Trick-or-Treat: Dietary Lipids and Host Resistance to Infectious Disease

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Abstract: The increased intake of omega-6 fatty acids together with the widely use of omega-3 supplements in Western diets can affect the host defence against infectious diseases. In the present review we focused on the impact of these fatty acids on salmonella and mycobacteria infection models in animals or in cell cultures. Particular attention was given to the molecular mechanisms involved.

Key Words: Omega-3 or -6 fatty acids, tuberculosis, inflammation, malnutrition, intracellular pathogens, actin assembly.

INTRODUCTION

The incidence of certain infectious diseases such as tuberculosis and diarrhoea has reportedly been shown to be increased in Eskimos and Inuit populations [1-3]. This was correlated with a diet rich in oily fish, the best-characterized source of omega-3 fatty acids [1,2], and other sources of this lipids such as seal and whale meat. However, because many socioeconomic and genetic factors contribute to the incidence of infectious diseases, it is difficult to establish the direct contribution of dietary (n-3) fatty acids to this phenomenon [4]. Parallel studies by Bang and Dyerberg [3] showed the importance of the dietary intake of omega-3 polyunsaturated fatty acids for reducing ischaemic heart disease and polyunsaturated fatty acids are now accepted as being essential constituents of a healthy diet. Modern Western diets have low levels of omega-3 fatty acids and have excessive amounts of omega-6 fatty acids. This is very different to the diet on which human beings have evolved and this is reflected as a genetic pattern [5]. This high omega-6/omega-3 ratio promote the pathogenesis of many diseases and, consequently a large number of studies argue for the beneficial effects of omega-3 supplements, or eating more fatty fish instead of meat [6,7]. An increasing percentage of people in the world are supplementing their diets with omega-3 fatty acids, typically in the form of fish oil pills. The motivations for this lies in the potential beneficial effects that omega-3 fatty acids have on risk factors associated with cardiovascular disease and to treat inflammatory conditions such as Crohn's disease, arthritis and psoriasis. Evidence from clinical studies with hospitalized trauma and cancer patients has led several researchers to suggest that the immuno-modulatory effects of omega-3 fatty acids increases pathogens resistance [8].

Given this increasing tendency to advise and use omega-3 dietary supplements, which have beneficial effects among uninfected individuals [9,10] it is important to evaluate their effect on patients suffering from tuberculosis and other infections since their impact on disease evolution is unknown. A literature search on the effects of omega-3 and -6 lipids on a variety of different pathogens, especially in animal models, the conclusion suggest that polyunsaturated fatty acids are not generally beneficial and are often detrimental. In a critical evaluation of all the experiments done to test the effects of omega-3 lipids in the context of infectious diseases Anderson and Fritsche [8] concluded that there were "an equal number of papers published that report an adverse effect of omega-3 fatty acids on host infectious disease resistance as those that do not show an effect or show a beneficial effect".

During the last five years we have followed the effects of lipids and their molecular effectors on the outcome of infection by intracellular pathogens: mycobacteria [11-18]. Collectively, our data suggests that a high omega-6 fatty acids diet might be beneficial against mycobacteriosis, while a high omega-3 fatty acids diet might be detrimental. However, depending on the molecular effectors and, on the time window during infection that the lipid is supplemented, opposite signalling effects were observed. The data with tuberculosis bacilli raise an important public health question: are those individuals latently infected with *Mycobacterium tuberculosis* (*Mtb*), but asymptomatic at increased risk of reactivating the disease upon omega-3 fatty acids supplementation?

TUBERCULOSIS AND SALMONELLOSIS

Tuberculosis in man is caused by bacteria of the *Mycobacterium tuberculosis complex* while most human diarrhoea cases are a result of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) infection.

Mycobacterium tuberculosis causes tuberculosis in millions of people worldwide and is responsible for 3 million deaths annually [19]. Under normal circumstances, infection begins by inhaling bacilli that are then internalized by alveolar macrophages and dendritic cells and set up infection in the lungs. During this initial phase, the bacteria often transiently infect the bloodstream and metastasize to other organs. The colonizing bacteria are then contained and walled-off within granulomas that are the product of the host's immune-response to infection. The disease is characterized by a

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long incubation period, a protracted disease course and dormancy. It has been estimated that one third of the worlds' population is infected with *Mtb.* Most of these individuals harbour *Mtb* within granulomas in their lungs, held in check by T cells and macrophages. Such carriers remain asymptomatic until the immune system weakens and reactivation of dormant *Mtb* occurs [20].

Tuberculosis has long been considered a disease of malnutrition [21] and it is widely recognized that malnutrition is the commonest cause of immunodeficiency worldwide [22]. In fact, a recent investigation has determined that food intake increases the levels of gamma interferon (IFN-γ) but not interleukin-4 (IL-4) production, whereas starvation enhances the IL-4 response but not IFN-γ levels of T lymphocytes [23,24]. While IFN-γis a very potent pro-inflammatory determinant for bacteria clearance, IL-4 is anti-inflammatory leading to bacteria protection. Therefore, the interactions between certain nutrients and immunity exert a crucial role that should be analyzed from a biological and a clinical point of view. Several Th1-type cytokines, such as IL-12 and IFN-γ, have been shown to promote the immune response against *Mtb* infection in both humans and mice [25]. The production of these cytokines were shown to be impaired in mice taking supplements of omega-3 fatty acids thus mimicking what is seen in malnutrition [26].

S. Typhimurium is a remarkably versatile pathogen, with the ability to infect multiple hosts and cause different diseases [27]. In humans, it is a leading cause of diarrhoea and in mice it causes a systemic disease resembling typhoid fever.

During infection, *S.* Typhimurium invades and proliferates within both phagocytic and nonphagocytic cells of the host. After crossing the intestinal mucosa through M cells *Salmonella* first encounters dendritic cells concentrated in the Peyer's patches which could play an essential role in triggering the immune response [28]. *Salmonella* colonise Peyer's patches [29,30] and trigger the recruitment of macrophages in response to the pro-inflammatory IL-8 released by infected enterocytes. Infected macrophages then spread *via* the blood stream to the liver and spleen where *Salmonella* is later found in large quantities [31].

