Nutrición Hospitalaria

Immunonutrition: methodology and applications

Belén Zapatera, Andreu Prados, Sonia Gómez-Martínez and Ascensión Marcos

Immunonutrition Research Group. Department of Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid. Spain.

Resumen

La Inmunonutrición es una materia emergente e interdisciplinar, ya que abarca distintos aspectos relacionados con la Nutrición, la Inmunidad, la Infección, la Inflamación y la Injuria o daño tisular, lo que se ha denominado como la Nutrición y las 4 "Ies". En estas interacciones se encuentran implicados los sistemas endocrino, nervioso e inmune, formando parte la microbiota de este último. Actualmente la microbiota intestinal tiene un papel fundamental no solo a nivel del tracto gastrointestinal sino que presenta además un eje de conexión bilateral con el sistema nervioso

Para el estudio de la Inmunonutrición existen diferentes biomarcadores del sistema inmune que proporcionan información acerca del estado nutricional del individuo. Sin embargo, se debe tener en cuenta que no existe un solo parámetro para evaluar la relación causa-efecto de la nutrición sobre el sistema inmunitario, sino que es un conjunto de biomarcadores a tener en cuenta dependiendo de los distintas situaciones nutricionales.

Si bien está claro que se trata de una materia multidisciplinar, no solo se deben focalizar los estudios sobre las interacciones entre la nutrición y el sistema inmune de manera aislada, sino sobre otros sistemas del organismo teniendo en cuenta un gran abanico de factores de confusión y determinantes derivados de las condiciones idiosincrásicas de cada individuo, su genética y su estilo de vida.

Por todo ello, la Inmunonutrición permite llevar a cabo una serie de estudios basados fundamentalmente en cuatro líneas de investigación: 1) Evaluación de poblaciones supuestamente sanas pero con riesgo de malnutrición (niños, adolescentes, adultos, gestantes, lactantes, personas mayores y deportistas), 2) Estudio de la evolución de pacientes con enfermedades relacionadas con la nutrición y el sistema inmunitario, 3) Estudio de los efectos de nutrientes, compuestos bioactivos y alimentos convencionales y funcionales sobre el sistema inmunitario; 4) Estudio del impacto del estilo de vida sobre el comportamiento del sistema inmunitario, teniendo como determinantes principales la dieta, el comportamiento alimentario, la actividad física, el sedentarismo, la calidad y cantidad de sueño, y como factor clave, el estrés.

(*Nutr Hosp* 2015;31(Supl. 3):145-154)

DOI:10.3305/nh.2015.31.sup3.8762

Key words: *Diet. Food. Food habits. Health. Lifestyles. Nutrients.*

Correspondence: Ascensión Marcos.
Immunonutrition Research Group. Department of Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC).
José Antonio Novais Street 10, 28040 Madrid, Spain.
E-mail: amarcos@ictan.csic.es

INMUNONUTRICIÓN: METODOLOGÍA Y APLICACIONES

Abstract

Immunonutrition is an emergent and interdisciplinary subject, since it comprises several aspects related to Nutrition, Immunity, Infection, Inflammation, and Injury or tissue damage, what is known as Nutrition and 4 "Is". Within these interactions the endocrine, nervous and immune systems are involved, microbiota being a part of the last one. Nowadays, gut microbiota has been shown to play an essential role, not only in the gastrointestinal tract but also into the nervous system, because of its bilateral connection.

There are several methods to study Immunonutrition, which allow measuring different immunological biomarkers to provide information about the nutritional status. However, it should be taken into account that there is not a single gold standard parameter to evaluate the cause-effect relationship between nutrition and the immune system. On the contrary, a combination of biomarkers have to be assessed depending on the different nutritional situations.

Since Immunonutrition is a multidisciplinary matter as mentioned above, the study on the interactions between nutrition and the immune system has not been exclusively focused as such, but bearing in mind other systems of the organisms as well as a wide range of confounding factors and determinants coming from idiosyncratic features, genes and lifestyle of each individual.

Therefore, Immunonutrition allows to study the following research fields: 1) Evaluation of nutritional status in presumably healthy people with risk of malnutrition (children, adolescents, adults, pregnant women, elderly, and sportspeople); 2) Assessment of the evolution and progress of patients with nutrition and immune-related diseases, such as food allergies, eating and metabolic disorders; 3) Evaluation of the effects of nutrients, bioactive compounds and both conventional and functional foods on the immune system; 4) Evaluation of impact of lifestyle determinants on the immune system, such as diet, food behaviour, physical activity, sedentariness, sleep quality and quantity, and as a key factor, stress.

(*Nutr Hosp* 2015;31(Supl. 3):145-154)

DOI:10.3305/nh.2015.31.sup3.8762

Palabras clave: *Immunonutrition. Biomarkers. Microbiota. Lifestyle. Malnutrition.*

State of the art: nutrition and immunity

The Immune System

The immune system is a complex defence network that protects body against potentially harmful agents and has the capacity to respond to millions of antigens. Through orchestrated reactions, that is, the immune response, the immune system can recognize and remove pathogens[1]. It serves to distinguish "non-self" from "self" and acts to ensure tolerance of "self", food and other environment components and commensal bacteria inherent in the body. However, any mistake or alteration in tolerance pathways may lead to an immunological disordered process. Although there are plenty of factors involved in the aetiology of disea-se, most conditions are related to the immune system, they frequently being infectious, inflammatory, or autoimmune processes[1]. In the last few years, research keeps confirming how an inappropriate inflammatory response actually plays a crucial role in the onset, progression and severity of many chronic conditions such as Alzheimer's disease, heart attacks, obesity, cancers or autoimmune pathologies (different types of allergies, psoriasis, systemic lupus erythematosus, Crohn's disease, celiac disease, multiple sclerosis, or rheumatoid arthritis)[1,2].

