Development of Monoclonal Antibodies against Caprine α_{S2}-Casein and Their Potential for Detecting the Substitution of Ovine Milk by Caprine Milk by an Indirect ELISA

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Monoclonal antibodies (MAbs) have been generated against purified caprine α_{S2}-casein recovered from goat's milk casein by cation-exchange chromatography. Using hybridoma technology, eight MAbs reactive against whole caprine caseins were identified and characterized. All of them failed to show a significant cross-reactivity when tested against sheep and cow whole caseins and beef, horse, porcine, chicken, and soya proteins, as well as gelatin and bovine serum albumin. One of the monoclonal antibodies produced by the hybridoma cell line B2B appeared to be species monospecific and reacted only with the caprine α_{S2}-casein fraction by immunoblotting analysis. Subsequently, MAb B2B was used in an indirect ELISA format for detection of defined amounts of goat's milk (0.5−15%) in ewe's milk (v/v) mixtures.

Keywords: *Monoclonal antibodies; caprine α_{S2}-casein; goat's milk detection; enzyme-linked immunosorbent assay (ELISA)*

INTRODUCTION

The production of ewe's and goat's milk has gained considerable economic importance in certain Mediterranean countries as a result of the widespread acceptance of traditional cheeses, many made exclusively of pure ewe's milk. Most traditional ewe's cheeses, such as manchego, roquefort, feta, or pecorino, must be made with pure ewe's milk. However, substitution of ewe's milk by goat's milk is a common fraudulent practice, arising mainly in response to seasonal fluctuations in ewe's milk production and to differences in price between them. For both economic and ethical reasons there is a need to develop sensitive methods for the quantitative detection of goat's milk in dairy products made from ewe's milk.

Among ruminants, interspecies comparison of the primary structures of homologous caseins reveals great similarity between ovine and caprine caseins. Total casein content as well as the relative proportions of each casein are characteristic of the species (Jennes, 1980). The α_{S1}-, α_{S2}-, β-, and κ- caseins are present in bovine milk in the approximate proportions 4:1:4:1, respectively, whereas ovine (2:2:5:1) and caprine (1:2:5:2) milks can be considered as β-casein-rich milks (Assenat, 1985; Remeuf and Lenoir, 1986).

The genetic polymorphism of milk proteins has been shown to be responsible for changes in milk composition and to influence milk's technological properties. These remarks also apply to goat's milk, for which qualitative as well as quantitative variability is observed, especially for the α_{S1}-casein (Grosclaude et al., 1987; Grosclaude, 1988). In addition, the variation in the relative amount of α_{S1}-casein in goat's milk produces a considerable variation in the relative amounts of the other caseins. Generally, as the relative amount of α_{S1}-casein decreases, the relative amounts of α_{S2}-, β-, and κ-caseins tend to increase (Law and Tziboula, 1992).

Several analytical methods have been proposed for detecting adulteration of milks of closely related species (Ramos and Juárez, 1984). Most of the immunological methods currently available for detection of milk mixtures use polyclonal antibodies against whey proteins, crude caseins, short peptides from milk proteins, or immunoglobulins (Aranda et al., 1988, 1993; Rodriguez et al., 1991, 1994; García et al., 1993, 1994; Rolland et al., 1993, 1995). Recently, the European Commission has established a reference method for detection of cow's milk casein in cheese made from ewe's milk by isoelectric focusing of the milk casein fraction (*Official Journal of the European Communities*, 1992). However, the interest in the immunological methods lies in their advantages of sensitivity, specificity, and cost-effectiveness, with ELISA being the most widely used immunoassay in food analysis (Allen, 1990).

Although immunological methods are accurate and sensitive, they require the production of specific antisera with high titers. The development of hybridoma technology (Köhler and Milstein, 1975) has provided the means for continuous production of monospecific antibodies of consistent specificity. Although hybridoma technology does not necessarily require highly purified antigens, selected antigens should produce a higher yield of monoclonal antibodies of interest. To obtain monoclonal antibodies specific for caprine caseins, it should be convenient to select as immunogen the casein fraction with the largest number of epitopes specific to goat's milk. In a previous work of our group (Haza et al., 1995), the goat's milk casein was fractionated by ion-exchange chromatography and the resulting fractions were characterized immunologically by an indirect ELISA. The most immunoreactive caprine casein fraction was the α_{S2}-casein purified by cation-exchange chromatography.

