of fusion, they still fail to control selective fusion of an aimed B lymphocyte with a myeloma cell to solely generate hybridoma cells secreting the desired monoclonal antibodies.

Next generation of hybridoma technology

B-cell targeting

To achieve selective fusion of a desired B lymphocyte with a myeloma cell, a new hybridoma technology was developed, as first described by Lo *et al.* [6]. The completely different approach comprises the three critical steps, outlined in FIGURE 4. The first is antigen-based preselection of sensitized B lymphocytes based on immunoglobulin receptors. Second, the antigen-selected B lymphocytes are brought into contact with myeloma cells by strong and specific binding between biotin and avidin. Finally, B lymphocyte–myeloma cell complexes are selectively fused by electrical pulses. These three critical steps realize selective production of hybridoma cells secreting novel monoclonal antibodies against antigens of interest. This advanced technology is termed as pulsed electric field method [7–9] or B-cell targeting (BCT) [10–14]. Recently, the three steps have been clarified on the basis of immunofluorescence analysis [11].

Two protocols of BCT have been developed, depending on the conjugates for selecting B lymphocytes, either antigen–biotin (FIGURE 4A) or antigen–avidin (FIGURE 4B). In the latter case, antigen–streptavidin is also available.

Antigen–biotin selection

After B lymphocytes are selected by antigen–biotin conjugates, B cell–B cell complexes are sometimes formed by addition of avidin or streptavidin. Since each of these complexes harbors four binding sites for biotin [15,16], antigen-selected B lymphocytes can bind to themselves through avidin or streptavidin, giving rise to B cell–antigen–biotin–avidin (streptavidin)–biotin–antigen–B cell complexes. The formation of B cell–B cell complexes may cause a decrease in the fusion efficiency between antigen-selected B lymphocytes and myeloma cells. This could be partly overcome by an addition of excess molar ratio of avidin or streptavidin to the biotinylated antigen.

Even with the formation of such complexes, this technique (FIGURE 4A) has certain advantages over the conventional methods. When the F_o subunit of F_oF_1-ATPase from bovine heart mitochondria was employed as an antigen, fusion efficiency was more than 16.5%, whereas that obtained by a PEG-mediated method was only 1.1–5.6% [8]. The fusion efficiency indicates the percentage of ELISA-positive wells to hybridoma-positive wells. Other groups also substantiated the suitability of BCT [17–19].

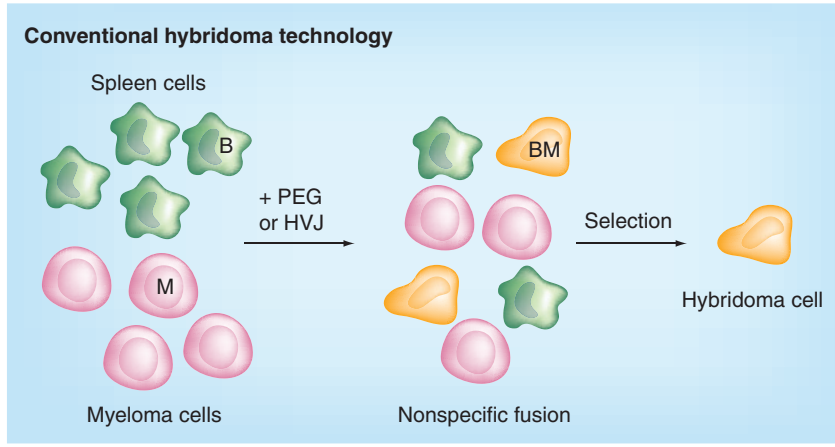


Figure 1. Conventional hybridoma technology based on polyethyleneglycol and hemagglutinating virus of Japan.
B: B lymphocyte; BM: Hybridoma cell; HVJ: Hemagglutinating virus of Japan; M: Myeloma cell; PEG: polyethyleneglycol.

There is another important point for this protocol. The use of streptavidin is most reasonable because of its acidic isoelectric point (pI) [15,16], with retention of a negative charge in the neutral pH region. This property inhibits nonspecific binding of streptavidin to negatively charged B lymphocytes by static interaction. By contrast, avidin has a pI value in the alkaline region (pI = 10) [20], harboring a positive charge at neutral pH. Considering the effectiveness of biotinylation by *N*-hydroxysuccinimido (NHS)-biotin, protein molecules are most suitable for this protocol.

