Journal of Chromatography, 563 (1991) 243–255
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5642

Chromatographic methods for large-scale preparation of immunoglobulin G_{2a} monoclonal antibodies Lym-1 and TNT-1 $F(ab')_2$ fragments

FENG-MING CHEN[a,*], CHANG-ZHENG LIU, SHAIK A. GAFFAR and ALAN L. EPSTEIN

Department of Pathology, University of Southern California School of Medicine, Los Angeles, CA 90033 (U.S.A.)

(First received May 1st, 1990; revised manuscript received September 12th, 1990)

ABSTRACT

Lym-1 and TNT-1 are two murine immunoglobulin G_{2a} monoclonal antibodies (MAbs) which have been used for clinical trials in cancer patients. This paper describes methods for large-scale preparation of $F(ab')_2$ fragments from 50 mg to 4 g of MAbs Lym-1 and TNT-1. Digestion of MAbs with pepsin was optimized and performed at pH 3.8, a pepsin/antibody ratio of 1:250, and 3–4 h of incubation at 37°C. The $F(ab')_2$ fragments were purified by tandem column procedures using fast protein liquid chromatography. Quality control analyses of the products included protein purity, isoelectric point, immunoreactivity, and endotoxin level. The results revealed that the chromatographic procedures are practical, simple, and effective, and can be used to produce gram quantities of clinical-grade $F(ab')_2$ fragments for the diagnosis of cancer in patients.

INTRODUCTION

Monoclonal antibodies (MAbs) have been shown to have great potential for the detection and treatment of tumors in both experimental animals and human [1–3]. Although intact antibody has been successfully used for the immunoscintigraphy of tumors, the antibody is cleared relatively slowly from blood stream, and significant background activity remains for several days after injection [2,4]. This problem may be corrected by computer-aided background subtraction methods using a second non-specific radioactive agent. However, it exposes the patient to additional doses and adds several sources of error [5,6].

Recent work with MAbs have demonstrated that $F(ab')_2$ fragments are cleared more rapidly from the circulation and are superior to intact antibody for the radioimmunodetection of tumors [4,7–10]. The advantages of using $F(ab')_2$ fragments are (1) markedly reduced background in bone marrow, liver, and spleen, (2) higher tumor/non-tumor ratios within 48 h, (3) a decrease in anti-antibody

[a] Address for correspondence: Department of Radiology Research, City of Hope National Medical Center, 1500 E. Duarte Road, Duarte, CA 91010-0269, U.S.A.

0378-4347/91/$03.50

response in patients receiving a foreign immunoglobulin (*i.e.* human anti-mouse antibody, HAMA), and (4) possible use of short-lived isotopes with optimal γ-energies, such as ^{123}I and ^{99m}Tc, for imaging studies.

The preparation of $F(ab')_2$ fragments from immunoglobulin G (IgG) MAbs is a well established technique [11]. Conventional-size exclusion chromatography has been used to purify $F(ab')_2$ fragments from the crude antibody digest. This method, however, is limited for large-scale application because of low capacity and poor resolution. Therefore, simple and effective scale-up procedures are needed to fulfill the demand for the large quantity of pure and immunoreactive MAb $F(ab')_2$ fragments for clinical applications. We report here the development of large-scale column chromatographic method for the preparation of $F(ab')_2$ fragments from two IgG_{2a} MAbs. Lym-1 is a MAb reactive with the cell surface of B-lymphocytes [12] and has been shown to give successful imaging and therapy in Raji lymphoma bearing-nude mice as well as lymphoma patients in Phase I/II clinical trials [3]. TNT-1 is a MAb with specificity for nuclear histones. It has been demonstrated that TNT-1 can bind to degenerating cells and necrotic areas of tumors and is useful for the imaging and treatment of a variety of transplantable tumors [13,14]. The large-scale preparation of $F(ab')_2$ fragments from these two MAbs and the evaluation of the final bulk products are described and discussed.

EXPERIMENTAL

Materials and instruments

Fast protein liquid chromatography (FPLC) columns and prepacked columns (Pd-10, Superose-12 HR 10/30, Mono-Q HR 5/5 and Mono-S HR 5/5) were purchased from Pharmacia (Uppsala, Sweden). S-Sepharose fast-flow and Q-Sepharose fast-flow ion-exchange matrices were obtained from Pharmacia and ABx 40 μ was purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). r-Protein-A Avid Gel F and buffers for antibody binding and elution were obtained from Bioprobe International (Tustin, CA, U.S.A.). Lym-1 and TNT-1 MAbs [10–13 mg/ml in phosphate-buffered saline (PBS)] were obtained from Techniclone International (Tustin, CA, U.S.A.). Pepsin, from porcine stomach mucosa, purified by crystallization followed by chromatography, was purchased from Sigma (St. Louis, MO, U.S.A.).