This paper describes the identification and partial characterization of monoclonal antibodies generated

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against a purified goat α_{S2}-casein recovered from goat's milk casein by cation-exchange chromatography on a column containing an S-Sepharose Fast Flow matrix and the evaluation of one of them on the detection and quantification of defined amounts of goat's milk in ewe's milk by an indirect ELISA.

MATERIALS AND METHODS

Preparation of Casein Samples. Fat was removed from goat's milk by centrifugation at 3000*g* for 10 min at 4 °C and filtration through glass wool. Skim milk was heated to 45 °C and acidified to pH 4.7 with 0.2 M HCl. The acidified milk was kept at 40 °C for 30 min and centrifuged at 6000*g* for 10 min to recover the precipitated caseins. The pellet was washed three times with distilled water to eliminate remaining whey proteins and centrifuged at 6000*g* for 30 min (Rodríguez et al., 1991). The supernatant was discarded, and the pellet containing the precipitated caseins was lyophilized, placed in an airtight container, and stored at −20 °C until use.

Purification of the Caprine α_{S2}-Casein Fraction. The lyophilized caprine caseins were fractionated by cation-exchange chromatography essentially as described by Jaubert and Martín (1992). Briefly, 1 g of lyophilized caprine casein in 25 mL of 0.8 mM DTT, 25 mM sodium formate, and 7.5 M urea buffer (pH 4.0) was reduced for 2 h at 24 °C and then applied to a column (2.5 × 30 cm) containing 150 mL of S-Sepharose Fast Flow cation exchanger (Pharmacia). The column was previously equilibrated with 0.064 mM DTT, 75 mM sodium formate, and7.5 M urea buffer (pH 4.0) while the elution was performed at room temperature at a flow rate of 1 mL/min using a linear NaCl gradient from 0 to 0.3 M for 18 h. Casein fractions were collected with a FRAC-100 collector (Pharmacia LKB). Absorbance of the samples was determined at 280 nm in a Hitachi model U-2000 spectrophotometer.

Mice Immunization. Six female BALB/c mice (6−8 weeks old) were immunized intraperitoneally with 100 μg of purified caprine α_{S2}-casein fraction in 0.1 mL of sterilized physiological saline (0.85%) emulsified with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, MI). Subsequent injections made in Freund's incomplete adjuvant were applied intraperitoneally at 1-week intervals for 2 months, while blood samples were obtained from the retrobulbar plexus of each mouse to determine serum titer and antibody specificity by an indirect ELISA. Three days prior to fusion, the mouse showing the highest immunization titer was boosted by intraperitoneal injection of 100 μg of caprine α_{S2}-casein fraction in 0.2 mL of distilled water.

Production of Caprine α_{S2}-Casein Specific MAbs. Four days after the booster injection, the mouse selected for producing antibodies against the caprine α_{S2}-casein was anesthetized by chloroform and its spleen removed and mechanically dissociated by passage through a sterile nylon mesh. Spleen cells were mixed with myeloma cells (P3X63-Ag8.653) in a 5:1 ratio and fused in poly(ethylene glycol) (molecular weight 1.500, Böehringer Mannheim GmbH, Germany) according to the protocol of Köhler and Milstein (1975).

Hybridoma cell growth was apparent in 2 weeks, after which time the supernatants of interest were tested by an indirect ELISA against lyophilized whole goat caseins. Hybridoma supernatants from positive wells were also tested against whole sheep and cow caseins as well as lyophilized soluble muscle proteins from beef, horse, pork and chicken, gelatin, soya proteins, and bovine serum albumin. Hybridomas producing antibodies specific to the caprine α_{S2}-casein were cloned by limiting dilution in 96-well plates and expanded to produce approximately 50 mL of culture medium containing antibody before they were frozen in liquid nitrogen.

Two hybridomas producing antibodies specific to caprine α_{S2}-casein were grown as ascites tumors in BALB/c mice pretreated by intraperitoneal injection with 0.2 mL of pristane (Sigma Chemical Co., St. Louis, MO) 10 days prior to injection of 1×10^6 hybridoma cells. The ascites fluids were centrifuged at 1000*g* for 10 min, and the supernatants were stored at −20 °C.

