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A comparison of IgG and IgG1 activity in an early milk concentrate from non-immunised cows and a milk from hyperimmunised animals

Michelle A. McConnell [a,*], Glenn Buchan [a], Michail V. Borissenko [b], Heather J.L. Brooks [a]

[a] *Microbiology Department, University of Otago, PO Box 56, Dunedin, New Zealand*
[b] *New Zealand Dairy Ingredients, PO Box 140, Te Puke, New Zealand*

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Abstract

This paper reports on the activity of bovine immunoglobulins IgG and IgG1 directed towards 19 microbial pathogens in an early milk concentrate from non-immunised cows and in a hyperimmune milk using an ELISA technique. Both products contained IgG and IgG1 which bound to all the microbial antigens tested. The early milk concentrate demonstrated a significantly higher IgG and IgG1 antibody titre ($P < 0.05$) against all of the antigens tested than the hyperimmune milk. In addition, the early milk concentrate was shown to stimulate the accumulation of immune cells in the peritoneum of Balb/c mice. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Passive immunisation and immunotherapy using bovine colostral preparations is an area of increasing interest. The importance of colostrum and milk in the transmission of immunity from mother to offspring was first postulated by Ehrlich in 1892 (Silverstein, 1996). Subsequent studies have demonstrated that the passive transfer of maternal antibodies (IgG) in humans occurs prior to birth via the placenta and after birth via the colostrum and milk. In cattle, pigs and sheep passive transfer of maternal antibodies occurs in the first 20–48 h after birth via the colostrum. During the immediate postnatal period the gastrointestinal tract undergoes rapid growth with morphological changes and functional maturation related to colostrum ingestion (Xu, 1996). It has been postulated that many animal species and perhaps pre-term infants have enhanced intestinal permeability to macromolecules, which may facilitate the absorption of colostral immunoglobulins during the early neonatal period (Burin, 1997).

The first recorded advocate for the therapeutic use of the high antibody content of colostrum is L. M. Spolverini who, in 1952, suggested using bovine colostrum as an infant food to protect the infant against shared human and bovine diseases (Campbell & Peterson, 1963). In a further development Campbell and Peterson immunised cows with a mixture of non-viable pathogens (hyperimmunisation) several times prior to parturition and collected the colostrum fraction which they referred to as immune or hyperimmune milk. Subsequently a milk product (Stolle) was developed by repeated immunisation of lactating cows. The manufacturers of this product (Stolle Milk Biologics Inc.) claim the milk increases resistance to bacterial infection, improves the immune system and has an anti-inflammatory effect.

During the last two decades interest in the use of immune milk as an immunotherapy agent has increased. Prophylactic and therapeutic use of immune milks has been reported to successfully prevent and treat enteropathogenic *Escherichia coli* infections in infants and adults (Hilpert et al., 1977; Tacket et al., 1988), rotavirus gastroenteritis in infants (Bogstedt et al., 1996; Hilpert, Brussöw, Mietens, Sidoti, Lerner, & Werchau, 1987), cryptosporidiosis and diarrhea in AIDs and other immunodeficient patients (Tzipori, Robertson, &

* Corresponding author. Fax +64-3479-8540.
E-mail address: michelle.mcconnell@stonebow.otago.ac.nz (M.A. McConnell).

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albicans to determine if consistent levels of IgG activity could be obtained. The five extra batches of Product A tested against *H. pylori*, *E. coli* 0157:H7 and *C. albicans* had similar levels of IgG activity to the product used in the rest of the study (Table 3).

Table 1
Antibody titres of IgG against whole microbial cell antigens or lipopolysaccharide fractions (LPS) in Immulac 15 and Stolle as measured by ELISA

Microbe	Product[a]	
	A	B
Bacillus cereus	2178±615.73	144.73±32.26
Streptococcus pyogenes	323±25.77	91.23±29.11
Streptococcus agalactiae	2245.21±439.42	322.71±98.88
Streptococcus pneumoniae	2139.14±554.25	143.54±26.97
Streptococcus mutans	4957.45±98.91	676.77±18.00
Staphylococcus epidermidis	2689.96±697.90	127.79±27.42
Staphylococcus aureus	3840.04±910.12	74.08±9.48
Listeria monocytogenes	5964.98±184.01	348.91±37.95
Yersinia enterocolitica	960.92±141.52	163.90±44.14
Escherichia coli	3968.77±658.06	301.42±14.79
E. coli 0157:H7	3879.38±811.25	321.48±33.54
Haemophilus influenzae	2087.07±170.62	181.50±28.15
Campylobacter jejuni	2729.67±499.47	200.36±48.27
Helicobacter pylori	3219.58±148.31	306.79±49.10
Salmonella enteritidis (LPS)	676.80±17.98	421.65±2.80
Salmonella typhimurium (LPS)	592.82±15.22	204.79±26.78
Klebsiella pneumoniae (LPS)	631.04±5.93	111.59±21.32
Propionibacterium acnes	3557.10±1226.15	242.61±20.14
Candida albicans	2811.45±318.09	280.98±20.30

