

Anti-inflammatory and Anti-Allergic Properties of Donkey's and Goat's Milk

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Abstract: Nowadays, donkey's and goat's milk consumption has been reevaluated for its potential benefits to human health. For example, in infants with intolerance to cow's milk, donkey's milk represents a good alternative due to its chemical characteristics similar to those of human milk. On the other hand, goat's milk in virtue of its higher content in short chain, medium chain, mono and polyunsaturated fatty acids than that of cow's milk, is more digestible than the bovine counterpart. From an immunological point of view, donkey's milk is able to induce release of inflammatory and anti-inflammatory cytokines from normal human peripheral blood lymphomononuclear cells, thus maintaining a condition of immune homeostasis. Similarly, goat's milk has been shown to trigger innate and adaptive immune responses in an *in vitro* human system, also inhibiting the endotoxin-induced activation of monocytes. Finally, in these milks the presence of their own microbiota may normalize the human intestinal microbiota with a cascade of protective effects at intestinal mucosal sites, even including triggering of intestinal T regulatory cells. In the light of the above considerations, donkey's and goat's milk should be recommended as a dietary supplement in individuals with inflammatory and allergic conditions, even including elderly people.

Keywords: Allergy, cow, donkey, elderly, goat, microbiota, milk.

INTRODUCTION

In western world, duration of human breastfeeding has dramatically dropped and in most cases, infant formula is used to reduce some discomforts related to breastfeeding by the mother's side. Among them, one should include waking many times a night for breastfeeding, restriction of time available for breastfeeding due to working outside the home, nipple pain, and modifications in the shape of the breasts [1]. However, formula milk is not free of side effects such as anaphylaxis [2] and cow's milk allergy (CMA) which is very common among young children with tolerance to milk starting by school age, and increasing through adolescence [3, 4]. In this respect, rates of allergy resolution have been reported by Skripack and associates [5]: 19% by 4 years, 42% by 8 years, 64% by 12 years, and 79% by 16 years. In the case of persistent allergy, patients exhibited higher levels of cow milk Immunoglobulin (Ig)E at all ages up to 16 years. These results are in contrast to a previous study [6] according to which 75% of children with cow milk IgE-mediated allergy became tolerant by 3 years of age. In conclusion, these epidemiological data suggest that the natural history of CMA tends to change with persistence until later ages [7].

IgE-mediated allergy accounts for about 60% of cow's milk-induced allergy, even if a form of milk allergy without cow's milk specific IgE [8], the so-called non-IgE-mediated cow's milk protein intolerance (CMPI), has been described

[9-12]. Both the gastrointestinal system (*e.g.*, nausea, bloating, intestinal discomfort, and diarrhea) and the skin [13, 14] are involved in the course of CPML.

From an immunological point of view, in CMA, symptoms may be caused by cow's milk-specific T helper (h)1 or Th17 cell responses, however, mechanisms such as antibody-dependent cell-mediated cytotoxicity or complement activation cannot be excluded [15, 16].

With special emphasis to the adaptive immune response in the course of CMPI [17], T cell responses were evaluated in 21 allergic children who, after a milk-free period of 2 months, were re-exposed to cow's milk. In tolerant children, higher frequencies of circulating CD4+CD25+ T regulatory (Treg) cells and decreased *in vitro* proliferative responses to bovine β -lactoglobulin in peripheral blood mononuclear cells (PBMCs) were noted in comparison with children with clinically active allergy. Quite interestingly, depletion of CD25 cells from PBMCs of tolerant children caused a fivefold increase in *in vitro* proliferation against β -lactoglobulin, thus suggesting the intervention of circulating Treg cells that may suppress the function of effector (e)T cells generated in response to the reintroduced cow's milk.

With regard to the humoral immunity, in CMPI patients [9, 18-23], studies are quite controversial. For instance, increased levels of milk-specific IgG1 and IgG4 in children with atopic dermatitis [24]; high IgG1, IgG4, and IgA to α -lactalbumin in atopic children [25]; similar levels of IgA and IgG to cow's milk proteins in healthy individuals and in patients with CMA [26,27] and low cow's milk-specific IgG

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levels in CMA patients [28] were reported. Furthermore, higher IgG4 to β -lactoglobulin were detected in atopic children [29], while higher IgG1, IgG4 and IgA levels to α -casein, β -casein, j-casein, α -lactalbumin, and β -lactoglobulin were reported in IgE-mediated cow's milk allergic patients in comparison to patients with non-IgE-mediated disorders and controls [30].

