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Mechanisms underlying chronic fatigue, a symptom too often overlooked

I- Deregulated immunity at the origin of chronic fatigue

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Acute fatigue after exertion, like acute inflammation after injury, is useful for our body. By contrast, chronic fatigue like chronic inflammation are deleterious, and they are associated in many pathologies. In this first part, we will analyze different immune phenomena (excessive collateral damage, memory of the innate immune system, link with the intestinal microbiota) involved in triggering chronic inflammation. This review aims at looking for links between different signs and symptoms associated with chronic fatigue, as well as between different pathologies in which severe chronic fatigue can manifest. On this basis, possible underlying mechanisms for these phenomena are discussed. This is a proposal made by a researcher, with no clinical experience, to doctors confronted with an entity that still remains largely mysterious. The connection between chronic inflammation, neuroinflammation and fatigue will be examined in a second part.

Chronic fatigue is a syndrome associated with many pathologies such as cancer, neurodegenerative diseases, post-infectious syndromes or chronic inflammatory rheumatism. As chronic fatigue is difficult to measure and is not specific to a disease, it is considered unclassifiable and too often neglected, despite the significant suffering it causes in those who are affected by it.

Our starting postulate is to consider that the fatigue associated with these different pathologies, even with different starting points, brings into play common mechanisms that need to be clarified. We will first seek to clarify the common points and differences between chronic pathological fatigue and physiological fatigue experienced after significant physical or intellectual effort. To understand the impact of these phenomena on chronic fatigue, we will examine, in a second part, the possible consequences on the brain of a chronic peripheral inflammation.

Acute fatigue versus chronic fatigue

The physiological fatigue felt after intense physical exertion is due to fairly well known phenomena. Muscle function requires the abundant production of ATP (adenosine triphosphate) by mitochondria, in all living cells, that ususally need glucose and oxygen. Lack of any of these causes a decrease in muscle contractile strength. Thus, anemia can be a common cause of fatigue. When it is due to vitamin B12 or erythropoietin (EPO) deficiency, it is easily treated by taking vitamin B12 or EPO (something professional cyclists are well aware of).

Incidentally, one can wonder if intellectual fatigue proceeds from similar mechanisms, operating in the brain instead of muscles. There is no clear answer

to this question today. We can nevertheless make clear that during a sustained cerebral effort, it is possible to measure an increase in glucose consumption of up to 12%, without an overall variation in oxygen consumption [1]. Remember that, although the weight of an average adult brain only represents 2% of the body's weight, in the absence of physical or intellectual effort, it consumes 20% of the available energy. Intense intellectual activity does little to modify this already very important basic consumption. This is true at least globally, because, during a specific intellectual task, one can observe by fMRI (*functional magnetic resonance imaging*) local increases or decreases in the BOLD (*blood oxygen level-dependent*) signal [2]. We cannot therefore exclude that intellectual fatigue is, alike muscle fatigue, linked to a *locally* insufficient production of ATP, but it would not be surprising if intellectual fatigue corresponded to more complex mechanisms.

Physiological fatigue is normally resolved with rest and restful sleep. As it is transient, we can speak of acute fatigue, by analogy with acute inflammation, which is also transient.

However, there is another fatigue, seen even in the absence of physical exertion. Chronic: It is felt by most people with neurodegenerative diseases, or commonly seen after different infections (e.g., infectious mononucleosis, influenza [flu], Covid-19). The pathology giving rise to chronic fatigue which has been the best studied is the chronic fatigue syndrome (CFS) or ME/CFS (*myalgic encephalomyelitis / chronic fatigue syndrome*), a name that underlines its association with neuroinflammation and musculoskeletal pain. The definition and even the reality of CFS have been the subject of significant debate for over 30 years. The main elements considered today to be characteristic of CFS are 1) debilitating chronic fatigue, a source of major mental suffering for the patient, lasting for at least 6 months; 2) post-exercise discomfort with worsening of other symptoms after exercise or stress, and unusually slow recovery. This trait, also called SEID (*systemic exercise intolerance disease*) is sometimes used as an alternative name to ME/CFS; 3) non-restorative sleep; 4) cognitive disorders (concerning memory, ability to concentrate), and 5) sometimes orthostatic intolerance occurring during prolonged upright position (for recent reviews on CFS, see [3–5]).

Acute and chronic fatigue share certain characteristics. In particular, it is possible to distinguish in both cases, a peripheral fatigue and a central fatigue. The first appears as a diminished response to direct muscle stimulation. The second is deduced from the reduced amplitude of myopotentials evoked by the transcranial magnetic stimulation of the motor cortex [6].

