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Bovine IgG1, but not IgG2, binds to human B cells and inhibits antibody secretion

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SUMMARY

We previously observed that milk-derived bovine IgG, but not serum-derived bovine IgG, strongly inhibits antibody secretion by pokeweed mitogen (PWM)-stimulated human peripheral blood mononuclear cells (PBMC). Bovine milk contains a greater percentage of IgG1 (90%) than does bovine serum (53%). To determine whether bovine IgG subclasses have different functional capabilities, we have examined the effects of bovine IgG1 and IgG2 subclasses upon not only antibody secretion but also mitogenesis by human PBMC. Both bovine IgG subclasses markedly inhibited PWM-stimulated mitogenesis. However, only bovine IgG1, and not IgG2, inhibited antibody secretion during a 14-day *in vitro* culture period. Also, antibody secretion was inhibited following a 24-hr preincubation of human PBMC with bovine IgG1, but not with IgG2. To determine whether these differences corresponded to specificities of human Fcγ receptors on subsets of mononuclear cells, fluorescence-activated cell sorter (FACS) analyses were preformed. Both bovine IgG subclasses bound to human monocytes. However, only bovine IgG1 bound to human B cells, and bovine IgG1 bound more avidly to human B cells than did human IgG. One model to explain these findings is that inhibition of mitogenesis may be due to the binding of both bovine IgG1 and IgG2 subclasses to monocytes; whereas subclass-specific inhibition of antibody secretion may result from the selective binding of bovine IgG1, but not bovine IgG2, to B cells. The observation that bovine IgG1 has a greater avidity for human B lymphocyte Fc receptors than human IgG may have important implications for future studies of Fcγ receptors on human leucocytes.

INTRODUCTION

Humans are exposed to significant amounts of bovine IgG from dietary sources and, in certain circumstances, human absorption of bovine IgG has been demonstrated (Cunningham-Rundles, Carr & Good, 1984; Bock, Remigio & Gordon, 1983). Dietary exposure to bovine IgG can have several effects on the human immune system. First, bovine IgG (as any foreign protein) may represent a potent antigen and may stimulate production of antibodies (May *et al.*, 1977; Lowenstein *et al.*, 1977; Tomasi & Katz, 1971). Second, the ability of bovine IgG, unlike other foreign proteins, to bind to some human Fc receptors (Kulczycki, 1987) provides it with a potential mechanism for interaction with and regulation of the human immune system apart from antigen-specific mechanisms. We have previously found that bovine IgG from milk inhibits the mitogenic response of human peripheral blood mononuclear cells to Con A (Kulczycki & MacDermott, 1985), and inhibits PWM-induced antibody secretion *in vitro* by human PBMC (Kulczycki *et al.*, 1987). In contrast, bovine IgG separated from serum inhibits mitogenesis but has little effect on antibody secretion (Kulczycki & MacDermott, 1985; Kulczycki *et al.*, 1987).

Milk from cows and other ruminants differs in antibody composition from non-ruminant milk. Although IgA is the predominant immunoglobulin in most mammalian milk, including human milk, IgG is the major immunoglobulin present in ruminant milk (Butler, 1981; Butler *et al.*, 1972). In bovine milk, the concentration of IgG averages 0·7 mg/ml, with IgG1 representing 90% or more of the total (Butler, 1981). In contrast, bovine serum contains about 19 mg/ml of IgG, with approximately 53% present as IgG1 (Butler, 1981). In this study, we have examined whether differences in subclass composition might explain the differing functional effects of bovine milk IgG and bovine serum IgG. Using purified preparations of both bovine IgG1 and IgG2, we have compared the subclasses in their abilities to inhibit PWM-stimulated *in vitro* mitogenesis, to inhibit antibody secretion, and to bind to human peripheral blood mononuclear cell subpopulations.