Noteworthy, milk-specific basophil reactivity was examined as a ratio with nonspecific (anti-IgE-mediated) basophil activation to account for the higher IgE receptor density observed in atopic subjects compared with that detected in healthy control subjects [31]. These studies were done in patients with seasonal and other environmental allergies [32] who could not avoid the specific allergen. Since up-regulation of Fc epsilon receptor I (Fc ϵ RI) is mediated by its interaction with IgE [33] and spontaneous histamine release was much lower in patients with atopic dermatitis and food allergy who strictly avoided their allergens compared with those who did not [34], it is conceivable that Fc ϵ RI could not be detected in patients under strict allergen avoidance. On the other hand, subjects with food allergy, and atopic dermatitis, who were not under strict avoidance of allergens, exhibited greater histamine release than patients with atopic dermatitis with no food allergies. This would support the hypothesis that subjects with milk allergy may have greater histamine release which could also be explained by production of the histamine releasing factor (HRF) [34]. In turn, HRF is able to interact with IgE bound to the surface of basophils, thus leading to the release of histamine.

Over the past few years, other substitutes of cow's milk have been exploited for child feeding but due to their scarce production they are known as "niche" milks. This is the case of donkey's and goat's milk whose biological properties and potential clinical applications will be illustrated in this review. Beside their lower allergenic potential in comparison to that of cow's milk, donkey's and goat's milk is also endowed with anti-inflammatory and immunomodulating activities which can be exploited in different clinical settings.

DONKEY'S AND GOAT'S MILK CHARACTERISTICS

Donkey's and goat's milk consumption is wide spread in the Mediterranean area where these animals have been adapting to the local vegetation and, therefore, dairy production is higher than in other areas in the world [35]. Nowadays, research on the properties of donkey's and goat's milk has become more popular than in the past and major highlights will be described in the next paragraphs.

Donkey's milk composition is affected by the stage of lactation [36-40] and, in particular, its fat content which

tends to decrease during lactation. Saturated Fatty Acids (SFAs) represent the most representative fatty acids which are comparable to the composition of mare [41] and human milk [42]. Of note, their content is lower than that observed in ruminant milk [36]. Palmitic acid (C16:0) is present at the highest concentrations but its content is less than that of cow's and human milk [43,44]. On the other hand, long-chain fatty acids, such as stearic acid (C18:0), are present in modest amounts, while it has been detected at higher levels in cow's and human milk (7-13%) [42-44]. The SFA content tends to decrease during lactation, while small changes in the SFA content have been reported in human milk and rather an increase in these fatty acids has been detected in cow's milk [45]. The unsaturated fatty acid of donkey's milk is similar to that of mare and human milk but higher than that observed in ruminant's milk (23-32%) [42, 46]. The monounsaturated fatty acids content increases during the lactation period and their increased content in the human diet seems to exert beneficial effects by lowering plasma low density lipoprotein (LDL) cholesterol and total cholesterol levels as well as the fibrinolytic activity of circulating plasma [47].

In Table 1 a comparison between major constituents of human, cow, donkey and goat milks has been made for a better appreciation of their differences.

One-quarter of goat's milk production in the world derives from the Mediterranean basin in comparison to 16% of cow's milk and 4% of buffalo's milk [48]. In general terms, as far as the quality of products is concerned 3 types of qualities can be distinguished [48]: i. hygienic and sanitary quality; ii. dietetic and nutritional quality; iii. gustative and gastronomic quality. In this respect, the benefits of goat's milk in terms of human digestion and metabolism have been ascribed to [49]: i. the small milk fat globules, the so-called naturally homogenized goat milk; ii. the polymorphisms of milk proteins different from those of cow's milk which generate a softer curd in digestion and in cheese-making; iii. higher contents in short chain, medium chain, mono- and poly unsaturated fatty acids than cow's milk and its cheese. In particular, goat's milk seems to be more easily digested than bovine milk also because of its higher content in essential fatty acids and, in comparison with human milk, it exhibits a higher content in conjugated linoleic acid and a lower percentage of α -casein [50] (see Table 1).

