ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2000) 18: 53-61

Production of Anti-CD14 Monoclonal Antibodies Using CD14 Expressing COS Cells as Immunizing Antigen

Seangduen Moonsom and Watchara Kasinrerk

SUMMARY **CD14 is a leukocyte surface molecule expressed on monocytes but not on lymphocytes. Recently, CD14 molecule was demonstrated to function as a receptor for endotoxin. CD14 specific monoclonal antibody (MAb), therefore, can be used to identify monocytes and study the host defense mechanism to bacterial endotoxin. To produce MAb against CD14 protein, in this study cDNA encoding CD14 protein and COS cell expression systems were used to prepare CD14 expressing COS cells. The CD14 transfectants were then used as antigen for mouse immunization. The spleen cells of the immunized mouse were then fused with myeloma cells by conventional hybridoma technique. By using this strategy, 5 hybridroma clones secreting antibody specific for CD14 molecule were generated within one fusion. The generated CD14 MAbs were strongly positive with monocytes, weakly positive with neutrophils but negative with lymphocytes. In addition, the generated CD14 MAb blocked the binding of lipopolysaccharide (LPS) to the CD14 molecules. These CD14 MAbs could be used to enumerate peripheral blood monocytes as well as using referent CD14 MAb. We, therefore, introduce an alternative method for preparation of antigen for production of monoclonal antibody. This type of antigen is a very effective antigen for the production of monoclonal antibodies against cell surface molecules.**

Monocytes belong to the mononuclear phagocytic system, which is a cell lineage that originates in bone marrow and eventually transforms into tissue macrophages. The stages of development are: committed stem cell, monoblast, promonocyte, monocyte and macrophage.[1] Differentiation which gives rise to monocytes takes place in the bone marrow. The newly formed monocytes leave the bone marrow within 24 hours. They remain in the blood for about 36-104 hours and then leave the circulation to become tissue macrophages which may live for many months.[2] Monocytes and macrophages play an important role in both natural immunity and acquired immunity.[3,4,5] They perform many of their functions in host defense prior to the development of specific immunity, including phagocytosis and cytokine production. These cells also function as both accessory and effector cells in acquired immune responses. They display foreign antigens on their surface in a form that can be recognized by antigen-specific T lymphocytes and express proteins and secrete various cytokines that promote T cell activation. Thus, monocytes/macrophages function as accessory cells in lymphocyte activation. After T lymphocyte activation, antigen-stimulated T lymphocytes secrete lymphokines that activate monocytes/macrophages. Such activated mononuclear phagocytes are more efficient at performing phagocytic, degradative and cytocidal functions than unstimulated cells, and are thus better able to destroy phagocytosed anti-

From the Department of Clinical Immunology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand
Correspondence: Watchara Kasinrerk

gens. In the humoral immune responses, foreign antigens, such as microbes, become coated, or opsonized by antibody molecules and complement proteins. Monocytes and macrophages bind and phagocytose opsonized particles much more avidly than uncoated particles. Thus, mononuclear phagocytes also participate in the elimination of foreign antigens by humoral immune responses.

To identify monocytes, simple staining of cells with Giemsa or Romanovsky stains was used in routine laboratory work. However, small monocytes may be difficult to distinguish from lymphocytes. Monocytes also can be identified by using cytochemical methods. The most widely used cytochemical methods for identifying monocytes are reactions for nonspecific esterases, NSE.[6,7] However, NSE is present not only in all cells of the mononuclear phagocytic lineage but also in many other cell types including some T lymphocytes.[6] In recent years, various monoclonal antibodies specific to monocytes have been generated.[8,9] These monoclonal antibodies were used to stain peripheral blood cells by immunofluorescent technique and the stained cells were analyzed by flow cytometry. According to both size and granularity and the reactivity of the stained monoclonal antibody, monocytes present in the blood sample were easily identified by flow cytometer.[10]

The CD14 antigen is a glycosyl-phosphatidylinositol-linked single chain surface membrane glycoprotein with the molecular weight of 53-55 kDa.[11] It is strongly expressed on monocytes, macrophages, and weakly on neutrophils.[11] However, it is absent from T cells, null cells, red blood cells and platelets. Therefore, antibody to CD14 antigen was routinely used for identification of monocytes by flow cytometry.[10,12] Recently, the CD14 molecule was demonstrated to function as a receptor for endotoxin (lipopolysaccharide; LPS).[13,14] LPS binds to a serum protein, LBP (LPS-binding protein) which facilitates the binding of LPS to the CD14 molecule.[13,14,15] When LPS binds to CD14 expressed by monocytes or neutrophils, the cells become activated and release cytokines such as TNF, IL-6 and IL-8[13,15,16,17,18,19] and up-regulate cell surface molecules, including adhesion molecules.[14,20] CD14 specific MAbs, therefore, can be used to identify monocytes and study the host defense mechanism to bacterial endotoxin.