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MATERIALS AND METHODS

Human peripheral blood mononuclear cells (PBMC)

PBMC were isolated from normal healthy volunteers as described previously (Kulczycki *et al.*, 1987). Peripheral blood, diluted 1:1 with Hanks' balanced saline solution (HBSS) (without Ca^{2+} or Mg^{2+}) containing 0·75 mM EDTA, was layered over Ficoll–Hypaque (specific gravity = 1·077), and was centrifuged at 400 *g* for 40 min. Cells were washed twice with HBSS without Ca^{2+} or Mg^{2+}; once with HBSS containing Ca^{2+} and Mg^{2+} and 5% fetal calf serum (FCS); and then washed four times through 5-ml FCS gradients. Cells were washed and resuspended in assay media: RPMI-1640 with 2 mM L-glutamine, 2% penicillin-streptomycin (10,000 U/10,000 μg per ml; Gibco, Grand Island, NY), and 10% FCS (Hyclone, Logan, UT).

Bovine gammaglobulins and monoclonal antibodies to surface markers

Bovine IgG1 and IgG2 were obtained from Jackson Labs, West Groves, PA. The IgG2 was isolated from serum, the IgG1 was derived either from milk or serum. Unconjugated IgG1 and IgG2 were used in mitogenesis and antibody secretion assays. FITC-labelled IgG1 or IgG2 were used for fluorescence-activated cell sorting (FACS) analyses. In the FACS analyses, phycoerythrin-conjugated monoclonal antibodies Leu 12, Leu 4, Leu M3, and Leu 11c (Becton-Dickinson, Mountain View, CA) were used to distinguish cell types. For controls, fluorescein-labelled mouse immunoglobulin controls (Becton-Dickinson) were used in the same concentrations as the monoclonals above and did not show significant binding to cells.

Mitogenesis

Cells were incubated, as previously described (Kulczycki & MacDermott, 1985), at 1×10^6/cc in 0·2 cc of RPMI-1640 assay media in 96-well microtitre plates at 37° and 5% CO_2 95% air in the presence of media or PWM (1/100). After 3 days, the wells were pulsed for 4 hr with tritiated [^{3}H]thymidine, harvested onto glass fibre filter strips using a MASH harvester (Microbiological Associates; Bethesda, MD), and counted in a Packard PRIAS Beta counter (Packard, Downers Grove, IL). Results are expressed as percentage inhibition of [^{3}H]thymidine incorporation in comparison to the control wells.

In vitro *antibody secretion assay*

Cells were incubated, as previously described (Kulczycki *et al.*, 1987), at 2×10^6/cc in 0·2 cc of RPMI-1640 assay media in 96-well microtitre plates, at 37° (5% CO_2) in the presence of media or PWM (1/100) with or without bovine gammaglobulins. At the end of the 14-day incubation, the cells were resuspended, centrifuged and the supernatants were assayed for human immunoglobulins (IgG, IgM, IgA).

Radioimmunoassay of Human Immunoglobulins

Immunoglobulin concentrations in supernatants from cultured cells were assayed by solid-phase RIA as previously described (Kulczycki *et al.*, 1987; Nash *et al.*, 1982). Briefly, affinity-purified immunoglobulins were radioiodinated using Iodobeads (Pierce Chemical Co., Rockford, IL). Rabbit anti-IgG, IgA or IgM covalently bound to polyacrylamide beads were commercially obtained (Bio-Rad, Richmond, CA). ^{125}I-labelled immunoglobulins (25,000 c.p.m., 0·05 ml), standards, or culture supernatants, and appropriate antisera bound to beads (0·05 ml) were added to wells of a 96-well microtitre plate and incubated at 25° overnight. Well contents were harvested onto glass fibre filter strips and individual discs counted in an LKB Clinigamma gamma counter. The concentrations (in ng/ml of antibody) were calculated by linear regression analyses of the points on the standard curve. Percentage inhibition was calculated using PWM-stimulated immunoglobulin secretion on Day 14 of culture as the 100% value.

FACS analysis

Isolated human PBMC were incubated for 1 hr at 37° in RPMI-1640 media containing 0·1% ovalbumin (FACS media) and were washed and resuspended at a concentration of 20×10^6 cells/ml. Tubes containing 0·05 ml of cells (1×10^6) and 0·05 ml of fluoresceinated protein at 1 mg/ml were incubated for 90 min at 4°, with 0·02 ml of monoclonal antibody (phycoerythrin-conjugated) added during the last 30 min of incubation. Cells were washed three times and resuspended in 0·5 ml of cold FACS media before dual label assay in a FACS 440 flow cytometer (Becton-Dickinson). In determinations of NK cell binding, binding of Leu 11c to cells was somewhat inhibited by human IgG since both bind FcγRIII (resulting in a shift of the Leu 11c-positive population toward background fluorescence). Leu 11c binding was not inhibited by bovine IgG1 or bovine IgG2. For standardization of the binding assays, beads with known concentrations of FITC (Flow Cytometry Standards Corporation, Research Triangle Park, NC) were run in parallel.

