Microbiota Modulation to Reduce Cardiovascular Risk in HIV Patients: A Systematic Review

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TITLE PAGE

MICROBIOTA MODULATION TO REDUCE CARDIOVASCULAR RISK IN HIV PATIENTS: A SYSTEMATIC REVIEW

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ABSTRACT

Background: human immunodeficiency virus (HIV) infection is associated with premature and accelerated aging linked to the development of several co-morbidities, such as cardiovascular events, even after controlling for traditional cardiovascular risk factors. Since both traditional and "novel" factors are involved in the increased cardiovascular risk (CVR) observed in HIV-infected patients, new biomarkers could be needed to diagnose, evaluate and prevent the evolution of these events. Furthermore, since microbiota disturbance is involved in HIV-associated co-morbidities, all strategies focused on reversing or modulating gut microbiota (GM) composition and/or functionality could be of interest. In this context, a solid scientific evidence based on well-designed clinical trials is needed to support the use of prebiotics, probiotics, symbiotics and fecal transplantation (FT) to modify GM in HIV-infected patients and, therefore, to reduce the incidence of cardiovascular disease (CVD) in this population.

Methods: a search in *PubMed* has been carried out covering all clinical trials (*Clinical Trial* filter) including adult humans (19+ years) and whose results have been published in the last 10 years. Besides, the results obtained from ClinicalTrials.gob have been included and described in the present review Thus, we have reviewed the main results obtained from 3 clinical trials concerning the effects of prebiotics, 25 concerning probiotics, 6 concerning symbiotics and 4 concerning FT.

Results: none of the trials included in this review investigated if these compounds were able to reduce cardiovascular events in HIV patients.

Conclusions: the huge variability observed in the type of compound used, the dose and the length of administration, makes difficult to analyse the results as a proper meta-

analysis and, therefore, to adopt general recommendations. Thus, there is an urgent need to investigate in this direction through robust and well-designed clinical trials.

KEYWORDS

HIV infection, aging, cardiovascular risk, gut microbiota, prebiotics, probiotics, symbiotics, fecal transplantation.

BACKGROUND

In last decades, life expectancy in human immunodeficiency virus (HIV) infection has significantly increased thanks to the improvement in clinical management and, specially, by the extended use of antiretroviral therapy (ART). Indeed, to date, HIV infection is considered a chronic disease, although life expectative is not as long as that observed in non-infected people or general population (1). This fact has been associated with premature and accelerated aging, among other factors (2). The acceleration of the aging process leads to immunosenescence, which is characterized by continuous activation of the immune system and a low-grade chronic inflammation (3). Thus, HIV patients are predisposed to co-morbidities and natural aging symptoms more frequently seen in elderly people. Therefore, HIV patients have higher rates of cardiovascular disease (CVD), mainly atherosclerosis, and also non-AIDS (acquired immune deficiency syndrome) cancers, frailty (loss of muscle mass, osteoporosis and muscle weakness), kidney or liver diseases and neurologic complications such as dementia, compared to uninfected subjects of the same age (4–10). As an example, several studies have demonstrated that HIV infection is associated with a 50% increased risk of acute myocardial infarction (AMI) beyond that explained by recognized/"classical" risk factors (4,11–13). In fact, recent studies have looked above traditional CVR factors expanding the view towards immunology, intestinal microbiome, red blood cells and endothelial function (14–17) disorders among others. Thus, the presence of factors others than "classical" cardiovascular risk (CVR) factors makes necessary to study in depth the physiopathology of these processes in the context of HIV infection. In addition, interventions to modify both traditional and "novel" risk factors are urgently needed in HIV people in order to reduce future adverse events and, therefore, to counteract the consequences of the accelerated aging and to improve their quality of life (18).

In this context, one "novel" factor recently associated with cardiovascular events in HIV-infected patients but also in the general population is gut microbiota (GM). Microbiota is the community of microorganisms which coexist in a specific environment, including bacteria, archaea, viruses and some unicellular eukaryotes (19,20). There are lots of studies that have demonstrated that alterations in GM play an important role in health and disease (21,22). Nowadays, the development of next-generation sequencing technologies allows the identification of new markers of the physiological and pathological state of each individual, which enables an approximation and a description of GM in different medical disorders (23–26). However, it is still not well established if modifications in GM are cause or consequence of the alterations in health and, therefore, every disorder should be separately and deeply analyzed. In the context of HIV, this infection is characterized by a set of structural changes of the gut epithelial barrier, immunological shifts and also modifications in the composition and functionality of the GM. In normal physiological conditions, the microorganisms are in the intestinal lumen interacting with the intestinal cells in a state of symbiosis but, when HIV infection occurs, depletion of lymphocytes T CD4 takes place in the gut-associated lymphoid tissue (GALT). This is accompanied by a rupture of the epithelial barrier, which triggers alterations in the intestinal lumen and also in the composition of the microbiota (at least at bacteria level) (27). This dysbiosis favors the passage of microorganisms and their components to the *lamina propia* and, hence, to the circulation, which is known as bacterial translocation (BT), which undergoes subsequent intestinal and systemic inflammation (28) (**Fig1).**

Dysbiosis is observed in HIV infection. Thus, all strategies focused on reversing or modulating GM composition and/or functionality could be of interest. Prebiotics, probiotics, symbiotics and even fecal transplantation (FT) have emerged as "novel"

promising therapies to control/reverse the changes observed in GM composition in HIV infection and to reduce the incidence of co-morbidities such as cardiovascular events or obesity (29–32). Nevertheless and despite the good reputation of these interventions in several recurrent diseases others than HIV infection, such as non-alcoholic fatty liver disease (NAFLD) (33), obesity (34,35), irritable bowel syndrome () (36) and *Clostridioides difficile* infection (37), it is necessary to show solid scientific evidences in order to recommend their use in a specific population such as HIV-infected people. Also, it is necessary to know which are the optimal dose, route, administration frequency and exact composition. In fact, in 2001 an expert commission gathered by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recognised the need of establishing guidelines for the evaluation of the efficacy and safety of probiotics (38), taking into account the evaluation of the toxicity and functionality of the strain using both *in vitro*, *ex vivo* and *in vivo* trials, and especially phase 2 and phase 3 clinical trials [34-35]. Concerning prebiotics, the regulation is far less strict because they are natural components. In the case of FT, the Food and Drug Administration (FDA) has to firstly answer a serious problem about if stool is a drug, a body tissue or a little of both (39) for a proper regulation, which seems to be different depending on the country. In addition, on June 13, 2019, the FDA issued a FT warning after 2 immunocompromised adults who received investigational FT developed invasive infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli*. To prevent similar problems in the future, the FDA now recommends screening donors in order to see if they are at risk of carrying multi-drug-resistant organisms (MDROs) (40). Thus, more studies are needed to control all these variables and to make clear recommendations to the general population or to specific groups, such as HIV-infected people.

METHODS

The objectives of this review were, *i*) to summarize the link between HIV infection and CVDs with special emphasis on GM involvement; ii) to describe "novel" biomarkers for cardiovascular risk in HIV infected patients; *iii*) to investigate if there is a solid scientific evidence (based on well-designed clinical trials) that supports the use of prebiotics, probiotics, symbiotics and FT to modify GM in HIV-infected patients. To achieve the last objective a search in *PubMed* has been carried out covering all clinical trials (*Clinical Trial* filter) including adult humans (19+ years) and whose results have been published in the last 10 years. We have limited the search to clinical trials because they are in the top of the "pyramid of scientific evidence", just below meta-analysis and systematic reviews (41). Meta-analyses and systematic reviews have also been consulted to confirm the clinical trials chosen for this review. A ten-years period has been chosen because from the 688 results obtained in *PubMed* with the keywords "microbiota HIV", 657 were published in the last 10 years, which means a huge and representative proportion (95.49%) of the total investigation developed in this field. Besides, a search in ClinicalTrials.gob has also been carried out and these studies have also been included and described in the present review (42).

RESULTS

1. CARDIOVASCULAR DISEASE IN HIV-INFECTED PATIENTS: FOCUS ON ATHEROSCLEROSIS

In this review, we have focused on atherosclerosis due to the fact that it constitutes a risk "*per se"* of developing other CVDs such as heart attack or stroke both in the general population as well as in HIV-infected patients. There are few differences in the histology of the atheromatous plaques of necropsy studies between HIV patients and uninfected patients, which suggests that the physiopathology of the development of the atheromatous plaque is quite similar (43) and consists on the well-known 3 phases: i) leukocyte infiltration into the subendothelial space and endothelial dysfunction, ii) formation of the lipid core of the atheromatous plaque, and iii) destabilization of the atheromatous plaque (44). Thus, we assume that the same CVR factors that contribute to de development of atherosclerosis affecting general population will also affect HIV-infected patients. The role of these traditional CVR factors in the development of cardiovascular events in HIV-infected people have been brilliantly reviewed by others (45).