INTRACELLULAR LIFE-STYLE

Mtb and *S.* Typhimurium are both facultative intracellular pathogens. After infection of professional phagocytes, macrophages and dendritic cells, both species reside in a membrane-bound compartment known as phagosome. The invasion of non-professional phagocytes, such as intestinal epithelial cells, by salmonella, leads to the formation of the salmonella-containing vacuole (SCV).

Macrophages are a particularly hostile environment for intracellular bacteria such as mycobacteria and salmonella. The bacteria have consequently developed different mechanisms to survive inside these cells.

Pathogenic mycobacteria modulate phagosome maturation in order to prevent the fusion of endosomes with lysosomes [32]. This blocks its delivery to lysosomes, the *Mycobacterium* is thereby able to avoid the acidic proteases of the lysosomes, avoid exposure to the bactericidal mechanisms that operate within lysosomes, prevent degradation and hence processing and presentation of mycobacterial antigens to the immune system [33,34]. By contrast, mycobacterial access to transferrin-bound iron [35] and apparent accumulation of transferrin receptors (TfR) on mycobacterial phagosomes [36] have been reported, suggesting that the mycobacterial phagosome is not a static organelle despite a block in the acquisition of lysosomal constituents. The mechanism by which *Mtb* is able to finely adjust the intracellular environment to its preference involves both host proteins and mycobacterial effectors. Among these are mycobacterial lipid products, which mimic mammalian phosphatidylinositols [37], targeting host cell membrane trafficking processes. These products interfere with membrane trafficking and organelle biogenesis processes initiated by Ca^{2+} fluxes [38], and ending with host cell Rab GTP-binding proteins and their effectors that modulate membrane traffic. The block includes phosphatidylinositol 3-kinase and membrane tethering molecules that prepare phagosomes for fusion with other organelles [37].

In contrast to mycobacteria, salmonella resides in two different macrophage vacuolar compartments. One is known to undergo normal phagolysosomal fusion, leading to microbial growth inhibition. The other is a more "spacious" vacuole that is modified by the bacteria, possibly for replication [39]. How the bacteria do this is not known. The spacious vacuoles have been shown to contain lysosome- associated membrane markers usually found in the phagosome, although delivery of the late lysosomal marker cathepsin L is delayed.

Salmonella invasion of epithelial cells leads to the formation of SCV, a phagosome-like compartment. Immediately after formation, the SCV undergoes a maturation process that includes transient interactions with early endosomes [40] followed by the acquisition of a subset of late endosomal markers [41] and an enrichment in cholesterol [42]. During this process, fusion of the SCV with lysosomes is thought to be avoided [43]. After a lag period of 3–4 h, the SCV begins to elongate into tubular structures called Salmonella-induced filaments (Sifs) [41] an event that coincides with the initiation of bacterial replication.

More recent studies show that the maturation of SCV in HeLa cells and in primary or cultivated macrophages is similar. Macrophages differ from HeLa cells or mouse fibroblasts by their inability to form Sifs. The reason why Sifs are not observed in macrophages which possess tubular lysosomes [44] remains obscure [45].

In contrast to *Shigella*, *Listeria* and *Rickettsiae*, which escape from their nascent membrane-bound compartment and replicate in the cytoplasm, the maintenance of a endolysosomal membrane for *Salmonella* enclosure is crucial for virulence. The fate of *S.* Typhimurium in the cytosol is dependent on the type of cell that is infected; the bacteria die in the cytosol of macrophages but replicate rapidly in the cytosol of epithelial cells [46,47]. The factor(s) that restrict bacterial growth in the cytosol of macrophages remain unknown [48]. For mycobacteria, two recent reports describe the ability of pathogenic mycobacteria to escape to the cytosol

[49,50]. The later study describes a similar ability of *Mtb* and *M. leprae* to escape from phagolysosomes of human dendritic cells and macrophages during later stages of infection. We, and others consistently find, even at latter stages of infection, that pathogenic mycobacteria reside inside a membrane bound compartment [15,51]. However, it is not clear whether cytosolic mycobacteria within human phagocytic cells are alive or death thus, or whether escape from a phagolysosome constitutes a survival mechanism.

MODULATION OF ACTIN ASSEMBLY AND INTRACELLULAR SURVIVAL OF PATHOGENS BY OMEGA-3 OR -6 FATTY ACIDS

In recent studies, using latex beads phagosomes (LBPs), we provided evidence for links between the ability of phagosomes to assemble actin filaments *de novo* on their cytoplasmic surface and late fusion events between phagosomes and late endocytic organelles [11,12]. Indeed, under a set of conditions that stimulate actin assembly in macrophages, the ability of phagosomes to assemble actin correlates well with both their increased fusion with later endocytic organelles and their acidification as well as with an increased ability of infected macrophages to kill non-pathogenic and pathogenic mycobacteria [11,12]. In contrast, conditions that inhibit phagosomal actin assembly are correlated with less fusion/acidification but increased replication of the mycobacteria [11-13,52,53].

A striking effect on phagosomal-actin assembly, late fusion and killing of mycobacteria was seen when a number of pro-inflammatory lipids, especially the omega-6 fatty acid, arachidonic acid (AA) and sphingosin, were added to J774 macrophages infected with the non-pathogenic *M. smegmatis* or with virulent strains of *Mtb* [11]. In contrast, the anti-inflammatory omega-3 fatty acid eicosapentaenoic acid (EPA) induced an increase in mycobacterial growth in macrophages [11]. These results were exciting because they fitted nicely into a general pattern seen with these groups of lipids in whole organisms. In support of this, three earlier studies showed that diets rich in omega-3 fatty acids led to a significant increases in growth of *Salmonella* in mice [54] and *Mtb* in guinea pigs [55,56].