IMMUNOMODULATING EFFECTS EXERTED BY VARIOUS MILKS

In this framework, it is worthwhile describing the immune potential of human milk and, then, comparing it with that of other natural substitutes. Human milk contains

Table 1. Major constituents of various milks.

Milk Composition	Human	Cow	Donkey	Goat
Fat g/100 g	3.5-4.0	3.5-3.9	0.3-1.8	3.5-3.8
Protein g/100 g	0.9-1.7	3.1-3.8	1.5-1.8	3.1-3.5
Lactose g/100g	6.3-7.0	4.4-4.9	5.8-7.4	4.4-4.8

Table 2. Immunological properties of donkey's and goat's milk.

Donkey's Milk	Goat's Milk
<i>In vitro</i> release of NO from human monocytes	<i>In vitro</i> release of NO from human monocytes and PMNs
<i>In vitro</i> release of IL-12, IL-1 β , IL-10 and TNF- α as evaluated by a cytofluorimetric bead array method [37]	<i>In vitro</i> release of IL-6, IL-10 and TNF- α as evaluated by an ELISA assay [57]
<i>In vitro</i> release of IgG and IgA as evaluated by ELISPOT method [37]	<i>In vitro</i> release of IgG and IgA was not evaluated

antimicrobial substances, and, in particular, secretory IgA, which prevent penetration and passage of pathogens into tissue; interferon (IFN)- γ , which potentiates the antimicrobial activity of macrophages; natural killer cells and T cytotoxic cells as well as mucins, oligosaccharides, fibronectin, lysozyme and lactoferrin (LF) [51-53]. In particular, LF, the most representative protein in milk, [54], exhibits antibacterial, antifungal, antiviral, antiparasitic, and antitumoral activities and binds to lipid A of Gram-negative bacteria, neutralizing their toxic activities [55]. LF also protects the intestinal epithelium, promotes bone growth, as well as immune recovery in immunocompromised animals [55]. Further evidence has been provided that oligosaccharides from human milk are able to inhibit monocyte, lymphocyte and, neutrophil adhesion to endothelial cells, thus protecting the host in the course of inflammatory diseases and, especially, human milk-fed infants [56].

Over recent years, the immune properties of donkey's and goat's milk have been elucidated, thus suggesting their potential exploitation in human nutrition. In our *in vitro* studies, donkey's milk has been shown to produce nitric oxide (NO), and pro-inflammatory cytokines from human PBMCs [37] (see Table 2). Our parallel studies have demonstrated that goat's milk is also able to induce NO release from both monocytes and polymorphonuclear cells (PMNs) as well as adaptive immunity as evidenced by cytokine production [57, 58] (see Table 2).

NO may exert cardioprotective, anti-atherogenic and antibacterial effects in donkey's and goat's milk consumers, thus promoting longevity and preventing infections in the aged host. Quite interestingly, co-treatment of PMNs with goat's milk and lipopolysaccharide (LPS) dramatically diminished NO total production. This last effect may depend on the content of LF that can bind to Lipid A of LPS, thus blocking its toxic activity while exerting a potential beneficial effect in the prevention of septic conditions [59].

In terms of cytokine release, goat's milk, used as a stimulant for normal human PBMCs, was able to induce release of tumor necrosis factor (TNF)- α , interleukin (IL)-6 and mostly IL-10 among a broad spectrum of cytokines screened in an ELISA assay. However, in a previous study [37], using a cytofluorimetric bead array method, donkey's milk induced higher amounts of human cytokines than those detected using the ELISA test as in the case of goat's milk (see Table 2). In any case, release of IL-10 promoted by donkey's and goat's milk may contribute to maintaining immune homeostasis in the host. This contention seems to be supported by two reports on the ability of goat's milk to alleviate both hapten- and dextran sodium sulphate-induced

colitis in rats [60, 61]. Additionally, goat's milk was less allergenic than cow's milk in mice, likely *via* down-regulation of Th2 cell activation [62].