To produce monoclonal antibody against CD14 protein, in general, CD14 expressing cell lines or freshly isolated monocytes were used as antigen for mouse immunization and the spleen cells of immunized mouse were fused with myeloma for the generation of specific monoclonal antibody.[16,21,22,23] Alternatively, in the present study, we used the COS expression system[24] to generate CD14 expressing COS cells. The CD14-COS cells were used as immunizing antigen for a generation of CD14 MAbs. By this technique, 5 hybridomas producing CD14 MAbs were generated within one fusion. The produced CD14 MAbs strongly react with human monocytes, weakly react with granulocytes, but do not react with lymphocytes. These CD14 MAbs can be used to enumerate monocytes as well as referent CD14 MAb.

MATERIALS AND METHODS

Plasmid DNA and antibodies

cDNA encoding CD14 protein, which was constructed into an eukaryotic expression vector, pCDM8 (designated CD14-DNA), was kindly donated by Dr. H. Stockinger, University of Vienna, Vienna, Austria. cDNA encoding CD147 protein, designated M6-DNA, was cloned in our laboratory.[25] Referent CD14 MAb, MEM18, was kindly provided by Dr. Horejsi, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Praha, Czech Republic. PE-labeled CD14 (Leu-M3) MAb and FITC-labeled CD45 (HLe-1) MAb were purchased from Becton Dickinson (San Jose, CA). FITC-conjugated sheep F(ab')2 anti-mouse immunoglobulins antibodies were purchased from Immunotech (Immunotech/Coulter Corporation, Miami, FL).

DNA preparations

For large scale preparation of CD14-DNA, the plasmid DNA were transformed into competent *E. coli* MC1061/p3 and the resulting bacteria were grown with vigorous shaking in 250 ml LB broth per 1-liter flask. After overnight cultivation, cells were harvested and lysed by the alkaline lysis procedure according to the QIAGEN protocol (QIAGEN, Hilden, Germany). DNA were then purified by QIAGEN ion exchange column (QIAGEN). The concentration and purity of DNA preparation was determined by OD260/280 reading.

Expression of CD14 protein on COS cells

To express the CD14 protein on COS cells, the CD14-DNA was transfected into COS cells using the modified DEAE-Dextran transfection method.[25] Briefly, 1 x 10^6 COS cells were transferred to 6 cm tissue culture dishes (NUNC, Roskilde, Denmark) on the day before transfection. Cells were transfected in 2 ml of MEM containing 250 μg/ml DEAE-Dextran, 400 μM chloroquine diphosphate and 2 μg DNA. After 3 hours at 37°C, the transfection mixture was removed and the cells were treated with 10% DMSO in PBS for 2 minutes at room temperature. COS cells were then cultured overnight in MEM containing 10% FCS, washed once, and re-cultured with the same medium for another 2 days to allow expression of the encoded proteins.

Production of monoclonal antibody to CD14 protein

A BALB/c mouse was intraperitoneally immunized three times at two-week intervals with 1 x 10^7 CD14-DNA transfected COS cells. A booster immunization was followed two-weeks after the third immunization by intravenous injection of 1 x 10^6 CD14-DNA transfected COS cells. The animal was sacrificed 3 days after the booster and the spleen was removed. Spleen cells were then fused with myeloma cells X63-Ag8.653 using 50% PEG. After that, cells were resuspended with HAT medium containing 10% BM combimed medium (Boehringer Mannheim, Germany) and distributed into 960 wells of 96 well-plates. Two weeks later, hybridomas were identified by an inverted microscope. Cell culture supernatants from hybridoma-containing wells were screened for antibody against CD14 protein. The positive clone was re-cloned two rounds by limiting dilution.

Screening for CD14 specific monoclonal antibody

Hybridoma cell culture supernatants were firstly analyzed by indirect immunofluorescence using peripheral blood mononuclear cells (PBMCs) as antigens. The stained cells were analyzed by flow cytometry. The supernatants which had shown positive reactivity with monocytes but negative or weakly positive with lymphocytes were screened further for antibody specific to CD14 protein by the same technique but using CD14-DNA transfected COS cells as antigens and analyzed by a fluorescent microscope. In all experiments, COS cells transfected with cDNA encoding unrelated protein (CD147) were used as negative control.