Chronic fatigue, however, has many unique characteristics, starting with a metabolic component. In particular, it has been shown that the PBMC (*peripheral blood mononuclear cells*), of people suffering from CFS have abnormalities in the expression of several mitochondrial proteins that are fundamental for the production of ATP. These anomalies fit well with the hypothesis of a deficit in the production of ATP, and with a partial compensation which leads, paradoxically, to an increase in oxidative stress before an effort, and even more after it [7,8]. This hypothesis is fully consistent with the results of an analysis of the respiratory function of PBMCs isolated from patients with CFS [9]. This excessive oxidative stress undoubtedly reflects a dysfunction of the mitochondria, but also an insufficient expression of molecules that are key for limiting this stress: the heat-shock proteins [10]. The existence of such

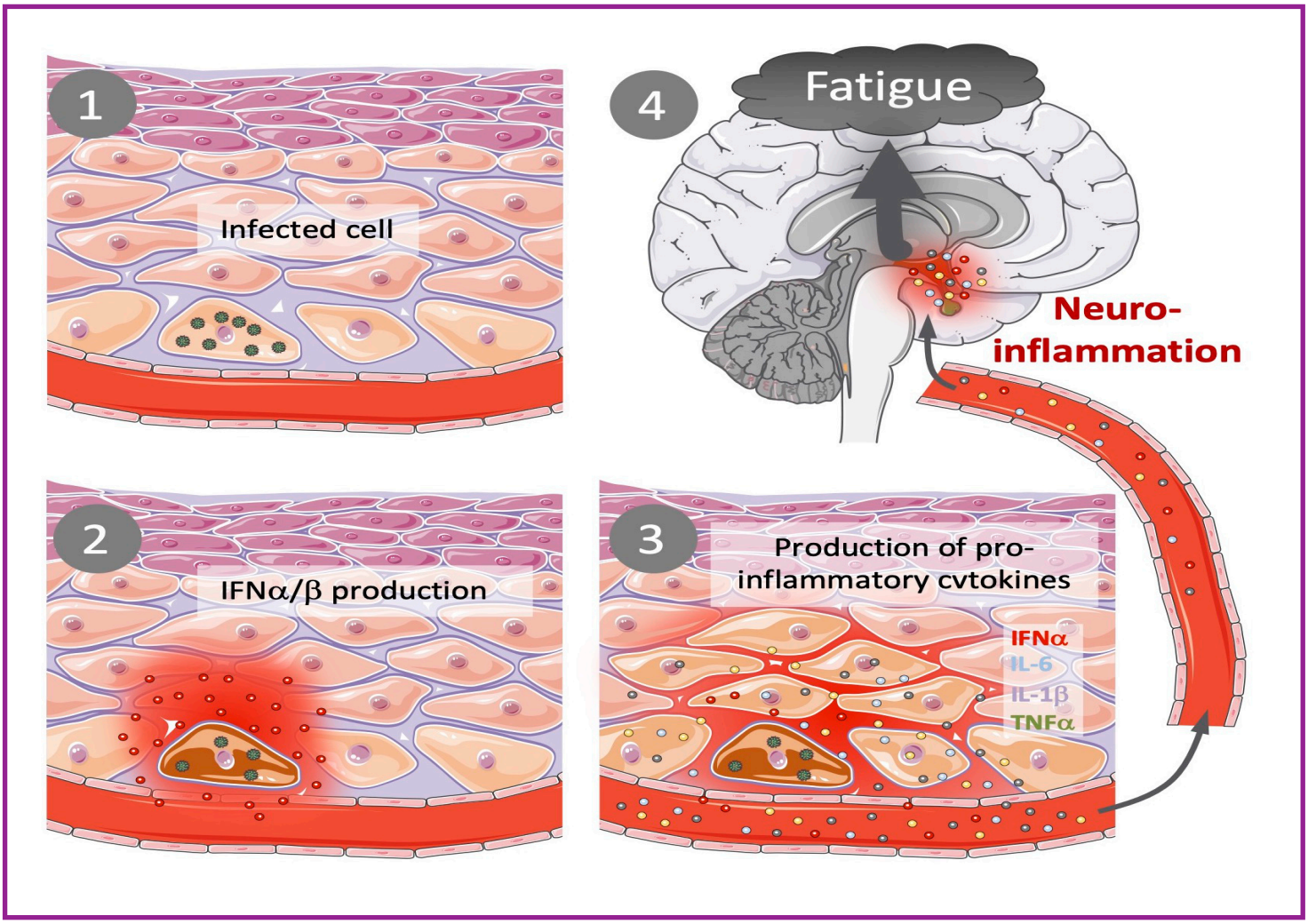

Figure 1. (1) A cell infected with a replicating virus produces IFNα/β. (2) IFN, which induces the transcription of specific genes, ISGs (interferon-stimulated genes), inhibits protein synthesis by the cell and thus blocks viral replication, not only in the infected cell but also in neighboring ones. (3) IFN locally triggers an inflammatory response. Pro-inflammatory cytokines thus produced can pass into the bloodstream and (4) eventually reach the brain, where they trigger neuroinflammation and fatigue.

excessive oxidative stress can be easily measured by measuring serum factors, such as TBARS (*thiobarbituric acid reactive substances*) or lipid peroxides, the abundance of which means excessive oxidation, and by evaluating, in parallel, the level of antioxidant molecules, such as RAA (*reduced ascorbic acid*) [10]. There may therefore be a partly peripheral origin (outside the central nervous system) of chronic fatigue, but many additional elements need to be taken into account in order to understand the origin of this fatigue.

Chronic post-infectious fatigue and the importance of IFNα/β (*Figure 1*)

Marked fatigue associated with infection is a common experience. It frequently involves type I interferons (IFN) (IFNα and IFNβ). During a viral infection, all cells, including the most differentiated ones such as neurons or hepatocytes, are able to produce some IFNα/β. These molecules induce a considerable slowing down of the transcription of DNA into RNA, and therefore of the production of proteins by the cell. By such a global slowing down, the cell prevents the virus from rapidly replicating and being transmitted to neighboring cells. An analysis of more than 600 metabolites, present in