On the other hand, donkey's milk has been used as the major substitute of cow's milk in the attenuation of allergy state and inflammation [63-65], *e.g.*, in infants with intolerance to bovine milk [66, 67]. In this framework, a population of 46 children with cow's milk allergy received donkey's milk as a substitute [68]. Thirty-eight children (82.6%) liked and tolerated this milk and a significant growth was observed in all subjects who underwent growth deficit during cow's milk assumption. The degree of cross-reactivity of IgE with donkey's milk proteins was negligible and not specific.

Another potential application of donkey's and goat's milk can be represented by allergic diseases in the elderly. In fact, in the dietary regimen of the elderly cow's milk assumption has become very frequent but not free of potential side effects in allergy-prone aged individuals. For this reason, in the next paragraphs, some information on the aged immune system will be provided. In terms of IFN- γ and IL-4 production in elderly, an age-associated increase [69-71], no modification [72, 73] or even a decrease have been found [74-77]. An accumulation of memory-like CD45RO Treg cells, as well as peripheral Treg cell production have been reported in aged individuals [78-80]. Also "inflamm-aging" may promote the occurrence of Treg cells, thus contributing to the outcome of inflammatory-allergic respiratory diseases in the elderly population [76-83]. However, some studies have reported a normal Treg cell frequency in blood [84] or their functional depression in aged people [85].

An impairment of phagocytic and killing capacities of neutrophils as well as production of free radicals [86-90] and an increased neutrophilic apoptosis have been reported in the elderly [91]. Also a decreased degranulation of eosinophils may impair their antiviral defenses in old people [92]. Natural killer cells increase in their absolute numbers with age, while their cytotoxic functions seem to be preserved in immune-senescence [93].

Asthma is a very common pathology in the elderly and evaluation of immune response in aged asthmatic has revealed: a reduced proliferative response [94]; a decrease in CD8+ T cell levels [95]; a shift of Th1 to Th2 cytokine profiles [96]; a decline in Fas-mediated T-cell apoptosis [97]; and an increased human leukocyte antigen-DR expression on T-cells [98]. An increased proportion of FoxP3+ CD4+ Treg cells has been detected in the peripheral blood of elderly

subjects, thus accounting for the immune abnormalities described in asthma [99, 100], however, others did not confirm this finding [101].

In the light of the above considerations, niche milk should be more investigated also in terms of re-modulation of the impaired immune responsiveness, thus allowing its exploitation in aged related diseases.

FERMENTED MILK

Over recent years, probiotics [*e.g.* *Lactobacillus* (*L.*) *rhamnosus* LGG[®], *L. reuteri*, *Bifidobacteria* and certain strains of *L. casei* or the *L. acidophilus*-group] have been employed as fermented milk products. Probiotics are defined as live microbial food supplements, which beneficially influence human health [102-105]. Nowadays, the concept of functional foods, even including probiotics and dietary supplements, is based on their ability to beneficially influence body functions, thus improving the state of well-being and health and reduce the risk of disease [106]. A prebiotic is "a selectively fermented ingredient that modifies both the composition and/or activity in the intestinal microbiota, thus conferring benefits to human health", whereas synergistic combinations of pro- and prebiotics are known as symbiotics [107]. Major intestinal effects of probiotics include: hydrolysis of antigenic proteins and limitation of their systemic spreading; increased local IgA production; attenuation of local inflammation as well as stimulation of epithelial cell growth and differentiation [108-110]. Systemically, probiotics exert anti-inflammatory effects mediated by Toll-like receptors (TLRs), Th1 polarization in response to allergens and activation of tolerogenic dendritic cells with Treg cell generation and tolerance induction [111, 112]. It is becoming evident that completely different effects may be observed, depending on the species and the strain of the microorganism used [113]. Recent *in vivo* studies in healthy human volunteers measured the changes in gene transcription profiles to determine the molecular responses that occur in the human duodenal mucosa following consumption of the probiotic *Lactobacillus* spp. [114, 115]. These effects may be fundamental in early development of immune tolerance in the newborn [116]. In BALB/c mice treated with *L. casei* DN114001 or the probiotic fermented milk, enhanced release of IL-6, increase in T lymphocytes and IgA+ B lymphocytes and expression of biomarkers of macrophages were reported [117].