[a] A, Immulac 15; B, Stolle. Mean plus standard error of the mean of four estimations.

Table 2
Antibody titres of IgG1 against whole microbial cell antigens or the lipopolysaccharide fractions (LPS) in Immulac 15 and Stolle as measured by ELISA

Microbe	Product[a]	
	A	B
Bacillus cereus	1283.59±411.10[2]	55.49±2.85
Streptococcus pyogenes	271.74±42.86	49.37±5.45
Streptococcus agalactiae	1261.43±305.27	55.65±7.27
Streptococcus pneumoniae	602.30±144.35	47.63±1.21
Streptococcus mutans	2001.49±690.36	128.09±22.02
Staphylococcus epidermidis	1275.51±252.22	63.37±7.37
Staphylococcus aureus	1766.49±371.89	36.77±4.66
Listeria monocytogenes	2785.93±697.12	255.88±7.42
Yersinia enterocolitica	1916.62±606.82	95.74±16.37
Escherichia coli	1232.08±505.33	51.92±4.72
E.coli 0157:H7	464.24±87.42	145.05±46.53
Haemophilus influenzae	801.72±218.53	43.19±0.94
Camplobacter jejuni	2182.54±104.98	83.60±19.75
Helicobacter pylori	2343.61±352.53	67.37±6.11
Salmonella enteritidis (LPS)	445.78±60.51	151.82±15.09
Salmonella typhimurium (LPS)	465.27±99.26	51.53±2.99
Klebsiella pneumoniae (LPS)	420.20±16.45	42.77±1.80
Propionibacterium acnes	1203.51±266.43	65.76±7.86
Candida albicans	773.82±126.20	57.00±4.25

[a] A, Immulac 15; B, Stolle. Mean and standard error of the mean of four estimations.

3.2. Mouse bioassay

The milk products did not demonstrate anti-inflammatory activity 18 h after the application of an inflammatory stimulus to the peritoneum of mice which had been fed the products for seven days (Table 4). However, when an inflammatory stimulus was applied to the peritoneum of mice after 7 days of milk consumption and then white blood cells in the peritoneum were counted 72 h later it appeared that mice fed Product A had higher numbers of white blood cells in the peritoneum than mice fed Product B or the control mice (Table 5). The unstimulated mice fed Product A in their drinking water for 10 days, with no inflammatory stimulus applied, appeared to have more white blood cells present in the peritoneum than mice fed Product B for 10 days or control mice (Table 5). There was an increase in the number of white blood cells in the peritoneum of mice fed Product B 72 h after the application of an inflammatory stimulus but not with mice fed Product A

Table 3
Titres of IgG antibody in six batches of Product A against *Helicobacter pylori*, *Escherichia coli* 0157:H7 and *Candida albicans* measured by ELISA (absorbance measured at 490 nm)

Batch number	Organism[a]		
	H. pylori	*E. coli* 0157:H7	*C. albicans*
Batch 530	531.00±113.44[1]	460.44±66.49	648.34±238.85
Batch 535	828.00±266.22	412.97±67.50	1071.90±200.50
Batch 538	769.98±328.14	374.12±78.62	536.19±149.85
Batch 539	809.13±321.96	442.59±189.46	488.99±169.95
Batch 541	1015.02±417.67	668.66±123.55	529.60±143.51
Product A[b]	1748.44±486.77	714.63±240.48	1807.54±343.70

[a] Mean (standard error of the mean) of four estimations.
[b] A, Immulac 15.