RESULTS

Immunoglobulin secretion by human peripheral blood mononuclear cells stimulated with PWM was markedly inhibited by bovine IgG1 at concentrations as low as 0·04 mg/ml (Fig. 1a). In contrast, bovine IgG2, even at concentrations as high as 1·2 mg/ml, did not significantly inhibit immunoglobulin secretion (Fig. 1b). The inhibition of antibody secretion resulting from a concentration of 1·2 mg/ml of bovine IgG1 (Fig. 1a) was 69·5% for IgA, 86·6% for IgG, and 96·9% for IgM (all P values $< 0{\cdot}001$).

The most likely explanation is that bovine IgG1, but not IgG2, inhibited human antibody secretion *in vitro* (Fig. 1). However, since the IgG1 tested had been isolated from cows' milk whereas the IgG2 had been purified from bovine serum, we considered an alternative possibility that a factor present only in milk [such as a stimulatory proline-rich protein that co-purified with IgG1 (Julius, Janusz & Lisowski, 1988) or lactose bound to IgG1], rather than IgG1 itself, may have produced the observed inhibition of antibody secretion. To examine this possibility, we simultaneously tested two different types of bovine IgG1, one isolated from milk and one from serum. Bovine IgG1 from both sources inhibited secretion of human IgG in a comparable dose-dependent manner. Inhibition by both proteins correlated (correlation coefficient of 0·88) over the concentrations used, ranging from 0·01 mg/ml ($31{\cdot}8 \pm 11{\cdot}8$; $33{\cdot}7 \pm 6{\cdot}1$) to 1·2 mg/ml ($92{\cdot}7 \pm 3{\cdot}5$; $96{\cdot}1 \pm 1{\cdot}0$) for milk and serum, respectively. Both milk- and serum-derived IgG1 also inhibited human IgA and IgM secretion (data not shown). Since the inhibition of antibody secretion was independent of the source of the bovine IgG1, it is

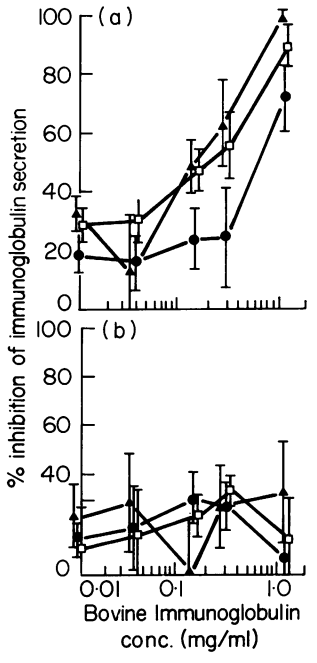

Figure 1. Inhibition of antibody secretion [(human IgG (□), IgA (●) and IgM (▲)] by PWM-stimulated human PBMC due to bovine IgG1 and bovine IgG2. Cells were cultured 14 days with PWM (1/100 dilution) in media with or without bovine IgG1 (a) or bovine IgG2 (b) over a concentration range of 0·01–1·2 mg/ml. Results are expressed as the mean ± SEM percentage inhibition of human immunoglobulin secretion for 10 experiments.

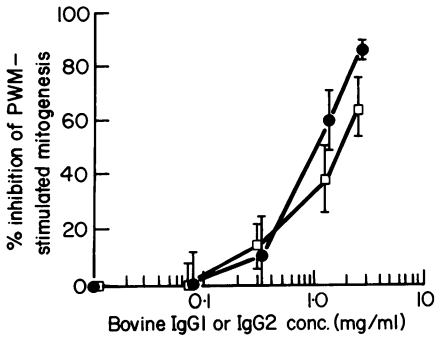

Figure 2. Inhibition of PWM-stimulated mitogenesis by human PBMC due to bovine IgG1 (●) or IgG2 (□). Cells were cultured 3 days with PWM (1/100 dilution) with media or bovine immunoglobulin over a concentration range of 0·01–1·2 mg/ml. Results are expressed as the mean ± SEM percentage inhibition of mitogenesis for 14 experiments.