Antigen–avidin selection

To prevent the formation of B cell–B cell complexes as described above, antigen–avidin (or antigen–streptavidin) conjugates can be

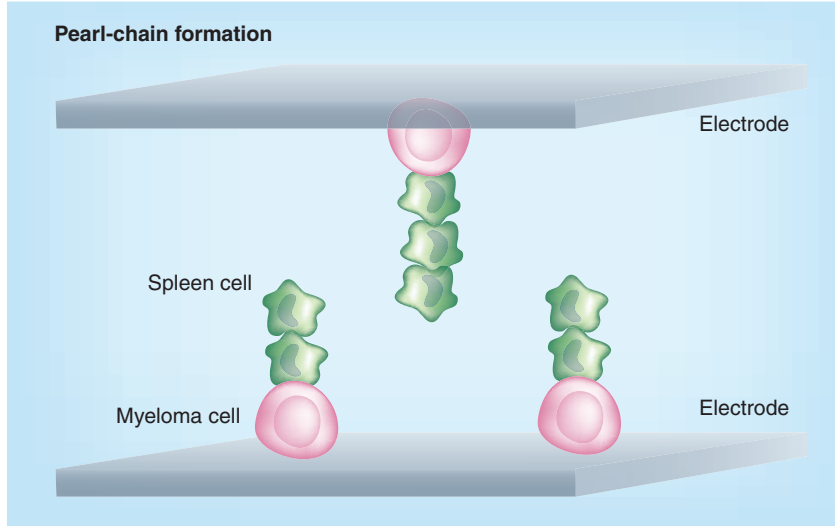


Figure 2. Improved hybridoma technology based on pearl-chain formation.

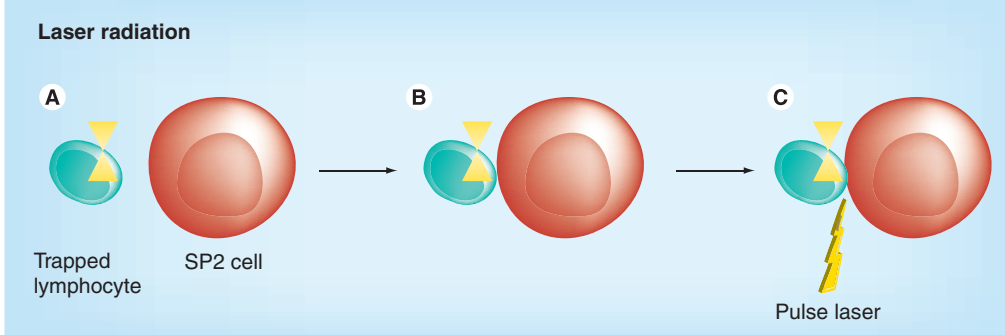


Figure 3. Improved hybridoma technology based on laser radiation.
SP2: Myeloma cell.

utilized for selecting B lymphocytes. When B lymphocytes are targeted by antigen–avidin conjugates, it is impossible to form B cell–B cell complexes through avidin. Antigen–avidin conjugates are prepared by iminobiotin Sepharose column chromatography using 1,5-difluoro-2,4-dinitrobenzene as a chemical modifier [6] so that Lys residues in both antigen and avidin are cross-linked.

Other cross-linkers such as *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and m-maleimidobenzoyl *N*-hydroxysuccinimide