In addition to the "classical" CVR factors, some authors consider that HIV infection acts as a CVR factor *"per se"*. At vascular level, during leukocyte (mainly lymphocytes and macrophages) infiltration into the subendothelial space, leukocytes from HIV-infected patients have greater penetration capacity (46), possibly due to the fact that viral protein TAT increase the permeability of the vascular endothelium (47). Once installed in the subendothelial space, the lymphocytes get in contact with the endothelial cells, and these induce a significant increase of the viral replication inside the lymphocytes (through major histocompatibility complex (MHC) class II – T-cell receptors (TCRs) interaction) and, therefore, of the expression of viral proteins (48). The endothelial cells in contact with the viral protein TAT show more expression of

macrophage attracting cytokines such as monocyte chemoattractant protein 1 (MCP-1/CCL2) (49) and adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) (50), which attracts macrophages towards the arterial wall and facilitates their penetration into the subendothelial space through fibronectin (51). Moreover, viral proteins TAT and GP120 induce a rise in the production of endothelin-1 in the vascular endothelium, the most powerful vasoconstrictor known (52,53). Once macrophages have been installed in the subendothelial space, they express the "scavenger" receptor CD36, which will recognize the oxidized lipoproteins (4). At this point, the macrophages start to phagocytize oxidized lipoproteins accumulating cholesterol esters inside and becoming a foam cell. These cells will die by various mechanisms and their cholesterol stores will merge forming the lipid core of the plaque (44). The reverse cholesterol transport mediated by HDL must be able to counteract this situation, but their receptor ABCA1 is inhibited by the viral protein NEF (54). Then, the atheromatous plaque can be eroded resulting in a desquamation of the endothelial cells, which uncovers the matrix and origins the thrombus formation (44). In this point, HIV "*per se"* may induce the expression of metalloproteases that promote the endothelial desquamation (55,56). The contact of the plaque core with the arterial space induces the platelet aggregation. When platelets are activated they liberate inflammatory cytokines such as RANTES (regulated on activation, normal T-cell expressed and secreted) that reactivate the process initiated by macrophages (44) **(Fig2).**

In addition, the immune activation and persistent chronic inflammation observed in HIV-infected patients despite ART, are two factors strongly predictive of atherosclerosis (57), and they are associated with the development of non-AIDS events, with an increase in morbidity and mortality and, therefore, with a substantial reduction of patients' quality of life (28,58–60). Besides, HIV infection induces GM dysbiosis and

ART is not able to completely reverse this situation (27). Only preliminary studies carried out with a small number of patients have showed an improvement in GM composition after treatment with integrase inhibitors (27).

2. CARDIOVASCULAR DISEASE RISK MARKERS IN HIV-INFECTED PATIENTS

Assessment of soluble and cellular proteins together with other approaches such as metagenomic or metabolomic could be used as biomarkers of cardiovascular events in HIV-infected people (61–67). Thus, we have herein classified these potential biomarkers as: (i) biomarkers dependent or partially dependent on microbiota; (ii) biomarkers independent on microbiota.

2.1. Biomarkers dependent on microbiota

2.1.1. Trimethylamine N-oxide

Choline (vitamin B), carnitine and betaine (trimethylglycine) present in diet (mainly in milk, red meat, fish, vegetables and mushrooms) are degraded by the action of trimethylamine lyases present in GM producing trimethylamine (TMA). TMA is subsequently processed by hepatic monooxygenases leading to trimethylamine N-oxide (TMAO) **(Fig3)**. TMAO is normally measured in blood by ultra-high pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), although there are also other methods developed to measure such metabolite (68). The relation between very high levels of TMAO and the development of future cardiovascular events such as atherosclerosis has been recently recognised in non-HIV patients (69–74). In fact, elevated blood levels of TMAO are associated with worse outcomes in patients with CVD, such as coronary artery disease or heart failure, and with higher mortality (70,74–78). In HIV patients, several studies have also demonstrated the relation between TMAO

and cardiovascular events (14,15,79,80). In one of these studies, high levels of TMAO were significantly associated with an increased risk of plaque incidence in the carotid artery (80). Furthermore, levels of TMAO were associated with higher levels of sCD14 and sCD163, two clusters of differentiation also involved in CVDs, as will be described later (80). Another study demonstrated an independent association between higher TMAO concentrations and higher CVR and between higher TMAO concentrations and multimorbidity (presenting ≥3 comorbidities) in HIV-infected patients (81). In the study from Haismman JM *et al.* (2017) no differences in TMAO blood levels were observed between naïve and ART-treated HIV patients, although high levels of its precursors, carnitine and betaine, were increased in untreated HIV patients compared to treated ones (82). It will be necessary to ensure if the lack of differences observed in that study between naïve and ART-treated people could be explained by diet. If diet were similar among both groups, it could be suggested that GM is different among both groups in terms of the ability to metabolize TMAO's precursors. In addition, the lack of differences observed in this study did not discard a significant increased cardiovascular risk in HIV-infected patients and other biomarkers of CVD should also be analysed. In fact, a very recent study has failed to find a strong association between TMAO, gut dysbiosis and inflammation in HIV infection (83). Therefore, the role of this metabolite as a marker of CVD in HIV population deserves further investigation.

2.1.2. Kynurenine/tryptophan ratio

Tryptophan is an essential amino acid (obtained from turkey, chicken, milk, cheese, fish, eggs, tofu, soybeans, sesame and pumpkin seeds, nuts, peanuts and peanut butter) indispensable to promote the liberation of serotonin, involved in the regulation of sleep and desire. Kynurenine is a metabolite resulting from degradation of tryptophan

through the action of GM (84). A study demonstrated that HIV patients (45-50 years old under protease inhibitors) presented a lipid profile that predispose to CVDs and also a higher kynurenine/tryptophan ratio (measured in plasma by UHPLC-MS/MS) than healthy controls (85). However, more studies are needed to validate this ratio as an easy plasma biomarker of CVR in HIV-infected patients.

2.1.3. sCD14 and sCD163

As mentioned before, sCD14 and sCD163 are two clusters of differentiation that act as monocyte activators and inflammation markers as well as indicators of BT. sCD14 (soluble CD14) is secreted by activated monocytes (by proteolytic processing) after the join of lipopolysaccharide (LPS) to the toll-like receptor 4/MD-2 (TLR-4/MD-2) complex mediated by lipopolysaccharide binding protein (LBP) and mCD14 (membrane CD14). sCD14 can join in turn to more LPS and transfer it to mCD14 triggering the activation cascade of a greater number of monocytes (86) **(Fig4)**. On the other hand, CD163, expressed in monocytes, has also two forms, mCD163 (membrane CD163) and sCD163 (soluble CD163). mCD163 is responsible of the removal of plasma hemoglobin through endocytosis of the very high-affinity complex hemoglobin–haptoglobin, thus, preventing the oxidative stress triggered by free hemoglobin through the release of the free iron, bilirubin and carbon monoxide. Moreover, sCD163 acts in the immune system but it is not yet clearly defined how. It is elevated in pathologies such as diabetes, obesity, liver disease and atherosclerosis, all of them characterized by a low-grade inflammation (87).

Regarding HIV-infection, only one study has reported a statistically significant relationship between the levels of sCD14 and sCD163 and new atheromatous plaque formation in HIV patients (88). Obviously, more studies are needed in this regard.

2.1.4. Other biomarkers related to immune activation, inflammation and bacterial translocation

D-dimer is a soluble fibrin degradation product that results from ordered breakdown of thrombin by the fibrinolytic system (89), whereas interleukin-6 (IL-6) is a glycoprotein with pleiotropic effects on inflammation, immune response and hematopoiesis (90). High levels of IL-6 and D-dimer are associated with increased CVR in HIV patients (91,92). Besides, these biomarkers remain high even under ART, which suggest immune activation even with successful suppression of HIV replication (93).

On the other hand, interleukin-1 (IL-1) is an inflammatory cytokine involved in the pathogenesis of multitude inflammatory disorders, including atherosclerosis (94). One study revealed that high levels of IL-1 receptor antagonist (IL-1Ra) in HIV-infected patients were associated with an increased risk of myocardial infarction, supporting the hypothesis that activating IL-1 signaling pathway could be a useful way to reduce CVR in HIV patients. (95).

Finally, when platelets are activated, they secrete other inflammatory cytokines such as RANTES (also known as CCL5), which reactivates the processes initiated by macrophages attracting more leukocytes (44). It has been demonstrated that platelets of HIV patients liberate more RANTES than healthy controls (96,97), although it should be of interest to investigate if this increased RANTES production could be associated with the increased CVR observed in these patients. One study reported that people with coronary atherosclerosis had significantly higher levels of RANTES than controls (98), but no studies have been published in the context of HIV-infection. It is also interesting to point out that CCR5, the receptor of CCL5, acts as a co-receptor of some HIV-1 strains (99) and it has been linked to atherosclerosis (100). Besides, Maraviroc, a CCR5 antagonist used in some HIV-infected patients (those in which HIV-1 has tropism towards

this receptor), has led to statistically significant improvements in several CVR markers (101,102) and inflammation (103).