Chromatography was performed with a Pharmacia FPLC system which consisted of two P-500 dual-piston pumps, a V-7 injection valve, solvent mixer, pre-filter, UV-1 monitor, and REC-482 dual-channel recorder. Buffers for FPLC procedures were filtered through a 0.22-μm Millex-GV filter units (Millipore, Bedford, MA, U.S.A.) prior to use.

Column clearing and pre-equilibration

The preparative ion-exchange columns were cleaned by passing one column volume of 0.5 *M* NaOH, followed by 5 column volumes of 0.1 *M* NaOH. These

columns were then thoroughly equilibrated with initial buffers to a constant pH as described in FPLC procedures.

The Sephadex G-25 column (30 cm × 5 cm I.D.) was washed with 3 column volumes of 0.2 *M* NaOH, followed by 3 column volumes of 0.1 *M* NaOH, and then thoroughly equilibrated with the solvent exchange buffer to constant pH.

The Protein-A column (10 cm × 5 cm I.D.) was cleaned by 10 column volumes of 70% undenatured ethanol (7 parts of 95% undenatured ethanol plus 3 parts of ultrafiltered water), followed by 5 column volumes of PBS.

Eluates from all the cleaned columns were collected for endotoxin testing. If the endotoxin level was greater than 1.0 E.U./ml, the columns were recleaned by the above procedures.

Pepsin digestion

Lym-1 and TNT-1 were digested with pepsin according to a modified procedure of Parham [11]. The effects of various experimental variables such as pH, reaction time, and the amount of pepsin were examined prior to the initiation of large-scale preparations. These have been done by digesting 2 mg of antibody with pepsin in different conditions and analyzing the digested mixture every half hour using either sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or Superose-12 FPLC methods. Based on the preliminary results, 50 mg to 4 g of MAb has been digested with pepsin as described below: MAbs (10–13 mg/ml in PBS) were adjusted to pH 4.3 by adding a small aliquot of 1 *M* sodium citrate (pH 3.0). Freshly prepared pepsin in 1 *M* sodium citrate (pH 3.8) was then added to the antibody solution at a weight ratio of 1:250 (pepsin/MAb). The pH of the mixture was finally adjusted to 3.8 by adding a small aliquot of 1 *M* sodium citrate (pH 3.0). Care was taken to avoid protein precipitation from solution due to highly acidic pH. The reaction mixture was incubated in a water bath at 37°C for 3–4 h. The digestion progress was monitored by Superose-12 FPLC method (see Fig. 1). At the end of incubation, 3.0 *M* Tris–HCl (pH 8.0) was added to the reaction mixture to bring it to pH 7.0. Alternately, the mixture was loaded onto a Sephadex G-25 column (30 cm × 5 cm I.D.) for buffer exchange.

Development of chromatographic methods

Pepsin, Lym-1 and TNT-1 MAbs, and their pepsin-digested mixtures were analyzed on analytical Mono-Q, Mono-S and ABx columns to investigate the binding and elution characteristics of each protein component. Various buffer systems and pH were tested to optimize protein binding, elution, and resolution of products and contaminates. Tris–HCl buffers, pH 7.0–8.5, were used with the Mono-Q column, and 2-(N-morpholino)ethanesulfonic acid (MES) buffers, pH 5.8–7.0, were employed with the Mono-S and ABx columns. The columns were generally eluted by a linear salt gradient. The resolution of the protein peaks was then optimized by appropriate step gradients. Data collected from these studies were summarized to establish large-scale procedures, which are described below.

Large-scale preparation of Lym-1 F(ab')$_2$ fragment

Lym-1 (55 mg), after digestion with pepsin, was loaded on a Sephadex G-25 column (30 cm × 5 cm I.D.) and the column was eluted (20 ml/min) with 50 m*M* Tris–HCl (pH 7.5). The fraction containing protein as detected by UV absorbance at 280 nm was collected and loaded onto a Q-Sepharose column (10 cm × 1 cm I.D.) which was then developed with Tris buffer. The eluate containing the F(ab')$_2$ fragment was directly passed through a second separation column (40-μm ABx, 10 cm × 1 cm I.D.) by connecting the column at the outlet of the UV detector. The ABx column, after connecting to the FPLC system, was washed thoroughly with 20 m*M* MES buffer (pH 6.3). The F(ab')$_2$ fragment was eluted from the ABx column by 0.30 *M* NaCl in 20 m*M* MES, pH 6.3 (see Fig. 4). MES buffer of Lym-1 F(ab')$_2$ product was exchanged with PBS by using a Sephadex G-25 column. The whole IgG bound in the first separation column (Q-Sepharose) was recovered by eluting with 0.4 *M* NaCl in Tris buffer (pH 7.5). The column, after washing with 1 *M* NaCl, was equilibrated thoroughly with the initial buffer.