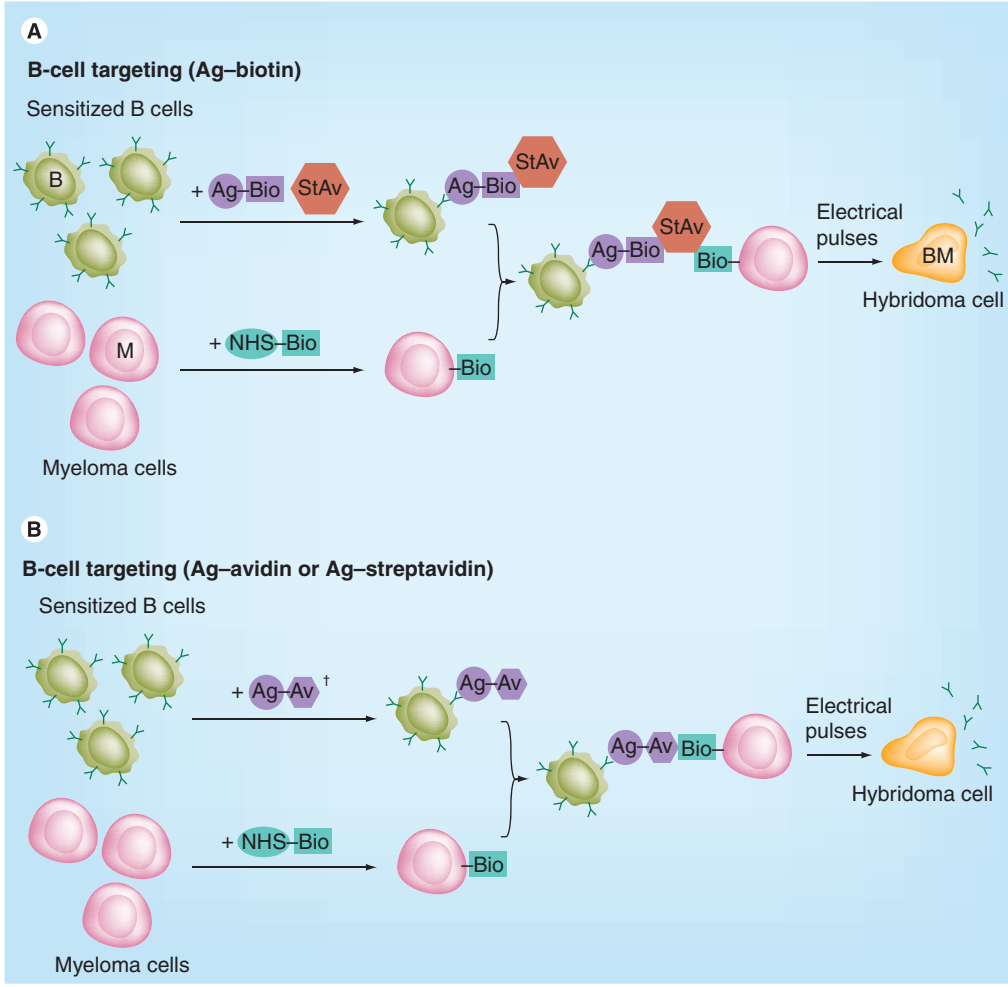


Figure 4. Next generation of hybridoma technology based on B-cell targeting. Selection of sensitized B lymphocytes was carried out with either **(A)** antigen–biotin or **(B)** antigen–avidin (or antigen–streptavidin) conjugates.
†Ag–StAv is also available.
Ag: Antigen; Av: Avidin; B: B lymphocyte; Bio: Biotin; BM: Hybridoma cell; M: Myeloma cell; NHS-Bio: *N*-hydroxysuccinimido-biotin; StAv: Streptavidin.

(MBS) have also been employed to improve the recovery of antigen–avidin conjugates and to regulate the modification of antigen and avidin. The characteristic of both cross-linkers is that they are heterobifunctional, making covalent bonds with Lys and Cys residues. Reactions can be carried out in solution with high recovery of antigen–avidin conjugates. A typical protocol for yielding antigen–avidin conjugates by SPDP or MBS is demonstrated in the foregoing study [8,10].

Formation of B lymphocyte & myeloma cell complexes

B lymphocytes, obtained from antigen-immunized mouse, are preselected by either antigen–biotin or antigen–avidin (or antigen–streptavidin) conjugates and combined together with myeloma cells by harnessing the power of biotin and avidin (or streptavidin), as shown in FIGURES 4A & B.

Applications of BCT

Long peptide

To evaluate the applicability of BCT, a long peptide composed of 49 amino acids containing three different aimed peptide sequences was first used for immunization, and each of three different peptide–avidin conjugates were employed for preselecting B lymphocytes according to FIGURE 4B. As a result, monoclonal antibodies were successfully generated with a high fusion efficiency of approximately 50.4% on average. This is 5–40-fold greater than that obtained with the PEG-mediated method (only 1.8–8.2%) [10]. Furthermore, each monoclonal antibody yielded by BCT displayed high specificity for the corresponding peptide sequence. No cross-reactivity towards other peptide sequences in the long peptide was detected. This was in clear contrast to the monoclonal antibodies generated by the PEG method, which had no specific reactivity, but showed cross-reactivity to unspecified regions. In these experiments, a long peptide linked to keyhole limpet hemocyanin was used for immunization to effectively enhance immune responses.