2.2. Biomarkers independent on microbiota

2.2.1. Endothelin-1

Endothelin-1 is synthesized by the vascular endothelium and presents vasoconstrictive, proinflammatory, profibrosis and promitogenic actions. It has been demonstrated that viral proteins TAT and GP120 are able to induce in the vascular endothelium an increase in the production of this molecule which leads to a higher vasoconstriction (52,53). Specifically, TAT regulates endothelin-1 at the transcriptional level through NF-κβ responsive sites in the promoter (52). The mechanism by which GP120 induces the production of endothelin-1 has not been described yet, but it is also at transcriptional level (104).

Elevated blood levels of endothelin-1 are independently associated with HIV-associated pulmonary arterial hypertension (PAH) maybe through the impairment of pulmonary endothelial function (105). Besides, it has been reported that plasma endothelin-1 levels are higher in HIV patients with PAH than in a non-infected population and also that levels increase with the severity of the PAH (106). The influence of ART on endothelin-1 deserves be further dilucidated.

2.2.2. Markers of oxidative stress

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences, which may lead to tissue injury (107). Besides, the relationship between oxidative stress, ART and

mitochondrial dysfunction has been documented ant it is related to alterations in the Krebs cycle and increased levels of ROS (108,109). This suggests that HIV infection and ART may induce oxidative stress (110,111). It has been demonstrated a decrease in the activity of paraoxonase-1 (PON-1), an antioxidant enzyme responsible for inhibiting the lipid peroxidation (112), in HIV-infected patients, which corroborates that HIV infection is accompanied by an increased oxidative stress. However, further studies are required to dilucidated the relation between HIV, ART and oxidative stress (113).

The marker of endothelial dysfunction asymmetric dimethylarginine (ADMA) is an inhibitor of nitric oxide synthase (NOS) and it has been proposed as a marker of CVR and oxidative stress in several pathologies. ADMA plasma levels are increased after HIV infection and they seem to be reduced during ART (114). It has been reported that HIV-infected patients with type 2 diabetes show higher ADMA plasma levels than HIV-infected patients without type 2 diabetes (115,116), and taking into account that type 2 diabetes is a CVR factor it is reasonable to assume that it could be a good CVR marker in HIV population.

2.2.3. Lipoprotein-associated phospholipase A2

Lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor acetylhydrolase (PAF-AH), is an enzyme produced by inflammatory cells (mainly macrophages) and responsible for hydrolyzing the oxidated phospholipids present in LDLs (117). It has been suggested that this enzyme could adequately and independently predict the onset of cardiovascular events in the general population (118–120). HIV patients showed higher levels of Lp-PLA2 in comparison with a healthy non-infected population (121,122), and these levels where associated with an elevated CVR in this population (122).

2.2.4. CD4/CD8 ratio

A low CD4/CD8 ratio is considered a hallmark of immunosenescence and is an independent predictor of mortality in the general population (123–125). Specifically, in HIV-infected patients under ART, it has been found a negative correlation between this ratio and immune activation and immunosenescence (126,127). Besides, CD4/CD8 is independently associated with markers of aging and, specifically, to carotid intima-media thickness (cIMT) and vascular stiffness (128). In this context, the analysis of the ICONA cohort showed that CD4/CD8 ratio below 0.30 compared to one above 0.45 was associated to mortality and to have non-AIDS events (129). Finally, another study demonstrated that this ratio was negatively correlated with progression of cIMT (130).

3. MICROBIOTA MODULATION TO REDUCE CARDIOVASCULAR RISK IN HIV PATIENTS: SCIENTIFIC EVIDENCE FROM CLINICAL TRIALS

3.1. Prebiotics

Prebiotics were firstly described in 1995 by Glenn Gibson and Marcel Ruberoid as "*a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health*" (131).

Eight studies have been published in *PubMed* concerning prebiotics in HIV-infected patients in the last 10 years. Only 5 are derived from clinical trials and of these, 3 have been discarded because they are related to symbiotics and, therefore, they will be incorporated in the appropriate section of this review. Of the 2 remaining articles, only one (NCT01838915) is registered in *ClinicalTrials.gov* and, interestingly, one of its objectives was to evaluate the effectiveness of the treatment to modify markers of endothelial dysfunction. However, the results concerning this issue have not been

presented yet. In addition, another clinical trial (NCT04058392) appears in this website and whose aim was to evaluate tolerance and changes in inflammation and microbe composition in stools of HIV-infected people and to assess changes of gut barrier by doing biopsies by colonoscopy. However, it is not completed yet. **Table 2** shows these 2 clinical trials registered in *ClinicalTrials.gov*, however, we have discussed the results obtained from all 3 clinical trials focused on prebiotics and HIV infection independently of its registration in the website.

Concerning the results published, the administration of prebiotics in HIV patients has proven to be able to modify microbiota composition at several taxonomical levels. However, this influence is only observed in naïve patients (132), possibly due to the fact that time since diagnosis of HIV infection is shorter in naïve patients in comparison with ART-treated patients. Thus, it is possible that the changes at intestinal level in naïve patients induced by the virus are mild and, therefore, can be more easily reversed whereas ART-treated patients could present a more established and stabilised GM. Specifically, naïve HIV patients treated during 6 or 12 weeks with different combinations of prebiotics (short chain galactooligosaccharides/long chain fructooligosaccharides/pectin hydrolysate-derived acidic oligosaccharides/glutamine (scGOS/lcFOS/pAOS/glutamine)) showed significant increases in *Bifidobacterium* genus (from 2.8% to 15.7%, p=0.0007) (133) and in the phyla Firmicutes (included *Faecalibacterium, Catenibacterium, Blautia* and *Eubacterium*) and Actinobacteria (included *Collinsella* and *Corinebacterium)* (132). In contrast, statistically significant decreases in *Clostridium lituseburense/Clostridium histolyctum* (including *Clostridium perfringens* and *Clostridium difficile*) (from 0.016% to 0.002%, p=0.009) (133) and in *Eubacterium rectale/Clostridium coccoides* (p=0.035) have also been demonstrated in these patients (132).

Moreover, the administration of scGOS/lcFOS/pAOS for 12 weeks has also been associated with decreases in BT markers such as sCD14 and LPS in naïve patients (133). However, using a similar mixture of prebiotics (scGOS/lcFOS/glutamine) for 6 weeks no statistically significant differences were observed in these BT markers (132). These discrepancies could be due to the different duration of the intervention (12 weeks *vs* 6 weeks). Thus, microbiota is rather stable in adulthood and it is necessary longer periods of administration in order to modify it.

It is also interesting to point out that although it has not been observed changes neither in the viral load nor in the number of lymphocytes T CD4 after prebiotic administration, a decrease in the activation of these lymphocytes has been observed after the administration for 12 weeks of scGOS/lcFOS/pAOS (from 0.52% to 0.27 % in naïve patients, $p<0.01$) (133) or after the intervention with scGOS/lcFOS/glutamine for 6 weeks (132). This could mean that, although the intervention is not able to restore the number of lymphocytes T CD4, it facilitates a reduction of the activation of the immune system, which could be beneficial for these patients, given the persistent chronic inflammation characteristic of HIV infection.

In these two studies, the administration of prebiotics turned out to be safe without being observed modifications in the hepatic or the renal function (132,133). Nevertheless, in terms of tolerability, one of the studies has reported complications (mainly diarrhea), which disappeared 4 weeks after start the intervention (133).

To sum up, the administration of prebiotics in HIV patients was able to modify microbiota composition and to reduce the activation of lymphocytes T CD4, while the effects on markers of inflammation and BT were not clear enough. Besides, there are not studies that analyze if these changes are able to reduce the incidence of co-morbidities in

this population and, specifically, if they are able to reduce CVR. Moreover, the type of compound and the time of administration is a variable to consider when drawing conclusions and making general recommendations. Therefore, it would be of interest to carry out more clinical trials to evaluate the effects of these compounds on CVR factors in HIV-infected patients and, specially, to carry out a follow-up study that could corroborate a significant reduction in cardiovascular events in those subjects with improvements in inflammation and intestinal dysbiosis.

3.2. Probiotics

The internationally endorsed definition of probiotics is "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*" (134). It has been suggested that the use of probiotics, such as *Lactobacillus*, *Bifidobacterium* and *Enterococcus* that can benefit the intestinal and immune system may be inexpensive and clinically important as coadjutants of ART in order to reduce HIV-related morbidity and mortality (135). The mechanisms by which probiotics may interfere with HIV have been brilliantly summarized by *D'Angelo et al*., 2017 (136). In essence, probiotics can rebalance microbiota by competition with pathogens, can improve the intestinal barrier reducing BT and, consequently, decreasing systemic inflammation and can restore the mucosal immune function lowering local inflammation (136).