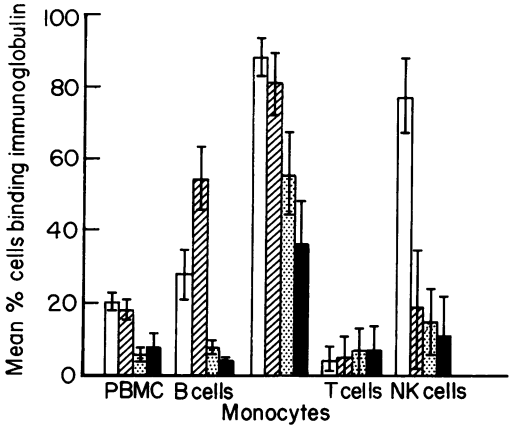

Figure 3. Binding of human IgG (□), bovine IgG1 (■), bovine IgG2 (▨), and bovine $F(ab')_2$ (■) to human PBMC subpopulations. Cells were incubated with fluoresceinated protein for 1 hr in media with ovalbumin, then with monoclonal antibodies to cell surface markers. Results of double label FACS experiments are expressed as the percentage of each cell population that bound each protein.

not due to a milk-specific contaminant but instead must represent a property unique to the bovine IgG1 subclass.

To determine whether inhibition required the presence of bovine IgG1 for the entire 14-day culture period, we examined the effects of pretreatment of PBMC with bovine IgG1 on antibody secretion. Pretreatment of human PBMC with 2·4 mg/ml of bovine IgG1 for 24 hr resulted in 71·3% ± 9·1% inhibition of IgA secretion, 60·9% ± 18·8% inhibition of IgG secretion, and 58·6% ± 20·8% inhibition of IgM secretion. Bovine IgG2 failed to inhibit and caused enhancement of antibody secretion in some pretreatment experiments.

Previously we had noted that both bovine milk and serum IgG were capable of inhibiting mitogenesis of Con A-stimulated PBMC (Kulczycki & MacDermott, 1985). To determine whether subclass-specific effects might be exerted during the proliferative phase of PWM-stimulation, we compared the effects of both subclasses and of unfractionated bovine IgG on mitogenesis. As we have shown previously, unfractionated milk IgG inhibited PWM induced mitogenesis by human PBMC (60·9% ± 7·7%). Results presented in Fig. 2 demonstrate that both bovine IgG1 and IgG2 also inhibited PWM-induced mitogenesis (85·1% ± 3·9% and 63·9% ± 10·5% inhibition, respectively). Inhibition of mitogenesis by bovine immunoglobulins was not due to direct cytotoxic effects since cell viabilities during the 3-day culture period, as determined by exclusion of trypan blue, were similar in the presence or absence of bovine gammaglobulins (data not shown).

To investigate possible mechanisms for bovine IgG1 inhibition of human antibody secretion *in vitro*, we examined the binding of the bovine IgG subclasses and human IgG to subpopulations of human PBMC using dual label FACS analysis (Fig. 3). Cell types were distinguished by using phycoerythrin-conjugated Leu 12 (B cells; CD19), Leu 4 (T cells; CD3), Leu M3 (monocytes; CD14) and Leu 11c (NK cells; CD16). Immunoglobulin binding was determined by incubating cells with FITC-conjugated human or bovine immunoglobulins at 1 mg/ml. Significant percentages of human B cells bound both human IgG and bovine IgG1 (28·5% and 54·5% of cells, respectively). Human NK cells bound human IgG (77·5% of cells) but interestingly did not significantly bind bovine IgG1. Bovine IgG2 failed to bind to either human B cells or NK cells [binding approximated the percentage binding of bovine IgG $F(ab')_2$ fragments]. In contrast, human monocytes (positively stained using Leu M3) bound all three immunoglobulins tested: human IgG > bovine IgG1 > bovine IgG2, (87·0%, 78·6% and 48·8% of cells, respectively). T cells, identified by Leu 4, did not bind any of the immunoglobulins (human IgG, 4·9%; bovine IgG1, 5·9%; bovine IgG2, 7·2%) when compared to the control bovine $F(ab')_2$ fragments (7·4%). To determine if differences in

