MINERVA

GASTROENTEROLOGICA E DIETOLOGICA

VOLUME 60 · N. 1 · MARZO 2014

EDIZIONI · MINERVA · MEDICA

PUBBLICAZIONE PERIODICA TRIMESTRALE - POSTE ITALIANE S.P.A. - SPED. IN A. P. D.L. 353/2003 (CONV. IN L. 27/02/2004 N° 46) ART. 1, COMMA 1, DCB/CN - ISSN 1121-421X TAXE PERÇUE

MINERVA GASTROENTEROL DIETOL 2014;60:71-8

COLOSTRO NONI administration effects on epithelial cells turn-over, inflammatory events and integrity of intestinal mucosa junctional systems

D. CARDANI

Dipartimento di Scienze Biomediche Per La Salute "Città Studi" Università degli Studi di Milano, Milan, Italy

***Aim*. In this work we evaluated the possibility for dietary supplement COLOSTRO NONI to be used as preventive and therapeutic agent in various diseases characterized by altered intestinal homeostasis with changes in the composition of the microbiota, alteration of the morphology and functionality, and also inflammation of the epithelium.**

***Methods*. Cellular activity of COLOSTRO NONI has been tested in an *in vitro* model of intestinal epithelium based on Caco-2 cell line. We tested the ability of COLOSTRO NONI to stimulate cellular turnover evaluating cell growth rate with WST-1 proliferation assay. We also tested the ability of COLOSTRO NONI to increase the gene expression of Interleukin-8 (IL-8) with a Real Time PCR assay. IL-8 is a fundamental chemotactic factor involved in inflammatory phenomena and in the control of tissue homeostasis.**

***Results*. COLOSTRO NONI is able to stimulate cell turnover in the proposed *in vitro* model and demonstrates active in increasing the gene expression of IL-8. Both aspects observed are fundamental for the establishment of mechanisms to repair tissue damage.**

***Conclusion*. Obtained results indicate that COLOSTRO NONI could find clinical application in treatment of gastrointestinal disorders characterized by impairment of proper intestinal permeability, in inflammatory bowel diseases, in dysenteric diseases, in gastritis and in forms of pathological alteration of the mucous layer as celiac disease and gluten sensitivity.**

KEY WORDS: Gastrointestinal tract - Gastric mucosa - Inflammation - Colostrum - Morinda.

Corresponding author: D. Cardani, via E. De Amicis 3, 20814 Varedo, Monza-Brianza, Italy.
E-mail: diegocardan@tiscali.it

The gastrointestinal tract is one of the most specialised organs of the human body and it is the largest area of bodily contact with the outside world (approx. 400 m²). Throughout its evolution, it has specialised in carrying out two fundamental and apparently contrasting functions: specialised filter able to guarantee optimal absorption of nutritional substances and selective barrier against pathogens of any nature.[1-3]

Mucous layer, intestinal microbiota, intercellular junctions and the intestine's immune system (gut associated lymphoid tissue, GALT) are the four fundamental levels of the intestinal barrier.[4, 5] The gastrointestinal tract is also a psycho-neuro-endocrine-immune (PNEI) system [6] able to secrete neuropeptides, neurohormones, hormones and cytokines contributing decisively to controlling both local and systemic physiological homeostasis, and able to react to various types of stimuli: goblet cells, for example, possess receptors for the corticotrophin-releasing hormone (CRH) and react to stress increasing the permeability of the intestinal epithelium [7] and the enteroendocrine cells are able to secrete tryptophan.[8] It can be claimed that the gastrointestinal tract is a neuro-immune-endocrine microcosm

with its own crucial role in controlling homeostasis, an organ central to homeostatic control. Acting on the gastrointestinal tract, preserving or re-establishing its histological integrity, its PNEI function and its ability to act as homeostatic controller means preventing or treating both local and systemic pathological alterations. Maintaining the homeostasis of the barrier, in particular the relationship between microbiota and PNEI system, is the key to a correctly functioning gastroenteric apparatus by maintaining that "physiological" or "controlled" inflammatory state which is the normal functioning condition of the digestive mucosa, essential for ensuring immune tolerance.[9] A break in the balance between neuro-immune-endocrine control of the mucosal function and composition of the microbiota is at the basis of the shift between physiological and pathologic inflammation and can trigger the gastro-intestinal pathology.