Thirty-five studies have been published in PubMed concerning probiotics in HIV-infected patients in the last 10 years. Only 15 are derived from clinical trials and of these, one has been discarded because it only contains results obtained from symbiotics and, therefore, it will be incorporate in the following section of the review. Another 3 publications have been dismissed because they do not include HIV-infected patients. Besides, we have added two more clinical trials that did not appear in the search carried

out in PubMed but meets the inclusion criteria (137,138) and from the unique meta-analysis and/or systematic review published (139), we have appended one more clinical trial that meets the selection criteria and have not been included previously (140). Of the 17 remaining articles, only 5 appear registered in *ClinicalTrials.gov*. In addition, in this website another 11 clinical trials different from the abovementioned appeared. Thus, **Table 3** shows all clinical trials registered in *ClinicalTrials.gov* although the discussion of all 25 clinical trials focused on probiotics and HIV infection has been included in this section.

The administration of probiotics in HIV infected patients has proved to be able to modify microbiota composition both in naïve patients and in ART-treated patients. Specifically, intervention of ART-treated patients with different combinations of probiotics was associated with an increase of the relative abundance of Actinobacteria and Firmicutes phyla [administration of 250 ml/day of fermented skimmed milk supplemented with *Lactobacillus rhamnosus* GG (10^8 CFU/ml), *Bifidobacterium animalis* subsp. *lactis* B-12 (10^8 CFU/ml) and *Lactobacillus acidophilus* La-5 (10^7 CFU/ml); 8 weeks] (141), and also with an increase of *Bifidobacteria spp* [administration of Visbiome®; twice a day; 24 weeks] (142). A statistically significant increase in the abundance of *Megamonas* and *Desulfovibrionales* levels after the administration for 12 weeks of capsules with 56.5 mg living *Saccharomyces boulardii* have also been observed (143). In contrast, a statistically significant reduction of Bacteroidetes phylum and some species of Clostridiales and *Catenibacterium* have also been demonstrated after the administration of 250 ml/day of fermented skimmed milk supplemented with *Lactobacillus rhamnosus* GG (10^8 CFU/ml), *Bifidobacterium animalis* subsp. *lactis* B-12 (10^8 CFU/ml) and *Lactobacillus acidophilus* La-5 (10^7 CFU/ml) for 8 weeks (141) and after the administration of capsules with 56.5 mg living *Saccharomyces boulardii*,

respectively (143). In a study carried out in naïve patients, a decrease in the total load of bacteria in stools with respect to the placebo group and an increase of the *Bifidobacterium* spp. concentration along with a decrease of the *Clostridium* spp. concentration was demonstrated after daily administration of *Lactobacillus rhamnosus* HN001 plus *Bifidobacterium lactis* Bi-07 (10^9 CFU/ml) for 16 weeks (144). Finally, in a study with both naïve and ART-treated patients, a reduction of the orders Enterobacteriales (p=0.018) and Erysipelotrichales (p=0.037), entirely driven by a reduction of the Enterobacteriaceae and Erysipelotrichaceae families were observed along with an increase in an uncultured genus from the Lachnospiraceae family (p=0.015) and *Ruminiclostridium* (P=0.023) from the Ruminicoccaceae family [administration of 2 capsules daily of *Lactobacillus rhamnosus* GG (10^{10} CFU); 8 weeks] (145).

Concerning inflammation, the administration twice a day for 48 weeks of 1g packet (from now indicated as packet A) containing *Streptococcus salivarius* ssp. *thermophilus* (at least $2.04x10^{14}$ CFU), Bifidobacteria represented by *B. breve*, *B. infantis* and *B. longum* (at least $9.3x10^{13}$), *Lactobacillus acidophilus* (at least 2 billion CFU), *Lactobacillus plantarum* (at least $2.20x10^8$ CFU), *Lactobacillus casei* (at least $2.20x10^8$ CFU), *Lactobacillus delbrueckii* ssp. *bulgaricus* (at least $3x10^8$ CFU) and *Streptococcus faecium* (at least $3x10^7$ CFU) was not able to reduce D-dimer levels (146). However, the administration of 250 ml/day of fermented skimmed milk supplemented with *Lactobacillus rhamnosus* GG (10^8 CFU/ml), *Bifidobacterium animalis* subsp. *lactis* B-12 (10^8 CFU/ml) and *Lactobacillus acidophilus* La-5 (10^7 CFU/ml) for 8 weeks was able to reduce both the levels of D-dimer (p=0.03) and IL-6 (p=0.06) compared to the placebo group (141). In addition, the administration for 12 weeks of *Saccharomyces boulardii* has also been able to reduce IL-6 levels respect to the placebo group (137). These discrepancies could be due to the different combinations used and, therefore, it makes

difficult to conclude the positive effects of probiotics on inflammation or at least on these parameters measured (D-dimer and/or IL-6). In relation to the effects on BT, different combinations of probiotics (capsules with 56.5 mg living *Saccharomyces boulardii*, packet A and *Saccharomyces boulardii*) have been able to decrease LBP levels with respect to the placebo group (137,143,146). However, no effects in sCD14 or sCD163 levels have been observed (141,143,145,146) despite the fact that sCD14 has been suggested to be a more relevant biomarker of disease progression as it reflects the host response to products of BT (147). These results make very difficult to confirm the beneficial effects of these probiotics on BT.

On the other hand, it has been observed an increase in the number of lymphocytes T CD4 in ART-treated patients after the administration of *L. rhamnosus* GR-1 and *L. reuteri* RC-14 for 25 weeks (148). Moreover, it has also been observed an increase in the percentage of lymphocytes T CD4 in the probiotic group after the administration of packet A (146). The administration *of Bacillus coagulans* GBI-30, 6086 (Ganeden BC^{30}; 2x1012 CFU) [once daily; 90 days] was also associated with a significant increase in the percentage of lymphocytes T CD4 compared to placebo (+2.8% vs. -1.8%, p=0.018), although their concentrations were generally unchanged inside each group (140). Nonetheless, no increases were observed after the administration of 125 ml yoghurt fortified with *L. rhamnosous* GR-1 (1.23x10^9 CFU/ml) [once a day; 4 weeks], possibly due to the lower duration of the intervention (149). It is also interesting the decrease observed in the activation markers CD38 and HLA-DR after the administration of packet A (146) and after the administration of Visbiome® for 24 weeks (150).

In all these studies, the administration of probiotics turned to be safe without side effects or modifications in hepatic or renal functions (138,140,146,148–152).

In summary, to date, we have some scientific evidence showing that the administration of probiotics in HIV patients is able to modify microbiota composition, increase the number of lymphocytes T CD4, decrease their activation and reduce LBP levels. There are more studies than in the case of prebiotics but, in general terms, these studies do not address the potential effects of these probiotics on CVR in HIV population. Moreover, it is necessary to assess if the changes observed in markers of inflammation and BT after the ingestion of probiotics could lead to improvements in the incidence of co-morbidities and, specifically, CVR, in this population. In addition, the huge variability in the type of compound used, the dose and the time of administration makes it impossible to obtain verified and confirmed conclusions. Therefore, the first step would be to make a good description using large cohorts of the bacterial species that are decreased in HIV-infected patients. Secondly, these depleted species would be good candidates to ingest as probiotics to restore GM, but they should pass the requirements imposed by FAO and WHO to be used with any doubt of their safety. And, finally, the effects of these probiotics should be assessed in HIV-infected patients taking into account markers of inflammation, BT but also CVR, among others.

3.3. Symbiotics

The term symbiotics was introduced by Gibson and Roberfroid in 1995 to describe a combination of synergistically acting prebiotics and probiotics (131).

Five studies have been published in *PubMed* concerning symbiotics in HIV-infected patients in the last 10 years. Only 4 were derived from clinical trials. Of the 4 remaining articles, 3 appear registered in *ClinicalTrials.gov*. In addition, 2 more clinical trials were included in this website. The first one (NCT03568812) is still recruiting patients and its objective was to assess changes in immunity and in inflammation and BT

markers. The objective of the second trial (NCT03542786), also in a recruiting state, was to evaluate inflammation and premature aging. It will be interesting to analyze the results of these clinical trials as soon as they are concluded. **Table 4** shows clinical trials concerning symbiotics registered in *ClinicalTrials.gov*. Again, the discussion of all 6 clinical trials has been included in this review.

The administration of symbiotics in HIV patients has proved to be able to modify GM composition. Specifically, the administration for 16 weeks of 10 g of agavins from *Agave tequilana* plus *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* Bi-07 (10^9 CFU/ml) in naïve patients induced a statistically significant decrease in total load of bacteria in stool with respect to the placebo group (144). Similarly, in another study carried out in ART-treated patients, an increase in the levels of *L. plantarum* and *P. pentosaceus* with respect to the placebo group was detected after the administration of Synbiotic 2000® during 4 weeks (153). However, the administration of PMT25431 during 48 weeks in naïve patients who initiated ART was not accompanied by significant changes in gut microbiota (154).