Immunoglobulins from the ascites fluids and the culture supernatants were recovered by ammonium sulfate precipitation to minimize further nonspecific interactions (Harlow and Lane, 1988). The class and subclass of the monoclonal antibodies specific to the purified caprine α_{S2}-casein and produced by the corresponding hybridoma cells in the culture supernatants and ascites fluids were determined by an indirect ELISA with commercial rabbit antisera in a kit format against mouse immunoglobulins IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA (Sigma).

ELISA Procedure. An indirect ELISA was used to determine the titer, sensitivity, and specificity of the generated monoclonal antibodies against the caprine α_{S2}-casein when they were present in mouse sera, culture supernatants, and ascites fluids.

Flat-bottom ELISA plates (Nunc, Roskilde, Denmark) were filled with 100 μL/well of whole goat's, sheep's, and cow's caseins, with soluble muscle proteins from beef, horse, pork, and chicken, with gelatin, soya proteins, and bovine serum albumin (20 μg/100 μL), with raw, pasteurized, and sterilized milk of goat, sheep, and cow (1/200), or with raw milk samples (1/25) diluted in phosphate-buffered saline (PBS) (pH 7.2) and incubated for 1 h at 37 °C. The wells were washed five times with PBST (PBS containing 5% Tween 20) and coated with 200 μL of 0.1% gelatin in PBS for 30 min at 37 °C. After another five washes with PBST, 100 μL of the mouse sera, ascites fluids, or culture supernatants containing antibodies diluted in PBST was added to the wells and the plates were incubated on a plate shaker (Pharmacia LKB) for 1 h at room temperature. After five more washes with PBST to remove free antibodies, 100 μL of rabbit anti-mouse immunoglobulins conjugated to peroxidase (Dakopatts, Denmark) diluted 1/1500 in PBST was added to the wells, and the plates were incubated with shaking for 1 h at room temperature. The wells were washed five more times with distilled water before addition of 100 μL of the substrate enzyme solution consisting of ABTS tablets dissolved in a buffer containing sodium perborate, citric acid, and disodium hydrogen phosphate (Böehringer, Mannheim). After 15 min of incubation, the green color developed by conversion of the substrate was measured at 405 nm with a Titertek Multiskan Plus spectrometer (Flow Laboratories, McLean, VA).

SDS−PAGE PhastSystem Electrophoresis and Phast-Transfer Immunoblotting. Whole goat, sheep, and cow caseins and selected caprine casein fractions recovered by cation-exchange chromatography (β-, κ-, α_{S1}-, and α_{S2}-) were analyzed by SDS−PAGE using PhastSystem electrophoresis equipment (Pharmacia LKB). Electrophoresis was performed on homogeneous 20% polyacrylamide gels according to the manufacturer's instructions.

After electrophoresis of the casein samples in the PhastSystem equipment, the proteins bands in the resulting gels were immediately transferred to a nitrocellulose membrane with a pore size of 0.45 μm by semidry electrophoretic transfer with PhastTransfer equipment (Pharmacia LKB). After the transfer, the nitrocellulose membrane was washed with a Tris buffer saline (TBS) for 10 min and blocked for 30 min with bovine serum albumin in TBS. The nitrocellulose membrane was washed with TBST (TBS with 0.05% Tween 20) and then probed with MAb B2B diluted 1/1000 in TBST on a plate shaker (Pharmacia LKB) for 1 h at room temperature. After blotting, the nitrocellulose membrane was washed one more time with TBST, and rabbit anti-mouse immunoglobulins conjugated to peroxidase (Dakopatts) diluted 1/2000 in TBST were applied to the blot. The membrane was incubated with the conjugate for 1 h at room temperature with shaking and washed as before; finally, the bound monoclonal antibody was detected using a peroxidase substrate solution that was prepared by mixing 0.6 mg/mL 3,3′-diaminobenzidine (Sigma) in TBS (pH 7.6) with H_2O_2 (110 volumes, 10 μL/mL buffer).

Reference Milk Samples. Goat's and ewe's milk samples were mixed at different percentages (1−100% v/v) and maintained frozen at −20 °C until use. Mixtures from three independent batches of milk were prepared to be tested by the indirect ELISA described.