Maturation and maintenance of the intracellular vacuole in which *Salmonella* replicates is controlled by virulence proteins including the type III secretion system, encoded by salmonella-pathogenicity island 2 (SPI-2). Several hours after bacterial uptake into different host cell types, salmonella induces the formation of an F-actin meshwork around bacterial vacuoles. This structure is assembled *de novo* from the cellular G-actin pool in close proximity to the salmonella vacuolar membrane [45]. It was demonstrated that the phenomenon does not require the Inv/Spa type III secretion system or cognate effector proteins, which induce actin polymerization during bacterial invasion. It does however require a functional SPI-2 type III secretion system, which plays an important role in intracellular replication and systemic infection in mice. Treatment with actin-depolymerizing agents significantly inhibited intra-macrophage replication of wild-type *S.* Typhimurium. Furthermore, after this treatment, wild-type bacteria were released into the host cell cytoplasm, whereas SPI-2 mutant bacteria remained within vacuoles. Taken together these results lead to the conclusion that actin assembly plays an important role in the establishment of an intracellular niche that sustains salmonella growth [45].

We found two intracellular pathogens for which the actin cytoskeleton plays opposite roles during the late stages of infection. For pathogenic mycobacteria the blockade of actin assembly by phagosomal membranes will prevent phagosome maturation and bacteria killing within phagolysosomes. For salmonella, the activation of actin assembly around the SVC will sustain an intracellular survival niche.

It would similarly be expected that actin blocking lipids will have a distinct role on the intracellular survival of these pathogens. Inhibition of actin assembly by an omega-3 fatty acid would be expected to strongly inhibit, the maturation of phagosomes containing mycobacteria. Consequently, this would either help *Mtb* growth or have no effect as the actin assembly is already blocked by the pathogen. For salmonella two kinds of situations could arise: 1) the lipid will not have any effect as an actin meshwork was already established around the SVC for salmonella survival or 2) a decreased amount of actin around the SVC will help bacteria killing.

Given this situation, we recently decided to test diets enriched in omega-3 or omega-6 fatty acids in mouse models of *S.* Typhimurium and *Mtb* infection [17,18]. The results obtained were surprising in that the opposite effects were seen in the animals, compared with observations in macrophages. For mycobacteria infected macrophages the omega-3 fatty acid, EPA, enhanced intracellular survival of *Mtb* while in infected mice an omega-3 fatty acid enriched diet promoted bacteria killing [17,18]. The lack of effect of an omega-3 fatty acid enriched diet on salmonella survival is in good agreement with our hypothesis but in total disagreement with previous published results [54].

When we looked at the effects of an omega-6 fatty acid enriched diet on the intracellular survival of these pathogens we found no effect on *Mtb* infected mice while for salmonella infected animals the bacteria survival was improved. From the perspective of actin assembly, our results were in agreement with the salmonella model because AA, by stimulating actin assembly will create an even higher actin meshwork around the SCV thus protecting the bacteria. For the mycobacteria model the results, in infected macrophages, also agree with our model as AA, by increasing actin around *Mtb* phagosomes will help bacteria killing.

In animals it may be the case that downstream breakdown products of the omega-6 fatty acid added to the diet could play opposite and more complex effects thus reflecting the lack of agreement regarding the outcome of the infection. This led us to explore, in more detail the cyclooxygenase (COX) and the 5- lipoxygenase (5-LO) pathways in the context of mycobacteria infection.

OMEGA-3 OR -6 FATTY ACIDS AND INFLAMMATION: COX AND 5-LO PATHWAYS

On the basis of the observations of Metchnikoff macrophages have classically been recognized as scavenger cells possessing phagocytic and intracellular digestive properties. The secretory function of macrophages however, maybe as

important as their phagocytic capacity. For example, depending on the nature of the stimulus and the local pulmonary environment, the alveolar macrophage can be triggered to selectively release AA (20:4 ω6) [57]. Free AA thus becomes the substrate for the COX and 5-LO enzyme cascades that convert 20:4 ω6 to a complex array of prostanoids and leukotrienes. These eicosanoids may contribute to the immunoregulatory and host defence functions exercised by the alveolar macrophage [57].

Eicosanoids are responsible for many of the effects found in acute inflammation [58]. The inflammatory response play a key role in shaping the adaptive response in large part through the secretion of an array of mediators such as interleukin 1 β (IL-1 β), tumor necrosis factor α (TNF-α), prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) in response to the pathogen. The last two mediators are final products of AA conversion by COX and 5-LO, respectively. EPA, another major source of eicosanoids present in membranes, inhibits these conversions and has a general anti-inflammatory role (Fig. (**1**)).

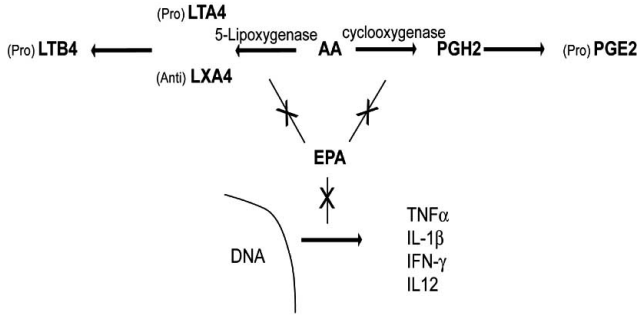

Fig. (1). Anti-inflammatory effects of EPA.

Recently was reported that the membrane of latex bead containing phagosome has the enzymes required to convert AA to a number of prostaglandins (PGs) (Griffiths G., EMBL-HD, personal communication). Moreover, some of these PGs, such as PGE2 inhibit actin assembly by LBP. In contrast, AA the precursor of PGE2, is a potent stimulator of phagosomal actin assembly. Here depending on whether there is accumulation of the precursor (AA) or the ending product (PGE2) opposite effects will be observed on the ability of phagosomal membranes to assemble actin. AA stimulating actin assembly will induce phagosome maturation and lysosomal pathogen killing while its product, with an inhibitory effect on actin assembly, will contribute to pathogen survival.