Short peptide

Next, the applicability of BCT for a relatively short peptide sequence, composed of 15 amino acids, was also confirmed. In this study, immunization was carried out with either peptide–(MBS)–protein or peptide–(SPDP)–protein conjugates. When peptide–(MBS)–protein conjugates were employed for immunization, the majority of polyclonal antibodies, except recognizing protein molecule, displayed reactivities with the region containing MBS moiety. This must be attributed to antigenicity of aromatic residue derived from MBS. The region including aromatic moiety of MBS might be recognized as an epitope. To address this problem, we utilized peptide–(SPDP)–protein conjugates for immunization. There is some risk with this usage as an antigen, because disulfide bonds between peptides and SPDP would be labile under reducing conditions in the mouse. However, it is reasonable that only a linear carbohydrate moiety from SPDP was included in the peptide–(SPDP)–protein conjugates after chemical modification. Consequently, we could obtain promising results to generate monoclonal antibodies specific to the peptide sequence based on BCT (FIGURE 4B) [TOMITA M & TSUMOTO K; UNPUBLISHED DATA].

Low-molecular-weight compound

The potential of BCT was also evaluated using a low-molecular-weight compound. With such materials as antigens, it is generally acknowledged that the number of sensitized B lymphocytes must be small. However, specific selection with the target antigen on the basis of immunoglobulin receptors helps isolation and concentration of sensitized B lymphocytes. For the purpose of our test, a chemical compound, di-(2-ethylhexyl) phthalate (DEHP), was chosen as the antigen. DEHP has long been utilized as a plasticizer all over the world but now is suspected of having endocrine disruptor potential. According to BCT as shown in FIGURE 4B, we could successfully generate novel high affinity monoclonal antibodies against DEHP showing a little cross-reactivity with other phthalate derivatives. After cloning of hybridoma cells, the dissociation constant (Kd) values were between 10^{-9} and 10^{-11} M [21]. It is worth mentioning that preselection of sensitized B lymphocytes by antigens can bring about preferential selection of maturated B lymphocytes expressing high-affinity immunoglobulin receptors on their surfaces.

Therefore, BCT can be considered applicable for efficient generation of monoclonal antibodies against peptides and low-molecular-weight compounds. In theory, all hybridoma cells yielded by BCT could secrete the desired monoclonal antibodies.

In vitro immunization

Immunization is a very important process to enhance differentiation of antibody-producing B lymphocytes into maturated forms. If

immunization is ideally carried out, it will successfully give rise to hypermaturated B lymphocytes that secrete specific and high-affinity antibodies against epitopes of antigens. However, for this purpose, immunization must usually be carried out *in vivo* and it will take a few months to be accomplished. If immunization could be completed within the short term, it would certainly contribute to swift generation of monoclonal antibodies. One focus is on immunization *in vitro* [22]. Spleen cells from a nonimmunized mouse were incubated in the coexistence of muramyldipeptide, IL-4, lipopolysaccharide and a target antigen in a CO_2 incubator at 37°C for 3–5 days [23–27]. It is interesting that even after short-term immunization *in vitro*, some antigen-selected B lymphocytes were detected on the basis of immunofluorescence analysis [11], and IgM- and IgD-double-positive maturated B lymphocytes were also found by Fluorescence-activated cell sorting analysis [26,27]. However, monoclonal antibodies generated by BCT after immunization *in vitro* displayed relatively wide cross-reactivity. This might be overcome by use of the ImmortoMouse® (Charles River, MA, USA) as described later under the 'Conclusion & future perspective' section. Hybridoma cells obtained by this technology after short-term immunization appear to have some advantages. Because they already express the genes encoding the desired antibodies, genetic information obtained from hybridoma cells may be one of several candidates as lead genes for generation of more specific and high-affinity monoclonal antibodies by exploiting the power of genetic engineering [28–34]. Genes from DNA libraries, after *in vitro* selection by the antigen, can often be employed for affinity maturation of monoclonal antibodies [31].