Concerning inflammation, a study with naïve patients demonstrated a statistically significant decrease in IL-6 levels with respect to the beginning of the intervention after the administration of 10 g of agavins from *Agave tequilana* plus *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* Bi-07 (10^9 CFU/ml) (144). However, the administration of PMT25431 during 48 weeks in naïve patients who initiate ART was not able to induce additional changes in IL-6 levels (154). Moreover, no effects were observed in the plasma levels of TNF-α, IL-1β, IL-10, sCD14 or sCD163 in naïve HIV-infected patients after the administration of 10 g of agavins from *Agave tequilana* plus *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* Bi-07 (10^9 CFU/ml) (144).

No actions of PMT25341 administration for 48 weeks were observed in naïve patients who initiated ART (154), and no effects were either observed in ART-treated patients after the administration of Synbiotic 2000® during 4 weeks (153).

An increase in the levels of lymphocytes T CD4 with respect to the beginning of the treatment was observed in naïve HIV-infected patients after the administration of 10 g of agavins (144). However, no effects were observed after 4 weeks of administration of Synbiotic 2000® in ART-treated patients (153). These discrepancies could be due to the different length of intervention, the different product used and the fact that in the second study, subjects were ART-treated and their GM could be more established, as previously mentioned. Finally, the administration of PMT25341® for 48 weeks in naïve HIV-infected patients who initiate ART did not induce additional changes to those exerted by ART at both levels and activation of T CD4 lymphocytes (154).

In all these studies, the administration of symbiotics was safe without side effects (144,153,155). Besides, the administration of LACTOFOS© twice daily for 30 weeks in naïve patients was able to significantly reduce diarrhea (reduction of 21.8% in incidents), nausea and/or vomits (reduction of 28.8% in incidents), constipation (reduction of 13.2% in incidents) and dyspepsia (reduction of 24.5% in incidents) (155).

In general terms, the results showed that the administration of symbiotics in HIV patients was able to modify microbiota composition, while the effects on the number of lymphocytes T CD4 and the markers of inflammation and BT were not as clear as in the case of probiotics, which results interesting considering that symbiotics are a mixture of probiotics and prebiotics. However, these discrepancies could be a consequence of the different probiotic used or/and the different length of administration. Similarly as what was observed in prebiotics and probiotics, there are not studies focused on the long-term

consequences of these changes. Moreover, there is a huge variability in the type of compound, the dose and the length of administration, so it is impossible to make conclusions and general recommendations.

3.4. Fecal transplantation

FT is the transfer of stools from a healthy donor into a patient with a disease characterized by a significant altered microbiome. Its objective is to restore the microbiota and, therefore, to treat the disease (156). FT is not new, in fact, there are reports of its use in ancient China for various purposes (157). It was firstly described as a treatment for pseudomembranous colitis in the 1950s (158) and in the recent years it has gained acceptance as a safe and effective treatment for recurrent *Clostridioides difficile* infection (CDI) associated diarrhea (159–163).

It has been observed that HIV patients treated for CDI by FT do not show any adverse events (164). However, in the same study, 14% of the inflammatory bowel disease (IBD)-patients experienced a disease flare requiring hospitalization in some cases (164). Therefore, FT involves an uncontrollable risk, particularly in immunocompromised patients if donor samples are not checked (165–167). In this context, the use of fecal filtrates could be a great alternative (168). Thus, despite all the advances in this field, the optimal protocol for FT is unknown and more well-designed studies and clinical trials are required because controversy issues are arising nowadays (169).

To our knowledge, 4 clinical trials have been registered up to date in *ClinicalTrials.gov*, while the results of these clinical trials have not been published yet nor uploaded to *PubMed*. Thus, it will be interesting to analyze the results of these clinical

trials in the future. **Table 5** shows clinical trials concerning FT registered in *ClinicalTrials.gov*.

DISCUSSION

Our review has revealed that among all the GM modulatory strategies analysed (prebiotics, probiotics, symbiotics and FT), probiotics emerge as the most beneficial therapies in HIV patients to improve their immune and inflammatory state. However, there are very few studies focused on their effects on specific CVR markers in HIV patients and focused on the long-term consequences of these effects on inflammation. In fact, follow-up studies to corroborate if the effects of probiotics on BT and inflammation lead to statistically significant reductions in cardiovascular events in those subjects with improvements in inflammation, intestinal dysbiosis and CVR factors are missing. In addition, a huge variability in the type of compound, the dose and the length of administration has been observed among the trials, although it seems that the most positive effects have been observed in long-term duration of the intervention. This could suggest that due to the fact that microbiota is quite stable in adulthood, a duration between 12 and 40 weeks is required to be able to modify/modulate it.

CONCLUSIONS

To sum up, HIV-infected patients have significant increased rates of cardiovascular events even after controlling for traditional CVR factors and this significantly affects their quality of life and represents a significant increase in healthcare costs. The usage of probiotics or other GM modulatory strategies along with the standard of care (i.e., ART) could be a good strategy to modify CVR factors in this population. However, more prospective controlled clinical trials with larger number of patients and with standardized dose, and duration of therapies are needed. Patients´ follow-up will be also required to confirm the results.

LIST OF ABBREVIATONS

ADMA: asymmetric dimethylarginine

AIDS: acquired immune deficiency syndrome

AMI: acute myocardial infarction

ART: antiretroviral therapy

BT: bacterial translocation

CDI: Clostridioides difficile infection

CFU: colony forming unit

cIMT: carotid intima-media thickness

CVD: cardiovascular disease

CVR: cardiovascular risk

FAO: Food and Agriculture Organization

FDA: Food and Drug Administration

FT: fecal transplantation

GALT: gut-associated lymphoid tissue

GM: gut microbiota

HIV: human immunodeficiency virus

HPV: human papillomavirus

IBD: inflammatory bowel disease

IBS: irritable bowel syndrome

IL-1: interleukin-1

IL-1Ra: interleukin-1 receptor antagonist

IL-6: iterleukin-6

INR: immunological non-responder

IR: immunological responder

LBP: lipopolysaccharide binding protein

lcFOS: long chain fructooligosaccharides

Lp-PLA2: lipoprotein-associated phospholipase A2

LPS: lipopolysaccharide

mCD14: membrane CD14

mCD163: membrane CD163

MCP-1: monocyte chemoattractant protein

MDROs: multi-drug-resistant organisms

MHC: major histocompatibility complex

MSM: men who have sex with men

NAFLD: non-alcoholic fatty liver disease

NOS: nitric oxide synthase

PAF-AH: platelet-activating factor acetylhydrolase

PAH: pulmonary arterial hypertension

pAOS: pectin hydrolysate-derived acidic oligosaccharides

PON-1: paraoxonase-1

RANTES: regulated on activation, normal T-cell expressed and secreted

sCD14: soluble CD14

sCD163: soluble CD163

scGOS: short chain galactooligosaccharides

TCRs: T-cell receptors

TLR-4: toll-like receptor 4

TMA: trimethylamine

TMAO: trimethylamine N-oxide

UHPLC-MS/MS: ultra-high pressure liquid chromatography coupled to tandem mass spectrometry

VCAM-1: vascular cell adhesion molecule-1

WHO: World Health Organization

DECLARATIONS

Ethics approval and consent to participate: not applicable.

Consent for publication: not applicable

Availability of data and materials: all data generated or analysed during this study are included in this published article.

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FIGURE LEGENDS

Fig1. In normal physiological conditions, the microorganisms are in the intestinal lumen interacting with the intestinal cells in a state of symbiosis but, when HIV infection occurs, depletion of lymphocytes T CD4 takes place in the GALT (gut-associated lymphoid tissue). This is accompanied by a rupture of the epithelial barrier, which triggers alterations in the intestinal lumen and also in the composition of the microbiota (at least at bacteria level) (27). This dysbiosis favors the passage of microorganisms and their components to the *lamina propia* and, hence, to the circulation, which is known as bacterial translocation (BT), which undergoes subsequent intestinal and systemic inflammation (28). **A)** Gut homeostasis. **B)** Gut dysbiosis after HIV infection.

Original. Made with *PowerPoint*.

Fig2. General scheme of atheroma plaque formation and effects of HIV infection (in Arabic numbers). Modified from *Alonso-Villaverde Lozano (2009)* (44). MHC II (major histocompatibility complex class II); TCRs (T-cell receptors); MCP-1 (monocyte chemoattractant protein 1); VCAM-1 (vascular cell adhesion molecule 1).

Original. Made with *Biorender*.

Fig3. Processing pathway of choline, carnitine and betaine from diet to produce TMAO and clinical effects of TMAO. TMA (trimethylamine); TMAO (trimethylamine N-oxide).

Original. Made with *Biorender*.

Fig4. sCD14 is secreted by activated monocytes after the join of LPS to the TLR-4/MD-2 complex mediated by LBP and mCD14. sCD14 can join in turn to more LPS and transfer it tomCD14 triggering the cascade activation of a greater number of monocytes. sCD163 acts in the immune system but it is not yet clearly defined how. LPS (lipopolysaccharide); TLR-4 (toll-like receptor 4); LBP (lipopolysaccharide binding protein); sCD14 (soluble CD14); mCD14 (membrane CD14); sCD163 (soluble CD163).