Determining a role in host defence against tuberculosis for the other class of eicosanoid mediators of inflammation, the leukotrienes (LTs), has received far less attention. As stated above LTs are metabolites of AA generated by the 5-LO pathway. Many studies have demonstrated that LTB4 is a powerful leukotropic, pro-inflammatory, and immunoregulatory mediator [59,60]. Moreover, it has been shown that these mediators are involved in antimicrobial host defence in a variety of infectious diseases [61,62]. LTs exert potent effects on phagocytosis and killing of microorganisms by both alveolar macrophages [63] and neutrophils [64]. Indeed, LTs have been reported to be necessary for optimal killing of *M. bovis* by neutrophils [65]. In light of these findings, we hypothesize that the absence of LTs during infection decreases the antimicrobial action of neutrophils and mononuclear cells, allowing persistence and growth of the bacilli in the lung. This may reflect indirect effects mediated by the modulation of Th1 cytokines. Other authors have shown that LTB4 is involved in T-cell activation and up-regulation of IL-2, IL-4, IFN-γ, IL-1β and TNF- α [66,67].

Replacement of AA by EPA in cellular membranes will prevent AA conversion into this group of potent pro-inflammatory modulators. Furthermore it will generate a class of PGs of the 3-series that are far less pro-inflammatory than those of the 2-series [68].

However the opposite effect can be also induced by EPA supplementation if the degradation of AA to LTs by the 5-LO pathway will be blocked. Lipoxins represent another class of 5-LO-derived eicosanoids, but in contrast to LTs, these mediators possess anti-inflammatory properties. It has been shown that high levels of lipoxin A4 (LXA4) are generated during infection with *Toxoplasma gondii* [69]. Bafica *et al.* [70] showed that mice genetically deficient in leukotriene production had improved bacterial clearance in a model of *Mtb*; these results were attributed to the abrogation of synthesis of the alternative 5-LO metabolite, LXA4, which has immunosuppressive and anti-inflammatory effects.

A decreased LT biosynthesis was associated with impairment of IL-12 and IFN-γ generation. *Mtb* infection induces local production of LTB4 in the lungs of mice. This endogenous production of LTs is important for the development of a protective host immune response. LTs likely contribute to such protection by virtue of their effects on phagocyte ingestion and killing of microbes, processes which are themselves influenced by the further ability of LTs to promote Th1 cytokine synthesis. Since LT production can be down-regulated by immunosuppressive circumstances such as HIV infection [71] and malnutrition [72], the recognition of the role of LTs in the immune response to TB may be clinically important and may define a potential target for immuno-modulatory therapy. Diets enriched in AA within this context would be expected to induce a protective immunity within *Mtb* infected lungs but *via* lipoxins pathway AA is expected to have the exactly opposite effect.

OMEGA-3 OR -6 FATTY ACIDS AND INFLAMMATION: TRANSCRIPTIONAL LEVEL REGULATION

A critical regulator of genes involved in inflammation is NF-κB [73]. This transcription factor consists of two subfamilies: the 'NF-κB' proteins and the 'Rel' proteins which are present in the cytoplasm as hetero-dimers, in a complex with an inhibitor, IκB [74]. When pro-inflammatory signalling occurs *via* activation of cell surface receptors such as the Toll-like receptors (TLR), IκB becomes phosphorylated. This releases the active subunits that enter the nucleus, where they up-regulate the transcription of hundreds of genes, a reflection of the complexity of this part of the inflammatory response [75]. In a recent study we showed that NF-κB is transiently activated early after infection of J774 cells and primary bone marrow derived macrophages with *M. smegmatis*. This activation is essential for mycobacterial

killing since when NF-κB is blocked *M. smegmatis* survives. One role of NF-κB in this system is to induce synthesis of a family of lysosomal enzymes and potential regulators of phago-lysosome fusion [16].

Many links have been described between lipids and NF-κB activation in different systems. For example, in Caco-2 cells AA activates NF-κB whereas EPA had no effect [76]. Moreover, in the same cells phosphatidylcholine, which inhibits LBP actin assembly, inhibited NF-κB activation induced by TNF-α [14]. In macrophages infected with pathogenic mycobacteria, AA potentiates NF-κB activation while EPA failed to activate these processes (Anes E., Gutierrez MG, Griffiths G., EMBL-HD, personal communication).

As stated above, NF-κB signalling could also be induced by TNF-α [14]. TNF-α signalling involves binding to members of TNF receptor super-family and recruitment of a complex of adapter proteins. Among these, TNF-receptor associated factors (TRAFs) activate several intracellular signall transduction pathways, in particular NF-κB and, Map kinases (MAPKs) that lead to modulation of gene expression by different transcription factors [77]. Pathogenic mycobacteria tends to inhibit all these pathways [16,78]

MAPKs are central players in cell signalling and much of their activities are localized on membranes [79]. There are three different classes of these kinases, namely ERK, JNK and p38 [80]. A number of studies have shown that these kinases are activated upon infection with mycobacteria [81]. Moreover, p38 and ERK are activated more during infection with non-pathogenic compared with pathogenic mycobacteria, implying that the pathogens inhibit these kinases [78,81]. The kinase p38 has been implicated in early endosome fusion; Fratti and colleagues also associated p38 activation with an inhibition of phagosome maturation in cells infected with *Mycobacterium bovis* (BCG) [82,83]. We additionally provided evidence for its importance in regulating phagosomal actin assembly, a process linked to some membrane fusion events [53]. By inhibiting this kinase, a block on phagosome actin assembly *in vitro* was observed. *In vivo* p38 inhibition, blocked phagosome maturation and, increased survival of *M. smegmatis* within J774 macrophages [12].

We also found that in *Mtb*-infected macrophages TNF-α secretion was stimulated by treatment with AA, whereas EPA inhibited this process [17,18]. In line with this, AA strongly activated the pro-inflammatory MAPK p38 in uninfected cells but *Mtb* infected cells blocked the ability of AA to activate p38 leading us to conclude that AA-dependent killing is therefore independent of p38.