Fusion efficiency

Although BCT elicits high fusion efficiency, there are still some areas for improvement. One is regulation of the directions of antigen-selected B lymphocyte–myeloma cell complexes to align in vertical positions at both electrodes based on dielectrophoresis. This would allow dissipation of the electric field more effectively as described later in the 'Conclusion & future perspective' section. Another is to increase attachment areas between B lymphocytes and myeloma cells, formed by biotin and avidin (or streptavidin) interactions. We have already demonstrated that addition of a low concentration of PEG may be effective for increasing fusion efficiency with electrical pulses [11,14]. The point is that the concentration of PEG must be sufficiently low not to itself elicit nonspecific cell fusion [35]. More recently, we have confirmed that usage of low hemagglutinating units (HAU) of HVJ is also effective for increasing fusion efficiency [Tomita M & Tsumoto K; Unpublished Data].

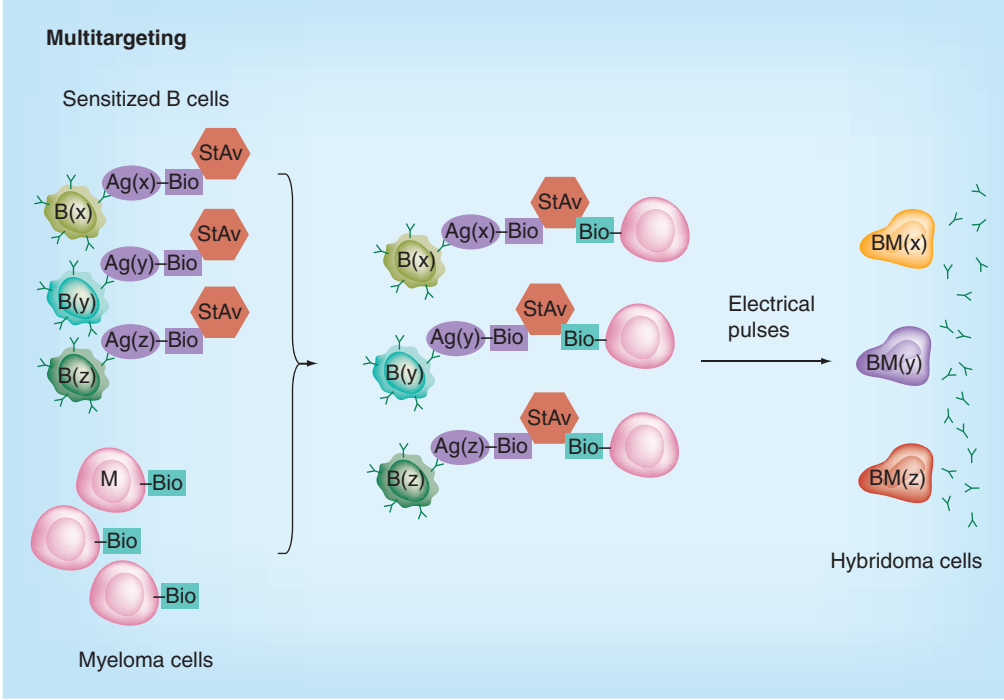


Figure 5. Next generation of hybridoma technology based on multitargeting.
Ag: Antigen; B: B lymphocyte; Bio: Biotin; BM: Hybridoma cell; M: Myeloma cell; StAv: Streptavidin.

The characteristic of cell fusion by electrical pulses is that only attached cells are preferentially fused. No fusion occurs between any nonattached free cells. An additional point for fusion to generate hybridoma cells is that the ratio of B lymphocytes to myeloma cells must be controlled at one to one. We have collected some evidence with regard to this. On specific binding of antigens to immunoglobulin receptors on B lymphocytes, antigen-bound receptors may gather together on parts of the cell surface. This may result in inhibition of multiple binding of biotinylated myeloma cells to antigen-selected B lymphocytes, as shown by immunofluorescence analysis [11].

Multitargeting

It is generally accepted that one mouse must be utilized for immunization with one antigen in order to raise monoclonal antibodies. This means that, in case of yielding monoclonal antibodies against multiple antigens, the same number of mice are needed. If monoclonal antibodies against multiple antigens could be generated using only one mouse, it would greatly contribute to mouse protection as well as reducing the laborious work necessary for the production of monoclonal antibodies. To realize this aim, a multitargeting technique was developed, as outlined in FIGURE 5. The point of this new technology is that each B lymphocyte sensitized by multiple antigens is selected in advance with the corresponding antigen based on immunoglobulin receptors on B lymphocytes. Since single B lymphocytes theoretically produce single specific antibodies against one epitope in an antigen, selection of sensitized B lymphocytes by each antigen is possible. This step is of critical importance in the entire pathway of the multitargeting technique. A corollary is whether each single B lymphocyte is specifically selected by the target antigen, even in the presence of many kinds of sensitized B lymphocytes. Our preliminary results indicate no interference of other sensitized B lymphocytes, specific selection of the desired B lymphocytes by the corresponding antigen being observed after immunization with multiple antigens [TOMITA M & TSUMOTO K; UNPUBLISHED DATA]. However, there is one question. If multiple antigens are used for immunization in one mouse, immune suppression might occur owing to concomitant immune stimulation with other antigens. In our study, use of at least three to five antigens appears possible for immunization in one mouse and monoclonal antibodies directed against each antigen could be generated successfully [36].