Large-scale preparation of TNT-1 F(ab')$_2$ fragment

The pepsin-digested mixture containing 90 mg of TNT-1 was passed through a Sephadex G-25 column (30 cm × 5 cm I.D.) for buffer exchange to 20 m*M* MES, pH 6.3. The protein fraction was then loaded on a S-Sepharose column (10 cm × 1.6 cm I.D.) and the column was washed with 2 column volumes of 20 m*M* MES, pH 6.3. The TNT-1 whole IgG, Fab' and F(ab')$_2$ fragments remained to the column. The proteins were eluted by a step gradient (0.15 *M* NaCl in 20 m*M* MES and 0.40 *M* NaCl in 20 m*M* MES). The protein fraction eluted with 0.4 *M* NaCl was diluted by adding 2 volumes of Protein-A-binding buffer and the pH of the sample was adjusted to 8.4–8.6 with 1 *M* NaOH. The mixture was then passed through a Protein-A column using the FPLC system. The unbound F(ab')$_2$ fraction was collected and exchanged to PBS (pH 7.0) using a Sephadex G-25 column. TNT-1 antibody was recovered from the Protein-A column by using the Protein-A elution buffer.

Quality control of F(ab')$_2$ products

Superose-12 FPLC. Aliquots of F(ab')$_2$ product (approximately 100 μg per 50 μl) were analyzed for protein purity using the Superose-12 FPLC method (see Fig. 6).

SDS-PAGE. Lym 1, TNT-1, and their F(ab')$_2$ fragments were analyzed under reducing and non-reducing conditions on a 10% gel which were run at a constant voltage of 23 V for 4 h. The proteins were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, Richmond, CA, U.S.A.) and the gel was dried under vacuum (see Fig. 7).

Immunoreactivity. Lym-1, TNT-1, and their F(ab')$_2$ fragments were radiolabeled with ^{125}I (New England Nuclear, Boston, MA, U.S.A.) using the chloramine-T method [14]. Immunoreactivities for each radiolabeled antibody

TABLE I

CHARACTERISTICS OF Lym-1, TNT-1, AND THEIR $F(ab')_2$ FRAGMENTS

	Whole IgG (kD)	H-Chain (kD)	L-Chain (kD)	Isoelectric point pH (bands)	Immunoreactivity[a] (%)
Lym-1	212	47.0	27.2	7.9–8.4 (6-7)	75–80
Lym-1 $F(ab')_2$	104	27.2	27.2	9.2 (1)	75–80
TNT-1	228	48.5	28.6	8.2–8.7 (5)	80–85
TNT-1 $F(ab')_2$	118	30.5	28.6	9.2 (1)	80–85

[a] Immunoreactivities of ^{125}I-radiolabeled antibodies were obtained by either a live cell radioimmunoassay [Lym-1 and Lym-1 $F(ab')_2$] or a paraformaldehyde–acetone fixed cell assay [TNT-1 and TNT-1 $F(ab')_2$] as described in the Experimental section.

preparation were determined by either a live cell radioimmunoassay [Lym-1 and Lym-1 $F(ab')_2$] [12], or a paraformaldehyde–acetone fixed cell assay [TNT-1 and TNT-1 $F(ab')_2$] [15]. Briefly, the radiolabeled antibodies were incubated for 30 min with the appropriate cell lines as follows: Lym-1 and Lym-1 $F(ab')_2$, live Raji cells; TNT-1 and TNT-1 $F(ab')_2$, paraformaldehyde-fixed and acetone-treated Raji cells. The cells were then washed three times with PBS and counted in a γ-counter (1282 Compugamma CS Model, LKB Instruments, Pleasant Hill, CA, U.S.A.). The percentage of immunoreactivity of ^{125}I-labeled antibodies was calculated by dividing the radioactivity bound in the cell pellet with the total radioactivity added in the assay (Table I).

Isoelectric focusing. Isoelectric focusing was performed in tubes using 5% acrylamide and Biolyte pH 3.0–10.0 (Bio-Rad) [16]. Isoelectric point of antibody or fragment was determined by comparing the migration with those of standard proteins.

Endotoxin examination. The Limulus Amoebocyte Lysate (LAL) gelatin test was employed for the quantitation of endotoxin [17]. This assay was performed with the Pyrogent kit (Whittaker Bioproducts, Walkersville, MD, U.S.A.) using the gel-clot method. The detection limit of the kit was 0.12 E.U./ml.

RESULTS AND DISCUSSION

Pepsin digestion

The pH of the antibody solution was adjusted to 4.0–4.5 prior to the addition of pepsin to avoid pepsin denaturation that may irreversibly occur at neutral pH. For large-scale preparation of Lym-1 and TNT-1 $F(ab')_2$ fragments, the protein concentration (greater than 10 mg/ml) was not diluted, and antibody–pepsin mixtures showed no visible precipitation after the pH was carefully adjusted as low as 3.6. The rate of peptic digestion was directly proportional to the pepsin/

antibody (w/w) ratio, but inversely proportional to pH of the reaction mixture. At a pH 3.8 and a pepsin/antibody ratio of 1:250, the digestion was completed in 3–4 h. When the progress of the reaction was monitored by Superose-12 FPLC or SDS-PAGE, the amount of IgG (Fig. 1, peak 1) decreased as the reaction progressed but the Fab′ and other small peptides increased. Thus, either incomplete or over-digestion may reduce the yield of $F(ab')_2$ fragments (Fig. 1, peak 2). Therefore, the reaction was terminated when 80–90% of whole IgG was digested. The reaction was not carried out to completion because the separation of $F(ab')_2$ from IgG is, in general, easier than the separation of $F(ab')_2$ from Fab′, and the recovered IgG was immunoreactive and can be reused.