A fundamental role in monitoring homeostasis (in particular inflammatory), and therefore of intestinal physiology, is exerted by the gut-brain axis (gut-brain axis, GBA):[9] conditions of psychic stress can trigger inflammation of the intestinal mucosa.

A primary role in this sense is played by the microbiota: the alteration of its physiological composition, induced by psychic stress which "travels" along the GBA axis, makes it lose its role of inflammatory homeostasis controller with a consequent increase in phlogosis indices. It is interesting to note that intestinal bacterial flora is integrated in the brain-gut axis in a two directional manner, or rather, not only is the microbiota affected by psychic stress conditions but it can in turn affect the central nervous system and behavior; for example, it can induce the production of metabolites in the epithelium which are able to directly affect the central nervous system (for instance, altering the metabolism of tryptophan with consequent manifestation of a depressive-like syndrome) [9] and promote the activation of the mucosal immune response (the expression of the Toll-like-2, -4 and 5 receptors is modulated by the fluctuations in the composition of the microbiota).[9]

Alteration of the brain-gut axis triggers the pathologies with a marked psychosomatic component such as irritable colon syndrome (a pathology with a high incidence rate in the more industrialised countries which strikes on average 15% of the population).[10, 11] Modifications in the function of GBA are also present in pathologies such as Crohn's Disease (it strikes between 27 and 48 inhabitants out of every 100,000) and in other acute and chronic (with a high rate in western countries) inflammatory pathologies and are also one of the most important signs of an incorrect diet (abuse of alcohol and/or "junk foods").

These pathological conditions having the loss of intestinal homeostasis among their concurrent causes result in a considerable modification of the intestinal barrier and, in particular, in the opening of the Tight Junctions (TJ) of the apical epithelial cells.[12-14]

Alteration of the junction systems, in the face of an inflammatory type manifestation damages the mucosal layer,[12, 15-18] modifying the typical morphology of the intestinal epithelium in terms of shape and structure (dimension and distribution of villi) and cellular composition of the tissue (alteration of the numerical relationships between the cellular types usually present); furthermore, activation of the immune response ensures that cells of the immune system under the form of inflammatory infiltrate [19, 20] are also found in the intestinal epithelium.

Among the physiological factors involved in regulating the junction systems, the diet is undoubtedly the most important since it represents a fundamental substratum for chronic inflammatory conditions; it has been demonstrated how several nutrients are able to modulate both positively (anti-oxidant substances such as vitamins C and E) and negatively (such as fatty acids in excessive amounts) the function of the intestinal barrier modulating the TJs, the production of mucus and epithelial cellular turnover.[21, 22] The typical diet of developed countries, the so-called Western Diet, unbalanced and excessively high in fats, salt, sugar and genetically modified foods (GMO), is considered decisive for the development of pathologies

typical of the industrialised western world. Diabetes, obesity and tumors of the colon-rectum, for example, are pathologies characterised by a latent and persistent subclinical inflammation which, as previously described, inevitably also affects the integrity of the intestinal epithelium and the composition of the commensal bacterial flora.[23, 24]

Various therapeutic strategies are available to manage pathological alterations of the intestinal barrier and the most appropriate should be chosen according to the barrier component which has been altered. One of the most important options is the recovery of the physiological composition of the microbiota through a selection of the commensal bacteria strains present, integrating the diet in an appropriate manner (as regards calorie intake and composition), preventing an unnecessary antibiotic therapy and limiting, where possible, the conditions which predispose to the alteration of the microbiota (diabetes, endocrine disorders, intestinal motility disorders, etc.). The use of a food supplement based on lyophilised bovine colostrum and *Morinda citrifolia* (Noni) like COLOSTRO NONI (Guna S.p.A., Milan, Italy), able to re-establish intestinal homeostasis with direct activity on the microbiota and on the integrity of the intestinal epithelium, has proven effective in preventing and treating episodes which alter both the direct (gastroenteritis with alteration of the structure and integrity of the mucosa, intestinal inflammatory pathologies, diarrhoea and dysenteric forms) and indirect (influenza and influenza-like conditions) gastrointestinal function, dysbiosis from antibiotic therapies and also conditions of psycho-physical stress which are secondary to the altered intestinal function. The integration of COLOSTRO NONI in the diet can also have a good preventive action in the aforementioned harmful effects of an unbalanced diet.