Table 1. Comparison of Cross-Reactivities among Several Proteins toward Goat α_{S2}-Casein Monoclonal Antibodies As Measured by an Indirect ELISA

	MAb[a]							
protein	A1H	B2B	B12B	C2H	C6C	C7G	F4E	A6F
caseins								
goat	2.167[b]	2.152	2.156	2.593	2.577	2.769	2.577	2.593
sheep	0.131	0.142	0.154	0.151	0.133	0.250	0.164	0.280
cow	0.124	0.117	0.117	0.149	0.129	0.170	0.164	0.229
soluble proteins								
pork	0.157	0.123	0.129	0.190	0.138	0.180	0.167	0.190
beef	0.136	0.109	0.141	0.153	0.122	0.167	0.164	0.211
horse	0.139	0.119	0.184	0.196	0.123	0.177	0.165	0.290
chicken	0.130	0.159	0.152	0.195	0.151	0.154	0.159	0.221
soya	0.123	0.194	0.169	0.192	0.146	0.170	0.142	0.250
gelatin	0.130	0.120	0.128	0.141	0.132	0.143	0.140	0.188
bovine serum albumin	0.115	0.142	0.125	0.175	0.132	0.155	0.125	0.181

[a] 100 mL of culture supernatant containing monoclonal antibodies (not diluted) was added to the assay wells. [b] Optical densities at 405 nm. Data points are the median values of triplicate assays from three independent protein preparations. Background absorbance values were not subtracted.

RESULTS

Antibodies against the caprine α_{S2}-casein purified from whole goat's casein by cation-exchange chromatography were raised in mice, and the antibody titer of each mouse serum was determined by an indirect ELISA (results not shown). The spleen of the mouse showing the highest immunization titer (1/16000) was chosen for its fusion with myeloma cells (P3X63-Ag8.653). The fusion efficiency (number of wells with growing colonies/number of wells seeded) was greater than 50%: from 400 wells with hybridoma growth, 25% produced monoclonal antibodies against goat's caseins as determined by the indirect ELISA.

The screening of the hybridoma cell supernatants by an indirect ELISA against goat, cow, and sheep caseins indicated that eight cell lines produced specific monoclonal antibodies against whole goat caseins. These eight cells lines were cloned by limiting dilution to obtain stabilized subclones designated A1H 4B (A1H), B2B 11C (B2B), B12B 9C (B12B), C2H 11H (C2H), C6C 7G (C6C), C7G 2A (C7G), F4E 2E (F4E), and A6F 5D (A6F). The specificity of the monoclonal antibodies produced by these eight subclones was further examined by evaluating their reactivity against muscle soluble proteins of beef, horse, pork, and chicken and also against soya proteins, gelatin, and bovine serum albumin. The results obtained (Table 1) indicated that none of these antibodies showed cross-reactivity against any of the proteins tested. The eight cell lines were expanded to produce 50 mL of supernatants, and two of them (B2B and C2H) were selected to produce ascites tumors in mice.

The class and subclass of the eight monoclonal antibodies were determined with a commercial kit in an indirect ELISA with six of them being IgG1 (B2B, B12B, C2H, C6C, C7G, and A6F) and two of them IgM (A1H and F4E). The immunoreactivity of the antibodies in the eight cell lines and two ascites fluids against 20 μg/100 μL of whole goat caseins was also determined by an indirect ELISA. The results obtained indicated that the most immunoreactive were the supernatants of cell lines B2B (1/3200), B12B (1/1600), and C2H (1/1600) and the ascites fluid of hybridoma B2B (1/1000), and thus they were selected for further studies.

The four monoclonal antibodies selected were partially purified by ammonium sulfate precipitation and tested against 20 μg/100 μL whole goat caseins and raw goat milk (1/200) by an indirect ELISA (Figure 1). MAb B2B (supernatant and ascites fluid) was the most immunoreactive. Table 2, shows the absorbance values at 405 nm of MAb B2B from supernatant and ascites fluid when tested against raw milk (1/200) and whole caseins (20 μg/100 μL) of goat, sheep, and cow. The results obtained indicate that MAb B2B from cell culture supernatants displays a much lower cross-reactivity than MAb B2B from ascites fluid. This MAb B2B supernatant was further tested against raw, pasteurized, and sterilized goat's milk (Table 3) to test the effect of processing temperatures on the response of MAb B2B against the goat's milk samples. The results obtained indicate that heating the milk samples does not inactivate epitopes involved in the binding of this monoclonal antibody.