Briefly, the immune system comprises three levels of defence: anatomical and physiological barriers, the innate or unspecific immunity and the adaptive or specific immunity (also termed acquired)[1,3,4]. Physico-chemical barriers include intact skin, ciliary clearance in respiratory tract, mucosal membranes, lysozyme in tears and saliva, stomach acid, and commensal microbiota in skin, mouth, gastrointestinal tract and genitourinary tract[4]. After infectious agents and other noxious insults have crossed this first line of defence, the immune system establishes active defence mechanisms which could be divided into two categories: the innate and the adaptive immune responses. Both immune responses include several blood-borne factors or soluble components and cells (Table I). The innate immune response represents the first defensive system in the organism, and it is particularly important for preventing the entry of infectious agents into the body and, if they enter, eliminating them rapidly[3,4]. The innate immune system includes cells (granulocytes -neutrophils, basophils, and eosinophils-, monocytes/macrophages, and natural killer -NK- cells), and soluble factors[1,3,4] (Table I). Innate immunity has no memory and is therefore not influenced by prior exposure to an organism[4]. This response is activated by any strange substance penetrating the organism, which will be eliminated by mechanisms of phagocytosis and cytotoxicity[1]. Although this first barrier represents a good defensive system, it is sometimes not sufficient to protect the organism, and thus the adaptive immune response is required. This response is more complex and sophisticated and its key feature is to be specifically effective for those antigens that triggered the response[4]. The adaptive response beco-

Table I
Components of the innate and adaptive immune system

	Innate immunity	Adaptive immunity
Cellular components	Granulocytes: neutrophils, basophils and eosinophils Monocytes/macrophages Mast cells Natural killer cells	B lymphocytes (B cells) T lymphocytes (T cells): T cytotoxic, T helper (T_h1, T_h2) cells
Soluble components	Complement factors Acute phase proteins Macrophage-derived cytokines	Antibodies Lymphocyte-derived cytokines

mes effective over several days after the initial activation, but it also persists for some time after the removal of the initiating antigen[4]. This persistence gives rise to immunological memory, which is the basis for a stronger, more effective immune response on reexposure to an antigen[4]. The adaptive response is mainly mediated by lymphocytes and classified into two types: humoral and cellular (Fig. 1). In general terms, the humoral response involves mainly B lymphocytes while T cells are in charge of the cell-mediated immune response[3,4]. Humoral immunity deals with extracellular pathogens, whereas cell-mediated immunity is directed towards intracellular pathogens -viruses and some bacteria-, which escape humoral immunity[4]. Both responses are linked and together result in a highly effective antigen-driven specific immune response. T helper (Th lymphocytes are characterized by their capacity to produce cytokines and participate in the initiation and development of the immune response. Th1 cells promote the cell-mediated immune response while Th2 stimulate the humoral response[1,3]. The innate and adaptive systems are communicated by direct cell-to-cell contact involving cell surface proteins (e.g., adhesion molecules) and by the production of chemical messengers such as cytokines[3]. On the other hand, gut microbiota do not only have a role as a physical barrier, but also interacts dynamically with both the intestinal innate and adaptive immune system, affecting various aspects of its development and function and therefore microbiota is a part of the immune system that should be taken into account to evaluate the nutritional status[5]. (Table I and Fig. 1).

Interest of immunonutrition

Relationships between nutrition and immunity

It is well-known that adequate nutrition is an important factor allowing the normal development of the immune system as well as its correct function throughout life, although the study of the relationship between nutrition and immune function is relatively recent. Immunonutrition is the science that studies interactions between nutrition and Immune system, Infection, Inflammation and Injury or tissue damage. Thus, Immunonutrition is also known as Nutrition and 4 "Is". Malnutrition is a condition that occurs when a person's diet does not contain the right amount of nutrients and can refer to both undernutrition and overnutrition. Traditionally, the study of the interaction between nutrition and infection (as *"the first I"*) has included the role of infection in defining nutritional status and the role of nutrition in determining host defence mechanisms[6]. The 1968 *World Health Organization* monograph about "Interactions between Nutrition and Infection" presented the mechanisms linking infection and poor nutritional status. Following the development of immunology as a science, increasing evidence was obtained as well to show how undernutrition may impaire resistance to infections and the immune response[7].