Development of chromatographic methods

The major components in the antibody digest were pepsin, IgG, $F(ab')_2$, Fab′, and Fc fragments, and other small peptides. Most small molecular mass peptides (M_r < 5 kD) could be separated by Sephadex G-25 column during the buffer exchange procedure. Any trace contaminants of the peptides were further separated in subsequent ion-exchange columns.

Pepsin has an isoelectric point less than pH 1 [18]; it is bound strongly to the

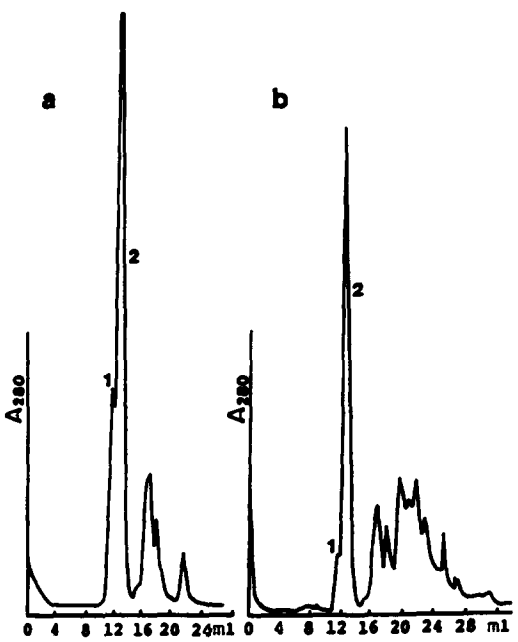

Fig. 1. Analytical-scale separation of Lym-1-pepsin digest. Digest conditions: pepsin/Lym-1 ratio = 1:250, pH 3.73, 37°C; (a) 2 h, (b) 3.5 h. Column, Superose-12 HR 10/30; eluent, PBS pH 7.5; sample volume, 50 μl (100 μg); flow-rate, 0.8 ml/min; a.u.f.s., 0.2 (UV 280 nm). Peaks: 1 = Lym-1 whole IgG; 2 = $F(ab')_2$ fragment.

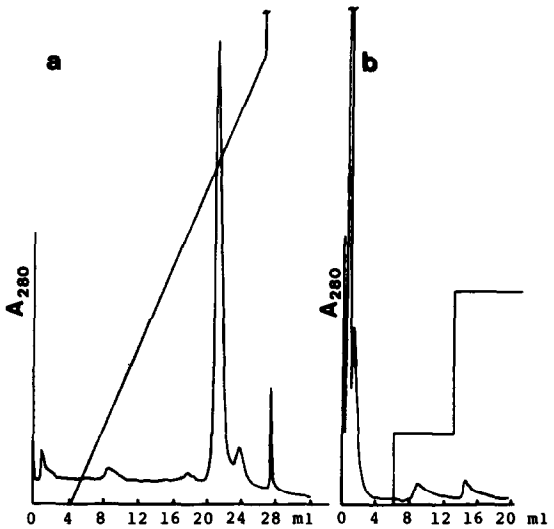

Fig. 2. Analytical-scale separation of pepsin (from Porcine stomach mucosa, Sigma, St. Louis, MO, U.S.A.). (a) Mono-Q HR 5/5; eluent A, 50 m*M* Tris–HCl (pH 7.5); eluent B, 1 *M* NaCl in 50 m*M* Tris–HCl (pH 7.5); linear gradient, 4–26 ml (0–60% B), 26–32 ml (100% B). (b) Mono-S HR 5/5; eluent A, 20 m*M* MES (pH 6.3); eluent B, 1 *M* NaCl in 20 m*M* MES (pH 6.3); step gradient, 6–14 ml (10% B), 14–20 ml (30% B). Sample volume, 100 µl of 4 mg pepsin per ml; flow-rate, 1.5 ml/min; a.u.f.s., 0.5 (UV 280 nm).

Mono-Q column, but not bound to the Mono-S column as shown in Fig. 2. This implies that pepsin can be easily separated from other protein components with higher isoelectric points by cation exchange column chromatography. The analyses of Lym-1-pepsin digest showed that Lym-1 IgG was bound to both Mono-Q and Mono-S columns while Lym-1 F(ab')$_2$ bound only to the Mono-S column (Fig. 3). On the other hand, both TNT-1 IgG and F(ab')$_2$ behaved similarly in Mono-S (both were bound) and Mono-Q (both were not bound) columns (data not shown). These results may be directly correlated with isoelectric points (Table I). Lym-1, TNT-1, and their F(ab')$_2$ fragments have high isoelectric points (greater than pH 8) and therefore tended to bind to the Mono-S column. They are not bound to the Mono-Q column except the Lym-1 which bound weakly because of its relatively low isoelectric point.