It was demonstrated that non-pathogenic mycobacteria strongly induces TNF-α secretion and therefore, the MAPKs [78]. Recently, using J774 macrophages infected with *M. smegmatis*, we showed alternate cycles of (pro-inflammatory) killing and (anti-inflammatory) growth before total bacterial clearance by macrophages [12]. Unexpectedly both AA and EPA could be either pro-inflammatory (more death) or anti-inflammatory (more growth) depending on when they were added during *M. smegmatis* infection [17]. Treatment with EPA between 12-16 h post-infection enhances bacteria killing, while the supplementation between 16-24 h enhances bacteria growth. When infected macrophages were treated with AA between 8-12 h an increase in bacteria survival was observed. The opposite result was observed when AA was added between 16-24 h (more killing). Moreover the time window between 12 h and 24 h is independent of p38 signalling in *M. smegmatis* infected cells, while the killing phase between 8-12 h is p38 dependent (Fig. (**2**)). Therefore, lipid signalling for boosting the macrophage bactericidal mechanisms is dependent on the course of the infection status, and on other mediators in addition to p38.

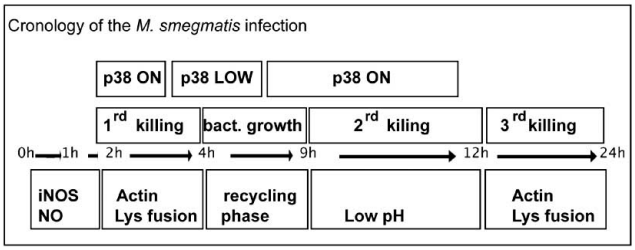

Fig. (2). Bactericidal effects induced by MAPK p38 during *Mycobacterium smegmatis* infection within J774 macrophages (adapted from [12]).

CONCLUDING REMARKS

Nutritional status is generally recognized as an essential factor involved in the modulation of immune response [22]. Hence, lymphocyte proliferation, cytokine production, phagocytic activity, adhesion molecule expression, and CD4/CD8 cell activity are susceptible to modification by the action of certain dietary lipids in both animals and humans. Given the several pro and anti-inflammatory mediators that can result from the same dietary lipid during the course of malnutrition infectious diseases such us tuberculosis, we suggest that further testing would be prudent. We recommend improving our understanding of how omega-3 fatty acids, omega-6 fatty acids and other dietary lipids affect host responses to human pathogens. Collectively our data argue against the idea of considering a simple recommended lipid-based diet against mycobacteria and other intracellular pathogens.

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ABBREVIATIONS

AA	=	Arachidonic acid
COX	=	Cyclooxygenase
EPA	=	Eicosapentaenoic acid
IL	=	Interleukin
IFN	=	Interferon
LBP	=	Latex beads phagosomes
LT	=	Leukotriene
LXA4	=	Lipoxin A4
5-LOX	=	5-Lipoxygenase

MAPK = Mitogen-activated protein kinase

Mtb = *Mycobacterium tuberculosis*

NF-κB = Nuclear factor-κB

PG = Prostaglandin

SCV = Salmonella containing vacuole

TNF-α = Tumour necrosis factor alpha

REFERENCES

[1] Kaplan, G. J.; Fraser, R. I.; Comstock, G. W. Tuberculosis in Alaska, 1970. The continued decline of the tuberculosis epidemic. *Am. Rev. Respir. Dis.,* **1972**, *105*, 920-6.

[2] Kromann, N.; Green, A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950-1974. *Acta Med. Scand.,* **1980**, *208*, 401-6.

[3] Dyerberg, J.; Bang, H. O. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet,* **1979**, *2*, 433-5.

[4] Grzybowski, S.; Dorken, E. Tuberculosis in Inuit. *Ecol. Dis.,* **1983**, *2*, 145-8.

[5] Simopoulos, A. P. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed. Pharmacother.,* **2006**, *60*, 502-7.

[6] He, K.; Song, Y. Risks and benefits of omega 3 fats: a few thoughts on systematic review. *BMJ,* **2006**, *332*, 915-6.

[7] Horrobin, D.; Fokkema, M. R.; Muskiet, F. A. The effects on plasma, red cell and platelet fatty acids of taking 12 g/day of ethyl-eicosapentaenoate for 16 months: dihomogammalinolenic, arachidonic and docosahexaenoic acids and relevance to Inuit metabolism. *Prostaglandins Leukot. Essent Fatty Acids,* **2003**, *68*, 301-4.

[8] Anderson, M.; Fritsche, K. L. (n-3) Fatty acids and infectious disease resistance. *J. Nutr.,* **2002**, *132*, 3566-76.

[9] Mori TA, B. L. Omega-3 fatty acids and inflammation. **2004**, *6*, 461-7.

[10] Siddiqui, R. A.; Shaikh, S. R.; Sech, L. A.; Yount, H. R.; Stillwell, W.; Zaloga, G. P. Omega 3-fatty acids: health benefits and cellular mechanisms of action. *Mini Rev. Med. Chem.,* **2004**, *4*, 859-71.

[11] Anes, E.; Kuhnel, M. P.; Bos, E.; Moniz-Pereira, J.; Habermann, A.; Griffiths, G. Selected lipids activate phagosome actin assembly and maturation resulting in killing of pathogenic mycobacteria. *Nat. Cell Biol.,* **2003**, *5*, 793-802.

[12] Anes, E.; Peyron, P.; Staali, L.; Jordao, L.; Gutierrez, M. G.; Kress, H.; Hagedorn, M.; Maridonneau-Parini, I.; Skinner, M. A.; Wildeman, A. G.; Kalamidas, S. A.; Kuehnel, M.; Griffiths, G. Dynamic life and death interactions between *Mycobacterium smegmatis* and J774 macrophages. *Cell Microbiol.,* **2006**, *8*, 939-60.