Original. Made with *PowerPoint*.

TABLES

Table 1. Summary of the main cardiovascular risk markers characterized in HIV patients. (↑) HIV patients present higher levels than general population. (↓) HIV patients present lower levels tan general population. (¿?) Controversial results.

Dependent on microbiota	Independent on microbiota
↑ TMAO ¿?	↑ Endothelin-1
↑ Carnitine	↓ PON-1 activity ¿?
↑ Betaine	↑ ADMA
↑ Kynurenine/Trp ratio	↑ Lp-PLA2 ¿?
↑ sCD14	↓ CD4/CD8 ratio
↑ sCD163	
↑ D-dimer	
↑ IL-6	
↑ IL-1Ra	
↑ RANTES	

BT (bacterial translocation); TMAO (trimethylamine N-oxide); Trp (tryptophan); sCD14 (soluble CD14), sCD163 (soluble CD163); IL-6 (interleukin-6); IL-1Ra (interleukin-1 receptor antagonist); PON-1 (paraoxonase-1); ADMA (asymmetric dimethylarginine); Lp-PLA2 (Lipoprotein-associated phospholipase A2).

Table 2. Clinical trials registered in *ClinicalTrials.gov* (42).

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
NCT01838915	Spain	(132)	Completed	scGOS/lcFOS + glutamine 6 weeks	45	18-70 years	To assess the effects in **naïve** and **ART-treated HIV-infected patients** on: • Safety • Gut microbiota composition • Bacterial translocation • Immune activation • Endothelial dysfunction	• Safe • Modification of gut microbiota composition only in naïve patients • Decrease of the activation of lymphocytes T CD4 in naïve and ART-treated patients
NCT04058392	Canada	No	Not yet recruiting	Camu Camu (2 capsules/day) 12 weeks	22 (enrolled)	18 years and olders	To evaluate the effects in **ART-treated HIV-infected patients** on: • Tolerance • Gut microbiota composition • Inflammation • Changes of gut barrier	

scGOS (short chain galactooligosaccharides); lcFOS (long chain fructooligosaccharides); HIV (human immunodeficiency virus); ART (antiretroviral treatment).

Table 3. Clinical trials registered in *ClinicalTrials.gov* (42).

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
NCT02164344	Italy	(146)	Unknown	*S. salivarius* spp. *thermophilus* 2'04x10^{14} CFU, *B. breve*, *B. infantis*, *B. longum* 9'3x10^{13} CFU , *L. acidophilus* 2x10^{12} CFU, *L. plantarum* 2'2x 10^{8} CFU, *L. casei* 2'2x10^{8} CFU, *L. delbrueckii* ssp. *Bulgaricus* 3x10^{8} CFU and *S. faecium* 3x10^{7} CFU 1 daily dose 48 weeks	30	18-75 years	To determine the effects in **ART-treated HIV-infected patients** on: • Microbial translocation induced inflammation • Immune function and activation	• Safe • Decrease of LBP levels • Increase of lymphocytes T CD4 • Decrease of the activation markers CD38 and HLA-DR presented on lymphocytes T CD4
NCT01439841	Norway and Sweden	(141)	Completed	Biola® (*L. rhamnosous* GG 10^{8} CFU, *L. acidophilus* La-5 10^{7} CFU and *B. animalis* subsp. *lactis* Bb-12 10^{8} CFU) 1 daily dose 8 weeks	32	18 years and olders	To investigate the effects in **ART-treated HIV-infected patients** on: • Safety and tolerability • Gut microbiota composition • Immune activation • Microbial translocation • Disease progression	• Modification of gut microbiota composition • Decrease of D-dimer and IL-6 levels
NCT00517803	Canada	No	Completed	Probiotic yogurt (175 g) 1 daily yogurt 16 weeks	24	18-85 years	To compare the effects of several levels of fortified probiotic yogurt *vs*. placebo in **HIV-infected patients with cancer** on: • Inflammation • Immune function	
NCT02448238	USA	No	Completed	VSL#3® (*S. thermophilus*, *B. breve*, *B. longum*, *B. infantis*, *L. plantarum*, *L. acidophilus*, *L. paracasei* and *L. bulgaricus* (9x10^{11} CFU)) 1 daily dose	23 (only females)	18 years and olders	To assess the effects in **naïve HIV-infected Malian women** on: • Safety, tolerability and level of stress • Inflammation	

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
				12 weeks			• Bacterial translocation • Immune function	
NCT02764684	Denmark	No	Completed	*L. rhamnosus* 2 daily doses 8 weeks	45	18 years and olders	To evaluate the effects in **naïve HIV-infected patients** on: • Gut microbiota composition • Microbial translocation • Lipid metabolism • Cardiovascular risk factors • Systemic and gut inflammation	
NCT02441231	Canada	No	Unknown	Visbiome® 2 daily doses ($9x10^{14}$ CFU/day) 48 weeks	36 (only males)	19 years and olders	To investigate the effects in **ART-treated HIV-infected men** on: • Microbial translocation • Immune cells function and activation	
NCT00536848	Tanzania	(138)	Unknown	*L. rhamnosous* GR-1 and *L. reuteri* RC-14 ($2x10^9$ CFU) 1 daily dose 24 weeks	65 (only females)	18-45 years	To determine the effects in **naïve HIV-infected women with vaginosis** on: • Diarrhea • Immune system • Bacterial vaginosis	• No improvements in bacterial vaginosis
NCT01908049	Spain	(137)	Unknown	*S. boulardii* 3 daily doses 12 weeks	44	18 years and olders	To assess the effects in **ART-treated HIV-infected patients** on: • Bacterial translocation • Gut microbiota composition	• Decrease of IL-6 and LBP levels

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
							• Immune function • Viral load	
NCT02706717	USA	No	Completed	Visbiome® 1 daily dose 24 weeks	93	18 years and olders	To evaluate the effects in **ART-treated HIV-infected patients** on: • Inflammation	
NCT02276326	Italy	(142)	Recruiting	VSL#3® (*S. thermophilus*, *B. breve*, *B. longum*, *B. infantis*, *L. plantarum*, *L. acidophilus*, *L. paracasei* and *L. bulgaricus* ($9x10^{11}$ CFU)) 4 daily doses 16 weeks	20	18 years and olders	To determine the effects in **ART-treated HIV-infected patients** on: • Neurocognitive profile	• Modification of microbiota composition • Improvement of neurocognitive profile
NCT02640625	Norway	No	Completed	*L. rhamnosus* GG, *L. acidophilus*, *L. bulgaricus*, *B. animalis* subsp. *lactis* and *S. thermophilus* 1 daily dose 10 weeks	60 (only males)	25-65 years	To assess the effects in **ART-treated HIV-infected men** on: • Safety • Biological effects To investigate differences between **IR** and **INR** in: • Microbial composition • Mucosal barrier function	
NCT01184456	USA	No	Completed	GanedenBC30® (*Bacillus coagulans* GBI-30 andPTA-6086 ($2x10^{12}$ CFU)) 1 daily dose 12 weeks	24	18 years and olders	To evaluate the effects in **ART-treated HIV-infected patients** on: • Bacterial translocation	
NCT04297501	China	No	Completed	*Bifidobacterium* ($3x10^{12}$) and *Lactobacillus* (10^{12}) 1 daily dose	50	18-65 years	To investigate the effects in **ART-treated HIV-infected patients** on:	

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
				12 months			• Microbial composition and diversity • Immune recovery and activation • Gut damage • Microbial translocation • Inflammation	
NCT04297488	China	No	Not yet recruiting	*Bifidobacterium* ($3x10^{12}$) and *Lactobacillus* (10^{12}) 1 daily dose 6 months	20	18-65 years	To explore the effects in **INR HIV-infected patients** on: • Microbial composition and diversity • Immune recovery and activation • Gut damage • Microbial translocation • Inflammation	
NCT04175223	France	No	Not yet recruiting	Vivomixx® 2 daily doses 6 months	50	18 years and older	To evaluate the effects in **ART-treated HIV-infected patients** with neurocognitive disorders on: • Immune activation • Cognitive performance	
NCT0409943	Italy	No	Recruiting	Vivomixx® 4 daily doses 6 months			To assess the effects in **ART-treated HIV-infected patients** with HPV genital infection on: • Anal HPV clearance • Anal dysplasia	

CFU (colony forming unit); HIV (human immunodeficiency virus); ART (antiretroviral treatment); IR (immunological responder); INR (immunological non-responder); IL-6 (interleukin-6); HPV (human papillomavirus).

Table 4. Clinical trials registered in *ClinicalTrials.gov* (42).