the plasma of subjects suffering from CFS or of healthy subjects, made it possible to show that, despite the heterogeneity of the disease, a metabolic signature of the disease could be identified. It is characterized by *dauer-like* hypometabolism, a state resembling hibernation, which allows cells to survive in slow motion in a hostile environment [11]. The hypometabolism induced by IFNα/β, which is decisive for its anti-viral action, is based on the activation of hundreds of genes, the ISGs (*interferon-stimulated genes*), including the *Schlafen* (SLFN) family of proteins ("sleep", in German) [12]. The key role of IFNα/β in antiviral defense can be illustrated by the fact that, during the long co-evolution between viruses and mammals, most viruses have developed an anti-IFNα/β arsenal capable of weakening the antiviral defenses of the host they infect, a particularly effective arsenal in coronaviruses such as SARS-CoV-2, responsible for COVID-19 [13].

IFNα/β is also a powerful inducer of fatigue. Thus, in rats, the injection of a molecule causing the production of IFNα/β very quickly induces behavior changes: the animals move slowly and appear lethargic, as if they were suddenly tired. In addition, their body temperature rises [14]. In humans, treatment with IFNα/β of patients infected with the hepatitis C virus causes a severe fatigue which can persist long after the injections have been stopped. [15]. This treatment-induced fatigue is in addition to that due to the infection itself, in particular due to the production of IFNα/β by the body in response to this infection.

From an evolutionary point of view, one can look for possible links between the three effects triggered by IFNα/β: interruption of the production of proteins, fever and marked fatigue. In fact, all three contribute to antiviral control. Fever clearly increases the efficiency of the immune system and decreases viral replication (which is very slow above 39°C or 102°F). Fatigue prevents an infected person from spending in physical exertion an energy, which remains available for the functioning of the immune system. This “forced confinement” of the infected subject, induced by fatigue, also limits its interactions with other individuals, which slows down the spread of the virus in the population. It is certainly not proven, but conceivable that such a mechanism, being selected by evolution, has contributed to improving the resistance of mammals to viral infections. It is during the acute phase (infectious) that such a mechanism could have an evolutionary interest. On the other hand, it would not have any during the chronic phase (post-infectious), since at this stage the patient is no longer contagious.

As with an infection, IFNα/β could also be the cause of the fatigue experienced by people whose cancer is treated with chemo- or radiotherapy. Indeed, the effectiveness of these treatments seems to be associated with triggering the production of IFNα/β [16], which is more toxic to rapidly dividing cells, such as tumor cells, than to healthy cells [17]. The fatigue associated with the treatment of certain cancers could therefore be due to mechanisms similar to those involved in post-infectious fatigue, and add to the consequences of anemia often induced by anti-metabolic anti-cancer molecules.