Indirect immunofluorescence analysis

The specificity of antibody against CD14 protein was assessed by indirect immunofluorescence using fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse immunoglobulin antibodies (Immunotech). PBMCs were firstly isolated from heparinized blood of healthy donors by density gradient centrifugation over Ficoll-Hypaque solution (Sigma, St. Louis, MO). PBMCs were then washed 3 times with PBS and adjusted to 1 x 10^7 cells/ml with 1% BSA-PBS-0.02% sodium azide. To block the non-specific Fc receptor mediated binding of the antibodies, cells were incubated for 30 minutes at 4°C with 10% human AB serum before staining. Fifty microliters of blocked cells were then incubated for 30 minutes at 4°C with hybridoma culture supernatants or MAb. After washing twice, cells were incubated with the FITC-conjugate for another 30 minutes. Membrane fluorescence was analyzed by a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA). Individual populations of blood cells were gated according to their forward and side scatter characteristics.

Determination of isotypes of monoclonal antibody

The isotypes of MAbs were determined by capture ELISA (Sigma) in accordance with the recommended protocol. Goat anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgA and IgM were used as capture antibodies, and peroxidase conjugated rabbit anti-mouse immunoglobulins (Dako, Glostrup, Denmark) were as used as conjugate. The reactivity was visualized by using 3',3',5',5'-tetramethylbenzidine (TMB) as substrate.

Inhibition of referent CD14 monoclonal antibody binding by generated CD14 monoclonal antibodies

PBMCs were pre-incubated with supernatant containing CD14 MAbs or irrelevant MAb for 30 minutes on ice. PE-labeled CD14 MAb Leu-M3 (Becton Dickinson) or FITC-labeled CD45 MAb HLe-1 (Becton Dickinson) was then added to the pre-stained cells, and incubated for another 30 minutes. Membrane fluorescence was analyzed by a flow cytometer.

The percent inhibition of fluorescence intensity was calculated from the mean fluorescence intensity (MFI) of the sample in the presence and absence of the first unlabeled MAb.

Inhibition of LPS binding to CD14 molecule by generated CD14 monoclonal antibody

CD14 MAb, MT14/3, was purified from ascites by using anti-mouse IgG coated sepharose column (Zymed Laboratory Inc., San Francisco, CA) in accordance with the recommended protocol.

To study the effect of generated CD14 MAb on LPS binding, PBMCs were incubated with 50% autologous plasma for 30 minutes at 4°C. PBMCs were then preincubated with purified MT14/3 MAb or OKT3 (anti-CD3 MAb; Ortho Diagnostic System Inc., Raritan, NJ) for 30 minutes on ice. FITC-labeled LPS (Sigma) was then added, and incubated for another 60 minutes. The membrane fluorescence of monocytes was analyzed by a flow cytometer.

RESULTS

Preparation of plasmid DNA encoding CD14 protein and expression of CD14 on COS cell membrane

Plasmid DNA encoding CD14 protein, named CD14-DNA, were transformed into *E. coli* MC 1061/p3. The plasmid DNA were then isolated from the transformed bacteria by QIAGEN ion exchange chromatography and the DNA yields were determined by the OD 260/280 reading after completion of all the purification steps. The OD 260/280 ratios of isolated plasmid DNA was 1.82, indicating the purity of the isolated DNA.[26] The yield of isolated CD14-DNA was 2.8 mg/liter of starting bacteria.

To verify whether the isolated CD14-DNA can be expressed to the encoded protein in eukaryotic cells, the isolated DNA was transfected into COS cells and analyzed for CD14 protein expression by indirect immunofluorescence using referent CD14 MAbs, MEM-18 and Leu-M3. As shown in Table 1, COS cells which were transfected with CD14-DNA showed a very strong positive reaction with both MEM-18 and Leu-M3, but negative with an irrelevant CD147 MAb.[27] The percentage of CD14 expressing COS cells obtained from this CD14-DNA transfection was approximately 60-70%. These results indicated that the CD14-DNA and the transfection method used effectively induced CD14 protein expression on COS cell membrane.

Production of monoclonal antibodies to CD14 protein using CD14 expressing COS cells as antigen

To generate hybridoma producing CD14 MAb, we first prepared CD14 expressing COS

Table 1 Expression of CD14 protein on CD14-DNA transfected COS cells

COS transfectants	Immunofluorescent reactivity		
	Anti-CD14 MAb		Anti-CD147 MAb
	MEM-18	Leu-M3	M6-1D4
CD14 COS	+	+	-
M6 COS	-	-	+

COS cells were transfected with CD14-DNA or M6-DNA by DEAE-Dextran transfection technique. The transfected cells were stained with CD14 MAbs, MEM-18 and Leu-M3 or CD147 MAb, M6-1D4. Immunofluorescent reactivity was analyzed by a fluorescence microscope.