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
NCT03009032	Spain	(154)	Completed	PMT25341® 1 daily dose 48 weeks	77	18-80 years	To investigate the effects of the intervention in **naïve HIV-infected patients who initiate ART** on: • Safety • Immunological recovery • Inflammatory markers • Gut microbiota composition	• No effects on microbiota composition. • No effects on lymphocytes T CD4. • No effects on markers of inflammation and bacterial translocation.
NCT00688311	USA	(153)	Completed	Synbiotic 2000® (4 probiotic bacteria (10^{10} CFU/bacteria) + 4 dietary fibers (2.5 g/fiber)) 1 daily dose 4 weeks	34 (only females)	18 years and olders	To determine the effects in **ART-treated HIV-infected women** on: • Intestinal function • Immune system overactivation • Immune function	• Safe • Modification of gut microbiota composition
NCT03568812	Indonesia	No	Recruiting	Rillus® (*L. plantarum* 8'55 mg, *S. thermophilus* 8'55 mg and *B. bifidum* 2'55 mg (10^9 CFU) + FOS 480 mg) 1 daily dose 12 weeks	80	18-55 years	To assess the effects **INR HIV-infected patients** on: • Gut mucosal integrity and immunity • Bacterial translocation • Gut inflammation	
NCT03542786	Spain	No	Recruiting	I3.1® (*L. plantarum CECT7484, L. plantarum CECT7485* and *P. acidilactici)* + ProSheed® 1 daily dose 48 weeks	40	18 years and olders	To evaluate the effects in **ART-treated HIV-infected patients** on: • Inflammaging (premature aging)	
NCT02180035	Brazil	(155)	Completed	*Lactobacillus paracasei*, *Lactobacillus*	290	19 years and olders	To investigate the effects in **ART-treated HIV-infected patients** on: • Lipid metabolism	• Reduction of diarrhea, nausea and/or vomits, constipation and dyspepsia

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
				rhamnosus, *Lactobacillus acidophilus* and *Bifidobacterium lactis* + FOS 2 daily doses 24 weeks				

CFU (colony forming unit); HIV (human immunodeficiency virus); ART (antiretroviral treatment); INR (immunological non-responder).

Table 5. Clinical trials registered in *ClinicalTrials.gov* (42).

NCT number	Country	Associated article	State	Intervention	Number of participants	Age of participants	Objective	Main outcomes
NCT02256592	USA	No	Completed	300 ml of fecal suspension from a healthy donor delivered by colonoscopy and provided by OpenBiome	18	18-75 years	To examine the safety and durability of the intervention To determine the effects in **ART-treated HIV-infected patients** on: • Immune activation • Inflammatory biomarkers	
NCT03329560	USA	No	Active, not recruiting	PRIM-DJ2727 (capsules containing lyophilized microbiota derived from 150 g of healthy donor stool) 1 daily dose 6 weeks	6 (enrolled)	18 years and older	To evaluate if the intervention is safe for **ART-treated HIV-infected MSM**	
NCT03008941	Spain	No	Completed	FT capsules provided by OpenBiome 10 capsules first week + 5 capsules weekly 8 weeks	30	18-80 years	To assess the effects in **ART-treated HIV-infected patients** on: • Bacterial metabolism • Plasma metabolite fingerprint • Immune function and activation • Inflammatory markers • Markers of enterocyte barrier function	
NCT04165200	Mexico	No	Completed	FT through frozen capsules 15 capsules every 12 hours for four doses 7 days prior ART start and on weeks 0, 4, 8 and 12 after ART start	22	18 years and older	To monitor the effects in **naïve HIV-infected patients who initiate ART** on: • Lymphocytes T CD4 • Viral load during week 0, 4, 8, 12 and 24 after ART start	

FT (fecal transplantation); HIV (human immunodeficiency virus); ART (antiretroviral treatment); MSM (men who have sex with men).

Figures

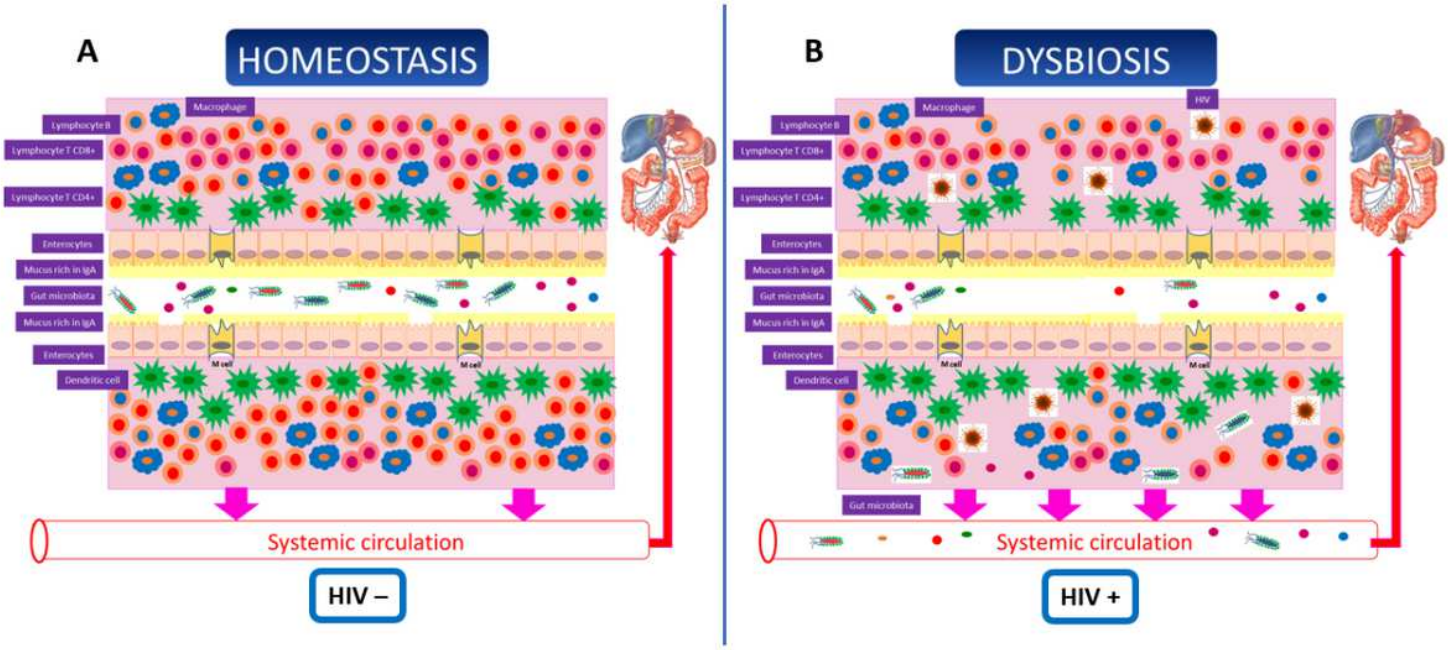

Figure 1

In normal physiological conditions, the microorganisms are in the intestinal lumen interacting with the intestinal cells in a state of symbiosis but, when HIV infection occurs, depletion of lymphocytes T CD4 takes place in the GALT (gut-associated lymphoid tissue). This is accompanied by a rupture of the epithelial barrier, which triggers alterations in the intestinal lumen and also in the composition of the microbiota (at least at bacteria level) (27). This dysbiosis favors the passage of microorganisms and their components to the lamina propia and, hence, to the circulation, which is known as bacterial translocation (BT), which undergoes subsequent intestinal and systemic inflammation (28). A) Gut homeostasis. B) Gut dysbiosis after HIV infection. Original. Made with PowerPoint.