In fact, nutrients play an important role in the development and functionality of the immune system and any deficiency, either single or multiple, is often the cause of a compromised immunity. The understanding that protein-energy malnutrition (PEM) is not only
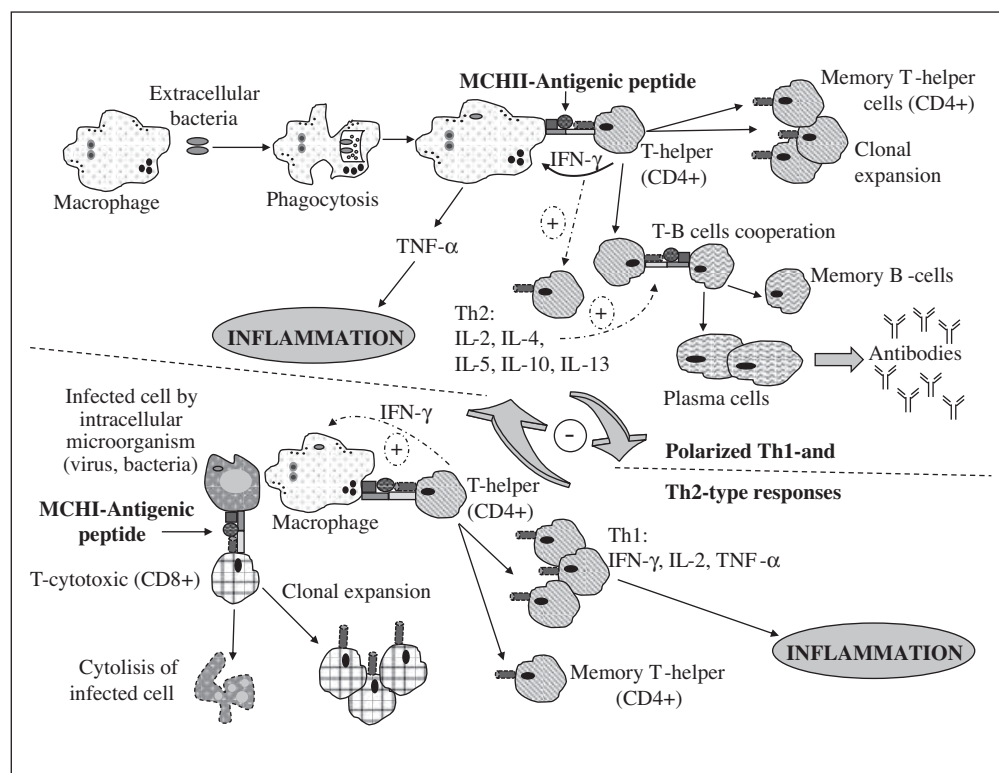

Fig. 1.—Schematic view of the adaptive immune response. Adapted from Marcos A, Nova E, Perdigón G, de Moreno A. Nutrición e Inmunidad. En: Nutrición y Salud Pública. Métodos, Bases Científicas y Aplicaciones. 2ª ed. Serra Ll, Aranceta J, Mataix J, eds. Barcelona: Masson; 2006. p.482-90.

protein and energy but also involves insufficiencies in the cellular supply of multiple micronutrients, serves to highlight the importance of specific micronutrients (vitamin A, Fe, Zn, and Cu) and their respective carrier proteins on specific and non-specific components of the immune response[6]. This knowledge led to the need to include immunity as the link within the relationship between nutrition and infection (*"the second I"*)[6]. Afterwards, the discovery that several nutrients, such as tocopherol, retinol, zinc and essential fatty acids, could modulate the intensity of the responses that define inflammatory process opened a field on the potential influence of nutrition on inflammatory processes[6]. The potential health gains from modulating the balance between n-3 and n-6 fatty acid intake paved the way for the inclusion of inflammation as *"the third I"* in the relationship between nutrition and infection[6]. Finally, the consideration that nutrients also modulate injury as an end point of hypoxic or toxin mediated cell damage led to include injury as *"the fourth I"*[6].

Controversies and limitations

As was mentioned before, study of immunological parameters allow helping to know the nutritional status of apparently healthy people. These parameters are not usually used in epidemiological studies, and their use is commonly limited by the necessity of experts on immunology who can correctly interpret the results, also of specialized laboratories and techniques, and the high costs of immunological tests[8]. In addition, it is important to highlight that no single biomarker is capable to predict malnutrition status and therefore it should be necessary to assess several biomarkers and their relationship with the nutritional status[9]. In this context, techniques should be standardized and based on updated laboratory protocols. It is also necessary to kick-off all the instruments.

There are several *In vivo* studies that are not possible to being performed in alive subjects because they use aggressive challenges. However, the evaluation of *in vivo* immune function can be determined through animal models or even by using analysis such as vaccines or delayed hypersensitivity skin tests (DHST)[9].

The immune system is affected by a variety of subject-specific and technical factors, which should be strictly controlled in order to reduce the variation in the outcome of immunological measurements (Table II). Furthermore, genetic polymorphisms, early life events, hormone status, and gut microbiota may be additional factors contributing to such variation. In addition, ethical constraints may restrict the use of specific markers in certain populations[9] (Table II).

With the aim to evaluate nutritional status through immunological parameters in human studies it is important to consider several aspects. First of all, the number of subjects should be sufficient in order to have strong statistical power and to extrapolate the outcomes to the general population[6]. In human nutritional studies there are several confounding factor that should be considered and they are: stress, physical activity/exercise, sleep time (quality/quantity), and food behaviour[9]. Besides, human studies are often limited by the ability to take samples, usually blood and external secretions such as saliva, tears or urine. In addition, normal ranges and reference values for immune cell number and function in specific populations (children, pregnant women, elderly, sports people, etc.) are not still well defined[3] (Fig. 2).

Moreover, studies should allow identifying changes in the prevalence of side effects. In addition, the design of the study should be randomized, double-blind, cross-over or parallel with adequate inclusion and exclusion criteria[6]. The final objective of the study should consider other aspects apart from adequate nutrition, in order to have an impact on overall health, reduction of both comorbidities and risk of mortality, and socio-economic determinants of life quality.