The action of IFNα/β can last, via the prolonged activation of certain ISGs. However, IFNα/β and the ISGs that it induces do not alone explain the post-infectious fatigue which is found not only in the case of viral infection but also in the case of bacterial infection, as in the persistent form of Lyme disease or long Lyme (***Box 1***). The mechanisms underlying fatigue following bacterial infection are poorly understood. It should be noted that an abnormally high level of IFNα/β has been measured in patients suffering from long Lyme [18], which is compatible with our hypothesis of a major role of IFNα/β in post-

Box 1.

While the majority of people who have developed Lyme disease after a bite from a tick infected with the *Borrelia* bacteria are cured by an appropriate antibiotic treatment, 10-20% of them [38] fail to do so and develop a chronic pathology called PTLDS (post-treatment Lyme disease syndrome).

PTLDS is defined by the conjunction of four elements [39,40]. 1) a polymorphic syndrome associating severe chronic fatigue, diffuse pain and cognitive impairment with in particular impairment of immediate memory and brain fog; 2) evidence of previous borreliosis that has been properly treated; 3) the absence of proven active borreliosis; and 4) the absence of other diagnoses (rheumatological, neurological, psychiatric). PTLDS is the subject of fierce controversy opposing infectious disease specialists who downplay its frequency and severity, and affirm the uselessness of treatment with antibiotics (ATB) [41–43], and those who emphasize its seriousness and recommend retreatment trials [40,44,45]. In France, SPPT, or persistent polymorphic symptomatology/syndrome after a possible tick bite [46], is equivalent to PTLDS, except for the second criterion which simply retains the possibility of a tick bite, even in the absence of certainty concerning a past borreliosis.

We will group here PTLDS and SPPT under the term long Lyme. The difficulties of its diagnosis are associated with the frequent absence of memory of the tick bite and/or a negative serology in more than 20% of neuroborreliosis, and finally with the involvement of co-infecting pathogens which have been injected by the tick [47].

Are persistent pathogens involved in long Lyme? Some are convinced of their absence, based on 1) the fact that the syndrome can resist a 1 to 3 month ATB treatment, and 2) the absence of pathogen detectable by PCR (polymerase chain reaction) in these patients. Based on these two notions, they propose that the pathology has an autoimmune origin [43].

To these and other facts, another interpretation can be given: 1) the ATBs currently used against *Borrelia* as monotherapy do not have an efficacy comparable to that of a cocktail of 3 or 4 ATBs efficiently used in the classical 6 month treatment of tuberculosis [48]. *Borrelia* have been detected in various tissues, including the brain at autopsy of patients treated with doxycycline [49,50] and in vitro, this ATB is totally ineffective against dormant forms of *Borrelia* [51]. 2) The detection sensitivity of *Borrelia* in the blood by PCR is low, if only because its abundance is much lower in the blood than in connective tissues for instance [52]. The effectiveness of ATB treatment has been shown to be greater at 3 months than at 1 month [53]. This result was criticized in an article with a questionable protocol [42], which, in addition, clearly shows the severity of long Lyme in people who have developed PTLDS despite an intravenous ATB treatment for 3 weeks. Finally, doctors convinced of the usefulness of prolonging ATB treatments do not publish their results, often for fear of prosecution. Only supervised and published clinical trials will make it possible to settle the question of the real usefulness or danger of such prolonged treatments.

Faced with a long Lyme, there is no certainty today, neither of the absence nor of the presence of *Borrelia* in these patients. If *Borrelia* is present, the patient should be improved by a new ATB treatment with effective ATB, and new ATBs have indeed been proposed [54,55]. However, a clinical improvement in response to ATB indicates the pathogenic presence of one or more bacteria, but it does not prove that it is necessarily *Borrelia*. Other bacteria can have a pathogenic effect following a tick bite, whether they are co-infecting bacteria, or bacteria brought in by a subsequent infection. Finally, there may be a pathological translocation of bacteria from the intestinal microbiota, which have left their initial niche [40]. Regardless of the correct interpretation, if a test ATB treatment can lead to clinical improvement, it should be possible to attempt (in the patient's interest), as part of a clinical trial, to supervise it and contribute to the advancement of knowledge.

infectious fatigue. Would the same apply to what is now called long Covid, this pathology observed in people who have been infected with SARS-CoV-2 and who show symptoms months later?