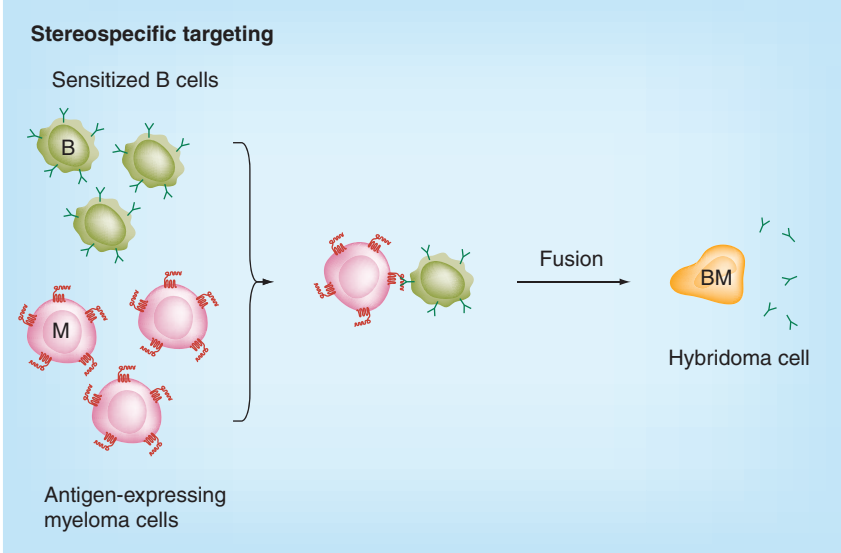


Figure 6. Next generation of hybridoma technology based on stereospecific targeting.
B: B lymphocyte; BM: Hybridoma cell; M: Myeloma cell.

Stereospecific targeting

Recently, conformation-specific monoclonal antibodies have become an extremely important focus, especially for the purpose of therapies for cancer and diseases caused by self-immunodeficiency. Demand for stereospecific monoclonal antibodies will surely increase in the future. However, no practical protocols have been reported until now. In order to address the problem of very low efficiency in raising monoclonal antibodies specific to tertiary structures of antigens, we developed a new technology [37], as outlined in FIGURE 6. The main feature of the new technology is employment of antigen-expressing myeloma cells for selecting sensitized B lymphocytes, where the transfected myeloma cells maintain high viability. The antigens expressed on the surfaces of intact cells retain their tertiary structures and antigen-selected B lymphocytes, which are myeloma cell–(antigen)–(immunoglobulin receptor)–B lymphocyte complexes, could be selectively fused by electrical pulses. In this step, even the use of PEG displayed relatively specific fusion for B lymphocytes selected by myeloma cells, possibly owing to attachment by specific interactions between antigens and antibodies. When the thyroid-stimulating hormone receptor was chosen as an antigen, monoclonal antibodies directed against thyroid-stimulating hormone receptor were able to compete for the same binding site targeted by TSH [38,39]. The frequency of producing hybridoma cells for stereospecific targeting would not be as high as that obtained by BCT, which may be attributed to the difference in binding affinities between B lymphocytes and

myeloma cells. For stereospecific targeting, these are based on the interactions between antigen and antibody (Kd = 10^{-7}–10^{-8} M), whereas stronger links may be available for BCT on the basis of biotin and avidin interactions (Kd = 10^{-15} M).

Conclusion & future perspective

Requirements of human monoclonal antibodies for medical purposes have been dramatically increasing, since they are very specific to target antigens, have a relatively long half-life and also exert limited side effects on the body. They can be expected to be an important component of the next generation of promising medicines. Stereospecific targeting technology will contribute greatly to this scenario because of the advantages of generation of specific monoclonal antibodies against tertiary structures of desired antigens. Combinations of stereospecific targeting and transgenic mice [40–42] will be unambiguously practical to yield conformation-specific human monoclonal antibodies against target antigens.