Table 4
Comparison of the anti-inflammatory effect of oral administration of Products A and B for 7 days on the number of white blood cells in the peritoneum of mice 18 h after exposure to an inflammatory stimulus[a]

Treatment	Number of white blood cells in peritoneal lavage ($\times 10^5$/ml)[b]
None (no milk, no TGC)	7.75 (0.43)
Product A + 1 ml 1% TGC	30.90 (0.167)
Product B +1 ml 1% TGC	30.10 (0.143)
1 ml 1% TGC followed by 50 μg dexamethasone (no milk)	9.5 (0.074)
1 ml TGC (no milk)	35.8 (0.082)
Skim milk + 1% TGC	30.10 (0.096)

[a] TGC, thioglycollate; Product A, Immulac; Product B, Stolle. Mice were fed the milk products for 7 days prior to the inflammatory stimulus and for the 18 h following stimulation.
[b] Mean and standard error of the mean in parentheses.

Table 5
Comparison of the anti-inflammatory effects of Products A and B (fed to mice for 7 days prior to being exposed to an inflammatory stimulus) on the number of white blood cells in the peritoneum of mice 72 h after exposure to the stimulus[a]

Treatment	Number of white blood cells in peritoneal lavage ($\times 10^5$/ml)[b]
None (no milk, no TGC)	7.88 (0.43)
Product A-no TGC	17.00 (0.09)
Product A + TGC	28.00 (0.43)
Product B-no TGC	9.40 (0.11)
Product B + TGC	16.00 (0.11)
TGC-no milk	16.2 (0.01)

[a] TGC, thioglycollate; Product A, Immulac; Product B, Stolle. Products A and B were fed to the animals for 7 days prior to stimulation and for the 72 h following stimulation. Control groups were fed for 10 days.
[b] Mean and standard error of the mean in parentheses.

(Table 5). Statistics have not been applied to this data because of the small group sizes.

4. Discussion

Colostrum, which is produced by cows immediately after parturition has a different composition to milk from cows in established lactation. The main difference is in the high level of IgG (predominantly IgG1 subclass). For several weeks prior to parturition until (or near) calving, IgG1 from the circulating IgG1 pool is actively concentrated into mammary gland secretions (Levieux & Ollier, 1999). Product A, containing immunoglobulin G (15% determined using affinity HPLC by the manufacturer; 9.57% by our ELISA) from non-immunised cows, had antibody activity (both IgG and IgG1) against all the bacterial pathogens tested in vitro by ELISA. This result confirms the observations made by a number of authors that there is IgG against pathogens in the early milks from non-immunised cows. By concentrating the milk using a spray drying technique it has been possible to produce a product with higher titres of antibody activity against 19 pathogenic organisms than milk produced from hyperimmunised animals.

Product B, derived from the milk of hyperimmunised cows (0.5% IgG as determined by manufacturer and 0.15% as determined by our ELISA) also had antibody activity (both IgG and IgG1) against the organisms tested. The mixture of 26 organisms used to immunise these cattle were predominantly members of the *Streptococcus* genus as well as staphylococci, salmonellae, *Klebsiella*, *Haemophilus* and others, all of which genera were tested in the current study.

The IgG contents of both products, as determined by ELISA, was lower than that claimed by both manufacturers. This may reflect our technique being less sensitive than that used by the manufacturers or that some IgG in both products has been degraded or lost biological activity on storage even though both products were still within their use by dates.

Six batches of Product A had similar IgG activity against the three pathogens selected showing that the presence of immunoglobulin against microbial pathogens in early milks is a natural occurrence. It is known that the greatest transfer of antibody from mother to young occurs in the first 48 h after birth in cattle so the presence of antibodies to a number of pathogens in these early milks is not unexpected. Some inconsistency in levels of IgG against various organisms may occur if there is a low prevalence of an organism in the environment to which these cows are exposed or if there is a low prevalence of cross-reacting serotypes in the cattle population (Hancock, Besser, Rice, Ebel, Herriot, & Carpenter, 1998).