Table 2 Flow cytometric immunophenotyping of monocyte population, comparison of results between MT14/1, MT14/2, MT14/3, MT14/4 and referent anti-CD14 MAb MEM-18

Donor no.	Monoclonal antibody				
	MT14/1	MT14/2	MT14/3	MT14/4	MEM-18
1	95	95	95	95	96
2	95	95	97	94	97
3	92	95	97	97	98
4	94	94	92	96	96
5	91	94	96	92	94

cells by transfection of COS cells with CD14-DNA. After 3 days, approximately 70% of the transfected COS cells were strongly positive with CD14 MAb, MEM-18. These CD14-expressing COS cells were then used as antigen for mouse immunization. Spleen cells of an immunized mouse were fused with myeloma cells as per the conventional hybridoma technique. A total of 612 hybridoma clones was identified from 960 wells. By indirect immunofluorescent technique, cell culture supernatants from 12 of 612 wells contained antibodies which reacted to monocytes, but did not, or reacted weakly to lymphocytes. To screen further for hybridomas that produced CD14 specific antibody, all 12 positive supernatants were tested again by the same technique, but CD14-DNA transfected COS cells were used as the antigen. Five out of 12 culture supernatants reacted with CD14 expressing COS cells, but not with mock CD147 transfectants. The hybridomas in these positive wells were then re-cloned two rounds by limiting dilution. The final clones which gave positive reactivity with CD14 expressing transfectants but negative to mock transfectants were propagated and re-named MT14/1, MT14/2, MT14/3, MT14/4 and MT14/5, respectively. MT14/1, MT14/2, MT14/3, MT14/4 and MT14/5 MAbs were IgG1, IgG2b, IgG1, IgG1 and IgM isotypes, respectively.

After single cell cloning, all generated hybridoma clones were grown at a high density and the culture supernatants were collected for further studies. However, a hybridoma clone, MT14/5, stopped producing antibody. Therefore, this MT14/5 clone was discarded.

Characterization of the specificity of generated CD14 MAbs

To confirm the specificity of generated MAbs, all MT14 MAbs were used to stained monocytes, granulocytes and lymphocytes isolated from 5 healthy donors by indirect immunofluorescence, and analyzed by flow cytometry. As predicted, all generated MT14 MAbs strongly reacted to monocytes, weakly reacted to granulocytes and were negative with lymphocytes. The reaction patterns were absolutely identical to those obtained by using reference CD14 MAb, MEM-18. The typical FASC profiles of MT14 and MEM-18

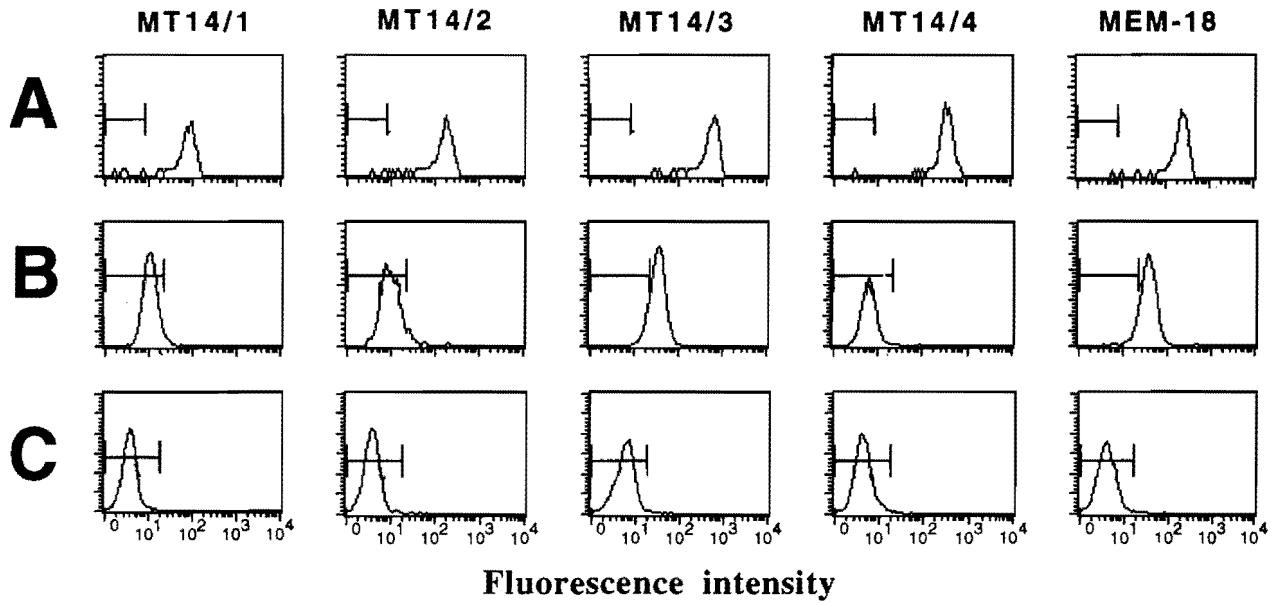

Fig. 1 Immunofluorescence analysis of the reactivity of MT14/1, MT14/2, MT14/3, MT14/4 and referent MEM-18 MAbs with monocytes (A), neutrophils (B) and lymphocytes (C). The marker represents background fluorescence of negative control MAb.