Deregulated immunity, autoimmunity and molecular mimicry

How can infection lead to long-lasting deregulated immunity? There are many examples of autoimmune conditions that have developed after an infection. However, it is not clear whether there is a causal link

between the two, and it is futile to seek to identify a specific pathogen and a pathogenic molecule as the origin of an autoimmune disease. An example concerns multiple sclerosis (MS) and the phenomenon of *molecular mimicry*. *(**Box 2**).*

One reason to rule out molecular mimicry in the development of an autoimmune disease like MS is that this hypothesis makes only T and B lymphocytes responsible for this disease. We now know that such a hypothesis is incorrect. It is established that every healthy person has a large number of autoreactive lymphocytes and antibodies. These participate in the normal immune system, its development and its maintenance in a functional state. [19]. Having autoreactive lymphocytes is therefore not a problem. It is only when these lymphocytes multiply massively and activate that the situation deteriorates. This degradation owes much to a third type of immune cells, myeloid cells, made up of inflammatory monocytes and macrophages.

The hypothesis that we favor for the development of autoimmune diseases, as an alternative to molecular mimicry, is that it is a collateral damage resulting from deregulated immunity and the phenomenon of *bystander activation*. According to this hypothesis, an autoimmune pathology is due to the excessive activation of a cellular and molecular network comprising inflammatory monocytes and macrophages, T and B lymphocytes and autoantibodies, and still other cells like mast cells. Within this network, each element would activate one or more other elements. Antibodies, or immunoglobulins, are made up of a variable part, the Fab, which allows, according to its sequence, the specific recognition of a wide variety of antigens, and of a non-variable part, called the Fc region. When a bacterium (or a virus) is recognized by antibodies specific for the antigens it expresses, it finds itself bristling with these immunoglobulins (generally isotype G, or IgG). Once “decorated”, the pathogen will be better recognized by the monocytes or macrophages which express on their surface specific receptors for the Fc part of IgG, the FcγR: this is called opsonization by antibody. This antibody-dependent binding to myeloid cells is at the origin of the phagocytosis of opsonized particles, bacteria or viruses, by these cells, which become cytotoxic by producing a quantity of toxic, nitrosylated radicals (such as nitrogen oxide, NO) and oxygenated ones (like H_2O_2), which causes a real "bleach" of the environment. These macrophages will also be stimulated by IFNγ, a cytokine produced by CD4+ T lymphocytes activated during infection (***Box 3***), themselves stimulated during the presentation by the macrophages of the antigens expressed at their surface after the phagocytosis of pathogens.

Opsonization, when it is effective, i.e., based on abundant antibodies having a good affinity towards

Box 2.

The sudden appearance of MS in the Faroe Islands in 1943 coincided with the presence of a cantonment of British troops during World War II. The presence of the first cases of MS near this cantonment led to the hypothesis of a link with a contagious infectious disease. A commonly accepted explanation, but very debatable as we shall see, is the occurrence of molecular mimicry-induced autoimmunity. The idea is that fragments of a pathogen can be identical to fragments of host proteins, such as myelin, and that antibodies initially directed against the pathogen turn against the proteins the host, causing abreakdown in tolerance to its own molecules, and therefore the disease. If the hypothesis is attractive, its demonstration has never been possible [56]. In the case of MS in the Faroe Islands, extensive investigations have been carried out to determine what could be the initial infectious agent. They never succeded [57]. The reason for this failure is very likely that there was no single initial infectious agent, but several possible ones. The linear explanation does not hold, which seeks a precise pathogen, a precise pathogenic molecule, and molecular mimicry with a molecule of the self.

bacterial or viral antigens, thus contributes powerfully to the elimination of pathogens. However, when it is ineffective (in certain infections or due, for instance, to a vaccine that induces weak and scarce antibodies), opsonization can be the source of a formidable mechanism: facilitation by antibody or ADE (*antibody-dependent stimulation*).

The consequence of ADE will be, in individuals presenting these antibodies which are non-neutralizing (not abundant enough or not very active against the pathogen), a more severe disease than in the subjects without antibodies, the latter giving the virus a route of entry. This is what has been observed with dengue hemorrhagic fever for some subjects who have developed a humoral response following a poorly effective vaccine. They were more likely to develop a severe disease sometimes resulting in fatal hemorrhages. This has been observed in the Philippines following a vaccination program against this same dengue virus with the Dengvaxia vaccine, which caused many deaths, and a collapse in the confidence of the population in the vaccination [20]. Two mechanisms are involved in ADE: 1) facilitating entry of the virus into cells that express FcγRs, such as macrophages, which help spread the virus to other cells in the body; and 2) activation of these macrophages by "immune complexes" formed between IgG and pathogens, kinds of virus clusters aggregated by antibodies [21]. Any disturbance of the immune system (infection, sometimes vaccination) is likely to activate this potentially dangerous network between antibodies and cells. And for that, there is no need for any particular molecular mimicry. A simple accidental, non-infectious trauma can, by causing the death of many cells, saturate the device for removing dead cells by macrophages. Such saturation can lead to the activation of this

Box 3.