The fact that immunoglobulins can be concentrated from early milk from non-immunised animals and still retain biological activity is important. Product A is produced as a very high protein powder with the intention of it being incorporated into other products or being ingested in small amounts in capsule form. The results obtained in this study would suggest that Product A, from non-immunised animals, has the potential to be used as a prophylactic and therapeutic agent in gastrointestinal infections. However, the dose of immunoglobulin required for oral prophylaxis or therapy has not yet been defined for any of the products currently on the market. In the human adult, approximately 5 g of IgA is produced and secreted at mucosal surfaces each day (Bogstedt et al., 1996). The IgG1 in bovine colostrum is thought to fulfil the same role in cattle as secretory IgA in humans (Ungar et al., 1990). When hyperimmune milk has been used as prophylaxis against *E. coli* challenge in human volunteers, doses of 5 g/day of bovine IgG were recorded (Tacket et al., 1988) and with *H. pylori* and *Cryptosporidium*, 3–15 g/day of bovine immunoglobulin were administered (Bogstedt et al.). The product derived from hyperimmunised animals used in this study has a recommended intake of 45 g/day of product (225 mg Ig). If this product is effective at this level it would follow that the non-immunised product should also be effective as it contains higher levels of immunoglobulin. Milk itself has long been recognised as being able to shorten periods of diarrhoeal illness e.g. in desert nomads (El Sayed, Agamy, Rupanner, Ismail, Champagne, & Assaf, 1992). However, the factor(s) in milk responsible for this effect is not known. In addition to immunoglobulins, milk also contains cytokines (IL-1, IL-6, IL-10, TNF-α, interferon γ), lysozyme, lactoferrin, and lactoperoxidase, all of which can aid in fighting infection. Further studies will have to be done on the product to determine if the immunoglobulins in it are resistant to digestion by gastric and intestinal enzymes.

Previous studies with hyperimmune milks and immunoglobulin concentrates suggests that partial digestion of the immunoglobulins occurs (Hilpert et al., 1987; Kelly et al., 1997; Warny et al., 1999). Further experiments, using known pathogens, will have to be carried out in animals to determine the amount of Product A which needs to be consumed to have a prophylactic or therapeutic effect and also the form in which it needs to be consumed to prevent degradation in the gastrointestinal tract.

Neither product demonstrated anti-inflammatory activity in terms of the assay system chosen. No anti-inflammatory effect was apparent with the milk products 18 h after application of an inflammatory stimulus. This is a period in which neutrophil infiltration predominates. More white blood cells were present in the peritoneum of mice fed Product A for 10 days compared with both control mice and mice fed Product B. However, 72 h after the application of an inflammatory stimulus an increased white blood cell infiltrate was obtained from mice fed Product B compared with mice fed Product A. Given the variety of cytokines and other immunomodulatory proteins in colostrum it is possible that this increase may be due to an as yet undefined chemokine in the product. At 72 h the neutrophil infiltrate has been replaced by a predominantly mononuclear infiltrate composed mainly of macrophages and lymphocytes. Mononuclear cells are important in the adaptive immune response and an increase in their number is necessary in dealing with a number of pathogens, particularly viruses and gram-negative bacteria.

Product A (Immulac 15) has the potential to be used as a prophylactic agent in humans and may be especially useful for immunocompromised patients. Further studies on the product to determine the levels of other factors e.g. cytokines, other classes of immunoglobulin etc., involved in fighting infection will also have to be carried out. In light of the current consumer resistance to modified foods a product prepared from a "natural" source is likely to give a good economic return to the company.

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Chapman, 1986; Ungar, Ward, Fayer, & Quinn, 1990), caries formation (Michalek et al., 1987; Takahashi, Eisenhuth, Lee, Laible, Binion, & Schatele, 1992), diarrhea in animals (Fayer, Guidry, & Blagburn, 1990), and to reduce blood cholesterol levels (Golay, Ferrara, Felper, & Schneider, 1990). In all the instances reported above the immune milks were prepared by immunising cows with specific antigens. It was also reported that, in some instances in the non-immunised control animals, there were low levels of antibody to the antigen(s) used in immunisation in the first one to two milkings after parturition. These included antibody against rotavirus (Bogstedt et al., 1996), enteropathogenic *E. coli* and its colonisation factor antigen (CFA I; Facon, Skura, & Nakai, 1995), a variety of bacterial strains associated with caries in humans (Takahashi et al., 1992) and against enterotoxigenic *E. coli* (Tacket et al., 1988).

The fact that the early milk from non-immunised cows contained specific antibodies led New Zealand Dairy Group (Specialty Ingredients Group) to develop a new product, called Immulac, from the first five milkings after parturition. In New Zealand, milk from the first 4 days (eight milkings) after parturition is not permitted to be used for the manufacture of traditional dairy products. The inclusion of colostrum can create problems for the dairy industry including low cheese yields, weak curd formation, off flavours in UHT milks and shorter shelf life times for both cheese and milks (Levieux & Ollier, 1999). Therefore, the product tested in this study makes use of what has previously been a waste product for the industry. The milks were processed using a proprietary process to produce a spray dried, skimmed colostrum powder with a high level of immunoglobulin. By concentrating the immunoglobulins it was postulated that the product might be of use in the nutraceutical and pharmaceutical industries for the prophylactic and therapeutic treatment of gastrointestinal diseases.