The chromatographic properties of Lym-1, TNT-1, and their F(ab')$_2$ fragments are similar on ABx and Mono-S columns. However, the ABx column showed a better separation for the F(ab')$_2$ fragment but poor capacity for protein binding. Based on all of the above information, the purification schemes for F(ab')$_2$ fragments were drawn as follows: Q-Sepharose and S-Sepharose (or ABx) for Lym-1 F(ab')$_2$; S-Sepharose and Protein-A for TNT-1 F(ab')$_2$.

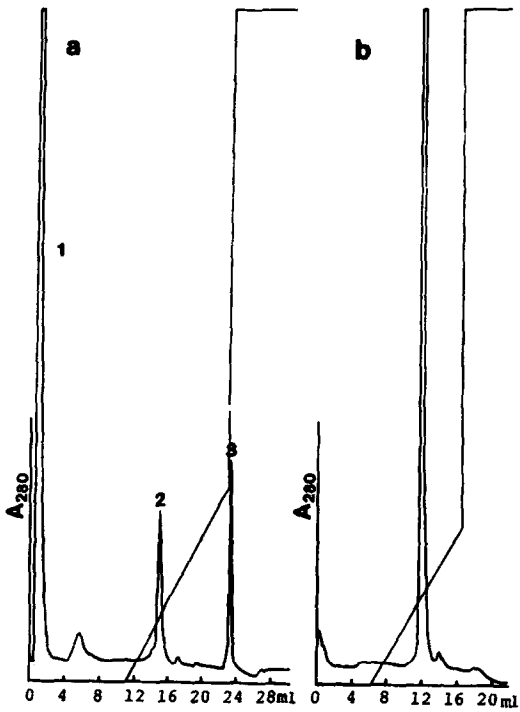

Fig. 3. Analytical-scale separation of Lym-1-pepsin digest. (a) Mono-Q HR 5/5; eluent A, 50 m*M* Tris–HCl (pH 7.5); eluent B, 1 *M* NaCl in 50 m*M* Tris–HCl (pH 7.5); linear gradient, 11–23 ml (0–28% B), 23–27 ml (100% B). Peak 1 contains Lym-1 $F(ab')_2$, peak 2 Lym-1 IgG and peak 3 pepsin. (b) Mono-S HR 5/5; eluent A, 20 m*M* MES (pH 6.3); eluent B, 1 *M* NaCl in 20 m*M* MES (pH 6.3); linear gradient, 6–16 ml (0–22% B), 16–20 ml (100% B). Sample volume, 50 μl (250 μg); flow-rate, 1.5 ml/min; a.u.f.s., 0.5 (UV 280 nm).

Large-scale chromatographic methods

We have demonstrated that the methods developed by analytical columns can be easily scaled up for preparative procedures without changing the elution conditions. Fig. 4 shows typical FPLC profiles for the preparation of $F(ab')_2$ fragment from pepsin digest of 55 mg Lym-1 MAb. It is important to note that the two preparative columns could easily be connected in the FPLC system. The whole IgG and pepsin were bound in the first column (Q-Sepharose) while the effluent containing $F(ab')_2$ was passed directly into the second column (ABx) (Fig. 4a peak 1). After loading, the ABx column was reconnected in the FPLC system so that the impurities were washed out and the $F(ab')_2$ was then eluted from the column with 0.30 *M* NaCl in MES buffer (Fig. 4b, peak 1). The ABx column not only gave a further purification for $F(ab')_2$, but also concentrated the volume about five-fold.