[13] Kalamidas, S. A.; Kuehnel, M. P.; Peyron, P.; Rybin, V.; Rauch, S.; Kotoulas, O. B.; Houslay, M.; Hemmings, B. A.; Gutierrez, M. G.; Anes, E.; Griffiths, G. cAMP synthesis and degradation by phagosomes regulate actin assembly and fusion events: consequences for mycobacteria. *J. Cell Sci.,* **2006**, *119*, 3686-94.

[14] Treede, I.; Braun, A.; Sparla, R.; Kuhnel, M.; Giese, T.; Turner, J. R.; Anes, E.; Kulaksiz, H.; Fullekrug, J.; Stremmel, W.; Griffiths, G.; Ehehalt, R. Anti-inflammatory effects of phosphatidylcholine. *J. Biol. Chem.,* **2007**, *282*, 27155-64.

[15] Jordao, L.; Bleck, C. K.; Mayorga, L.; Griffiths, G.; Anes, E. On the killing of mycobacteria by macrophages. *Cell Microbiol.,* **2008**, *10*, 529-48.

[16] Gutierrez, M. G.; Mishra, B. B.; Jordao, L.; Elliott, E.; Anes, E.; Griffiths, G. NF-kappaB activation controls phagolysosome fusion-mediated killing of mycobacteria by macrophages. *J. Immunol.,* **2008**, *181*, 2651-63.

[17] Jordao, L. *The links between membrane actinassembly by mycobacterial phagosomes and, phagosome maturation, macrophages activation and, pathogen killing.* PhD, University of Lisbon, 2008.

[18] Jordao, L.; Lengeling, A.; Bordat, Y.; Boudou, F.; Gicquel, B.; Neyrolles, O.; Becker, P. D.; Guzman, C. A.; Griffiths, G.; Anes, E. Effects of omega-3 and -6 fatty acids on *Mycobacterium tuberculosis* in macrophages and in mice. *Microbes Infect.,* **2008**, *doi:101016/micinf.2008.08.004*.

[19] WHO Annual Report 2004 - Stop TB partners. **2005**.

[20] Russell, D. G. *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat. Rev. Mol. Cell Biol.,* **2001**, *2*, 569-77.

[21] Schaible, U. E.; Kaufmann, S. H. Malnutrition and infection: complex mechanisms and global impacts. *PLoS Med.,* **2007**, *4*, e115.

[22] Chandra, R. K. Nutrition, immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. *Proc. Natl. Acad. Sci. USA,* **1996**, *93*, 14304-7.

[23] Najera, O.; Gonzalez, C.; Toledo, G.; Lopez, L.; Ortiz, R. Flow cytometry study of lymphocyte subsets in malnourished and well-nourished children with bacterial infections. *Clin. Diagn. Lab. Immunol.,* **2004**, *11*, 577-80.

[24] Rodriguez, L.; Gonzalez, C.; Flores, L.; Jimenez-Zamudio, L.; Graniel, J.; Ortiz, R. Assessment by flow cytometry of cytokine production in malnourished children. *Clin. Diagn. Lab. Immunol.,* **2005**, *12*, 502-7.

[25] Cooper, A. M.; Dalton, D. K.; Stewart, T. A.; Griffin, J. P.; Russell, D. G.; Orme, I. M. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J. Exp. Med.,* **1993**, *178*, 2243-7.

[26] Fritsche, K. L.; Anderson, M.; Feng, C. Consumption of eicosapentaenoic acid and docosahexaenoic acid impair murine interleukin-12 and interferon-gamma production *in vivo*. *J. Infect. Dis.,* **2000**, *182* (Suppl 1), S54-61.

[27] Tsolis, R. M.; Kingsley, R. A.; Townsend, S. M.; Ficht, T. A.; Adams, L. G.; Baumler, A. J. Of mice, calves, and men. Comparison of the mouse typhoid model with other *Salmonella* infections. *Adv. Exp. Med. Biol.,* **1999**, *473*, 261-74.

[28] Hopkins, S. A.; Niedergang, F.; Corthesy-Theulaz, I. E.; Kraehenbuhl, J. P. A recombinant *Salmonella typhimurium* vaccine strain is taken up and survives within murine Peyer's patch dendritic cells. *Cell Microbiol.,* **2000**, *2*, 59-68.

[29] Jones, B. D.; Ghori, N.; Falkow, S. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *J. Exp. Med.,* **1994**, *180*, 15-23.

[30] Jensen, V. B.; Harty, J. T.; Jones, B. D. Interactions of the invasive pathogens *Salmonella typhimurium*, *Listeria monocytogenes*, and *Shigella flexneri* with M cells and murine Peyer's patches. *Infect. Immun.,* **1998**, *66*, 3758-66.

[31] Richter-Dahlfors, A.; Buchan, A. M.; Finlay, B. B. Murine salmonellosis studied by confocal microscopy: *Salmonella typhimurium* resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes *in vivo*. *J. Exp. Med.,* **1997**, *186*, 569-80.

[32] Armstrong, J. A.; Hart, P. D. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J. Exp. Med.,* **1971**, *134*, 713-40.

[33] Pieters, J. Entry and survival of pathogenic mycobacteria in macrophages. *Microbes Infect.,* **2001**, *3*, 249-55.

[34] Pancholi, P.; Mirza, A.; Bhardwaj, N.; Steinman, R. M. Sequestration from immune CD4+ T cells of mycobacteria growing in human macrophages. *Science,* **1993**, *260*, 984-6.

[35] Sturgill-Koszycki, S.; Schaible, U. E.; Russell, D. G. *Mycobacterium*-containing phagosomes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis. *EMBO J.,* **1996**, *15*, 6960-8.

[36] Clemens, D. L.; Horwitz, M. A. The *Mycobacterium tuberculosis* phagosome interacts with early endosomes and is accessible to exogenously administered transferrin. *J. Exp. Med.,* **1996**, *184*, 1349-55.

[37] Fratti, R. A.; Backer, J. M.; Gruenberg, J.; Corvera, S.; Deretic, V. Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. *J. Cell Biol.,* **2001**, *154*, 631-44.