Increased lactose tolerance, a well-balanced intestinal microflora, antimicrobial activity, stimulation of the immune system and antitumoral, anticholesterolemic and

antioxidative properties have been reported in human subjects administered with fermented milk [118, 50]. In this respect, administration of goat's milk fermented with *L. fermentum* strain (ME-3) to human volunteers has led to the following results [119]:

i. anti-atherogenicity; ii. resistance of the lipoprotein fraction to oxidation, low levels of peroxidized lipoproteins, oxidized LDL, 8-isoprostanes and glutathione redox ratio, respectively; iii. enhancement of total antioxidative activity; iv. normalization of the intestinal microbiota.

In our own studies, fermented bovine milk containing *L. rhamnosus* and oligofructose (a prebiotic) was administered to free-living elderly people for one month as a symbiotic. Serum cytokines were evaluated before and after administration of the symbiotic and an increase in IL-1 β , IL-6 and IL-8 was detected [120]. However, reduced levels of IL-12, IL-10 and TNF- α were not modified by such a dietary treatment. In a parallel study [121], in another group of free-living elderly people, administration of donkey's milk led to the same results, namely an enhancement of innate immunity cytokines and, especially of IL-8. This cytokine, as a chemoattractant for neutrophils, may offer protection in the elderly host in winter infections, thus reducing inflammatory consequences which may complicate a condition of chronic allergic asthma. In the same clinical trial, in another group of elderly subjects, when administered with goat's milk, instead a decrease in serum cytokines (IL-1 β , IL-6 and IL-8) was observed, thus indicating that goat's milk may exert immune suppression in the elderly. These opposite effects are summarized in Table 3.

In conclusion, these data indicate that especially donkey's milk can also be used under form of fermented milk (*e.g.*, as a symbiotic) in elderly subjects for prevention of inflammatory and allergic conditions.

MICROBIOTA

According to recent studies, the natural history of CMA has been changing, with a trend of persistence until later ages. In this respect, an altered composition of intestinal microbiota has been invoked to explain the pathogenesis of CMA. In fact, there are qualitative and quantitative differences in the composition of intestinal microbiota between children affected by CMA and healthy children [122]. Therefore, in this section some information on the gut microbiota will be provided and the possibility to target it for the prevention of CMA will be emphasized.

Intestinal microbiota homeostasis keeps a condition of healthy status through a dynamic equilibrium with

Table 3. Different effects following *in vivo* administration of donkey's and goat's milk on serum cytokine profile in free-living elderly people.

Donkey's Milk	Goat's Milk
IL-12, IL-10, IL-1 β and TNF- α : no modifications observed after administration [121].	IL-12, IL-10, IL-1 β and TNF- α : no modifications observed after administration [121].
IL-6 and IL-8: increased levels after administration [121].	IL-6 and IL-8: decreased levels after administration [121].

immune host cells, and, in particular, with gut-associated lymphoreticular tissue cells [123, 124]. In this context, Peyer's patches (PP) represent the major inductive site of gut immune responsiveness [125]. Environmental factors, e.g., pollutants, dietary habits and stress may contribute to the interruption of the intestinal microbiota equilibrium [126]. *Firmacutes* and *Bacteroidetes* are the major components of intestinal microbiota and play a protective role in the host, modulating the immune response, inhibiting bacterial translocation and, ultimately, favoring digestion [127, 128].

Furthermore, there is evidence, that microbiota is deeply involved in the immune system maturation as well as oral tolerance [129, 130] and environmental factors able to modify the microbiota composition have been invoked in the development of allergic diseases [131] as well as of childhood dysbiosis [132]. In addition, obese ob/ob mice as well as obese patients possess a microbiota characterized by more numerous *Firmacutes* than *Bacteroidetes* [133]. For example, transfer of cecal bacteria from ob/ob mice to lean mice causes obesity in these animals [133-137]. In this framework, it is worthwhile mentioning that childhood obesity is increasing in both western and westernized countries and some studies have reported its association with an altered gut microbiota [138] as well with asthma [139]. Furthermore, evidence has been provided that, in ageing, a decrease in *Anaerobes* and *Bifidobacteria* with an increase in *Enterobacteria* [140], due to dietary changes, increased incidence of infectious events. A large study on 35,292 adults has shown that some faecal bacterial species (*Escherichia coli*, and *Enterococci* spp.) increased with age, while others decreased with age (*Bacteroides* spp.) or, in the case of *Lactobacilli* and *Bifidobacteria* remained stable [141].