MAbs stained monocytes, granulocytes and lymphocytes are shown in Fig. 1. The MT14 MAbs were used to enumerated monocytes in isolated PBMCs and compared to referent MEM-18 MAb. As shown in Table 2, within the monocyte population, a range from 91-97% showed positive reactivity with either MT14/1, MT14/2, MT14/3 and MT14/4 MAbs and again the percentage of positive cells obtained was virtually identical to those obtained from MEM-18 MAb.

To study the epitope on the CD14 molecule which reacted to the generated MAbs, MT14 MAbs were used to inhibit the binding of a referent CD14 MAb Leu-M3. Monocytes were, firstly, incubated with MT14. Then, PE labeled CD14 MAb Leu-M3 was added and the fluorescence intensity was determined by a flow cytometer. It was found that MT14/3 could inhibit the binding PE labeled Leu-M3 MAb (Table 3). In the same experiment, MT 14/3, however, did not inhibit anti-CD45 MAb (HLe-1) binding (data not shown). Interestingly, MT14/4 enhanced the staining intensity of Leu-M3 approximately 2-fold (Table 3). The MT14/1 and MT14/2 neither inhibited nor enhanced Leu-M3 binding (Table 3).

Inhibition of LPS binding by MT14/3 MAb

The CD14 molecule was recently demonstrated to be a receptor for LPS.[13,14,15] We therefore determined the effect of generated CD14 MAb, MT14/3, on LPS binding. Monocytes, in the presence of autologous plasma, were pre-incubated with various concentrations of purified MT14/3 or isotype matched OKT3 MAb. FITC-labeled LPS was then added and the membrane fluorescence was determined by a flow cytometer. As shown in Fig. 2, MT14/3 MAb as well as a positive CD14 MAb control, MEM-18, blocked LPS binding in a dose dependent manner. In contrast, the isotype matched control, purified OKT3 MAb, had no effect on LPS binding.

DISCUSSION

The CD14 molecule is a leukocyte surface molecule strongly expressed on monocytes but not on lymphocyte surface.[11] This molecule is, therefore, routinely used as a marker for identification of monocytes.[10,12] To produce monoclonal antibody against CD14 protein, generally, CD14 expressing cell lines or freshly isolated monocytes were used as immunizing agents and the spleen cells of immunized mice were fused with myeloma for generation of hybridoma produced specific monoclonal antibody.[11,21,22,23] As immunizing cells, either CD14 expressing cell lines, or freshly isolated monocytes also express a large number of other membrane proteins. The immunogenicity of the expressed proteins are different, induction of immune response to some antigens may be dominated and some are poorer. Using these types of antigens, failure to obtain hybridoma which have produced CD14 antibody, therefore, can occur. To overcome this problem, cells expressing every high level of CD14 molecules are required for use as immunizing antigen. This type of antigen, in theory, will increase the possibility of obtaining CD14 producing hybridoma clones.

Table 3 Inhibition of referent CD14 MAb Leu-M3 binding to monocytes by generated MT14 MAbs

Experiment no.	MFI (% inhibition)				
	Pre-incubated MAbs				
	MT14/1	MT14/2	MT14/3	MT14/4	no MAb
1	203 (4)	182 (14)	6 (97)	389 (–84)	211
2	127 (-14)	85 (24)	8 (93)	234 (-110)	111
3	259 (-13)	218 (5)	6 (97)	523 (-127)	230

Peripheral blood monocytes were pre-incubated with indicated MT14 MAbs or without MAb. PE-labeled CD14 MAb, Leu-M3, was added to the pre-stained cells. The mean fluorescence intensity (MFI) was analyzed by flow cytometry. Percent inhibition was calculated between with and without pre-incubated MAb.