[38] Kusner, D. J.; Barton, J. A. ATP stimulates human macrophages to kill intracellular virulent Mycobacterium tuberculosis *via* calcium-dependent phagosome-lysosome fusion. *J. Immunol.,* **2001**, *167*, 3308-15.

[39] Alpuche-Aranda, C. M.; Racoosin, E. L.; Swanson, J. A.; Miller, S. I. *Salmonella* stimulate macrophage macropinocytosis and persist within spacious phagosomes. *J. Exp. Med.,* **1994**, *179*, 601-8.

[40] Steele-Mortimer, O.; Meresse, S.; Gorvel, J. P.; Toh, B. H.; Finlay, B. B. Biogenesis of *Salmonella typhimurium*-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway. *Cell Microbiol.,* **1999**, *1*, 33-49.

[41] Garcia-del Portillo, F.; Zwick, M. B.; Leung, K. Y.; Finlay, B. B. *Salmonella* induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells. *Proc. Natl. Acad. Sci. USA,* **1993**, *90*, 10544-8.

[42] Brumell, J. H.; Tang, P.; Mills, S. D.; Finlay, B. B. Characterization of Salmonella-induced filaments (Sifs) reveals a delayed inter-

action between salmonella-containing vacuoles and late endocytic compartments. *Traffic,* **2001**, *2*, 643-53.

[43] Buchmeier, N. A.; Heffron, F. Inhibition of macrophage phagosomelysosome fusion by *Salmonella typhimurium*. *Infect. Immun.,* **1991**, *59*, 2232-8.

[44] Swanson, J.; Burke, E.; Silverstein, S. C. Tubular lysosomes accompany stimulated pinocytosis in macrophages. *J. Cell Biol.,* **1987**, *104*, 1217-22.

[45] Meresse, S.; Unsworth, K. E.; Habermann, A.; Griffiths, G.; Fang, F.; Martinez-Lorenzo, M. J.; Waterman, S. R.; Gorvel, J. P.; Holden, D. W. Remodelling of the actin cytoskeleton is essential for replication of intravacuolar salmonella. *Cell Microbiol.,* **2001**, *3*, 567-77.

[46] Beuzon, C. R.; Unsworth, K. E.; Holden, D. W. *In vivo* genetic analysis indicates that PhoP-PhoQ and the *Salmonella* pathogenicity island 2 type III secretion system contribute independently to *Salmonella enterica* serovar Typhimurium virulence. *Infect. Immun.,* **2001**, *69*, 7254-61.

[47] Beuzon, C. R.; Salcedo, S. P.; Holden, D. W. Growth and killing of a *Salmonella enterica* serovar Typhimurium sifA mutant strain in the cytosol of different host cell lines. *Microbiology,* **2002**, *148*, 2705-15.

[48] Brumell, J. H.; Grinstein, S. *Salmonella* redirects phagosomal maturation. *Curr. Opin. Microbiol.,* **2004**, *7*, 78-84.

[49] Stamm, L. M.; Brown, E. J. *Mycobacterium marinum*: the generalization and specialization of a pathogenic *Mycobacterium*. *Microbes Infect.,* **2004**, *6*, 1418-28.

[50] van der Wel, N.; Hava, D.; Houben, D.; Fluitsma, D.; van Zon, M.; Pierson, J.; Brenner, M.; Peters, P. J. *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell,* **2007**, *129*, 1287-98.

[51] Rohde, K.; Yates, R. M.; Purdy, G. E.; Russell, D. G. *Mycobacterium tuberculosis* and the environment within the phagosome. *Immunol. Rev.,* **2007**, *219*, 37-54.

[52] Jahraus, A.; Egeberg, M.; Hinner, B.; Habermann, A.; Sackman, E.; Pralle, A.; Faulstich, H.; Rybin, V.; Defacque, H.; Griffiths, G. ATP-dependent membrane assembly of F-actin facilitates membrane fusion. *Mol. Biol. Cell,* **2001**, *12*, 155-70.

[53] Kjeken, R.; Egeberg, M.; Habermann, A.; Kuehnel, M.; Peyron, P.; Floetenmeyer, M.; Walther, P.; Jahraus, A.; Defacque, H.; Kuznetsov, S. A.; Griffiths, G. Fusion between phagosomes, early and late endosomes: a role for actin in fusion between late, but not early endocytic organelles. *Mol. Biol. Cell,* **2004**, *15*, 345-58.

[54] Chang, H. R.; Dulloo, A. G.; Vladoianu, I. R.; Piguet, P. F.; Arsenijevic, D.; Girardier, L.; Pechere, J. C. Fish oil decreases natural resistance of mice to infection with *Salmonella typhimurium*. *Metabolism,* **1992**, *41*, 1-2.

[55] Mayatepek, E.; Paul, K.; Leichsenring, M.; Pfisterer, M.; Wagner, D.; Domann, M.; Sonntag, H. G.; Bremer, H. J. Influence of dietary (n-3)-polyunsaturated fatty acids on leukotriene B4 and prostaglandin E2 synthesis and course of experimental tuberculosis in guinea pigs. *Infection,* **1994**, *22*, 106-12.

[56] Paul, K. P.; Leichsenring, M.; Pfisterer, M.; Mayatepek, E.; Wagner, D.; Domann, M.; Sonntag, H. G.; Bremer, H. J. Influence of n-6 and n-3 polyunsaturated fatty acids on the resistance to experimental tuberculosis. *Metabolism,* **1997**, *46*, 619-24.

[57] Cochran, F. R.; Roddick, V. L.; Connor, J. R.; Thornburg, J. T.; Waite, M. Regulation of arachidonic acid metabolism in resident and BCG-activated alveolar macrophages: role of lyso(bis)phosphatidic acid. *J. Immunol.,* **1987**, *138*, 1877-83.

[58] Alexander, J. W. Immunonutrition: the role of omega-3 fatty acids. *Nutrition,* **1998**, *14*, 627-33.

[59] Ford-Hutchinson, A. W.; Bray, M. A.; Doig, M. V.; Shipley, M. E.; Smith, M. J. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature,* **1980**, *286*, 264-5.