Materials and methods

COLOSTRO NONI is a food supplement based on bovine colostrum lyophilised using a freeze-drying technique and *Morinda citrifolia* (juice of the fruit in powder) in a fast-acting mouth dissolving formulation thanks to the synergic activity of its components and characterises itself for its marked activity to maintain or restore intestinal homeostasis, with particular reference to its ability to re-establish the correct composition of bacterial flora and the physiological turnover of intestinal epithelial cells. The use of COLOSTRO NONI in an *in vitro* model of intestinal epithelium reveals its properties of physiological regulator of cellular turnover and of chemotaxis, mechanisms involved in the protection and re-epithelisation of damaged tissue. As further comparison, to assess the synergic role of NONI juice, a group in which the cells were treated with only bovine colostrum was also added to the tests.

Cell lines and reagents

The Caco-2 cell line (epithelial cells of human colon-rectal carcinoma) was obtained from ATCC-American Type Culture Collection (Manassas, VA, USA). The Caco-2 cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) with L-glutamine (Invitrogen/Life Technologies, Carlsbad, CA, USA), supplemented with 10% foetal bovine serum (FBS) (Invitrogen/Life Technologies), penicillin G sodium salt (200 U/mL), and streptomycin sulphate (200 µg/mL) (Sigma-Aldrich, St Louis, MO, USA), 1 mmol/L of sodium pyruvate, 1% of non-essential amino acids (all produced by Sigma-Aldrich). The cells were incubated at 37 °C with 5% CO_2 at controlled humidity.

Colostrum and COLOSTRO NONI (Guna S.p.A.) have been dissolved in a complete medium to obtain a solution containing 2% of both products.

The recombinant protein tumor necrosis factor-α (TNF-α) (Sigma-Aldrich) is prepared by dilution in a complete medium for a final concentration of 100 ng/mL.

WST-1 proliferation assay

The cellular growth curve is assessed with the WST-1 cellular proliferation test (Roche

Molecular Biochemicals, Penzberg, Germany), which measures the mitochondrial dehydrogenase activity, a system which is only active in living cells.

1×10^4 cellule Caco-2 are plated in wells of 96-well plates (Corning, Tewksbury, MA, USA). Once the monolayer state has been reached and after differentiation to enterocytes for 21 days, the cells are plated and treated in triplicate according to the following scheme for 24 hours:

— Group 1: STARVED: cells cultivated in DMEM medium with no complement;

— Group 2: UNTR: cells cultivated in a complete medium;

— Group 3: COLOSTRUM: cells cultivated in a complete medium with the addition of 2% Colostrum;

— Group 4: COLOSTRUM NONI: cells cultivated in a complete medium with the addition of 2% COLOSTRO NONI.

After 24 hours of treatment the WST-1 is added to each well of the 96-well plate with a final dilution of 1:10. It is left to incubate for 1 h at 37 °C, the absorption is then read at 440 m, using a Dynatech MR5000 microplate reader (Dynatech, Billinghurst, Sussex, UK). All the measurements were carried out in quadruple form.

Assessment of the IL-8 expression

2×10^5 Caco-2 cells are plated in wells of 24-well plates (Corning). Once the monolayer state has been reached and after differentiation to enterocytes for 21 days, the cells are plated and treated in triplicate according to the following scheme for 24 hours:

— Group 1: UNTR: cells cultivated in only a complete medium;

— Group 2: TNF-α: cells cultivated in a complete medium with 100 ng/mL TNF-α;

— Group 3: TNF-α + COL.: cells cultivated in a complete medium with the addition of 2% Colostrum and 100 ng/mL TNF-α;

— Group 4: TNF-α + COL. NONI: cells cultivated in a complete medium with the addition of 2% COLOSTRO NONI and 100 ng/mL TNF-α.

At the end of the treatment period the cells were lysed and the RNA fraction extracted using TRI-Reagent and reverse transcribed to cDNA with apposite kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions.

The assessment of the IL-8 gene expression was assessed by means of Real-Time PCR using the kit GoTaq® qPCR Master Mix Promega A6002 (Promega Corporation, Madison, WI, USA) and commercial primers for the IL-8 gene and for the GADPH and 18S (Applied Biosystems) housekeeping genes.

The PCR reaction was performed with Viia 7 thermal cycler (Applied Biosystems).

All samples were read in quadruple form.

Statistical analysis

To assess the statistical relevance of the results, the Student's *t*-test (paired two-tailed) was applied. To elaborate and assess the statistics of the data, GraphPad Prism software (GraphPad Prism Software Inc., San Diego, CA, USA) was used. The differences were considered statistically significant for values of $P<0.05$.