Type I (mainly IFNα/β) and type II (IFNγ) interferons are key molecules for our defense against pathogens. Besides the name, they share part of their intracellular signaling. However, their respective functional importance is distinct. IFNα/β is the key molecule for antiviral defense. Its action results in inhibiting all transcription, thereby slowing down the replication and propagation of viruses, and alerting the immune system to the existence of a local danger. IFNα/β can be produced by most cells undergoing viral infection, and exert ubiquitous effects.

On the other hand, the number of cells capable of producing IFNγ or of being its target is much more limited. This cytokine is produced, namely during infection, by a fraction of activated T lymphocytes and NK (natural killer) cells. A major target of this IFNγ are myeloid cells, (monocytes/macrophages and dendritic cells), which IFNγ helps to strongly activate. On an infectious or inflammatory site, in the dialogue between T lymphocytes and myeloid cells, the latter can stimulate the lymphocytes by presenting them with antigens, and in return be activated by the IFNγ produced by the T cells. This bidirectional exchange of information between lymphocytes and myeloid cells is very useful for the development of an anti-infective response. The same type of dialogue can however lead to deleterious effects on an inflammatory site where self-reactive T cells and activated monocytes can be found, each of these cells then maintaining the state of activation of the other, thus promoting a chronicization of inflammation. This could explain the abnormally high serum IFNγ level in people with CFS, at least in the first phase of the pathology [58]. It is also consistent with the fact that certain polymorphisms of the gene encoding IFNγ can be associated with severe CFS [59], as well as another category of polymorphism affecting the negative feedback of T cell activation, which normally prevents runaway of this response. Thus, a polymorphism that risks to prolong excessively the duration of the T response, via a dysfunction of the molecules PTPN22 (protein tyrosine phosphatase non-receptor type 22) and CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) increases the risk of developing CFS after an infectious episode [60].

Box 4.

ASIA (autoimmune/inflammatory syndrome induced by adjuvants) is a concept that has been introduced in 2011 by Yehuda Shoenfeld. It aims at proposing an explanation for a syndrome similar to CFS, which would be triggered, in some individuals by a strong immune response, but in the absence of infection [61]. Four pathologies have been grouped under this term: siliconosis, Gulf War syndrome, macrophage myofasciitis syndrome, and post-vaccination phenomena. In each of these cases, the activation of the myeloid cells by an adjuvant would cause a lasting deregulation of the immune system, in people with an appropriate genetic susceptibility and immune history. These four pathologies share a set of signs and symptoms that suggest the existence of a common denominator. Hence the concept of ASIA.

Siliconosis has been observed in a minority of patients who have had a breast implant, but it is a minority that cannot be overlooked. For Gulf War syndrome, we know that the American veterans had all received an anthrax vaccine, in 6 injections, containing as adjuvant aluminum salts and squalene. Almost all of these veterans had developed anti-squalene antibodies, although not all were ill. Macrophage myofasciitis [62] includes a local component (inflammation at the site of injection of a vaccine with aluminum salts, with an infiltrate of macrophages containing, years later, aluminum inclusions) [63], and a chronic inflammatory autoimmune disease with a myriad of symptoms, including major chronic fatigue and significant and lasting cognitive impairment [64]. As for chronic post-vaccination pathologies, although they are infrequent, they are nonetheless severe and lasting for patients who suffer from them. Faced with this, two risks of major errors exist: on the one hand a systematic anti-vax (anti-vaccine) attitude, on the other,, the dogmatic denial of the existence of such pathologies.

deleterious network, and thus cause the multiplication and stimulation of self-reactive T and B lymphocytes which preexist and are harmless when they are in low number. Simple adjuvants may be sufficient, sometimes, to provoke an immune response which becomes chronic and may for instance lead to ASIA syndrome (*autoimmune/inflammatory syndrome induced by adjuvants*), a syndrome resembling CFS appearing in subjects whose immune system has been strongly activated in the absence of any infection (***Box 4***). Inflammatory phenomena can also be observed in subjects whose macrophages have a dysfunction in their ability to eliminate intracellular pathogens by autophagy or xenophagy. Several polymorphisms of genes of the autophagy system (ATG, *autophagy-related genes*) have thus been implicated in the phenomena of autoinflammation. [22].