In the next paragraphs the pathogenesis of food allergy will be elucidated.

Oral tolerance originates from the interaction between intestinal microbiota and immune and non-immune cells which leads to the induction of IgA and Treg cells [130]. Therefore, the balance between the intestinal immune system and its antigenic load is maintained, thus preserving the integrity of the intestinal mucosa. Food allergy is the result of an alteration of these homeostatic processes [142]. An allergic reaction to food allergens involves their presentation to naïve Th0 cells and their differentiation in the presence of IL-4 into Th2 cells. In turn, activated Th2 cells produce IL-4 that leads to the production of allergen-specific IgE [143]. IgE then binds to mast cells via FcεRI and degranulation of mast cells occurs after further exposure to the same allergen with release of allergic mediators responsible for clinical manifestations of allergy. In this context, studies on germ-free mice demonstrated a failure of tolerance to food proteins and mice with food allergy exhibited a specific profile of gut microbiota able to transmit disease susceptibility. On the other hand, transplantation of healthy infant microbiota resulted to be protective on sensitization and CMA in mice. Also polymorphisms or deficiency of endotoxin sensors, such as TLR-4 seem to account for food allergy [144-146].

Colonization of mice by certain *Clostridium* strains led to production of transforming growth factor (TGF)-β and

influenced FoxP3+ Treg number and function in the colon. Oral administration of *Clostridium* in the early life of conventionally mice induced resistance to allergic colitis and IgE responses in adult mice [147]. In this direction, four weeks treatment with *L. rhamnosus* LGG® led to a significant increase in clostridia in milk-hypersensitive subjects [148].

In asthmatic subjects, both microbiota burden and bacterial diversity were significantly higher than in control subjects and correlated with bronchial hyperresponsiveness [149]. The development of atopy and atopic dermatitis is associated with altered early postnatal microbiota [150, 151]. In the case of food allergy, one study reported alterations in the microbiota of infants with milk allergy [152], while in other studies in which atopic dermatitis cohorts were examined for microbiota modifications in association with food sensitization no relationship was found [153, 154].

Human milk is a secretion belonging to the mucosal immune system, containing protective substances which are passively transferred from mother to neonate [155, 156]. This is the case of secretory IgA which migrate from PP to mammary gland via lymphatic and hematic vessels [157]. This represents a compensatory mechanism of the transitory immunodeficiency in the first year of life. The *Bifidus* factor, present in human milk, promotes the growth of *L. bifidus*, thus affecting intestinal microbiota and shaping immune response [158]. Also maternal passage of IFN-γ to the child seems to represent an additional mechanism of protection [155, 156]. Quite interestingly, breastfeeding seems to afford protection against asthma via development of Treg cells and Th2 response down-regulation [159, 160]. Furthermore, human milk IgA is able to induce immune tolerance [161], while IgG immune complexes in human milk have poorly been investigated [162]. Conversely, in mouse milk transfer of IgG immune complexes from allergen sensitized mothers to neonates gave rise to antigen-specific Treg cells, which, in turn, abrogate asthma outcome in the progeny [163]. Donkey's milk, which has been reported to be less allergenic than cow's milk [164, 165], contains lactose that contributes to intestinal microbiota growth, and microbioma homeostasis [38].

CONCLUSION

In the previous sections, we have provided evidence for the ability of donkey's and goat's milk to prevent allergic reactions to bovine milk, rendering tolerogenic the immune response against cow's milk proteins [37]. Also on the bases

Table 4. Immunoregulation exerted by Treg cells.