Results

Treatment with COLOSTRO NONI influences the cellular proliferation curve

The Caco-2 epithelial cells were cultivated in the different experimental conditions for 24 hours and the growth rate was assessed using the WST-1 colorimetric method. The Caco-2 cells represent the basic experimental model for the in vitro study of the small intestine epithelium, since 21 days after sowing they spontaneously differentiate into enterocytes, forming a single layer and keep themselves stable.

The cells cultivated in a medium deprived of nutritious substances show a stop in the growth rate (STARVED: 100±5%) and represent the comparison group for the other treatments. The cells cultivated in a normal medium show a slight growth increase (UNTR: 115±3%). The treatment with co-

lostrum alone shows a significant increase in the proliferative abilities (COLOSTRO: 130±1%; COLOSTRO *vs.* STARVED** P<0.01). The cells cultivated with COLOSTRO NONI show a greater increase in the growth rate if compared with the other experimental groups (COLOSTRO NONI: 225±15%; COLOSTRO NONI *vs.* STARVED**/UNTR°°/COLOSTRO^^ P<0.01) (Figure 1). All the readings were performed in quadruple form.

Treatment with COLOSTRO NONI influences the IL-8 gene expression

The Caco-2 epithelial cells were cultivated in different experimental conditions for 24 hours. The cellular DNA was extracted and upon its reverse transcription, the IL-8 expression was subsequently assessed by Real Time PCR. All the readings were performed in quadruple form.

Treatment with TNF-α (100 ng/mL) induces a significant increase in the level of IL-8 (TNF-α: +5 folds *vs.* UNTR) expression. The treatment with COLOSTRO NONI (2%) influences the IL-8 expression in association with TNF-α increasing its expression (TNF-α+COL NONI *vs.* TNF-α+25 folds). The addition of Colostrum alone (2%) to the cultivation medium in the presence of TNF-α (TNF-α+COL) does not significantly modify the mRNA level of IL-8 (Figure 2) statistically speaking.

Discussion

The term "colostrum" describes the first milk produced by all mammals after giving birth. It is the first and most important source of nutrition for the offspring since it contains all the essential and non-essential amino acids, hormones and growth factors, vitamins A, C, D and E, all group B Vitamins, folic acid, enzymes, metals and mineral salts. It also carries out the function of "primary vaccination" since it is rich in immunoglobulins (IgA, IgG, IgM), proteins (in particular lactoferrin) and antimicrobial, bacteriostatic peptides and able to select the microbiota for the strains of commensal bacteria more useful to the wellness of the intestinal tract.[25-27]

Morinda citrifolia (better known as Noni) is a plant which has been used for centuries by the populations of the South Pacific and has considerable phytotherapeutic properties. The most used part is the juice from the fruit, with important anti-inflammatory properties and stimulation of the organism's immune system. Substances like xeronine are also present which contribute to

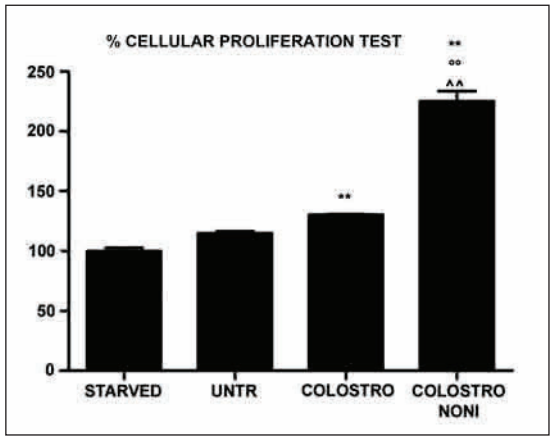

Figure 1.—Cellular growth curve analysis in the different treatment conditions. The ability of COLOSTRO NONI to stimulate cellular turnover on cultivated Caco-2 cells clearly emerges, an essential mechanism in the repair of epithelial damage.