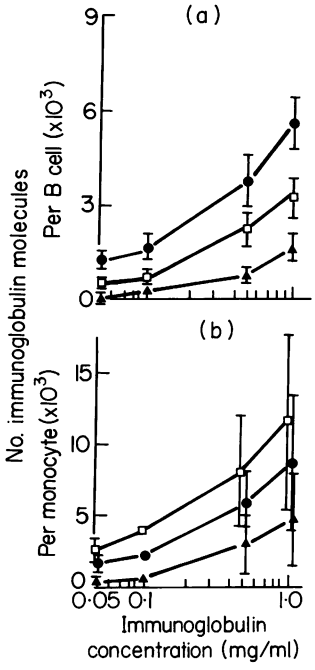

Figure 4. Effects of varying concentrations of human IgG (□), bovine IgG1 (●), and bovine IgG2 (▲) on the number of molecules bound to human B cells (a) and monocytes (b) as measured by two-colour FACS.

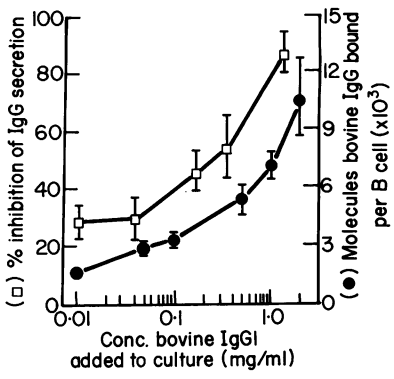

Figure 5. Correlation between binding of bovine IgG1 molecules (●) and inhibition (□) of antibody synthesis. The *x*-axis displays the concentration of bovine IgG1 added, the right *y*-axis shows the number of molecules bound per B cell (FACS analysis), and the left *y*-axis the percentage inhibition of the PWM-stimulated antibody secretion.

aggregation of the bovine immunoglobulin subclasses in these preparations could account for the different binding patterns of bovine IgG1 and IgG2 observed, we removed aggregates from our preparations by centrifugation in a Beckman airfuge (149,000 *g* for 15 min) immediately prior to binding experiments. The use of supernatants from this process produced similar results to those using unseparated proteins (data not shown).

Because of the marked differences in binding of bovine IgG1 and IgG2 to B cells, which paralleled their differences in inhibition of antibody synthesis, we examined the numbers of Ig molecules bound to B cells after incubation with various concentrations of human IgG, bovine IgG1 and bovine IgG2. In these experiments, a series of bead preparations containing known amounts of fluorescein molecules was used to standardize the assays. Measurement of the number of bound FITC molecules and the fluorescein to protein (F/P) ratio for each protein permitted the calculation of the number of bound immunoglobulin molecules. We have used fluorescent labelled proteins (rather than iodinated) in these experiments to enable us to examine the binding of the proteins to individual subpopulations of cells in a mixed population of normal peripherial blood cells. Bovine $F(ab')_2$ fragments were used as the control for non-specific protein binding and monocytes were also examined, since all of the immunoglobulins had been able to bind to monocytes. Bovine IgG1 bound more avidly to B cells than human IgG over the entire concentration range from 0·05 to 1 mg/ml ($P < 0{\cdot}05$ at concentrations $\geq 0{\cdot}5$ mg/ml), with a maximum binding of 5500 ± 800 molecules/cell for bovine IgG1 versus 3200 ± 600 molecules per cell for human IgG (Fig. 4a). On the other hand, bovine IgG2 (Fig. 4a) did not bind efficiently to human B cells [a maximum of only 1600 ± 400 molecules per cell were bound at 1 mg/ml, approximating the binding of bovine $F(ab')_2$ fragments, 1500 ± 200]. In comparison, human IgG was the immunoglobulin that bound most avidly to monocytes over the concentration range of 0·05–1 mg/ml, with a maximum of $11{,}600 \pm 6100$ molecules per cell (Fig. 4b), while maximum bovine IgG1 binding to monocytes was 8800 ± 4700. Bovine IgG2 bound less well to monocytes than either human IgG or bovine IgG1 (the maximum number of molecules bound was 4800 ± 900 at 1 mg/ml).