Upregulation of CTLA-4
Deletion of antigen presenting cells
Deletion of T cells
Exhaustion of IL-2
Generation of suppressive cytokines such as: IL-10; TGF-β; IL-35; Galectin-1

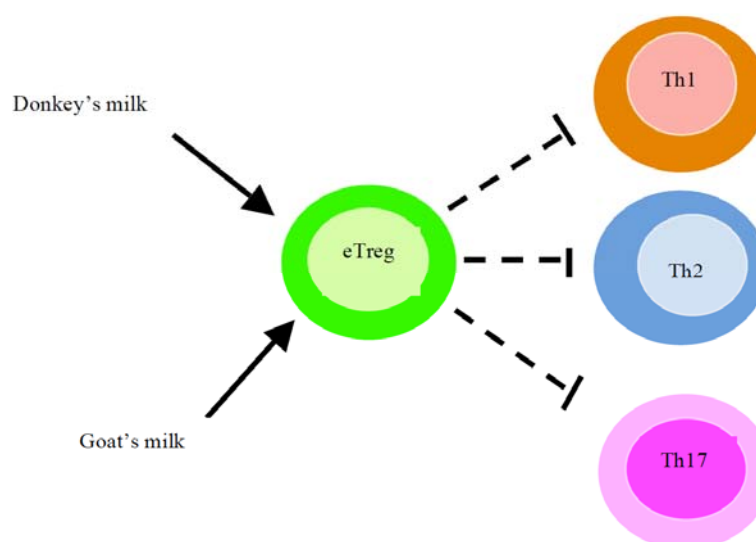


Fig. (1). Activation of eTreg cells by administration of donkey's and goat's milk and putative suppression of undesired inflammatory/allergic reaction in the host.

of our previous findings [58], normal human PBMCs stimulated with donkey's and goat's milk release IL-10 in their supernatants, thus suggesting the possible involvement of eTreg cells. In this framework, it is worthwhile illustrating the new vistas on the functional plasticity of eTreg cells. When compared to naïve Treg cells ($\text{FoxP3}^{\text{lo}} \text{CD45RA}^+ \text{CD45RO}^-$) eTreg cells are $\text{FoxP3}^{\text{hi}} \text{CD45RA}^- \text{CD45RO}^+$, also expressing the Fas receptor and the cytotoxic T lymphocyte antigen-4 (CTLA-4) [166]. Major regulatory functions of Treg cells are expressed in Table 4.

As recently reviewed by Cretney and associates [167], the distinction between naïve Treg cells and eTreg cells is too simplistic and a more complex differentiation seems to be more realistic.

Accordingly, naïve Treg cells in response to the initial stimulus can differentiate into various types of eTreg cells: Th1-Treg, Th2-Treg, Th17-Treg, T follicular regulation (TFR) and fat Treg cells, respectively. This event has been shown to occur because naïve Treg cells are able to co-opt transcription factors from conventional T cells such as T-bet, which is essential for Th1 cell differentiation, GATA-3, which serves for Th2 cell differentiation, and STAT3 which is needed for Th17 cell differentiation [168-170]. These new subsets of Th1-, Th2-, ThFR and Th17-like eTreg cells have been identified on the basis of chemokine receptor expression and all express IL-10 and B lymphocyte-induced maturation protein (Blimp-1) [171]. IL-10 represents a suppressive cytokine for eTreg cells which are committed to down regulate Th1-, Th2-, Th17-, TFR and fat Treg cells, while Blimp-1 constitutes a common marker for eTreg cells of different types.

In conclusion, this new definition of eTreg cells should help in understanding their functions in different clinical settings and, consequentially, their therapeutic manipulation. In this framework, further studies with donkey's and goat's milk should address this important aspect, namely how they may drive differentiation and function of eTreg cells in the host (see Fig. 1).

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

CMA	=	Cow's milk allergy
CMPI	=	Cow's milk protein intolerance
CTLA	=	Cytotoxic T lymphocyte antigen
eTreg	=	effector Treg cells
FcεRI	=	Fc epsilon receptor I
HFR	=	Histamine releasing factor
Ig	=	Immunoglobulin
IL	=	Interleukin
IFN	=	Interferon
LDL	=	Low density lipoprotein
LF	=	Lactoferrin
LPS	=	Lipopolysaccharide
NO	=	Nitric oxide
PBMCs	=	Peripheral blood mononuclear cells
PMNs	=	Polymorphonuclear cells
SFAs	=	Saturated Fatty Acids
PP	=	Peyer patches
TNF	=	Tumor necrosis factor
TLRs	=	Toll like receptors

TFR	=	T follicular regulation
TGF	=	Transforming growth factor
Treg	=	T regulatory cells

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