New Strategies to Establish Human Monoclonal Antibodies

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1992, 168 (2), 323-327 — In order to establish human monoclonal antibodies to
any sort of antigens efficiently, we have made following two approaches. Our first
approach is to improve cell fusion frequency. By improving our previous method
for production of human hybridomas, we obtained higher frequency (1/700 vs. 1/
5500) compared with our previous method by adding irradiated myeloma cells to
culture of fusion cells and modifying the selective medium. Our second approach
is to use a SCID-hu mouse for immunization. Since the injection of human PBL
can result in the stable long-term reconstitution of a human immune system in
SCID mouse, we tried to immune SCID-hu mouse with KLH. In the serum of
immunized SCID-hu mouse, we obtained human IgG antibodies to KLH. Addition-
ally, we succeeded in establishing human B lymphoblastoid cell lines which
produced antibodies specific to KLH. These methods will open new prospects for
the detection and therapy of cancer. ————— human monoclonal antibody;
SCID-hu mouse; GIT medium; cell fusion

The need for the efficient production of human monoclonal antibodies to tumor-specific antigens is increasing. Compared with the establishment of the regular mouse monoclonal antibody, that of human monoclonal antibody to any sort of antigens is technically very difficult. This is mainly because (1) fusion frequency to establish human hybridomas is very low, and (2) immunization of humans with any sort of antigens is dangerous. In order to break down these obstacles, as a first approach, we tried to improve fusion frequency. As a second approach, we used SCID-hu mouse for immunization, as SCID-hu mouse could reconstitute a human immune system.

MATERIALS AND METHODS

(*Mouse* × *human*) heteromyeloma (SHM-D33) and GIT culture medium were mainly used for cell fusion experiments (Kudo et al. 1988).

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Selective medium. GIT-HAT selective medium was composed of a GIT medium supplemented with 1/50 vol. of concentrated HAT solution, 2-mercaptoethanol (2×10^{-5} M), dihydroxyethylglycine (1.8 mg/ml) and L-glutamine (2 mM). GIT-HAT-O was made by the addition of ouabain (2×10^{-6} M) to the GIT-HAT medium. The GIT-HAT-O^L (low ouabain) selective medium was made by the addition of ouabain (1×10^{-6} M) to GIT-HAT medium (Kudo et al. 1991).

Cell fusion and hybridoma selection. Approximately 1×10^6 EBV-lymphoblastoid cells and an equal number of myeloma cells were fused using PEG as described previously (Kudo et al. 1988). In order to determine fusion frequency, various numbers of fused cells were put in each well of a 96-well plate. In the our new culture schedule, the fused cells were resuspended in warm GIT medium after cell fusion. After overnight cultivation, 50 μ l of the selective medium (GIT-HAT-O) were added to each well. The cells were cultured in GIT-HAT-O medium for 5 days and then refed GIT-HAT selective medium.

SCID-hu mouse. The FOX CHASE SCID C.B-17/Icr-scid mice (8 weeks old, female) were purchased from Japan Clea (Tokyo). SCID-hu mice were established by intraperitoneal injection of peripheral blood mononuclear cells (5×10^7) from healthy volunteers.

Measurement of specific antibody activity. Human antibody specific to KLH was measured by ELISA using KLH-coated plate and peroxidase conjugated anti-human Ig as a second antibody.

RESULTS

Re-examination of the composition of selective medium

In order to obtain better fusion frequency, ouabain concentration in the selective medium was examined. Two kinds of selective media (GIT-HAT-O and GIT-HAT-O^L) were tested for hybridoma growth. Use of GIT-HAT-O^L resulted in better outgrowth of hybridoma (46.7% in exp. 1, 51.7% in exp. 2) than the use of GIT-HAT-O medium (25.0% in exp. 1, 33.3% in exp. 2).

Use of irradiated myeloma cells as feeder cells greatly enhanced hybridoma growth

Fused cells (myeloma plus EBV-lymphoblastoid cells) of either 5×10^3 or 1×10^4 cells were put in each well in the presence of various number of irradiated (30 Gy) SHM-D33 myeloma cells. Among four fusion experiments, the best in fusion frequency was obtained when 2×10^4 irradiated myeloma cells were put in each well as feeder cells (98.8–100%). Use of 1×10^4 cells/well, 4×10^3 cells/well and 8×10^4 cells/well resulted in worse growth of hybridomas (33.3–62.3%, 78.8–83.8% and 0–3.3%, respectively).