Fig. 5 shows the S-Sepharose FPLC profile for the preparation of $F(ab')_2$ from

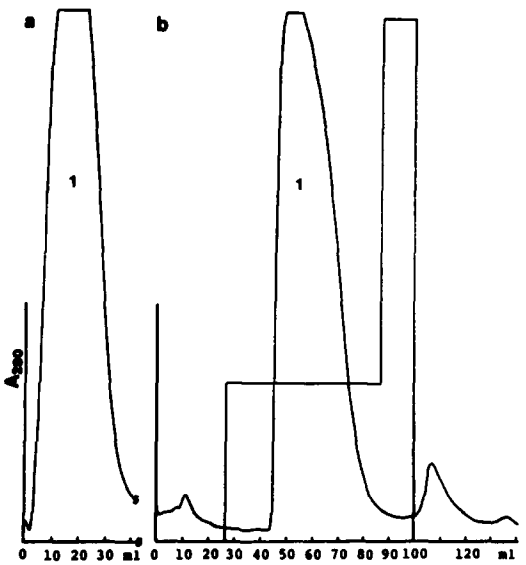

Fig. 4. Large-scale preparation of Lym-1 F(ab')$_2$ fragment from 55 mg of Lym-1 MAb. (a) Q-Sepharose (10 cm × 1 cm I.D.); 50 m*M* Tris–HCl (pH 7.5); peak 1 containing F(ab')$_2$ was directly passed through the second column (ABx, 40 μm, 10 cm × 1 cm I.D.). (b) The ABx column was reconnected to FPLC system and eluted with step gradient; eluent A, 20 m*M* MES (pH 6.3); eluent B, 1 *M* NaCl in 20 m*M* MES (pH 6.3); 0–26 ml (0% B), 26–86 ml (30% B), 86–100 ml (100% B). Flow-rate, 2.0 ml/min; a.u.f.s., 2.0 (UV 280 nm). F(ab')$_2$ product was eluted at peak 1.

the pepsin digest of 90 mg of TNT-1 MAb. Both F(ab')$_2$ and whole IgG showed higher binding to ion-exchange matrix than other proteins, and were eluted as a mixture in peak 3 (Fig. 5). Pepsin was eluted in peak 1 while Fab' fragment was eluted in peak 2. The possible separation of Fab' and F(ab')$_2$ fragments revealed that the binding and elution of proteins from the column are governed not only by the charge, but also by the three-dimensional interaction with the matrix. Final separation of F(ab')$_2$ and whole IgG was carried out by a Protein-A column because Protein-A has affinity for the Fc region of the IgG molecules. The tandem column procedures have also been performed reversely and similar results have been obtained with respect to purity and yield for the TNT-1 F(ab')$_2$ fragment. One advantage of the latter method is that the F(ab')$_2$ product had higher protein concentrations.

The above chromatographic methods have been scale-up to 550 mg of Lym-1, and 500 mg and 4 g of TNT-1 by merely replacing the columns with larger preparative columns. The yields of F(ab')$_2$ were high (80–90%) because the reaction with pepsin was terminated prior to completion to prevent over-digestion, and the undigested IgG antibodies were reused.

Quality control of the F(ab')$_2$ products

Superose-12 FPLC profiles as shown in Fig. 6 demonstrate the large-scale

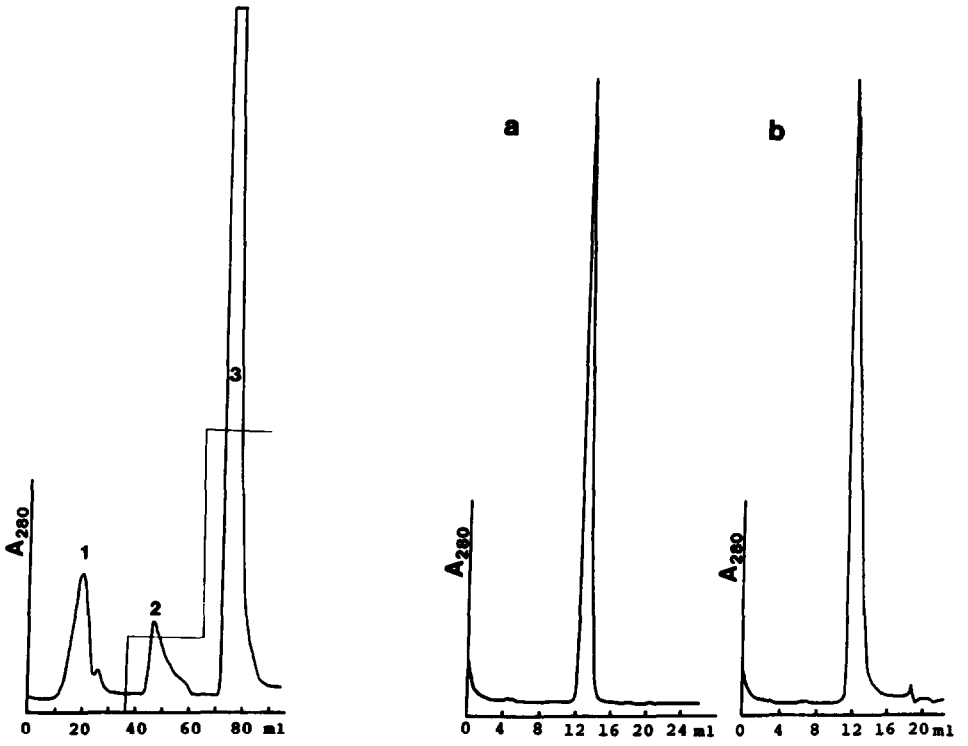

Fig. 5. Large-scale preparation of TNT-1 $F(ab')_2$ fragment from 90 mg of TNT-1 MAb. Column, S-Sepharose (10 cm × 1.6 cm I.D.); eluent A, 20 m*M* MES (pH 6.3); eluent B, 1 *M* NaCl in 20 m*M* MES (pH 6.3); step gradient, 0–36 ml (0% B), 36–66 ml (15% B), 66–96 ml (40% B); flow cell S-2. Peak 1 contains pepsin and other impurities, peak 2 contains mainly TNT-1 $F(ab')_2$, and peak 3 contains TNT-1 whole IgG and $F(ab')_2$ fragment which were further separated by Protein-A column (see text).