The relationship of bovine IgG1 binding to B cells and inhibition of antibody secretion by PWM-stimulated PBMC is shown in Fig. 5. Over the concentration range tested, changes in inhibition of antibody secretion corresponded very closely with changes in the binding of bovine IgG1.

DISCUSSION

Most types of human leucocytes express one (or more) of three distinct Fcγ receptors (Anderson & Looney, 1986). Human monocytes express a high-affinity 60,000–68,000 MW 'FcγRI' as well as a lower affinity 43,000 MW 'FcγRII' (Anderson, 1982; Cohen, Sharp & Kulczycki, 1983). Human B cells display only the 43,000 MW 'FcγRII' (Cohen *et al.*, 1983; Kulczycki, Solanki & Cohen, 1981), Human NK cells only express a third 50,000–70,000 MW Fcγ receptor (Perussia *et al.*, 1984), now termed 'FcγRIII'. These three Fcγ receptors are distinguishable not only by size but also by monoclonal antibodies (Perussia & Tinchieri, 1984; Anderson & Looney, 1986; Perussia *et al.*, 1984) and by specificity for mouse IgG subclasses (Lubeck *et al.*, 1985; Jones, Looney & Anderson, 1985). Also, at least one gene encoding each of these receptors has been cloned (Allen & Seed, 1989; Stengelin, Stamenkovic & Seed, 1988; Simmons & Seed, 1988; Peltz *et al.*, 1989).

In this study we have demonstrated that human cell types vary in their specificity for binding *bovine* IgG subclasses: (i) human monocytes (expressing FcγRI and FcγRII) bind both bovine IgG1 and IgG2; (ii) human B cells (presumably FcγRII) bind only bovine IgG1; and (iii) human NK cells (FcγRIII) bind neither bovine IgG1 nor IgG2. Therefore, the FcγRI on monocytes confers the ability to bind bovine IgG2 (and may bind IgG1 as well). Furthermore, we have demonstrated that B lymphocyte Fcγ receptors bind bovine IgG1 more avidly than

human IgG, which is consistent with our previous finding that isolated B-cell Fcγ receptors (43,000 MW) bound better to insolubilized bovine IgG than to insolubilized human IgG (Kulczycki, 1987). The observed binding of human (and bovine) immunoglobulins to receptors other than the FcγRI might be due to the presence of aggregates; however, similar binding of ultracentrifuged proteins suggests aggregation is unlikely to account for this binding. Using radiolabelled IgG, others have found some monomeric IgG binding to non-monocytic cells, albeit considerably less than aggregated IgG (Kurlander & Batker, 1982).

The ability of human B cells to bind only the bovine IgG1 subclass (and bind it avidly) provides an explanation for our observation that only bovine IgG1 inhibits human IgG synthesis *in vitro*. In fact, the increasing number of bovine IgG1 molecules bound to human B cells corresponds closely to the increasing inhibition of human immunoglobulin synthesis over the entire concentration range examined (Fig. 5). Bovine IgG2, which does not bind significantly to human B cells, does not inhibit antibody secretion. Bovine $F(ab')_2$ also does not bind to B cells (demonstrating that binding ability is contained in the Fc fragment), and also does not inhibit immunoglobulin synthesis (Kulczycki *et al.*, 1987). These results also extend our previous studies of the inhibitory effects of bovine milk and serum IgG on the human immune system (Kulczycki & MacDermott, 1985; Kulczycki *et al.*, 1987), and provide an explanation for the finding that bovine milk IgG inhibited antibody secretion to a significantly greater extent than did bovine serum IgG (Kulczycki *et al.*, 1987), while both subclasses were equally capable of inhibiting human PBMC mitogenesis by Con A (Kulczycki & MacDermott, 1985). This disparity in functional effects appears to be related to the differences in IgG subclass compositions (bovine milk IgG is 90% IgG1 whereas bovine serum is about 53% IgG1).