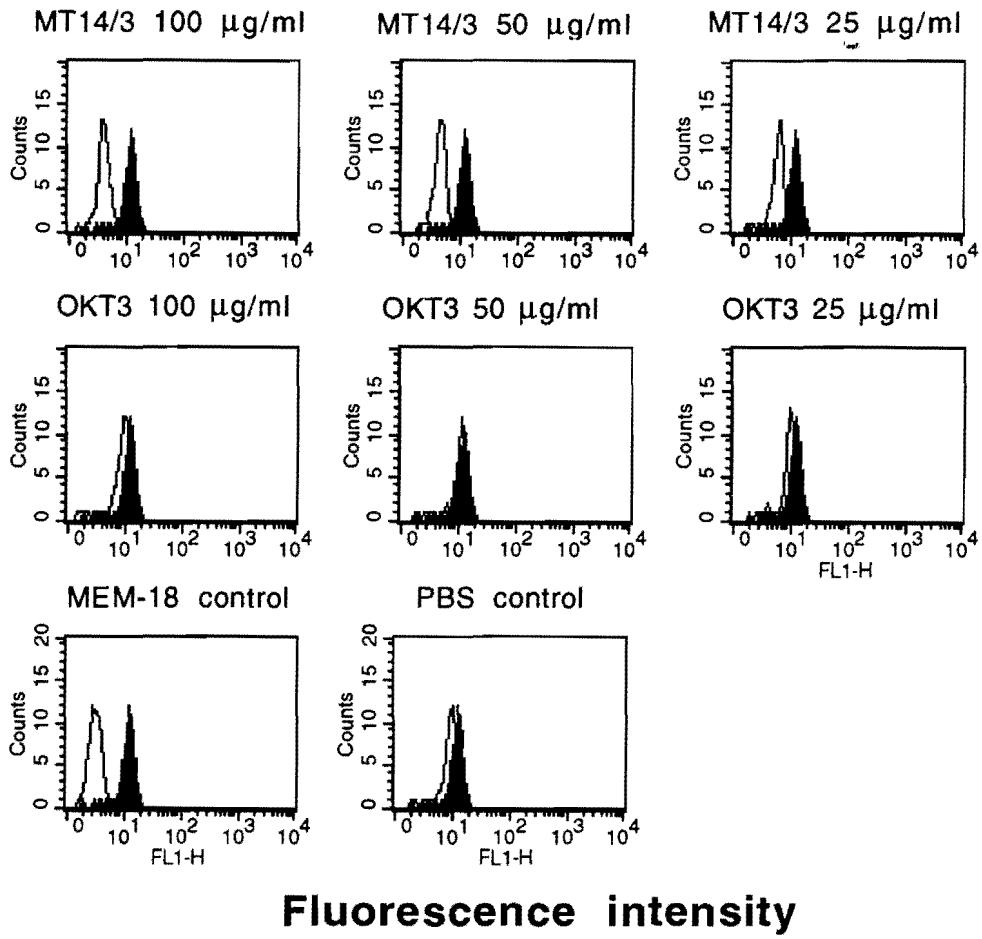

Fig. 2 Inhibition of LPS binding to monocytes by MT14/3 MAb. Monocytes were incubated with PBS (shaded peaks) or with various concentrations of indicated MAbs (un-shaded peaks) and FITC labeled LPS. MEM-18 ascites 1:100 and PBS were used as positive and negative controls, respectively.

To generate cells expressing the large number of CD14 molecules, in the present report, the high-efficiency COS cell expression system was used.[24] In this system, cDNA encoding CD14 molecules were first constructed into an eukaryotic expression vector, pCDM8.[28] The pCDM8 vector contains SV40 replication origin, a cytomegalovirus/T7 RNA polymerase promoter, the SV40 small tumor (t) antigen splice, and early region polyadenylylation signals derived from pSV2.[28] These features of the vector allow high-level expression in COS cells of coding sequences placed under its control. To generate CD14 expressing COS cells, in our experiment, cDNA encoding CD14 protein which was inserted in pCDM8 was first transfected into COS cells by using the optimized DEAE-Dextran transfection.[25,29] This optimized DEAE-Dextran transfection has been demonstrated to produce outstanding transfection efficiency.[25,29] In the present study, approximately 70% of CD14-DNA transfected COS cells were positive with CD14 MAb, indicating that the transfection method used effectively introduced plasmid DNA into COS cells. The fluorescent intensity observed on each positive transfected cell was very strong compared to monocytes, indicating a large number of CD14 molecules were expressed on the transfectant surface. Therefore, these CD14 transfected COS cells should be a better immunogen, compare to CD14 positive cell lines or monocytes, for eliciting immune responses to CD14 molecule.

CD14 transfectants were then used to immunize mouse and spleen cells were then fused with myeloma by conventional hybridoma technique. After fusion, the hybridoma culture supernatants were screened using CD14 transfected COS cells and mock transfected cells as antigens. By this screening procedure, supernatants from 5 hybridoma clones showed positive reactivity with CD14 transfectants but not to mock transfectants. With peripheral blood cells, these five antibodies strongly reacted to monocytes, weakly reacted to neutrophils, but did not react to lymphocytes. The staining patterns, together with the transfectant experiments, indicated that the generated MAbs are specific to CD14 protein. Obtaining 5 hybridoma clones produced antibodies to a surface molecule within one fusion is unusual. Our experiment, therefore, demonstrated that the high efficiency COS cell expression system can be used to produce very good antigen for production of monoclonal antibody. In addition to CD14 MAbs, using the same strategy, 3 clones of anti-CD99 MAbs were also produced in our laboratory (data not shown).