Fig. 6. Superose-12 FPLC profiles of (a) Lym-1 $F(ab')_2$ and (b) TNT-1 $F(ab')_2$ obtained from large-scale preparation procedures. Column, Superose-12 HR 10/30; eluent, PBS (pH 7.5); sample volume, 50 μl (100 μg); flow-rate, 0.8 ml/min; a.u.f.s., 0.5 (UV 280 nm).

preparation achieved for Lym-1 $F(ab')_2$ (Fig. 6a) and TNT-1 $F(ab')_2$ (Fig. 6b). The SDS-PAGE (Fig. 7a) in lanes 2 and 4 demonstrates that both Lym-1 and TNT-1 $F(ab')_2$ preparations had high protein purity. Under reducing conditions, Lym-1, TNT-1, and their $F(ab')_2$ fragments showed similar molecular masses for the light chains (Fig. 7b). The $F(ab')_2$ fragments showed a smaller heavy chain than their intact antibodies. Fig. 7b, lane 2 shows an overlap of the heavy chain and light chain of Lym-1 $F(ab')_2$. We therefore treated Lym-1 $F(ab')_2$ with 2-mercaptoethanol and injected a small aliquot into the Mono-S HR 5/5 column. The presence of heavy and light chains were demonstrated as shown in Fig. 8, peaks 2 and 3. Isoelectric focusing showed that both Lym-1 and TNT-1 had multiple bands because of microheterogeneity of the Fc regions [19]. Their

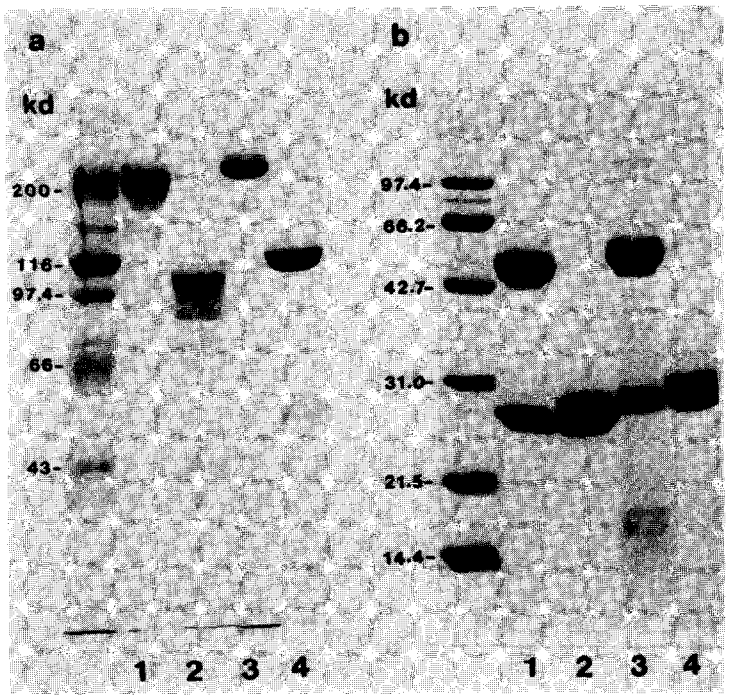

Fig. 7. SDS-PAGE of Lym-1, TNT-1, and their F(ab')$_2$ fragments prepared by large-scale methods. Approximately 20 μg of protein were loaded in each track of the gel. (a) 10% non-reducing gel; lane 1, Lym-1; lane 2, Lym 1 F(ab')$_2$; lane 3, TNT-1; lane 4, TNT-1 F(ab')$_2$. (b) 10% reducing gel; lane 1, Lym-1; lane 2, Lym-1 F(ab')$_2$; lane 3, TNT-1; lane 4, TNT-1 F(ab')$_2$.

F(ab')$_2$ fragments had a single band. Immunoreactivities of F(ab')$_2$ fragments were examined by radioimmunoassay and showed no decrease in comparison to the intact antibodies. The F(ab')$_2$ products also showed low endotoxin levels and were suitable for clinical application. Table I summarizes the data of the molecular masses, isoelectric points, and immunoreactivities of Lym-1, TNT-1, and their F(ab')$_2$ fragments.

CONCLUSIONS

Our results demonstrate that the large-scale procedures for Lym-1 and TNT-1 F(ab')$_2$ fragments are simple and effective. Yields of F(ab')$_2$ were high and the products were suitable for clinical trials in cancer patients. Development of methods include the optimization of the pepsin digestion, chromatographic procedures, and scale-up purification techniques. The process used here should find similar applications for the fragmentation and purification of other MAbs.