Determination of fusion frequency by limiting dilution (Fig. 1)

Since the precursor of parental cells forming hybrid cells are independently distributed throughout the wells, the number of growing hybrids per well follows a Poisson distribution. Thus, interpolating at the level of $F_0 = 0.37$, which corresponds to one hybridoma per well, it was calculated that the hybrid cell growth was 1 in 700 in our revised system using irradiated myeloma cells as feeder (2×10^4 /well). This frequency is much better than our previous result (1/5,500).

Fig. 1. Limiting dilution of parental myeloma cells for calculation of fusion frequency.

Human IgG antibody specific to KLH was demonstrated in the serum of SCID-hu mouse immunized with KLH

The demonstration that a functionally intact human immune system can survive in SCID-hu mouse is of fundamental importance. We immunized SCID-hu mouse with KLH (100 μ g) intraperitoneally, twice, and measured the antibody response 2 weeks later. By ELISA, specific human IgG antibody to KLH was detected in the serum of the SCID-hu mouse. No mouse antibody to KLH was detected in the mouse.

Human B lymphoblastoid cell lines established from the immunized SCID-hu mouse showed production of human antibodies specific to KLH in the culture supernatant

From the SCID-hu mouse, we tried to establish human B lymphoblastoid cell lines. When the SCID-hu mouse immunized with KLH became sick, remarkably enlarged spleen, mesenteric lymphnodes and thymus were observed. From these lymphoid organs, we could establish 18 lymphoblastoid cell lines. Chromosomal analysis demonstrated that these cell lines were originated from human cells. They produced various classes of human immunoglobulins. Specific human antibodies to KLH in their culture supernatant were examined by ELISA using KLH-coated plate and peroxidase-conjugated goat anti-human mu, gamma or alpha chain as second antibodies (Fig. 2). The specific antibody activity to KLH was expressed as the values of OD₄₉₂ in the ordinate. Almost every cell line showed the production of both IgM and IgA antibodies to KLH. Five cell lines out of 13 cell lines showed the production of IgG antibodies specific to KLH.

Fig. 2. Specific human antibodies to KLH in the culture supernatant of the lymphoblastoid cell lines established from SCID-hu mouse.

Absorption test showed that human antibody in the culture supernatant LCL8-9 was specific to KLH

LCL8-9 was the culture supernatant of the lymphoblastoid cell line established from SCID-hu mouse which had been immunized with KLH. The LCL8-9 sample was absorbed with different concentration (0.3125–10 mg/ml) of KLH or OVA, by mixing 1 : 1, and the residual antibody activity was tested by ELISA using KLH-coated plate and peroxidase-conjugated rabbit anti-human IgM, IgG and IgA as a second antibody. While the antibody activity in LCL8-9 supernatant was apparently absorbed with KLH, that was not absorbed with OVA even at the concentration of 10 mg/ml.

DISCUSSION

There is an increasing demand for the establishment of human monoclonal antibodies to tumor-specific antigens, since they may be applicable to humans for detection and therapy of cancer with far less side effects than the mouse monoclonal antibodies. Most human monoclonal antibodies so far obtained were established from human B cells which had been sensitized in pathologic state without any deliberate immunization. We established more than 30 human monoclonal antibodies which reacted with lung cancer cells from the regional lymphnodes of lung cancer, but most of them also reacted with normal human tissues. Therefore, we envisioned developing such a method to establish human

monoclonal antibodies to any sort of antigens without causing any harm to the immunized volunteers as the method already established in mouse monoclonal antibody production has shown.

A new experimental mouse which was created by Dr. Bosma gave us a hint. When human peripheral blood lymphocytes were injected to the mice with severe combined immunodeficiency (SCID), reconstitution of human immune system was successful, for we could detect a considerable amount (500–1,000 $\mu\text{g/ml}$) of human IgG in the sera obtained from the non-immunized SCID-hu mice. We could also detect human IgG specific to KLH by injecting KLH, twice, to a SCID-hu mouse. This result spurred us into establishing culture cell lines which produced human antibodies from the immunized SCID-hu mice. After several trials, we succeeded in establishing human B lymphoblastoid cell lines from them. As expected, these cell lines continued to produce human antibodies with high titer specific to KLH. This in vivo immunization system using SCID-hu mice should be applicable to any sort of antigens, provided that the antigens served for immunization were recognized by human immune system. This system may be more reliable than the method of in vitro sensitization, as stable long-term reconstitution of human immune system which enables the repeated administration of antigens is obtained in SCHI-hu mice, while only short-term cultivation of human lymphocytes is possible with in vitro sensitization method.

Taken together, by combining in vivo immunization system using SCID-hu mice with our efficient procedure to establish human hybridomas presented as a first approach in this paper, human monoclonal antibodies to any sort of antigens will be easily available.

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