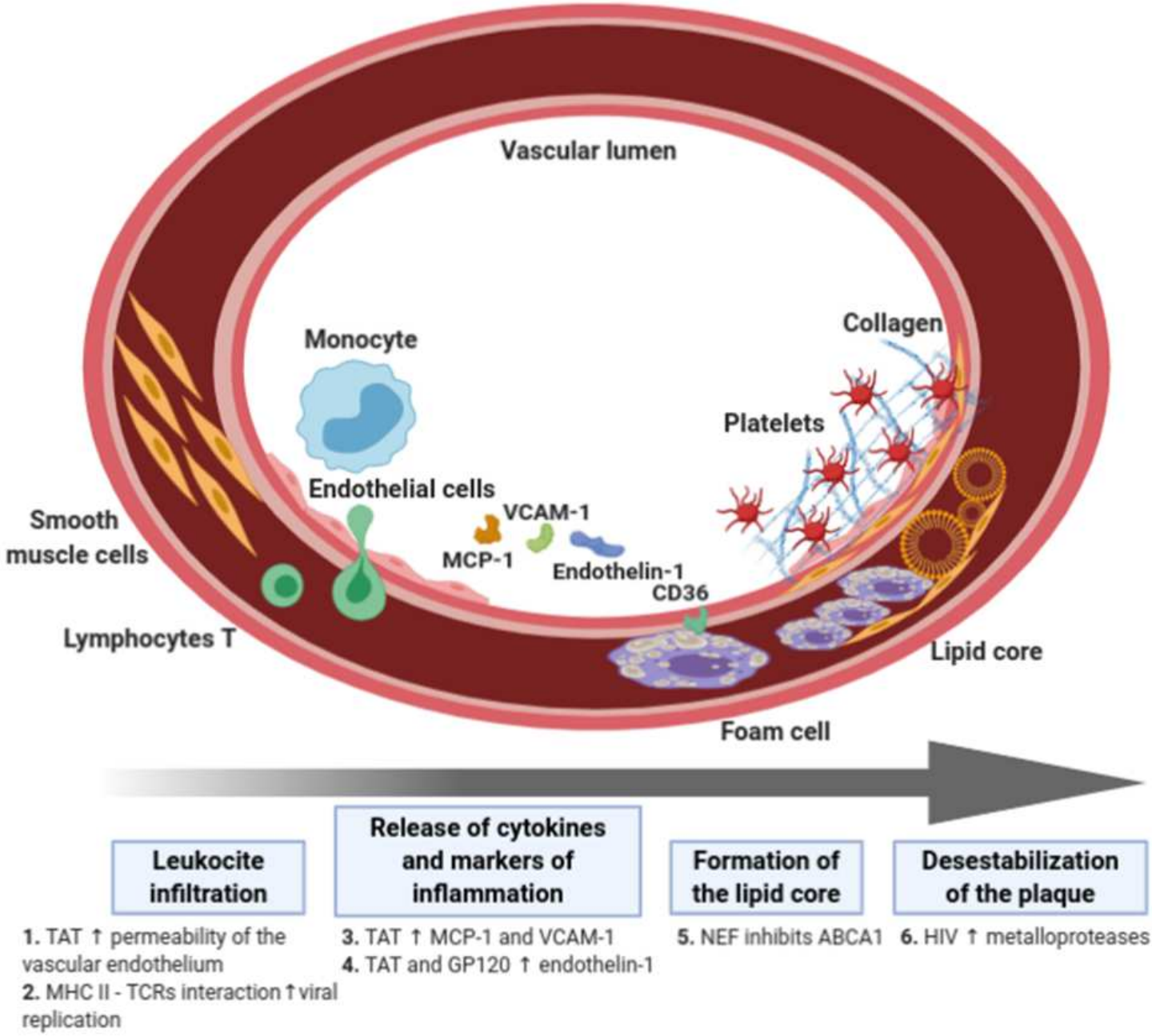

Figure 2

General scheme of atheroma plaque formation and effects of HIV infection (in Arabic numbers). Modified from Alonso-Villaverde Lozano (2009) (44). MHC II (major histocompatibility complex class II); TCRs (T-cell receptors); MCP-1 (monocyte chemoattractant protein 1); VCAM-1 (vascular cell adhesion molecule 1). Original. Made with Biorender.

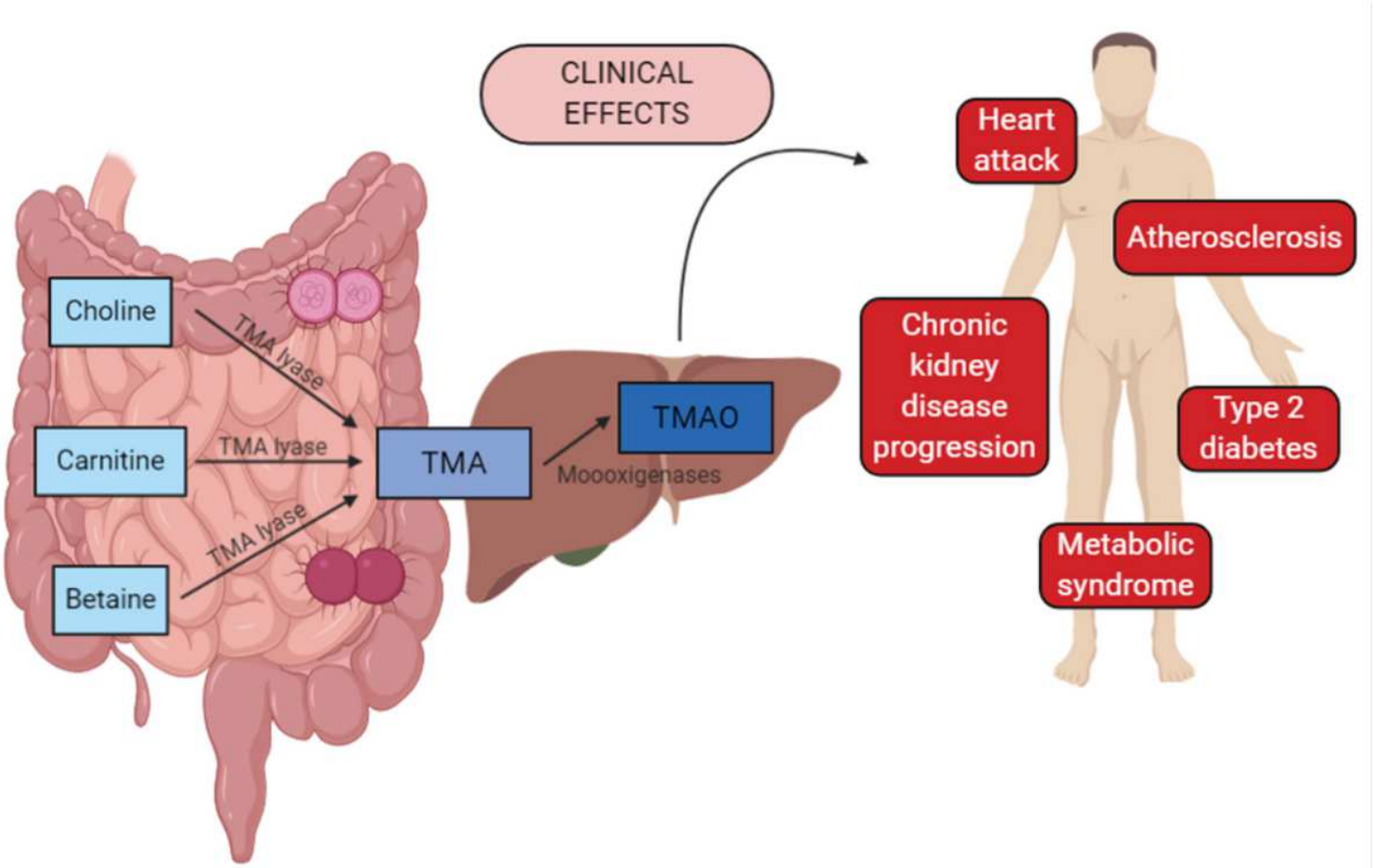

Figure 3

Processing pathway of choline, carnitine and betaine from diet to produce TMAO and clinical effects of TMAO. TMA (trimethylamine); TMAO (trimethylamine N-oxide). Original. Made with Biorender.

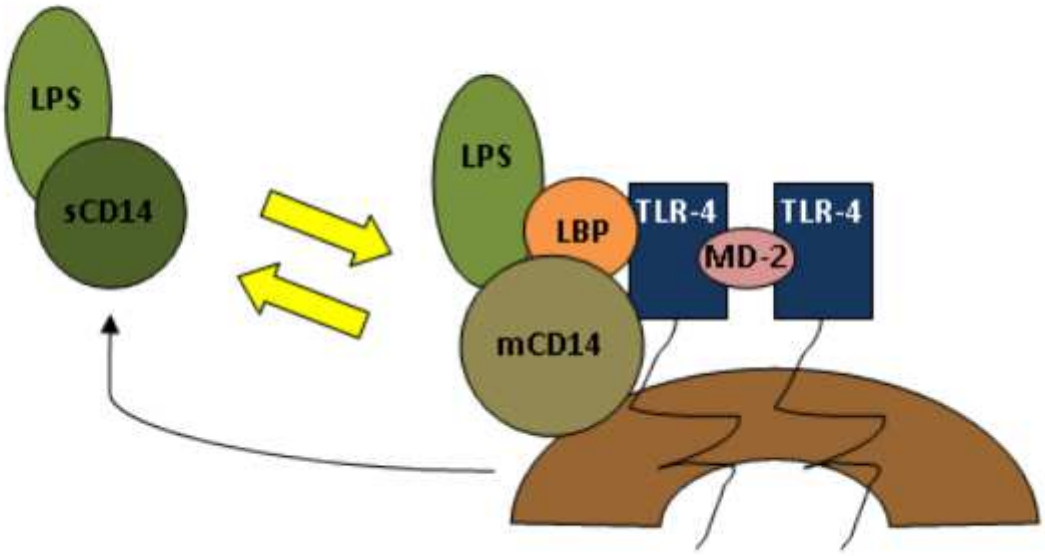

Figure 4

sCD14 is secreted by activated monocytes after the join of LPS to the TLR-4/MD-2 complex mediated by LBP and mCD14. sCD14 can join in turn to more LPS and transfer it tomCD14 triggering the cascade activation of a greater number of monocytes. sCD163 acts in the immune system but it is not yet clearly defined how. LPS (lipopolysaccharide); TLR-4 (toll-like receptor 4); LBP (lipopolysaccharide binding protein); sCD14 (soluble CD14); mCD14 (membrane CD14); sCD163 (soluble CD163). Original. Made with PowerPoint.