Table II
Confounding subject-specific and technical factors modulating immune function in human studies[10]

Subject-specific factors	Technical factors
Age	Selection of the population and appropriate controls to be evaluated and compared with
Sex (hormones, menstrual cycle)	
BMI	Time of sample collection (circadian rhythm)
Background diet and nutrient supplementation before and during intervention	Seasonality
	Time since last meal (fasting period)
Physical activity and exercise	Use of washout periods
Smoking	Length of intervention period
Hydration status	Appropriate selection of immunological biomarkers
Genetics (low/high responder)	Correct interpretation of the outcome by experts on
Presence of infections or other diseases	Immunonutrition
Psychological stress	
Sleep deprivation	
Alcohol, drug and medications	
Vaccination and infection history	
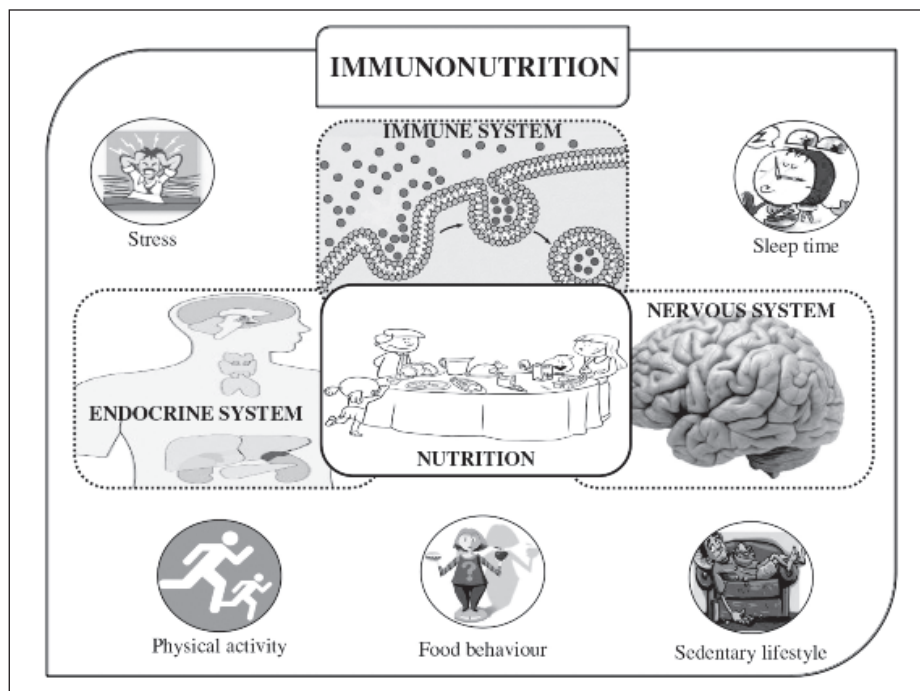
Current status and perspectives

The assessment of immunological parameters can point towards a subclinical malnutrition status and therefore acquires great interest as a useful tool to evaluate the nutritional status. When analysing immune parameters, it is necessary to bear in mind the complex interactions and reciprocal control among the immune system, the endocrine system and the central nervous system, as already cited above[10].

Tissues and organs

Malnutrition may lead to decreased cellular proliferation, decreased of protein synthesis, and deficiency of nutrients involved in metabolic pathways. These alterations can modify the immune response by affecting lymphoid tissues and immune cell number and function[11]. Specifically, protein-energy malnutrition (PEM) produces generalized atrophy of lymphoid tissues. In these situations, thymus, spleen, tonsils, Peyer's patches, and lymphatic nodes are seriously affected, with histological evidence of significant specific atrophy in T lymphocyte areas affecting mainly the adaptive immune system[12].

Circulating factors

Molecules

Immunoglobulins (Ig)

Immunoglobulins are proteins found in different fluids in the organism including blood and secretions such as saliva, tears, and breast milk. They are produced by plasma cells and their main function is to recognize the antigens or strange substances in order to neutralize them. Immunoglobulins (Ig) are also known as antibodies and there are five primary types of them (IgG, IgA, IgM, IgD and IgE) and each type has different isotypes (IgG1, IgG4…). Their determination could be possible through different immunoassays (ELISA, ELISPOT, standard analysis by nephelometry). Concentration of these antibodies in blood allows analysing basal status of any subject. However, the study of Ig concentrations after stimulation (in *ex vivo* cultures induced by a mitogene such as lipopolysaccharide (LPS) or *in vivo* with a specific antigen) allows getting more information about the response capacity of B cells.

Acute-phase proteins: C-reactive protein, complement factors C3 and C4 and ceruloplasmin

C-reactive protein (CRP) is a very sensitive marker of inflammation, and its concentration increases rapidly in response to a wide range of stimuli. Belonging to the innate immune response, high CRP levels have no specificity in differentiating disease entities from one another, but despite its lack of specificity, CRP has now emerged as one of the most powerful predictors of cardiovascular risk. Indeed, CRP is usually increased in overweight/obese people, as obesity involves a low-grade chronic inflammation state not only in adults, but also in children[13].

Regarding complement factors C3 and C4 and ceruloplasmin, these are proteins that strongly predict cardiovascular events in adults. Complement factors C3 and C4 are part of the innate immune system and

stimulate phagocytosis of foreign components and active some inflammatory processes in the organism. On the other hand, ceruloplasmin is another protein produced in the liver during the acute phase response. It is a major plasma protein and functions as a copper transporter. However, its high levels are associated with atherosclerosis. C3, C4, highly sensitive CRP (hs-CRP), and ceruloplasmin are measured in serum by using immunoturbidimetry[13].