Since human IgG binds to the three types of human Fcγ receptors, bovine IgG1 apparently binds to two human FcγRs, and bovine IgG2 binds to only one human Fcγ receptor, the bovine subclasses may be useful in assessing the functional roles of the various human Fcγ receptors. For example, one may have attributed the inhibition of human antibody secretion by bovine immunoglobulins that we observed either to specific inhibition of B-cell development and transformation into antibody-secreting cells or to a more generalized inhibition of the activity of multiple interacting cells culminating in reduced antibody secretion. However, bovine IgG2 inhibits the more generalized phenomenon of [^{3}H]thymidine incorporation into PWM-stimulated lymphocytes, but has no effect on immunoglobulin secretion. Therefore, it appears quite likely that the inhibition of immunoglobulin secretion by bovine IgG1 must involve the B cell more specifically and more directly. In fact, we observed that bovine IgG is specifically bound to B cell Fcγ receptors. Thus, these results define two distinct pathways by which bovine IgG may potentially modulate the human immune system. First, both subclasses may be effective in more generalized inhibitory phenomena that are initiated by binding to the FcγRI of monocytes. Second, B-cell development into immunoglobulin secreting cells may be preferentially blocked by the binding of IgG1, but not by IgG2, to the Fcγ receptor of the B cell (presumably FcγRII).

The binding of human IgG (or its fragments) to B cells has a role in the modulation of B-cell functions, potentially involving both stimulation of the immune response (Morgan *et al.*, 1986; Mannhalter *et al.*, 1986) and feedback inhibition of antibody-producing cells (Uher & Dickler, 1986; Ohno *et al.*, 1987). This regulation may facilitate responses to specific antigenic challenges, yet inhibit excessive production of antibodies. Interestingly, bovine IgG1 binds to human B cells at even lower concentrations and to a greater extent than human IgG. This apparent B-cell Fcγ receptor preference for a non-homologous ligand is analogous to the heteroclitic phenomenon of antibody preference for non-immunizing antigens (Kimball, 1986). Based upon its preferential B-cell binding characteristics, bovine IgG1 might be more potent in modulating human antibody secretion via Fc binding than human IgG, suggesting the possibility that bovine IgG1 may be capable of such a function in the presence of human IgG.

It is not known to what extent these findings can be applied to *in vivo* immune responses in man. Nevertheless, humans often consume large quantities of dairy and beef products which contain bovine IgG subclasses, e.g. three 8 oz glasses of cow's milk (720 ml) contain over 500 mg of bovine IgG, mostly IgG1 (Butler, 1981; Butler *et al.*, 1972). Absorption of bovine IgG has been demonstrated both in serum of IgA-deficient individuals (Cunningham-Rundles *et al.*, 1984) as well as in milk from normal nursing mothers (P. Clyne and A. Kulczycki, manuscript submitted for publication). Inhibition of local immune responses due to bovine IgG1 could be particularly important at the mucosal surface where concentrations of bovine IgG would be greatest and where normal mucosal immune responses predominantly involve IgA. Under certain circumstances, bovine IgG effects on the immune system might also be important systemically: e.g. (i) in IgA-deficient individuals where the mucosal IgA barrier may be compromised and absorption of bovine IgG can be demonstrated (Cunningham-Rundles *et al.*, 1984); (ii) in children, while the mucosal barrier is immature; or (iii) in individuals where the gastrointestinal mucosal barrier has been damaged due to inflammation or intestinal tissue injury.

In conclusion, we have extended our previous observations of bovine milk IgG (but not serum IgG) inhibiting human antibody secretion by demonstrating an inhibition of *in vitro* human antibody secretion that occurs in the presence of bovine IgG1 but not IgG2. We have related this inhibition to the binding of bovine IgG1, but not IgG2, to the Fc receptor of human B cells. We have also shown that both subclasses are capable of binding to Fc receptors on monocytes, and correlate this binding to the inhibition of mitogenesis. These results suggest that at least two distinct pathways exist by which the binding of human IgG, or differential binding of ingested bovine IgG subclasses, to human mononuclear cells may influence human mucosal and systemic immune responses.

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