The non-immunised animals from which the early milk was obtained for this study had been maintained on pasture under typical New Zealand husbandry practices and as a result the animals might have been naturally exposed to many of the microorganisms used to hyperimmunise animals in the production of Stolle milk.

Claims have been made that some hyperimmune milks have anti-inflammatory properties in addition to their anti-microbial activities (Omrod & Miller, 1991; Owens & Nickerson, 1989; Wolf & Eibl, 1994). The nature of the anti-inflammatory action is not known. It may be due to the presence of immunoglobulin (Wolf & Eibl, 1994) or to other proteins present in the milk (Omrod & Miller, 1991; Owens, & Nickerson, 1989).

This study sets out to compare the IgG and IgG1 binding ability of Immulac 15 (an immunoglobulin enriched colostrum powder) to 19 human microbial pathogens with Stolle milk (a milk powder obtained from animals which receive regular immunisation with a cocktail of organisms). IgG and IgG1 were chosen as the predominant antibody in bovine milk is IgG with the subclass IgG1 thought to fulfill the same role as IgA in human milk. In addition, the anti-inflammatory properties of these products were tested using a mouse bioassay system.

2. Materials and methods

All chemicals used were of AnalaR grade quality and were purchased from BDH Chemicals (Palmerston North, NZ) or from Sigma Chemical Company (St Louis, MO, USA). ELISA plates (Nunc immunoplate maxisorp #442404) and defibrinated horse blood were purchased from Life Technologies (Gaithersburg, MD, USA). Media for bacterial and yeast culture were purchased from Difco (Detroit, MI, USA). Bacterial and yeast cultures were obtained from the Environmental and Scientific Research Culture Collection (Porirua, NZ) with the exception of *E. coli* which was obtained from the Microbiology Department, University of Otago, collection. Immulac 15 (Product A- protein 74.6%, moisture 3.1%, IgG 15.3%) was provided by the manufacturer (Specialty Ingredients Group, New Zealand Dairy Group, Cambridge, NZ). Stolle (Product B- protein 37.7%, IgG 0.5%) was purchased over the counter in Taiwan and skim milk (Anchor brand, Morrinsville, NZ) was purchased from a local supermarket.

2.1. ELISA

The IgG content of the milk derived products was measured using ELISA. ELISA plates were coated with 100 μl of anti-bovine IgG (2.5 ng/ml, Sigma), incubated for 2 h at 37°C and then at 4°C overnight. Plates were washed 6× in wash buffer [phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7) + 0.05% Tween 20]. To inhibit non-specific binding, wells were blocked using 100 μl of wash buffer containing 0.3% skimmed milk at 37°C for 1 h. Milk products were reconstituted in sterile distilled water at 1 g per 10 ml (10^{-1}) and serially diluted in wash buffer. After washing, 100 μl volumes of milk product dilutions were added in duplicate to each plate which was incubated for 1 h at 37°C. A standard curve constructed using bovine IgG (Sigma) was included in each plate. Duplicate plates were prepared. Plates were washed as before and then 50 μl of rabbit antibovine IgG-HRPO (Sigma) were added and plates incubated for a further hour. Plates were washed and 100 μl of substrate (*o*-phenylenediamine + hydrogen peroxide + citric acid + Na_2HPO_4) were added to each well and plates were incubated at room temperature for 20 min.

The reaction was stopped by the addition of 50 μl of 2M H_2SO_4. Absorbance was measured at 490 nm using a Bio Rad 450 ELISA plate reader (Hercules, CA, USA).