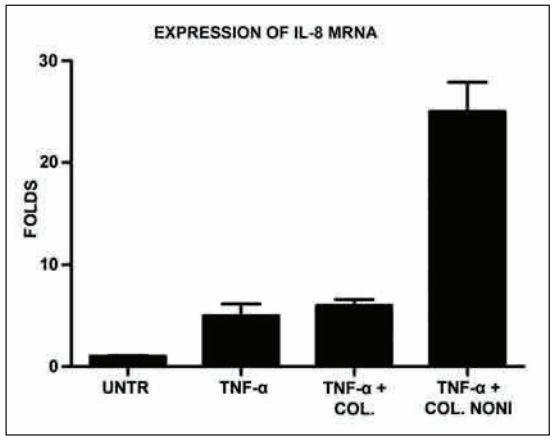

Figure 2.—Graphic representation of the IL-8 gene expression in face of the different treatments described. The use of COLOSTRO NONI allows to increase the IL-8 expression maintaining the levels (+25 folds approx.) well below those typically produced by a pathological inflammatory response (+300/600 folds approx.).

TABLE I.—*This table lists and briefly describes the key active components of COLOSTRO NONI.*

Immunoglobulins and other immunomodulatory components	IgA/IgM/IgG, PRP (Proline-rich polypeptides), lactoferrin, cytokine pool (IL-10 and IL-2), glycoproteins and trypsin inhibitors, lymphokines, lysozyme, oligo-polysaccharides and glycoconjugates.
Hormones, amino acids, growth factors	Estrogens, androgens and hormones of calcium metabolism, of the full amino acid pool, and growth factors such as IGF-1, EGF, FGF, TGF stimulate the growth and repair of the intestinal mucosa with a clear improvement of the barrier effect of the same.
Vitamins, co-enzymes, trace elements	Vit. A, Vit. C, Vit. D, Vit. E, Vit. B, Co-enzyme Q10, trace elements (Zn, Se, Cu, etc.) guarantee excellent cellular metabolism and ensure effective protection against free radicals.
Minerals	Na, K, Ca, Mg, Fe, Cu, Zn, Cr, Se, P, S restore the osmotic balance and hydrosaline pattern.

strengthening and supporting the entire organism, ensuring a faster psycho-physical recovery and an overall feeling of wellbeing.[28, 29] Table I shows the principal active ingredients of COLOSTRO NONI.

The results presented illustrate the activity of COLOSTRO NONI in an *in vitro* model of the intestinal epithelium. At the cellular level the colostrum demonstrates its ability to physiologically regulate the turn-over of enterocytes mimicking *in vitro* its natural activity of regulating and stimulating the development of the epithelium of the child at the neonatal phase. The results illustrated in Figure 1 show how COLOSTRO NONI is able to stimulate epithelial cell turnover in an experimental model *in vitro*. The regulation of cellular proliferation is central to supporting the protection and re-epithelisation of the gastrointestinal tract following damage of the epithelium and is mediated by lactoferrin, a protein present in the colostrum and involved in numerous cellular processes including the immune response from bacterial and viral infection and the stimulation of cellular proliferation and differentiation.[30-32] In literature there are no known secondary collateral effects in using lyophilised bovine colostrum and *Morinda citrifolia* juice; indeed, preliminary data on their postoperative prophylactic use in subjects affected by Hirschsprung disease verify their safety even in developing subjects such as children.[33] The antioxidising and protective effect of Noni juice on epithelial cells is essential and synergic to achieve this result in strengthening the effect of the colostrums.[34-36] Much literature exists on the antioxidising, blood-glucose and lipid-lowering properties of Noni juice. Taking Noni leads to an improvement in the plasma glucose levels regulating the expression of enzymes involved in gluconeogenesis and in glycogenolysis by phosphorylation of Fox01 and also affects the absorption of lipids in a particularly fat-rich diet providing effective cardio-protection.[37-39]

COLOSTRO NONI has demonstrated its ability to induce the expression of the IL-8 cytokine by enterocytes (Figure 2) with levels of expression (+25 folds) which remain well below those usually recorded in the presence of infections (+300/600 folds).[40] It is widely known that if, on one hand, the over-expression of IL-8 in response to aggression by a pathogen (virus or bacterium) at intestinal level has as a main symptom the increase in intestinal permeability with consequent diarrhoeic phenomena, on the other, the physiological expression of IL-8 performs two essential protective functions: it is a neutrophil chemotactic factor (and for other granulocytes) and a promoter of angiogenesis, mechanism of essential regeneration to recover damaged tissues and part of the physiological responses to epithelial damage. If, on one hand, IL-8 has no direct effect on the proliferation of intestinal epithelial cells, it nevertheless significantly stimulates the migration of epithelial cells re-establishing normal cellular turnover.[41] Both processes described carry out an important role in maintaining and remodelling the intestinal epithelial barrier in physiological and

pathological conditions with particular reference to gastrointestinal pathologies characterised by alteration of the epithelial barrier, such as all the acute and chronic inflammations of the digestive tract (IBD and IBS), celiac disease and hyper-sensitivity to gluten, but also secondary mucosal damage caused by chemotherapy and/or radiotherapy.

