The role of physiological elements in the future therapies of rheumatoid arthritis. II. The relevance of energy redistribution in the process of chronic inflammation*

Michał Gajewski, Przemysław Rzodkiewicz, Elżbieta Wojtecka-Łukasik

Department of Biochemistry and Molecular Biology, Institute of Rheumatology, Warsaw, Poland

Abstract

The reasons for development of chronic inflammation are complex and not fully understood. One of the factors affecting the prolongation of inflammation is changes in cell metabolism, occurring at the center of the inflammatory process. In chronic inflammation there is an imbalance between the processes of storage and consumption of energy reserves. Hypoxia that is a consequence of edema results in transition of white blood cells to anaerobic metabolism. Neutrophils, lymphocytes and macrophages produce active oxygen metabolites which on one hand facilitate the elimination of pathogens, and on the other hand, can cause damage to healthy cells located in the inflamed tissue. In this paper, we discuss the importance of disturbed redistribution of energy as one of the main reasons for transformation of the acute inflammatory process into the chronic one.

Key words: chronic inflammation, energy metabolism, leukocytes.

Energy metabolism in the process of evolution

"Dzisiaj czuję się jak C w CH. Niesłyszalna"
– Cuca Canals

Diseases of affluence are virtually always accompanied by a chronic inflammatory process. The reasons for transition of acute to chronic inflammation are not yet clear. It has been suggested recently that the transition point of the acute to the chronic inflammatory phase is the point of total consumption of available energy resources [1].

Throughout the evolution of mammals (65 million years), the amount of energy resources in the body has been relatively constant, and sufficient to meet the body's energy demands for 2–4 weeks. It should be noted that at the very beginning of the 20th century, there was a sharp increase of energy resources accumulated in the human body. Still at the end of the 19th century, energy resources in females of *Australopithecus africanus* held out for 20.3 days, *Homo habilis* – 21.4 days, and *Homo sapiens* – for 28.3 days. Energy resources accumulated in the body of a contemporary woman (USA, since 1900) hold out for as many as 43 days. For males of the mentioned species, the figures are 25.3, 22.2, 31.8 vs. 41 days respectively [1].

Paradoxically, the fact results from the environmental impact rather than from the "inside" of the body. The sign of our times is common obesity caused not only by hypercaloric nutrition or lack of physical exercise but also from the increasing ambient temperature of the human environment. In our homes and workplaces (where we spend most of the time), the temperature is close to the thermic neutral point (TNP) of 23°C. It is the point at which the human body does not require any energy to keep the body temperature stable. Every degree Celsius below the TNP means a necessity of spending some energy in order to maintain the thermal equilibrium of ongoing enzymatic processes. This considerable amount of energy which had to be exploited by our ancestors freezing in their natural environment is now transformed into body fat. Thus, obesity results not only from energy con-

---

sumed with food but mainly from energy of the contemporary, much-changed environment [2].

While our ancestors spent most energy on thermoregulation and physical work, contemporary humans spend their energy resources on the activity of the nervous and immune systems in the first place. The inflammatory process accompanying diseases of affluence seems to be associated with the widely understood psychophysiological stress of modern society, which is surprisingly strongly affected by biological, social, behavioral, psychological and religious factors. These factors also seem to affect the development of the most serious epidemic of modern times, which is chronic inflammation called the "metabolic syndrome", and people of today are still more often called "inflammacitizens" [3].

Regulation of energy flow in homeostasis of the body

Changes in energy flow and production are very important homeostatic regulators of physiological processes. Due to their function, factors which regulate energy metabolism can be divided into factors providing high-energy substrates to energy-storing organs (primarily, to the parasympathetic nervous system – PSNS) and those providing such substrates to energy-consuming organs (primarily, to the sympathetic nervous system – SNS) [1, 3].

After food is consumed and absorbed, energy-rich metabolites are stored in the liver and skeletal muscles (as glycogen and proteins) or adipose tissue (as triglycerides). Energy resources accumulated in the adipose tissue are about 13 kg (equivalent of 500 000 kJ, which theoretically would be sufficient for 2.4 months of fasting), and in the liver 150 g, which would hold out for half of a day only (2500 kJ). Stocks in the muscles amount to 300 g, which is equivalent of 5000 kJ but can be used up only locally. The main system controlling energy storage in the adipose tissue, liver and muscles is the PSNS [4].

The activity of energy "consumers" (brain, muscles and immune system) and energy "suppliers" (liver, adipose tissue) is regulated for 24 hours; energy is delivered to the brain and muscles during the day, and to the immune system at night. The regulation is strictly accurate, permitting the body's homeostasis to be maintained. Upon waking up, release of energy stored in tissues is increased due to activation of the SNS and the hypothalamic-pituitary-adrenal axis. Hormones secreted at that time of the day inhibit various activities of the immune system [4, 5].