Bacillus cereus (NCTC 8035), *Streptococcus pyogenes* (NCTC 8198), *Streptococcus agalactiae* (NCTC 8181), *Streptococcus pneumoniae* (NCTC 7465), *Streptococcus mutans* (ATCC 25175), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Listeria monocytogenes* (NCTC 7973), *Yersinia enterocolitica* (ATCC 9610), *E. coli* and *E. coli* 0157:H7 (ATCC 35130) were grown overnight at 37°C in tryptic soy broth (TSB). *Candida albicans* (ATCC 10261) was grown overnight at 37°C in Sabouraud broth. *Propionibacterium acnes* (NCTC 737) was grown for 3 days at 37°C in TSB in an anaerobic glovebox (Forma Scientific, Marietta, OH, USA). *Haemophilus influenzae* (ATCC 49247) was grown on chocolate agar plates for 5 days in a 5% CO_2 incubator (Forma Scientific, Marietta, OH, USA). *Campylobacter jejuni* (NCTC 11351) and *Helicobacter pylori* (NCTC 11637) were grown on 5% defibrinated horse blood chocolate agar plates for 5 days at 37°C in a 5% CO_2 incubator. Growth from plates was scraped into 10 ml PBS. All broth cultures and PBS were spun at 6000 ***g*** for 10 min. Pellets were washed twice with PBS and resuspended to an optical density of 0.4 in carbonate buffer (15 mM Na_2CO_3, 34.5 mM $NaHCO_3$, pH 9.6). Lipopolysaccharide fractions of *Salmonella typhimurium* (Sigma L6511), *Salmonella enteritidis* (Sigma L6011) and *Klebsiella pneumoniae* (Sigma L4268) were resuspended in Hanks' balanced salt solution (Gibco BRL, NY, USA) and diluted in carbonate buffer to give a concentration of 5 μg/ml. ELISA plates were coated with 100 μl/well of antigen as previously described. Plates were washed and blocked as previously described. Milk products were reconstituted in sterile distilled water at 1 g per 10 ml (10^{-1}) and serially diluted in wash buffer. After washing, 100 μl volumes of milk product dilutions were added in duplicate to each plate which was incubated for 1 h at 37°. Skimmed milk, which did not contain immunoglobulin, was included as a negative control in each plate. Duplicate plates were prepared for each organism. Plates were washed as before and then 50 μl of either rabbit antibovine IgG-HRPO (Sigma) or rabbit antibovine IgG1-HRPO (Bethyl Laboratories, Montgomery, Tx, USA) were added and plates incubated for a further hour. Plates were washed and substrate added as previously described. The reaction was stopped as previously described and absorbance was measured at 490 nm using a Bio Rad 450 ELISA plate reader (Hercules, CA, USA). For each assay, the dilution at which half the maximal OD was obtained was calculated. At this point all samples have the same amount of IgG.

Five further batches of Product A were tested against *C. albicans, H. pylori* and *E. coli* O157:H7.

2.2. Mouse bioassay

Groups of five Balb/c mice were fed the milk products (reconstituted at 10 g/100 ml) ad libitum in their drinking water for 7 days. Both drinking bottles and water were sterilised and fresh milk supplied daily to minimise bacterial contamination. An inflammatory stimulus in the form of 1 ml of 3% thioglycollate was then injected into the peritoneum of the mice. After 18 h mice were sacrificed by CO_2 anaesthesia followed by cervical dislocation. Peritoneal lavage using 5 ml of ice cold PBS was carried out. White blood cells were counted in a Neubauer counting chamber. Control mice were fed water and injected with thioglycollate (TGC) as described or with TGC immediately followed by an injection of 50 μg of dexamethasone (a commonly used anti-inflammatory drug). Resident white blood cell populations in the peritoneum were determined as a control. In a second experiment, groups of six mice were fed each milk product. After 7 days feeding, the mice were separated into two groups of three. One group, from each feeding regimen, had an inflammatory stimulus in the form of 1 ml of 3% thiogycollate injected into the peritoneum. Milk products were fed for a further 72 h at which time animals were sacrificed and peritoneal lavage carried out as previously described.

2.3. Statistics

Results were analysed by Student's *t*-test using the software package Instat for Macintosh Version 2.01. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. ELISA

The IgG content of Immulac 15 was 9.57% and that of Stolle 0.15% when determined by ELISA; these values are lower than those claimed by the manufacturers.

The ELISA results indicated that both products contained IgG and IgG1 which was able to bind to all the microbial antigens tested. The antibody titres of the two products against the microbial antigens tested were determined at half the maximal optical density for each assay. Differences in titre of the two products were compared statistically using Student's *t*-test. In all cases Immulac 15 demonstrated significantly higher titres ($P < 0.05$) of IgG and IgG1 antibody to the antigens tested than Stolle (Tables 1 and 2). Both products had higher titres of IgG against the antigens tested than IgG1.

A further five batches of Product A were assayed for IgG activity against *H. pylori*, *E. coli* O157:H7 and *C.*