Conclusions

Incorrect diet, non-optimal lifestyles, psychic stress, pathological states and pharmacological therapies are connected in a vicious circle which has direct or indirect repercussions on the gastrointestinal tract altering the homeostasis.

From the data presented and from the abundant literature available it can be stated that the use of COLOSTRO NONI, in the presence of pathological inflammation (toxic, autoimmune or psychosomatic origin) of the gastrointestinal tract, allows to rebalance and preserve the delicate relationship between bacterial flora and correct intestinal permeability while re-establishing the proper inflammatory homeostasis of the mucosa and the physiological harmony of the brain-gut axis, which are crucial elements for an optimally functioning digestive tract.

The data emerged from the study suggest the clinical use of COLOSTRO NONI in gastrointestinal pathologies characterised by an increased permeability of the mucosa due to the altered function of junction systems (Leaky Gut Syndrome), in IBD, in dysenteric forms, in gastritis from erosion and its use can also be hypothesised in forms of pathological alteration of the mucus layer such as celiac disease and gluten sensitivity.

Riassunto

Effetti di COLOSTRO NONI sul turn-over delle cellule epiteliali, sugli stati infiammatori e sull'integrità dei sistemi giunzionali della mucosa intestinale

Obiettivo. In questo lavoro si è valutata la possibilità di utilizzo dell'integratore alimentare COLOSTRO NONI a scopo preventivo e terapeutico in diversi stati patologici caratterizzati da alterazione dell'omeostasi intestinale con modificazione della composizione del microbiota, alterazione della morfologia e funzionalità dell'epitelio ed infiammazione dello stesso.

Metodi. L'attività di COLOSTRO NONI a livello cellulare è stata testata in un modello *in vitro* di epitelio intestinale basato sull'utilizzo della linea cellulare Caco-2. È stata valutata la capacità di COLOSTRO NONI di stimolare il turn-over cellulare mediante saggio del rate di crescita cellulare WST-1. Tramite Real-Time-PCR, si è invece testata la capacità di COLOSTRO NONI di incrementare l'espressione genica di Interleuchina-8 (IL-8), importante fattore chemotattico coinvolto nei fenomeni infiammatori e nel controllo dell'omeostasi tissutale.

Risultati. COLOSTRO NONI è in grado di stimolare il turn-over cellulare nel modello *in vitro* proposto e si dimostra attivo nell'incrementare l'espressione genica di IL-8. Entrambi gli aspetti osservati sono fondamentali per l'instaurazione di meccanismi di riparazione dei danni tissutali.

Conclusioni. I risultati ottenuti indicano che COLOSTRO NONI può trovare applicazione clinica nel trattamento delle patologie gastro-intestinali caratterizzate da compromissione della corretta permeabilità intestinale, nelle IBDs (Inflammatory Bowel Diseases), nelle forme dissenteriche, nelle gastriti ed in forme di patologica alterazione del layer mucoso come celiachia e gluten sensitivity.

PAROLE CHIAVE**:** Tratto gastro-intestinale - Mucosa gastrica - Infiammazione - Colostro - Morinda.

References

1. Pastorelli L, De Salvo C, Mercado JR, Vecchi M, Pizarro TT. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics. Front Immunol 2013;17:280.
2. McGuckin MA, Eri R, Simms LA, Florin TH, Radford-Smith G. Intestinal barrier dysfunction in inflammatory bowel diseases. Inflamm Bowel Dis 2009;15:100-13.
3. Randall-Demllo S, Chieppa M, Eri R. Intestinal epithelium and autophagy: partners in gut homeostasis. Front Immunol 2013;30:301.
4. Tsuji M, Suzuki K, Kinoshita K, Fagarasan S. Dynamic interactions between bacteria and immune cells leading to intestinal IgA synthesis. Semin Immunol 2008;20:59-66.
5. Cunningham-Rundles S, Ahrné S, Johann-Liang R, Abuav R, Dunn-Navarra AM, Grassey C *et al*. Effect of probiotic bacteria on microbial host defense, growth, and immune function in human immunodeficiency virus type-1 infection. Nutrients 2011;3:1042-70.
6. Ader R. Psychoneuroimmunology. IV ed. Vol. 1 e 2. Amsterdam: Academic Press; 2007.
7. Hart A, Kamm MA. Review article: mechanisms of initiation and perpetuation of gut inflammation by stress. Aliment Pharmacol Ther 2002;16:2017-28.
8. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E. Microflora modulates endocrine cells in the