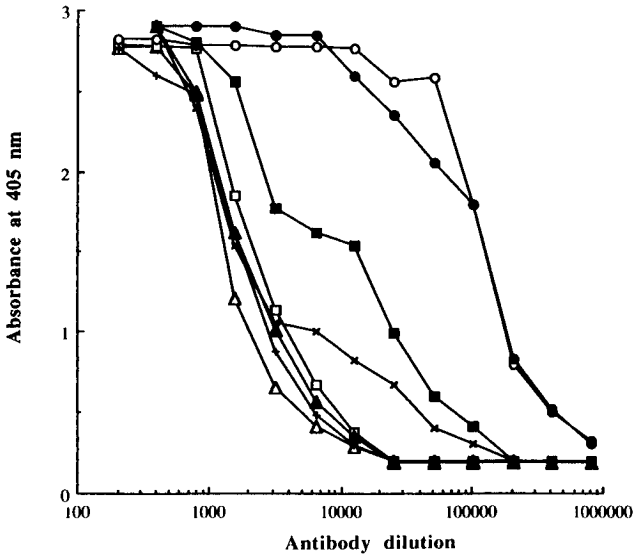

Figure 1. Immunoreactivity of partially purified monoclonal antibodies from supernatants of cell lines B2B (□), B12B (△), C2H (+), and the ascites fluid B2B (○) for whole goat's milk caseins and from the supernatants of cell lines B2B (■), B12B (▲), C2H (×), and the ascites fluid B2B (●) for goat's raw milk as determined by an indirect ELISA.

The specificity of MAb B2B for the goat α_{S2}-casein was confirmed by SDS−PAGE immunotransfer when purified caprine casein fractions by cation-exchange chromatography on a column containing an S-Sepharose Fast Flow matrix (β-, κ-, α_{S1}-, and α_{S2}-caseins) and whole goat, cow, and sheep caseins were probed with this monoclonal antibody (Figure 2). A strong positive interaction was visualized with the whole goat caseins and the goat α_{S2}-casein fraction, which was very weak in the goat α_{S1}-casein, suggesting it still contains

Table 2. Cross-Reactivities of MAb B2B, from Cell Culture Supernatants and Ascites Fluid, against Whole Raw Milk and Caseins of Different Animal Species As Measured by an Indirect ELISA

MAb B2B	antibody dilutions	caseins (20 mg/100 mL)			raw milk		
		goat	sheep	cow	goat	sheep	cow
ascites fluid	1/3200	2.781[a]	0.737	0.257	2.782	0.639	0.226
	1/6400	2.784	0.651	0.191	2.602	0.502	0.217
	1/12800	2.564	0.558	0.125	2.589	0.412	0.155
cell culture supernatant	1/3200	2.353	0.124	0.104	1.837	0.158	0.137
	1/6400	1.767	0.102	0.110	1.210	0.154	0.141
	1/12800	1.162	0.102	0.100	0.747	0.146	0.138

[a] Optical densities at 405 nm. Data points are the median values of triplicate assays from three independent batches.