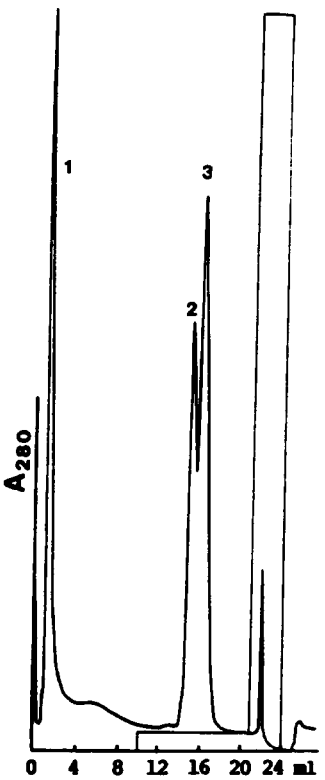

Fig. 8. Analytical separation of TNT-1 $F(ab')_2$ reduced with 2-mercaptoethanol. Peak 1,2-mercaptoethanol; peak 2 and 3, heavy and light chains of TNT-1 $F(ab')_2$. Column, Mono-S HR 5/5; eluent A, 20 m*M* MES (pH 6.3); eluent B, 1 *M* NaCl in 20 m*M* MES (pH 6.3); step gradient, 0–10 ml (0% B), 10–21 ml (5% B), 21–24 ml (100% B); sample volume, 50 μl (100 μg); flow-rate, 1.5 ml/min; a.u.f.s., 0.5 (UV 280 nm).

ACKNOWLEDGEMENTS

We wish to express our thanks to Mr. Kyril Dambuleff for his technical assistance and to Mrs. Sue-Jane Wang and Ms. Donnel Loftin for the typing of this manuscript. This work was supported by grant from Techniclone International (Tustin, CA, U.S.A.).

REFERENCES

1 C. C. Badger, K. A. Krohn, A. V. Peterson, H. Shulman and I. D. Berstein, *Cancer Res.*, 45 (1985) 1536.
2 R. M. Sharkey, M. J. Pykett, J. A. Siegel, E. A. Alger, F. J. Primus and D. M. Goldenberg, *Cancer Res.*, 47 (1987) 5672.
3 S. J. DeNardo, G. L. DeNardo, L. F. O'Grady, D. J. Macey, S. L. Mills, A. L. Epstein, J. S. Peng and J. P. McGahan, Intl. J. Biological Markers, 2 (1987) 49.

4 D. M. Goldenberg, in M. S. Mitchell (Editor), *Progress in Clinical and Biological Research*, Alan R. Liss, New York, 1989, p. 413.
5 M. V. Pimm, M. J. Embleton, A. C. Perkins, M. R. Price, R. A. Robins, G. R. Robinson and R. W. Baldwin, *Int. J. Cancer*, 30 (1982) 75.
6 F. H. Deland, E. Kim, G. Simmons and D. M. Goldenberg, *Cancer Res.*, 40 (1980) 3046.
7 G. L. Buraggi, L. Callegaro, G. Mariani, A. Turrin, N. Cascinelli, A. Attili, E. Bombardien, G. Terno, G. Plassio, M. Dovis, N. Mazzuca, P. G. Natali, G. A. Scassellati, U. Rosa and S. Ferrone, *Cancer Res.*, 45 (1985) 3378.
8 R. L. Wahl, C. W. Parker and G. W. Philpott, *J. Nucl. Med.*, 24 (1983) 316.
9 D. L. Munz, A. Alavi, H. Koprowski and D. Herlyn, *J. Nucl. Med.*, 27 (1986) 1739.
10 D. Herlyn, J. Powe, A. Alavi, J. A. Mattis, M. Herlyn, C. Ernst, R. Vaum and H. Koprowski, *Cancer Res.*, 43 (1983) 2895.
11 P. Parham, *J. Immunol.*, 131 (1983) 2895.
12 A. L. Epstein, R. J. Marder, J. N. Winter, E. Stathopoulos, F. M. Chen, J. W. Parker and C. R. Taylor, *Cancer Res.*, 47 (1987) 830.
13 A. L. Epstein, F. M. Chen and C. R. Taylor, *Cancer Res.*, 48 (1988) 5842.
14 F. M. Chen, C. R. Taylor and A. L. Epstein, *Cancer Res.*, 49 (1989) 4578.
15 S.A. Gaffar, F. M. Chen, C. Z. Liu and A. L. Epstein, *J. Immunoassay*, (1990) in press.
16 T. Lass and F. A. Johansson, in H. Peeters (Editor), *Proteins of the Biological Fluids*, Pergamon Press, Oxford, 1979, pp. 693–697.
17 R. J. McAuley, R. D. Ice and E. G. Curtis, *Am. J. Hosp. Pharm.*, 31 (1974) 688.
18 F. A. Bovey and S. S. Yanari, in P. D. Boyer, H. Lardy and K. Myrback (Editors), *The Enzymes*, Vol. 4, Academic Press, New York, 1960, Ch. 4, p. 63.
19 Z. L. Awdeh, A. R. Williamson and B.A. Askonas, *Biochem. J.*, 116 (1970) 241.