gastrointestinal mucosa of the rat. Gastroenterology 1994;107:1259-69.
9. Collins SM, Bercik P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology 2009;136:2003-14.
10. Salonen A, de Vos WM, Palva A. Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. Microbiology 2010;156(Pt 11):3205-15.
11. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology 2006;130:1480-91.
12. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci 2013;70:631-59.
13. Assimakopoulos SF, Papageorgiou I, Charonis A. Enterocytes' tight junctions: from molecules to diseases. World J Gastrointest Pathophysiol 2011;2:123-37.
14. Schneeberger EE, Lynch RD. The tight junction: a multifunctional complex. Am J Physiol Cell Physiol 2004;286:C1213-28.
15. Shin K, Fogg VC, Margolis B. Tight junctions and cell polarity. Annu Rev Cell Dev Biol 2006;22:207-35.
16. Keiper T, Santoso S, Nawroth PP, Orlova V, Chavakis T. The role of junctional adhesion molecules in cell-cell interactions. Histol Histopathol 2005;20:197-203.
17. Juric M, Xiao F, Amasheh S, May O, Wahl K, Bantel H *et al*. Increased epithelial permeability is the primary cause for bicarbonate loss in inflamed murine colon. Inflamm Bowel Dis 2013;19:904-11.
18. Ivanov AI. Structure and regulation of intestinal epithelial tight junctions: current concepts and unanswered questions. Adv Exp Med Biol 2012;763:132-48.
19. Martínez C, Lobo B, Pigrau M, Ramos L, González-Castro AM, Alonso C *et al*. Diarrhoea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. Gut 2013;62:1160-8.
20. Koch S, Nusrat A. The life and death of epithelia during inflammation: lessons learned from the gut. Annu Rev Pathol 2012;7:35-60.
21. Al-Shmgani HS, Moate RM, Macnaughton PD, Sneyd JR, Moody AJ. Effects of hyperoxia on the permeability of 16HBE14o- cell monolayers-the protective role of antioxidant vitamins E and C. FEBS J 2013;280:4512-21.
22. Kundumani-Sridharan V, Dyukova E, Hansen DE 3rd, Rao GN. 12/15-Lipoxygenase mediates high-fat diet-induced endothelial tight junction disruption and monocyte transmigration: a new role for 15(S)-hydroxyeicosatetraenoic acid in endothelial cell dysfunction. J Biol Chem 2013;288:15830-42.
23. Martinez-Medina M, Denizot J, Dreux N, Robin F, Billard E, Bonnet R *et al*. Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. Gut 2013 [Epub ahead of print].
24. Conlon MA, Kerr CA, McSweeney CS, Dunne RA, Shaw JM, Kang S *et al*. Resistant starches protect against colonic DNA damage and alter microbiota and gene expression in rats fed a Western diet. J Nutr 2012;142:832-40.
25. Khan Z, Macdonald C, Wicks AC, Holt MP, Floyd D, Ghosh S *et al*. Use of the 'nutriceutical', bovine colostrum, for the treatment of distal colitis: results from an initial study. Aliment Pharmacol Ther 2002;16:1917-22.
26. Sacerdote P, Mussano F, Franchi S, Panerai AE, Bussolati G, Carossa S *et al*. Biological components in a standardized derivative of bovine colostrum. J Dairy Sci 2013;96:1745-54.
27. Hagiwara K, Kataoka S, Yamanaka H, Kirisawa R, Iwai H. Detection of cytokines in bovine colostrum. Vet Immunol Immunopathol 2000;76:183-90.
28. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK *et al*. Morinda citrifolia (Noni): a literature review and recent advances in Noni research. Acta Pharmacol Sin 2002;23:1127-41.
29. Chan-Blanco Y, Vaillant F, Perez AM, Reynes M, Brillouet JM, Brat P. The Noni fruit (Morinda citrifolia L.): A review of agricultural research, nutritional and therapeutic properties. Journal of Food Composition and Analysis 2006;19:645-54.
30. Buccigrossi V, de Marco G, Bruzzese E, Ombrato L, Bracale I, Polito G *et al*. Lactoferrin induces concentration-dependent functional modulation of intestinal proliferation and differentiation. Pediatr Res 2007;61:410-4.
31. Liao Y, Jiang R, Lönnerdal B. Biochemical and molecular impacts of lactoferrin on small intestinal growth and development during early life. Biochem Cell Biol 2012;90:476-84.
32. Lönnerdal B, Jiang R, Du X. Bovine lactoferrin can be taken up by the human intestinal lactoferrin receptor and exert bioactivities. J Pediatr Gastroenterol Nutr 2011;53:606-14.
33. Grandi F, Betalli P, Midrio P, Fascetti-Leon F, Gamba PG. Enterocolitis complicating Hirschsprung's disease: preliminary results of post-operative prophylaxis with Colostrums. Abstract book and scientific programme of the 17th International Paediatric Colorectal Club Meeting; 2010 July 17-19; Padua, Italy.
34. Chang MS, Kim WN, Yang WM, Kim HY, Oh JH, Park SK. Cytoprotective effects of Morinda officinalis against hydrogen peroxide-induced oxidative stress in Leydig TM3 cells. Asian J Androl 2008;10:667-74.
35. Palu A, Su C, Zhou BN, West B, Jensen J. Wound healing effects of noni (Morinda citrifolia L.) leaves: a mechanism involving its PDGF/A2A receptor ligand binding and promotion of wound closure. Phytother Res 2010;24:1437-41.
36. Lin YL, Chang YY, Yang DJ, Tzang BS, Chen YC. Beneficial effects of noni (Morinda citrifolia L.) juice on livers of high-fat dietary hamsters. Food Chem 2013;140:31-8.
37. Wang MY, Peng L, Weidenbacher-Hoper V, Deng S, Anderson G, West BJ. Noni juice improves serum lipid profiles and other risk markers in cigarette smokers. ScientificWorldJournal 2012;2012:594657.
38. Palu AK, West BJ, Jensen J. Noni-based nutritional supplementation and exercise interventions influence body composition. N Am J Med Sci 2011;3:552-6.
39. Nerurkar PV, Nishioka A, Eck PO, Johns LM, Volper E, Nerurkar VR. Regulation of glucose metabolism via hepatic forkhead transcription factor 1 (FoxO1) by Morinda citrifolia (noni) in high-fat diet-induced obese mice. Br J Nutr 2012;108:218-28.
40. Wang H, Moon S, Wang Y, Jiang B. Multiple virus infection alters rotavirus replication and expression of cytokines and Toll-like receptors in intestinal epithelial cells. Virus Res 2012;167:48-55.
41. Sturm A, Baumgart DC, d'Heureuse JH, Hotz A, Wiedenmann B, Dignass AU. CXCL8 modulates human intestinal epithelial cells through a CXCR1 dependent pathway. Cytokine 2005;29:42-8.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Colostro Noni