What remains to be explained is how such deregulated immunity can become chronic and be involved in severe chronic pathologies, and in the fatigue associated with them.

Individual immunity has one history and several memories

The existence of chronic fatigue associated with long-lasting deregulated immunity requires an analysis of what immune memory is, and what are the cells in which this memory is located. Immune memory that appears as a result of infection is commonly attributed to memory T and B cells. This would be a memory relying primarily on adaptive immunity. However, if the importance of this concept and its long-established physiological reality remain unquestionable, another immune memory, independent of this immunity, and based on the innate immune system, has recently been revealed. This memory mechanism has been called *trained immunity* in the pioneering work of Mihai Netea [23,24]. It is sometimes called *innate memory*, a kind of oxymoron, since a memory is necessarily acquired. In this case, this memory is indeed acquired, although by cells which have so far been categorized as key to innate immunity, hence this shortcut.

This memory would have for support, after an infection and its resolution, persistent modifications of cells such as monocytes, macrophages, NK (natural killer) cells, endothelial cells, as well as modifications affecting their progenitors or stem cells. These late modifications would help understanding how this memory can last for months, well beyond the lifespan of innate immune cells which is of only a few days [24,25].

Innate memory is not based on chromosomal rearrangements, as is the case with adaptive memory, but on epigenetic modifications, with the opening or closing of chromatin which allows genes to be transcribed, and which can thus result in either a sensitized (open) state or a state of immune tolerance (closed). Thus, during a second infection, the innate memory will result in either an amplified response or an attenuated response. However, the mechanisms which allow tilting in one direction rather than the other remain poorly understood. This lasting changes in innate cells can impact the immune response against a second infection, not only when the second infectious pathogen is the same as the first, but well beyond. This cross-memorization of various infectious stresses makes it possible to understand how a vaccination with BCG (*Bacillus Calmette-Guérin*), used against tuberculosis, can improve the reactivity of the immune system against a wide variety of pathogens such as *Mycobacterium tuberculosis* but also *Candida albicans* or *Schistosoma mansoni* [24] and may be even SARS-CoV-2 [26].

This innate memory can be induced not only by pathogenic bacteria but also by commensal bacteria, or microbiota, this set of bacteria inhabiting our epithelia. In the intestine, when the integrity of the epithelium is altered by inflammation, for instance, bacteria can migrate from the lumen of the intestine to the intestinal vascularization, proximal lymph nodes and even beyond. This is called a bacterial translocation. In certain inflammatory diseases, typical intestinal bacteria, such as *Tropheryma Whipplei* have thus been detected… in the joints of patients [27].

Thus, the immune system has several memories. A long-term memory, which relies on T and B lymphocytes and the antibodies that B lymphocytes produce when they differentiate into plasma cells. This memory can sometimes last a few years, for example after a vaccination against tetanus toxin, which then necessitates regular booster shots. It can also last a lifetime, as is the case with childhood illnesses (measles, mumps, rubella). A shorter-term memory that probably lasts a few months also exists. It is innate memory. This innate memory of past infectious stresses persists when it is based on epigenetic modifications affecting stem cells, or long-lived cells, such as neurons. These two memories are not completely independent of each other, since autoantigens presented by myeloid cells have a role in the activation of autoreactive lymphocytes.

Mast cell activation syndrome

We have seen the important role of IFNα/β in persistent fatigue induced by viral infection. We also examined how an infection could deregulate the immune system through the *bystander activation* mechanism that involves lymphocytes and monocytes/macrophages. Such deregulation can also involve mast cells. These cells, which play a role of sentinels near the vessels of richly vascularized tissues, such as the lung, the intestinal mucosa, the skin and the brain, can be activated in multiple ways. First by TLRs (*Toll-like receptors*), detectors of pathogens expressed namely by monocytes/macrophages and mast

cells. Mast cells can also be activated by allergens, when allergens opsonized by IgEs are detected by FcεRI receptors and induce a cell activation, which is key to asthma, for instance. When activated, mast cells release many molecules, including histamine, lipid mediators (prostaglandins and leukotrienes), or TNFα (tumor necrosis factor alpha).

In an inflammatory context in which inflammatory cytokines or neuropeptides (such as substance P) are produced, mast cell activation functions as an amplifier of primary inflammation. Excessive uncontrolled activation of these cells can then be responsible for mast cell activation syndrome (MCAS), in which one can find the association of chronic inflammation, extreme fatigue, brain fog, and musculo-articular pain. This syndrome is difficult to diagnose and treat.