One of the most critical steps in BCT is selective fusion of an antigen-selected B lymphocyte with a myeloma cell. This is also true for multitargeting and stereospecific targeting. When we apply electric pulses, a strong electric field is vertically formed between electrodes arranged in parallel. Therefore, complexes of B lymphocytes–myeloma cells aligned along the electric field are effectively fused, whereas no electrical fusion occurs for complexes in any other direction. Unfortunately, there have been no investigations of the efficiency of electric fusion dependent on the angles of cell complexes. However, it is certain that the efficiency will be significantly improved by controlling their directional arrangement. Among the physical manipulation methods for cells such as laser manipulation, electrophoresis, magnetic force and microfluidic flow, dielectrophoresis has attracted much attention as a driving force for cell manipulation applied to form aligned structures of cells [43–48]. Therefore, the use of DEP techniques before applying electric fields for cell fusion may result in a significant improvement in the efficiency. In addition, DEP manipulation may be easily incorporated into cell fusion systems since it is based on applying alternating current voltage.

There are several important characteristics of *in vitro* immunization. Lower levels of antigen can be used, the process can be accomplished in 3–5 days and toxic or unstable antigens *in vivo*, are also usable. However, maturation of B lymphocytes may be insufficient owing to short-term immunization. Therefore, the transgenic ImmortoMouse may have clear advantages [49,50] since its cells live longer *in vitro* and can be sensitized for a longer time. If such B lymphocytes are fused, hybridoma cells are able to secrete more specific monoclonal antibodies than those obtained from a normal mouse. In addition, the genes obtained from such hybridoma cells may be more suitable for genetic production of high-affinity monoclonal antibodies.

Another possibility for hybridoma technology to raise monoclonal antibodies is the use of animal species besides the mouse. Bigger animals may have a greater repertoire of B lymphocytes, for example rabbits [51,52]. Although the generated heterohybridoma cells proved very unstable when immunized rabbit spleen cells were fused with mouse myeloma cells containing rabbit genes [53], isolation of genes encoding aimed antibodies from heterohybridoma cells before degradation and transfection into mammalian

Executive summary

Conventional hybridoma technology

- The original hybridoma technology was aimed to generate hybridoma cells.
- Although the technology is simple, large numbers of undesired fused cells are produced because of nonspecific fusion.

Improved hybridoma technology

- Improved hybridoma technology has the characteristic of eliciting relatively specific fusion between a spleen cell and a myeloma cell.
- The techniques still lacks selective fusion of antigen-immunized B lymphocytes with myeloma cells.

Next generation of hybridoma technology

- Next generation of hybridoma technology consist of three critical steps that feature selective fusion of antigen-selected B lymphocytes with myeloma cells by electrical pulses.
- Antigen-based preselection of B lymphocytes preferentially selects those producing high-affinity antibodies.
- Theoretically, all hybridoma cells could secrete monoclonal antibodies against antigens of interest.

Conclusion

- Utilizing innate functions of immune cells and cancerous cells is of critical importance for hybridoma technology.
- Selective fusion of sensitized B lymphocytes with myeloma cells by electrical pulses elicits efficient production of hybridoma cells secreting target novel monoclonal antibodies.
- B-cell targeting, multitargeting and stereospecific targeting may contribute to the next generation of hybridoma technology.

cells, resulted in recombinant, extremely high-affinity, monoclonal antibodies with a Kd value of approximately 10^{-14} M [51,52]. This technology could be very useful for the preparation of novel monoclonal antibodies, which cannot be obtained by the conventional techniques.

Monoclonal antibodies generated by hybridoma technology always retain their native structure, composed of Fab and Fc portions. The Fc portion may be critically important for their secondary functions because this region is related to antibody-dependent cellular cytotoxicity [54,55] and complement-dependent cytotoxicity [54,55]. Monoclonal antibodies, selectively reacting with aimed cells with high specificity and affinity produced by BCT, multitargeting and stereospecific targeting, will markedly contribute to other important functions.

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Financial & competing interests disclosure

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Ethical conduct of research

All experiments were conducted according to Mie University's guidelines for the care and treatment of experimental animals.

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