After 2 rounds of single cell cloning, however, a hybridoma clone termed MT14/5 stopped producing antibody. The cessation of antibody producing by hybridomas due to their having an unstable assortment of chromosomes has been described.[30] The epitope recognized by the generated CD14 MAbs was analyzed using the inhibition test. One of the generated MAbs, named MT14/3, completely inhibited the binding of referent CD14 MAb Leu-M3.[22,23] This result indicated that MT14/3 MAb may react to CD14 molecule at the same or at a very close epitope of that recognized by referent CD14, Leu-M3, MAb. Leu-M3 was an anti-CD14 MAb derived from the hybridization of mouse Sp2/0 myeloma cells with spleen cells of BALB/c mice immunized with peripheral blood monocytes from a patient with rheumatoid arthritis.[22] This MAb was now used as a LeukoGate reagent in SIMULTEST reagent kit developed by Becton Dickinson. In contrast to MT14/3, MT14/4 MAb enhanced the binding of Leu-M3 MAb with an unknown mechanism. This enhancement effect was also observed in the CD147 system.[31]

A series of studies was performed to analyze the function of the CD14 molecule and indicated that CD14 functioned as a receptor for LPS.[13,14,15] LPS was shown to bind to CD14, a process mediated by the plasma protein LBP, and blockade of the CD14 molecule with some anti-CD14 MAbs prevented LPS induced TNF release by monocytes.[13,15] We therefore raised the question whether our generated CD14 MAb bind to the epitope which is involved in LPS binding. To address this question, monocytes were incubated with autologous plasma, which used as a source of LBP, in the presence of CD14 MAb and FITC-labeled LPS. It was found that the generated CD14 MAb, MT14/3, inhibited LPS binding. In the same experiment, the isotype matched control showed no effect on LPS binding. These results indicated that MT14/3 MAb recognizes an epitope on CD14 which is involved in LPS binding.

In conclusion, in the present study, we introduce an alternative method for preparation of antigen for immunizing mice in the production of monoclonal antibody. We used the high-efficiency COS cell expression system to induce the over expression of the protein of interest on COS cell surface. This type of antigen was shown to be a very effective antigen for the production of monoclonal antibody against cell surface molecules. Using this type of antigen, we could produce 5 MAbs directed against CD14 protein within one fusion. This strategy is, therefore, very efficient in production of monoclonal antibody against any cell surface protein when cDNA clone is available.

ACKNOWLEDGEMENTS

This work was financially supported by the Thailand Research Fund, the National Science and Technology Development Agency of Thailand and by the Ministry of University Affairs. S. Moonsom is a research assistant supported by The National Science and Technology Development Agency of Thailand (NSTDA). The authors would like to thank Dr. H. Stockinger for providing the cDNA encoding CD14 protein.

REFERENCES

1. Lasser A. The mononuclear phagocytic system. Human Pathol 1983; 14: 108-26.
2. van Furth R. Mononuclear phagocytes in immunity, infection and pathology. Oxford: Blackwell Scientific, 1975.
3. Johnston RB. Current concepts: immunology. Monocytes and macrophages. N Engl J Med 1988; 318: 747-52.
4. Zembala M, Asherson GL. Human monocytes. London: Academic Press Limited, 1989.