The activation of mast cells, when it is due to allergens opsonized with IgE, can be treated with mast cell stabilizers, such as sodium cromoglycate. They act by blocking mast cell degranulation induced by the binding of IgE to their receptor. Unfortunately, if the effectiveness of many of these inhibitors has been demonstrated *in vitro*, their effectiveness *in vivo* remains very limited. These inhibitors do not interfere with the activation of mast cells through other pathways (TLRs or substance P). Some efficacy has been observed for inhibitors of H1-type histamine receptors, such as ketotifen, but it is far from inhibiting all of the consequences of massive mast cell activation.

Diet, inflammation and microbiota

We now know that diet can influence chronic inflammation. The existence of links between diet and inflammation is indeed recognized, in particular the association between obesity and inflammatory state. This diet is therefore likely to have an impact on the fatigue often associated with an inflammatory state. The mechanisms for explaining this association deserve to be recalled. To this end, let's take a detour by examining the processes involved in regulating blood glucose levels in mammals. The level of glucose in the blood is efficiently regulated, but it is far from constant: after a large meal, this rate can double; it will come down again in the hours following the meal to be normalized [28], in particular by mechanisms which allow the transformation of excess glucose into triglycerides which will be stored in adipocytes. The mass of adipose tissue, made up of these cells, is also regulated, in particular by TNFα [29]. In healthy subjects, this cytokine is present in small quantities in the adipose tissue. In obese subjects on the other hand, its adipose level increases sharply. The TNFα is then released into the peripheral blood where it contributes to the systemic inflammatory state seen in these individuals.

During a fast, the level of glucose in the blood decreases slightly [30] but this decrease is gradually offset by the appearance of another fuel: ketone bodies (KB). KB are produced primarily by the liver from lipids released by adipocytes [31]. KB represent an excellent fuel for the production of ATP by all cells, including in the brain where they represent an essential contribution in case of glucose deficit. In addition, they have an anti-inflammatory role, especially in the brain [32]. These KB are practically absent from the blood of a well-nourished individual. Their blood levels do not rise until after a few days of fasting. At this point, glucose has become scarce and it is no longer stored in the adipocytes. The production of TNFα is then reduced, and this contributes to the establishment of an anti-inflammatory state. These effects, pro-inflammatory of an abundant food rich in carbohydrates, and on

the contrary, anti-inflammatory of fasting, have been known for a very long time [33]. They are partly based on a change in the composition of the intestinal microbiota [34], whose bacteria ensure certain steps of digestion and assimilation of nutrients.

By their action on the immune cells present in the intestinal mucosa, the bacteria of the microbiota influence the immune system [35]. A very important category of molecules that these bacteria produce during the digestion of foods rich in fiber, is represented by SFCA (*short chain fatty acids*). Several of these molecules have anti-inflammatory properties. A recent study has shown the remarkable benefit, for patients with MS, of supplementing their food with propionic acid, one of these SCFAs [36].

We now know that the more diverse the bacterial populations inhabiting our microbiota, the more it is balanced and effective for the digestion of food and for the balance of our immune system. Fasting increases this bacterial diversity. Conversely, an excessively rich diet decreases it (for review, see [37]). These data could therefore argue in favor of the use of a supervised fast for its potentially anti-inflammatory properties. However, we must stress that most of the conclusions about the link between diet and inflammation have been obtained in mice. And men are not mice ...

Data on the effect of fasting in humans is still incomplete. It is therefore difficult to recommend this approach for its anti-inflammatory properties, especially since prolonged food deprivation can have many deleterious consequences, in particular by weakening the immune system. However, there is a scientific basis for recommending that people suffering from chronic inflammation eat a balanced diet without excess, low in carbohydrates and high in fiber, but studies are still needed to determine the merits of this type of diet.

Conclusion

In this first part, we reminded how infections could lead to persistent inflammation and fatigue, and how they can modify the functioning of the immune system, by bringing into play the phenomena of *bystander activation*, which can lead to pathological autoimmune phenomena, and memory of the innate immune system (*trained immunity*), all of which can be amplified by excessive activation of mast cells. The state of systemic, low-grade inflammation, can be influenced by diet and the composition of the gut microbiota. To understand the impact of these phenomena on chronic fatigue, it will then be necessary to examine how peripheral inflammation can cause neuroinflammation, and chronic activation of the hypothalamus-pituitary-adrenal gland. This will be the subject of the second part of this review.

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Links of interest

The author declares that he has no link of interest concerning the data and analyses published in this article.

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