In a representative sample of Spanish adolescents aged 13-18.5 years from the AVENA study, CRP, C3, and C4 levels were correlated with central obesity. Particularly, we found that central obesity is independently associated with C3 concentrations[13].

Concentrations of cytokines or of soluble cytokine receptors

Cytokines can be affected by malnutrition, both undernutrition and overnutrition. They can be classified as pro-inflammatory (IL-1, IL-6, TNF-a) and anti-inflammatory (IL-2, IL-4, IL-10). An excess of body fat have not an observed effect on the capacity of leukocytes for *in vitro* production of IL-6 and TNF-a in a sample of Spanish adolescents in the AVENA Study[13]. Conversely, in anorexia nervosa patients, the production of TNF-a and IL-6 was lower while the secretion of IL-1b was higher than in the control group when these levels were compared before and one month after admission to the hospital. This outcome could suggest that the immune function in anorexic patients could be preserved despite their severely malnourished condition[14]. In our group, he have found that excess weight and inadequate sleep duration are independently associated with the incidence of allergy symptoms in adolescents. Adequate sleep duration and weight during adolescence might be relevant for a decreased risk of suffering allergy symptoms[15].

The production of cytokines by lymphocytes and monocytes usually requires these immunocompetent cells to be stimulated by a mitogen such as phytohemagglutinin or bacterial lipopolysaccharide. Moreover, the spontaneous production of cytokines can also be measured by peripheral blood mononuclear cells (PBMCs). Cytokine protein concentrations in the cell culture medium are measured by ELISA or flow cytometry.

Actually, it is very useful the determination the soluble cytokine receptors in biological samples through immunoassays.

Immunocompetent cells

Neutrophils and monocytes counts and functionality

Neutrophils and monocytes are the main phagocytic cells and their counts help understand how is the immune system response to an infection. In order to complete the study, it is necessary to know not only their counts, but also their functionality. Only in extreme cases, such as severe denutrition, leukocyte counts themselves are physiological relevant. Thus, it is important to be familiarized with the significance of their normal reference values.

Total lymphocyte counts

Total lymphocyte count is a measure of nutritional status. Total lymphocyte normal range is over 2.000 cells/mm³. When it is between 1.200 and 2.000 cells/mm³ indicates mild denutrition; 800-1.200 cells/mm³ reflects moderate denutrition and counts under 800 cells/mm³ represents severe denutrition[16].

Lymphocyte subsets counts

Phenotypic analysis by flow cytometry allows define different types of lymphocytes. Furthermore, it gives information about redistribution of immunocompetent cells caused by several factors such as fasting and refeeding[15]. Study of lymphocyte subsets include: mature T-cells (CD3+), helper T-cells (CD4+), cytotoxic or suppressor (CD8+), natural killer (NK) cells (CD3-CD16+ CD56+), B-cells (CD19+), naïve and memory cells (CD45RA+ and CD45RO+, respectively). This analysis allows distinguish between active subsets using different markers such as CD69+, in order to be used in the functionality of the immune system. The ratio CD4+/CD8+is an index of nutritional status that reflects immunodeficiency secondary to malnutrition states[17,18]. A sleep duration of 8-8.9h/night was associated with a healthier immune profile in European adolescents[19].

Immune system functionality

In vivo measures

Vaccines

Vaccination responses are widely used in immunonutrition studies in the human population and allow high quality information about their immunomodulatory effects on the immune response. Vaccination responses may be influenced by a variety of factors other than environmental ones and they include psychological stress, nutrition, and (infectious) diseases, and lifestyle determinants (e.g., smoking)[20].

Delayed hypersensitivity skin test (DHST)

DHST is based on the reaction that occurs in response to the intradermal injection of an antigen (e.g., tuberculin). The histologic findings and immunologic mechanisms characterizing this form of immunologic response are based on mobilization of macrophages and other phagocyte cells causing an increased lymphocyte response. This measurement is useful because it represents a coordinated, integrated cell-mediated immune response to a relevant challenge. However, the test cannot be

repeated on the same are of skin, and recent vaccination may interfere with the outcome[3].

Ex vivo measures

Ex vivo measures allow the functional responses of specific immune cell types to be determined[3].

Phagocytosis by neutrophils and monocytes

Substrates for phagocytosis can be studied in the opsonized (i.e., complement- or antibody-coated) and unopsonized states. Some techniques (e.g., flow cytometry) allow identification of both the number of cells participating in phagocytosis and the phagocytic activity per cell[3]. Measures of phagocytosis can be coupled to measures of oxidative burst.

Oxidative (respiratory) burst (superoxide generation) by neutrophils and monocytes

Oxidative burst studies the percentage and activity of phagocytic cells (neutrophils and monocytes). Reactive oxygen species such as hydrogen peroxide are measured. This technique can oriented research on innate immune mechanisms and inflammation[3].

Chemotactic response of neutrophils or monocytes

This is the movement of these cells toward particular stimuli; stimuli used include leukotriene B4, and bacterial cell wall peptides[3].

Natural killer cell activity

NK cells induce direct cytotoxicity or secretion of cytokine/chemokine without recognizing a specific antigen as B and T cells. NK cytotoxicity acts against virus-infected cells and tumour cells[22].