[60] Ford-Hutchinson, A. W.; Bray, M. A.; Smith, M. J. Lipoxygenase products and the polymorphonuclear leucocyte. *Agents Actions,* **1980**, *10*, 548-50.

[61] Bailie, M. B.; Standiford, T. J.; Laichalk, L. L.; Coffey, M. J.; Strieter, R.; Peters-Golden, M. Leukotriene-deficient mice manifest enhanced lethality from *Klebsiella pneumonia* in association with decreased alveolar macrophage phagocytic and bactericidal activities. *J. Immunol.,* **1996**, *157*, 5221-4.

[62] Peters-Golden, M.; Henderson, W. R., Jr. The role of leukotrienes in allergic rhinitis. *Ann. Allergy Asthma Immunol.,* **2005**, *94*, 609-18; quiz 18-20, 69.

[63] Mancuso, P.; Standiford, T. J.; Marshall, T.; Peters-Golden, M. 5-Lipoxygenase reaction products modulate alveolar macrophage phagocytosis of *Klebsiella pneumoniae*. *Infect. Immun.,* **1998**, *66*, 5140-6.

[64] Mancuso, P.; Nana-Sinkam, P.; Peters-Golden, M. Leukotriene B4 augments neutrophil phagocytosis of *Klebsiella pneumoniae*. *Infect. Immun.,* **2001**, *69*, 2011-6.

[65] Coffey, M. J.; Phare, S. M.; Peters-Golden, M. Role of leukotrienes in killing of *Mycobacterium bovis* by neutrophils. *Prostaglandins Leukot. Essent. Fatty Acids,* **2004**, *71*, 185-90.

[66] Marcinkiewicz, J.; Grabowska, A.; Bryniarski, K.; Chain, B. M. Enhancement of CD4+ T-cell-dependent interleukin-2 production *in vitro* by murine alveolar macrophages: the role of leukotriene B4. *Immunology,* **1997**, *91*, 369-74.

[67] Larfars, G.; Lantoine, F.; Devynck, M. A.; Palmblad, J.; Gyllenhammar, H. Activation of nitric oxide release and oxidative metabolism by leukotrienes B4, C4, and D4 in human polymorphonuclear leukocytes. *Blood,* **1999**, *93*, 1399-405.

[68] Calder, P. C. Dietary modification of inflammation with lipids. *Proc. Nutr. Soc.,* **2002**, *61*, 345-58.

[69] Aliberti, J.; Serhan, C.; Sher, A. Parasite-induced lipoxin A4 is an endogenous regulator of IL-12 production and immunopathology in *Toxoplasma gondii* infection. *J. Exp. Med.,* **2002**, *196*, 1253-62.

[70] Bafica, A.; Scanga, C. A.; Serhan, C.; Machado, F.; White, S.; Sher, A.; Aliberti, J. Host control of *Mycobacterium tuberculosis* is regulated by 5-lipoxygenase-dependent lipoxin production. *J. Clin. Invest.,* **2005**, *115*, 1601-6.

[71] Coffey, M. J.; Phare, S. M.; Cinti, S.; Peters-Golden, M.; Kazanjian, P. H. Granulocyte-macrophage colony-stimulating factor upregulates reduced 5-lipoxygenase metabolism in peripheral blood monocytes and neutrophils in acquired immunodeficiency syndrome. *Blood,* **1999**, *94*, 3897-905.

[72] Skerrett, S. J.; Henderson, W. R.; Martin, T. R. Alveolar macrophage function in rats with severe protein calorie malnutrition. Arachidonic acid metabolism, cytokine release, and antimicrobial activity. *J. Immunol.,* **1990**, *144*, 1052-61.

[73] Caamano, J.; Hunter, C. A. NF-kappaB family of transcription factors: central regulators of innate and adaptive immune functions. *Clin. Microbiol. Rev.,* **2002**, *15*, 414-29.

[74] Delhalle, S.; Blasius, R.; Dicato, M.; Diederich, M. A beginner's guide to NF-kappaB signaling pathways. *Ann. N Y Acad. Sci.,* **2004**, *1030*, 1-13.

[75] Natoli, G.; Saccani, S.; Bosisio, D.; Marazzi, I. Interactions of NF-kappaB with chromatin: the art of being at the right place at the right time. *Nat. Immunol.,* **2005**, *6*, 439-45.

[76] Ramakers, J. D.; Mensink, R. P.; Schaart, G.; Plat, J. Arachidonic acid but not eicosapentaenoic acid (EPA) and oleic acid activates NF-kappaB and elevates ICAM-1 expression in Caco-2 cells. *Lipids,* **2007**, *42*, 687-98.

[77] Baud, V.; Karin, M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.,* **2001**, *11*, 372-7.

[78] Schorey, J. S.; Cooper, A. M. Macrophage signalling upon mycobacterial infection: the MAP kinases lead the way. *Cell Microbiol.,* **2003**, *5*, 133-42.

[79] Morrison, D. K.; Davis, R. J. Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell Dev. Biol.,* **2003**, *19*, 91-118.

[80] Martin-Blanco, E. p38 MAPK signalling cascades: ancient roles and new functions. *Bioessays,* **2000**, *22*, 637-45.

[81] Roach, S. K.; Schorey, J. S. Differential regulation of the mitogen-activated protein kinases by pathogenic and nonpathogenic mycobacteria. *Infect. Immun.,* **2002**, *70*, 3040-52.

[82] Fratti, R. A.; Chua, J.; Deretic, V. Induction of p38 mitogen-activated protein kinase reduces early endosome autoantigen 1 (EEA1) recruitment to phagosomal membranes. *J. Biol. Chem.,* **2003**, *278*, 46961-7.

[83] Vergne, I.; Chua, J.; Singh, S. B.; Deretic, V. Cell biology of *Mycobacterium tuberculosis* phagosome. *Annu. Rev. Cell Dev. Biol.,* **2004**, *20*, 367-94.

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