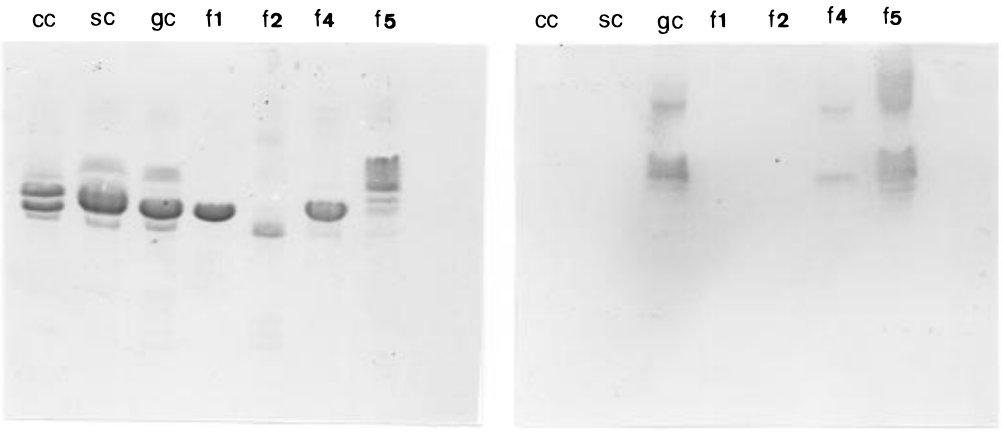

Figure 2. Specificity of MAb B2B for caprine milk caseins as determined by SDS−PAGE analysis and PhastTransfer immunoblotting: lane CC, whole cow's milk caseins; lane SC, whole sheep's milk caseins; lane GC, whole goat's milk caseins. Purified caprine casein fractions by cation-exchange chromatography: lane F1, fraction 1 (β-casein); lane F2, fraction 2 (κ-casein); lane F4, fraction 4 (α_{S1}-casein with residual α_{S2}-casein, β-casein, and κ-casein); lane F5, fraction 5 (mostly α_{S2}-casein).

Table 3. Effect of Processing Temperatures on Response of MAb B2B against Goat's Milk Samples As Measured by an Indirect ELISA

dilutions of MAb B2B	goat's milk (1/200)		
	raw	pasteurized	sterilized
1/400	2.773[a]	2.782	2.765
1/800	2.766	2.773	2.767
1/1600	2.602	2.766	2.484
1/3200	1.837	1.962	2.041
1/6400	1.210	1.222	1.308
1/12800	0.747	0.812	0.889

[a] Optical densities at 405 nm. Data points are the median values of triplicate assays from three independent batches.

residual quantities of the α_{S2}-casein, while no other interactions were visualized between MAb B2B and protein bands corresponding to whole cow casein, whole sheep casein, and purified β- and κ-casein from goat's milk.

MAb B2B was also used to detect and quantify the substitution of ovine milk by caprine milk in raw milk mixtures by an indirect ELISA format. Extensive checkerboard titrations were performed to determine the dilutions of antigens and antibody required to optimize the assay. Optimum conditions for the indirect ELISA developed in this work were obtained using the reference milk samples diluted 1/25 and partially purified MAb B2B (0.6 mg/mL) diluted 1/1000, while the commercial rabbit anti-mouse immunoglobulins conjugated to horseradish peroxidase (1.3 mg/mL) were diluted 1/1500.

Three independent batches of reference goat's and ewe's milk mixtures prepared to include 0, 0.5, 1, 2.5, 5, 10, and 15% of goat's milk in 100 mL were tested when diluted 1:25. Results from Figure 3 show that the absorbance values increase linearly until 15% substitution of goat's milk by ewe's milk. The lower detection

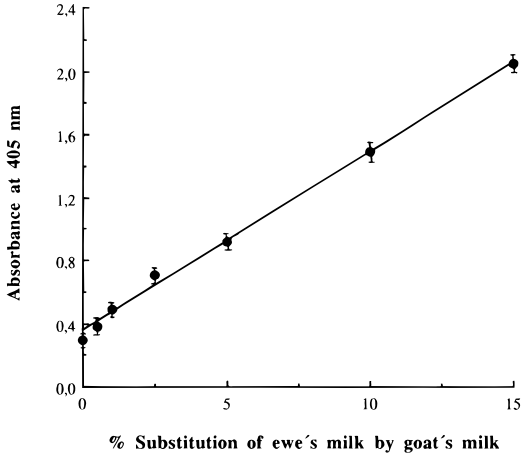

Figure 3. Indirect ELISA response to substitution of ewe's milk by goat's milk. Each symbol represents the mean of duplicate determinations from three independent milk mixtures.

limit of the assay is in the range of 0.5−1% substitution. Assay precision was estimated from replicates of the standard curve, and absorbance was related to goat's milk content (%G) in the range 0−15% by the equation $A_{405} = 0.35047 + 0.11379(\%G)$, with a correlation coefficient of $r^2 = 0.996$ and a $p < 0.0001$.

DISCUSSION

A number of monoclonal antibodies against bovine κ-casein (Feng and Cunningham-Rundles, 1989; Kuzmanoff et al., 1990), α_{S1}-casein (Kuzmanoff et al., 1991), α_{S2}-casein (Leung et al., 1991), and β-casein (Nagaune et al., 1988; Kuzmanoff et al., 1991; Oudshoorn et al., 1994) have been obtained. They have been utilized in studies of hormonal and developmental regulation of

casein and whey proteins during lactogenesis, to investigate structural, topological, and conformational aspects of those proteins, to evaluate cross-reactivity interactions with other proteins, and to quantitate their synthesis during lactogenesis.