NK cells activity is one of the most sensitive functions affected by diet. It can be due to the fact that NK cells are high dependent on cytokines. Killing can be expressed in various ways, such as percentage target cells killed or "lytic ratio", which is the ratio of killer to target cells required to kill a particular percentage of target cells[3].

Lymphocyte proliferation

This is the increase in number of lymphocyte in response to a stimulus. Most often this is measured as the incorporation of radioactively labelled thymidine into the DNA of the dividing lymphocytes, although a number of other measures, not involving the use of radioactivity, are available[3].

Measures of mucosal immune responses

The collectively called mucosa-associated lymphoid tissue (MALT) is found along mucosal linings in the human body and constitutes the most extensive component of human lymphoid tissue. These surfaces protect the body from an enormous quantity and variety of antigens. MALT includes gut-associated lymphoid tissue (GALT), bronchial-associated lymphoid tissue (BALT), nasopharynx-associated lymphoid tissue (NALT), vulvovaginal-associated lymphoid tissue (VALT), and lacrimal drainage-associated lymphoid tissue (LDALT)[23]. The basic architecture of MALT includes discrete areas where B lymphocytes are localized next to areas in which T lymphocytes predominate. Lymphoid tissues of MALT have the ability to concentrate and respond to local antigens. Concentration of total and antigen-specific secretory IgA is a useful measure of mucosal immune responses[3].

GALT accounts for up to 80% of the mucosal immune system and is distributed along the intestine in two forms: as organized GALT, which includes Peyer's patches, isolated follicles and mesenteric lymph nodes, and as diffuse GALT, consisting of lymphocytes scattered in the epithelium and the lamina propria. Both compartments are part of a regulatory system with specific roles; organized GALT is the inductor site of the immune response and diffuse GALT is the effector site. GALT has a role in both innate and acquired immune responses. Another component of the mucosal immune system consists of regulatory T-cells, which mediate peripheral T-cell tolerance to antigens derived from the dietary origin or from the commensal microbiota. Systemic immune system biomarkers can be found in lamina propria and they can be studied by the same methods mentioned before[23].

Microbiota

A healthy gut microbiota may be viewed as a positive attribute, while dysbiosis (gut microbiota alteration) is associated with altered health states. The current understanding of the gut microbiota provides information essential for efficiently dealing with well-being and diseases such as obesity, the metabolic syndrome, food intolerance, inflammatory bowel disease and irritable bowel syndrome. Furthermore, the central nervous system is also affected through gut-brain communication pathways[24] (Fig. 3).

In human studies, gut microbiota could act as a biomarker to assess dietary and lifestyle interventions. In the comprehensive programme EVASYON (Development, implementation and evaluation of the efficacy of a therapeutic programme for overweight/ obese adolescents), which includes diet, physical activity and psychology interventions in 13-16 years old overweight adolescents after intervention there were two groups: low weight-loss (< 2 kg) and high weight-loss (> 4 kg) group. We observed that intervention were only successful in the high weight-loss group, maybe due to a different microbiota between both groups. Therefore, individual's gut microbiota
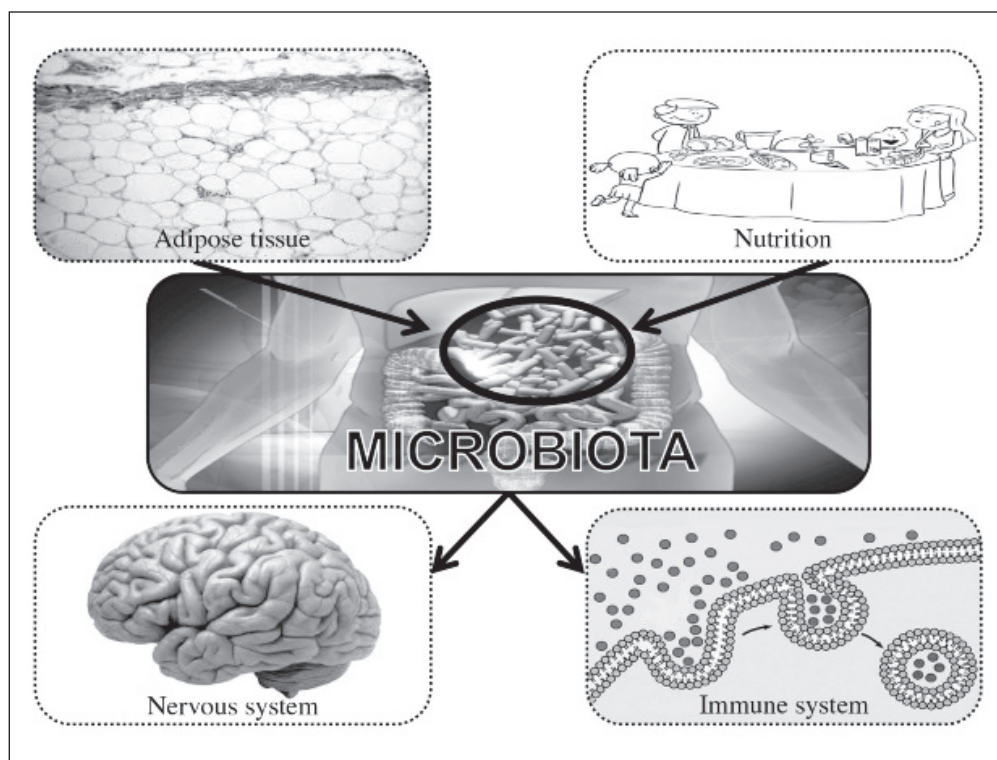
Fig. 3.— Interrelationships between confounding factors and microbiota.

composition is more important to achieve weight loss than expected[25]. On the other hand, childhood microbial colonization patterns are different depending on maternal Body Mass Index (BMI). This evidence suggests that microbiota may act as a marker to predict the possible risk of obesity[26].