5. Tizard IR. Immunology. 4th ed. New York: Saunder College Publishing, 1995.
6. Stachura J. Cytochemistry of monocytes and macrophage. In: Zembala M, Asherson GL, eds, Human monocytes. London: Academic Press Limited, 1989; p. 27-36.
7. Koski IR, Poplack DG, Blaese RM. A non-specific esterase stain for the identification of monocytes and macrophages. In: Bloom BR, David JR, eds, *In vitro* methods in cell-mediated and tumor immunity. New York: Academic Press, 1976; p. 359-62.
8. Knapp W, Dorken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, von dem Borne AEGK. Leukocyte typing IV. Oxford: Oxford University Press, 1989.
9. Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein RL, Springer TA, Tedder TF, Todd RF. Leukocyte typing V. New York: Oxford University Press, 1994.
10. Montaner LJ, Collin M, Herbein G. Human monocytes: Isolation, cultivation, and applications. In: Herzenberg LA, Weir DM, Herzenberg LA, Blackwell C, eds, Weir's handbook of experimental immunology. Volume IV: The integrated immune system. 5th ed. Oxford: Blackwell Science, 1996; 155: 1-11.
11. Gadd S. Cluster report: CD14. In: Knapp W, Dorken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, von dem Borne AEGK, eds, Leukocyte typing IV. Oxford: Oxford University Press, 1989; p. 787-9.
12. Kasinrerk W, Baumruker T, Majdic O, Knapp W, Stockinger H. CD1 molecule expression on human monocytes induced by granulocyte-macrophage colony-stimulating factor. J Immunol 1993; 150: 579-84.
13. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990; 249: 1431-3.
14. Hailman E, Lichensrein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, Busse LA, Zukowski MM, Wright SD. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. J Exp Med 1994; 179: 269-77.
15. Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ. Structure and function of lipopolysaccharide binding protein. Science 1990; 249: 1429-31.
16. Goyert SM, Haziot A, Jiao D, Katz IR, Kruger C, Ross J, Schtt C. CD14 cluster workshop report. In: Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein RL, Springer TA, Tedder TF, Todd RF, eds, Leukocyte typing V. New York: Oxford University Press, 1994, p. 778-90.
17. Baggiolini M, Dewald B, Walz A. Interleukin-8 and related cytokines. In: Gallin JI, Goldstein IM, Synderman R, eds, Inflammation: basic principles and clinical correlates. 2nd ed. New York: Raven Press, 1992, p. 247-63.
18. Hirono T, Akira S, Taga T, Kishimoto T. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. Immunol Today 1990; 11: 443-9.
19. Koj A. Initiation of acute phase response and synthesis of cytokines. Biophys Acta 1996; 1317: 84-94.
20. Pohlman TH, Harlan JM. Endotoxin-endothelial cell intreraction. In: Morrison DC, Ryan JR, eds, Bacterial endotoxic lipopolysaccharide. Vol 1: Molecular biochemistry and cellular biology. Boca Raton: CRC Press, 1992, p. 347-71.
21. Goyert S, Tesio L, Ashman L, Ball E, Basil V, Garrido F, Hogg N, Horejsi V, Howell A, Jayaram Y, Look AT, Ruiz-Cabllo F, Schmitt D, Schneider M, van der Schoot CE. Report on CD14 cluster Workshop. In: Knapp W, Dorken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, von dem Borne AEGK, eds, Leukocyte typing IV. Oxford: Oxford University Press, 1989, p. 789-94.
22. Dimitriu-Bona A, Burmester G, Waters S, Winchester R. Human mononuclear phagocyte differentiation antigens. I. Patterns of antigenic expression on the surface of human monocytes and macrophages defined by monoclonal antibodies. J Immunol 1983; 130:145-52.
23. Herrmann F, Komischke B, Odenwald E, Ludwid WD. Use of monoclonal antibodies as a diagnostic tool in human leukemia. I. Acute myeloid leukemia and acute phase of chronic myeloid leukemia. Blut 1983; 47: 157-63.
24. Aruffo A, Seed B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. Proc Natl Acd Sci USA 1987; 84: 8573-7.
25. Kasinrerk W, Fiebinger E, Stefanova I, Baumruker T, Knapp W, Stockinger H. Human leukocyte activation antigen M6, a member of the immunoglobulin superfamily, is the species homologue of rat OX-47, mouse basigin and chicken HT7 molecule. J Immunol 1992; 149:847-54.
26. Sambrook J, Fritsch EF, Maiatis T. Molecular cloning: A laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
27. Kasinrerk W, Tokrasinwit N, Phunpae P. CD147 monoclonal antibodies induce homotypic cell aggregation of monocytic cell line U937 via LFA-1/ICAM-1 pathway. Immunol 1999; 96: 184-92.
28. Seed B. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2. Nature 1987; 329: 840-42.
29. Kasinrerk W. Transfection of gene into eukaryotic cells by DEAE-Dextran transfection. J Med Tech Assoc Thailand 1990; 18: 105-8.
30. Harlow E, Lane D. Monoclonal antibodies. In: Harlow E, Lane D, eds, Antibodies: A laboratory manual. New York: Cold Spring Harbor Laboratory, 1988, p. 139-242.
31. Stockinger H, Ebel T, Hansmann C, Koch C, Majdic O, Prager E, Patel DD, Fox DA, Horejsi V, Sagawa K, Shen DC. CD147 (neurothelin/basigin) workshop panel report. In: Kishimoto T, Kikutani H, Borne AEGK, Goyert SM, Mason D, Miyasaka M, Moretta L, Okumura K, Shaw S, Springer T, Sugamura K, Zola H, eds, Leukocyte typing VI. New York: Garland Publishing, 1997, p. 76-9.