Levieux and Venien (1994) have also obtained two monoclonal antibodies specific to bovine β-lactoglobulin and developed a sensitive two-site ELISA to detect and quantify the presence of cow's milk in ewe's milk. Until now, only monoclonal antibodies against bovine milk components have been used in sensitive enzyme immunoassays to detect the presence of cow's milk in goat's or ewe's milk. We are not aware of other monoclonal antibodies against goat's milk components. Since cheese manufacture in Mediterranean countries is often affected by fraudulent substitution of ovine milk by caprine milk due to their difference in price, we report here the development of monoclonal antibodies against a caprine casein fraction to detect and quantify the presence of goat's milk in ewe's milk.

Because interspecies comparison of the primary sequences of homologous caseins reveals great similarity, especially between caprine and ovine caseins, we considered it important to use partially purified species specific antigens to obtain monoclonal antibodies against goat caseins (Haza et al., 1995).

The use of purified goat α_{S2}-casein for immunization of mice resulted in the successful production of hybridoma cells secreting antibodies recognizing goat caseins. Eight of these hybridoma cells (A1H, B2B, B12B, C2H, C6C, C7G, F4E, and A6F) were specific for goat caseins (Table 1). After extensive analysis of the eight monoclonal antibodies (supernatants of the hybridomas and ascites fluids) against whole goat caseins and raw goat's milk by an indirect ELISA, MAb B2B was the most immunoreactive (Figure 1) and was further partially purified by ammonium sulfate precipitation.

Monoclonal antibodies are normally collected either as tissue culture supernatants or as ascites fluids. If large amounts of antibody are needed, it is generally easier to grow the cells as tumors in animals because ascites will usually contain around 1000 times the antibody concentration in cultures (Goding, 1986). However, for most immunochemical assays, tissue culture supernatants will be the most useful source of monoclonal antibodies since the supernatants are not contaminated with high levels of other antibodies and their concentration is high enough for most immunoassays (Harlow and Lane, 1988). We have tested the specificity of MAb B2B from ascites fluid and culture supernatant against whole caseins and raw milk of goat, ewe, and cow, and the results obtained (Table 2) indicate that antibodies from culture supernatants are more specific for goat caseins and raw milk than those from the ascites fluids, probably because between 2 and 10% of the antibodies from ascites fluids derive from the mouse's current antibody repertoire and not from the hybridoma cells (Harlow and Lane, 1988).

A control experiment was also performed to measure the response of the specific MAb B2B against goat's milk samples subjected to different heat treatments. The effect of processing temperatures on response of MAb B2B against goat's milk samples as measured by an indirect ELISA is shown in Table 3. The results obtained indicate that the antigenicity of goat's milk casein was not affected by heat treatment, as also reported by other investigators (Ramos and Juárez, 1984; Rolland et al., 1993).

The specificity and cross-reactivity of MAb B2B against different caseins and casein fractions were also analysed by SDS−PAGE analysis and PhastTransfer immunoblotting. As shown in Figure 2, MAb B2B bound only to slots containing whole goat's caseins and not to those containing whole sheep or cow caseins, while it also bound to the purified casein fractions containing α_{S2}-casein and the α_{S1}-casein with residual α_{S2}-, β-, and κ-caseins. The minor bands detected in the blots are attributed to genetic variants or degradation products. Thus, our MAb B2B appears to be highly monospecific for caprine α_{S2}-casein.

MAb B2B has also been used for the detection of defined amounts of goat's milk (0.5−15%) in ewe's milk mixtures by using an indirect ELISA (Figure 3). The immunoassay developed for the identification and quantification of goat's milk in ewe's milk is in the range of sensitivity reported by others (García et al., 1993; Rodriguez et al., 1994). Immunoassay techniques provide complementary and alternative approaches to reduce the use of costly sophisticated equipment and time, while still maintaining reliability and improved sensitivity. The indirect ELISA test using our MAb B2B was accurate, easy to perform, sensitive, and specific. This MAb could also be suitable for commercial production of stable kits for caprine milk identification in unknown milk mixtures in large or small scale inspection programs.

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