La naturale prevenzione dei disturbi gastro-intestinali

Protegge il tuo equilibrio intestinale naturalmente

Colostro Noni è una formulazione **orosolubile** innovativa, a base di **colostro bovino**, indicata come coadiuvante sia nei bambini che negli adulti. Grazie all'attività sinergica dei suoi principi attivi naturali, aiuta a proteggere il tratto gastro-intestinale preservandone l'ottimale funzionalità.
L'attività di Colostro Noni è anche sostenuta dalla presenza del **succo di Noni** (Morinda citrifolia), con proprietà protettive e stimolanti naturali. Colostro Noni per i suoi componenti rappresenta un valido supporto nella prevenzione dei disturbi gastro-intestinali anche legati ad episodi influenzali.

il nostro laboratorio è a
Impatto Zero®
GUNA S.p.a. aderisce al progetto Impatto Zero® di LifeGate. Compensate le emissioni di CO_2 con la creazione di nuove foreste.

LIFEGATE IMPATTO ZERO®

GUNA
terapie d'avanguardia

GUNA S.p.a.
Via Palmanova 71 – 20132 Milano

UNI EN ISO 9001:2008
CERTIQUALITY
SISTEMA DI GESTIONE QUALITÀ CERTIFICATO

AZIENDA CON SISTEMA
DI GESTIONE QUALITÀ
UNI EN ISO 9001:2008
CERTIFICATO DA CERTIQUALITY