In addition, the interaction of gut microbiota with immune cells in the mucosal environment has a principal role in a number of processes directly dependent on the MALT, such as oral tolerance induction, the modulation of cytokine and chemokine release and, in general, the regulation of immune responses in the intestinal mucosa which are important in the pathogenesis of inflammatory bowel disease[27].

Genetics

Nutritional genomics has tremendous potential to change the future of dietary guidelines and personal recommendations. Nutritional genomics covers nutrigenomics, which explores the effects of nutrients on the genome, proteome and metabolome, and nutrigenetics, the major goal of which is to elucidate the effect of genetic variation on the interaction between diet and disease[28].

Epigenetics refers to the study of changes on heredity patterns of gene expression that occur without changes in the DNA sequence. In recent years, epigenetic markers emerged as a new tool to understand the influence of lifestyle factors on obesity phenotypes. Indeed, we have seen in EVASYON study that methylation changes

may help to better understand the weight loss response in obese adolescents[29]. Secondly, telomeres (TL) are biomarkers of biological aging. Shorter telomeres have been associated with increased adiposity in adults but we found that a weight loss intervention is accompanied by a significant increase in TL in overweight/obese adolescents. Moreover, we suggest that initial longer TL could be a potential predictor for a better weight loss response[30].

Numerous gene variants that are associated with a greater or lesser risk of the different types of cardiovascular diseases (CVDs) and of intermediate phenotypes (i.e., hypercholesterolemia, hypertension, diabetes) have been successfully identified. However, despite the close link between aging and CVD, studies analyzing the genes related to human longevity have not obtained consistent results and there has been little coincidence in the genes identified in both fields. The APOE gene stands out as an exception, given that it has been identified as being relevant in CVD and longevity[30].

Immunonutrition applications

Immunonutrition is an emergent subject that carries out studies based mainly on four investigation lines:

Evaluation of nutritional status through immunological biomarkers in presumably healthy people with risk of malnutrition (children, adolescents, adults, pregnant women, elderly, and athletes).

Different immunological and stress biomarkers can be studied in serum and plasma samples of subjects

in different life stages in order to detect the risk of potential malnutrition states, both by defect and excess. Immune system development begins in the utero environment, depending on lifestyle and nutrition of mother, and continues with lactation. After, childhood and adolescence are critical periods in which healthy habits should be instaured and will have an impact on adult age. Finally, immunosenescence is linked to subclinical deficiencies and effects on immune system and cognitive functions are the major outcomes.

Study evolution of patients with diet and immune-related diseases, such as food allergies and other atopies, eating disorders, obesity, metabolic syndrome, diabetes, cardiovascular disease (CVD), different types of cancer, and autoimmune diseases including fibromyalgia, multiple sclerosis and Alzheimer's disease, among others.

Study of nutrients' effects, bioactive compounds and both conventional and functional foods on the immune system.

Assess the impact of lifestyle determinants, such as physical activity, exercise, sedentariness, food behaviour, sleep time and stress, on the immune response. Short sleep duration during adolescence might play an important and independent role in cardiovascular and metabolic diseases through C-reactive protein (CRP).

Recommendations and final remarks

The use of immunological parameters to assess nutritional status should be considered both an individual level and epidemiological studies. This subject offers a sensitive and useful tool for detect nutritional imbalances at a subclinical level, caused both by undernutrition or overnutrition.

References

1. Romeo J, Pérez de Heredia F, Gómez-Martínez S, Díaz LE, Pozo-Rubio T, Marcos A. Food Supplements and Immune Function in Humans. In: Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases. Watson R, Preedy V, eds. San Diego, CA, USA: *Academic Press* 2013. p.145-156.
2. Pérez de Heredia F, Gómez-Martínez S, Marcos A. Obesity, inflammation and the immune system. *Proc Nutr Soc* 2012; 71(2):332-338.
3. Calder PC. Immunological parameters: what do they mean? *J Nutr* 2007; 137(3 suppl 2):773S-780S.
4. Delves PJ, Seamus JM, Burton DR, Roitt IM. Essential Immunology. 12th ed. Oxford: Wiley-Blackwell; 2011.
5. Purchiaroni F, Tortora A, Gabrielli M, Bertucci F, Gigante G, Ianiro G, et al. The role of intestinal microbiota and the immune system. *Eur Rev Med Pharmacol Sci* 2013; 17(3):323-333.
6. Uauy R. Academic-industry partnerships in addressing nutrition-[infection-immunity-inflammation] interactions. *Br J Nutr* 2007; 98 Suppl 1:S17-23.
7. Scrimshaw, N. S., Taylor, C. E. & Gordon, J. E. (1968) Interactions of nutrition and infection. Monogr. Ser. World Health Organ. 57: 3–329.
8. Wärnberg J, Ruiz JR, Ortega FB, Romeo J, González-Gross M, Moreno LA, et al. On behalf on AVENA group. AVENA study. Food and Nutritional Evaluation in Adolescents. Results obtained 2003-2006. *Pediatr Integral* 2006; Supl (1): 50-55.
9. Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, et al. Markers to measure immunomodulation in human nutrition intervention studies. *Br J Nutr* 2005; 94(3):452-481.
10. Marcos A. Eating disorders: a situation of malnutrition with peculiar changes in the immune system. *Eur J Clin Nutr* 2000; 54 Suppl 1:S61-64.
11. Marcos A. The immune system in eating disorders: an overview. *Nutrition* 1997; 13:853-862.
12. Savino W, Dardenne M. Nutritional imbalances and infections affect the thymus: consequences on T-cell-mediated immune responses. *Proc Nutr Soc* 2010; 69(4):636-643.
13. Wärnberg J. Inflammatory status in adolescents; the impact of health determinants such as overweight and fitness. Thesis for doctoral degree, 2006. Stockholm: Karolinska University Press; 2006.
14. Nova E, Gómez-Martínez S, Morandé G, Marcos A. Cytokine production by blood mononuclear cells from inpatients with anorexia nervosa. *Br J Nutr* 2002; 88(2):183-188.
15. Nova E, Martínez-Gómez D, Gómez-Martínez S, Veses AM, Calle ME, Veiga OL, Marcos A. Influence of health behaviours on the incidence of infection and allergy in adolescents: the AFINOS cross-sectional study. *BMC Public Health* 2014;14:19.
16. Codificación de la Desnutrición Hospitalaria. En: Codificación Clínica con la CIE-9-MC. Unidad Técnica de la CIE-9-MC para el Sistema Nacional de Salud. Ministerio de Sanidad y Política Social. Madrid: 2008.
17. Walrand S, Moreau K, Caldefie F, Tridon A, Chassagne J, Portefaix G, CynoberL, Beaufrère B, Vasson MP, Boirie Y. Specific and nonspecific immune responses to fasting and refeeding differ in healthy young adult and elderly persons. *Am J Clin Nutr* 2001; 74(5):670-8.
18. Marcos A, Nova E, Montero A. Changes in the immune system are conditioned by nutrition. *Eur J Clin Nutr* 2003 Sep;57 Suppl 1:S66-9.
19. Pérez de Heredia F, Garaulet M, Gómez-Martínez S, Díaz LE, Wärnberg J, Androutsos O, Michels N, Breidenassel C, Cuenca-García M, Huybrechts I, Gottrand F, Ferrari M, Santaliestra-Pasías AM, Kafatos A, Molnár D, Sjöstrom M, Widhalm K, Moreno LA, Marcos A; HELENA Study Group. Self-reported sleep duration, white blood cell counts and cytokine profiles in European adolescents: the HELENA study. *Sleep Med* 2014 Oct;15(10):1251-8.
20. Van Loveren H, Van Amsterdam JG, Vandebriel RJ, Kimman TG, Rümke HC, Steerenberg PS, Vos JG. Vaccine-induced antibody responses as parameters of the influence of endogenous and environmental factors. *Environ Health Perspect* 2001;109(8):757-64.
21. Park KH, Park H, Kim M, Kim Y, Han K, Oh EJ. Evaluation of NK cell function by flowcytometric measurement and impedance based assay using real-time cell electronic sensing system. *Biomed Res Int* 2013:210726.
22. Brandtzaeg P. Function of mucosa-associated lymphoid tissue in antibody formation. *Immunol Invest* 2010; 39(4-5):303-355.
23. Granger N, Kevil CG & Grisham MB (2006) Recruitment of inflammatory and immune cells in the gut: physiology and pathophysiology. In Physiology of the gastrointestinal tract, pp. 1137–1162 [LR Johnson, KE Barrett, JL Merchant, FK Ghishan, HM Said and JD Wood, editors]. Amsterdam: Elsevier Academic Press.
24. Doré J, Simrén M, Buttle L, Guarner F. Hot topics in gut microbiota. *United European Gastroenterol J* 2013; 1(5):311-318.
25. Santacruz A, Marcos A, Wärnberg J, Martí A, Martin-Matillas M, Campoy C, et al. Interplay between weight loss and gut microbiota composition in overweight adolescents. *Obesity (Silver Spring)* 2009; 17(10):1906-1915.
26. Sanz Y, Moya-Pérez A. Microbiota, inflammation and obesity. *Adv Exp Med Biol* 2014; 817:291-317.

27. Nova E, Wärnberg J, Gómez-Martínez S, Díaz LE, Romeo J, Marcos A. Immunomodulatory effects of probiotics in different stages of life. *Br J Nutr* 2007; 98 Suppl 1:S90-95.

28. Corella D, Ordovás JM. Aging and cardiovascular diseases: The role of gene-diet interactions. *Ageing Res Rev* 2014 Aug 24;18C:53-73.

29. Moleres A, Campión J, Milagro FI, Marcos A, Campoy C, Garagorri JM, Gómez-Martínez S, Martínez JA, Azcona-Sanjulián MC, Martí A; EVASYON Study Group. Differential DNA methylation patterns between high and low responders to a weight loss intervention in overweight or obese adolescents: the EVASYON study. *FASEB J* 2013;27(6):2504-12.

30. García-Calzón S, Moleres A, Marcos A, Campoy C, Moreno LA, Azcona-Sanjulián MC, Martínez-González MA, Martínez JA, Zalba G, Marti A; EVASYON Study Group. Telomere length as a biomarker for adiposity changes after a multidisciplinary intervention in overweight/obese adolescents: the EVASYON study. PLoS One. 2014; 9(2):e89828.