Hormone levels begin to decrease in the evening, and reach a minimum in the middle of the night; thus the supply of energy to the brain and muscles is reduced,

and the immune system is not inhibited. During the night, energy is mainly used up by the immune system, and for body growth processes in the case of children. Shortly after we fall asleep, there is increased secretion of growth hormone which stimulates glycogenolysis and glucose release from the liver. Glucose, released to the blood, is the main source of energy for the nightly activated immune system, which is why for centuries, patients in all civilizations worldwide instinctively got into beds and tried to fall asleep [4, 5].

Energy flow in chronic inflammation

In chronic inflammation, the balance between energy storage and energy consumption processes is disturbed. Significant increase of the SNS activity with concomitant decrease of the PSNS activity leads to a major feature of chronic inflammation, that is the body's adaptation to continued redistribution of energy, from energy-storing organs to the activated immune system. Chronic inflammation, immune diseases, and even the metabolic syndrome, seem to be an effect of over-activation of the neuro-immuno-endocrine system [4, 5].

In the course of generalized inflammation, the nervous system and immune cells release pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin (IL) 1 and IL-6. The signals are like an "appeal" for energy-rich tissues and cells to engage in the supply of sufficient amounts of energy-rich substrates ("energy appeal reaction") [1]. A result of the energy appeal reaction is activation of the SNS and decrease in the PSNS activity at the same time [1, 5].

In chronic inflammation, dislocation of high-energy substrates to energy-consuming organs is notably disturbed. It should be noted that activation of the immune system is associated with a greater demand for energy (even by 2100 kJ/day, which makes up to 30% of the basal metabolic rate of 7000 kJ/day) [1].

Boundary between acute and chronic inflammation

It remains unclear at which point of transition acute inflammation becomes chronic. There is a distinct time correlation between the transition to chronic inflammation and exhaustion of energy resources in all mammals that have been studied up to now, that is 19–43 days [1, 2].

If the acute phase of a disease lasted beyond the time in which energy stores were exhausted, the affected individual would probably die of inanition or starvation. If the inflammatory response, in any modified form, lasted for a shorter time, the person might survive. In inflammation, dislocation of high-energy substrates can be observed, from energy-storing organs to the im-

mune system. It is only not clear whether the SNS and PSNS activity changes are the cause or the consequence of the pathological process [1, 2].

Acute inflammation is a self-limiting process, and it includes an innate immunity phase of 2 to 3 days. The subsequent phase of the adaptive immunity phase lasts 3 to 4 weeks. The acute phase of infectious diseases consumes considerable amounts of energy. Activation of the immunity system and infections lead to the so-called sickness behavior including decreased physical activity and extended sleeping time, which allows for greater allocation of energy to the immune system on the one hand, and for decreased energy intake from the environment (e.g. through loss of appetite) on the other hand.

If an immune response cannot end properly because energy stores have run empty and do not suffice to initiate an adequate repair process, a chronic inflammatory process will begin [3, 5].

The inflammatory process may be associated not only with pathological phenomena but also with "purely" physiological processes such as oogenesis or embryogenesis (interdigital webbing is resorbed due to widely understood inflammatory mechanisms) [1]. Moreover, it should be noted that the inflammatory process takes part not only in ontogenesis (development of an individual organism) but also in phylogenesis (development of species), so it may be deemed an important mechanism by which organisms adapt to environmental changes. It can be thus concluded that the diversified panel of chronic inflammatory diseases (hypertension, diabetes, coronary artery disease, obesity, neurodegenerative diseases) that we witness is ongoing evolutionary adaptation to a significant and presumably irreversible change of the living environment in the Eastern hemisphere, and the ultimate result of the change may be a far-reaching shakeup of society [3]. An interesting hypothesis formed in recent years suggests that chronic inflammation begins at the time point of total energy consumption, and it is anything but evolutionary adaptation to excess utilization of energy stores during acute inflammation [1].

Chronic inflammatory process – an attempt to redefine it

Each inflammatory process includes three phases in which the cellular energy metabolism is changed [6, 7].

During the first phase, the occurring edema leads to hypoxia. Therefore, the cells turn back to a metabolic pathway that repeats the evolutionary anaerobic phase of life – glycolysis occurring in the cytoplasm. Although decreased, oxidative phosphorylation still takes place in the mitochondria because they always contain some oxygen. It is hypoxia, not anoxia. The few oxygen molecules contact with an unchanged number of mitochon-

drial electrons "waiting" for oxygen, which – by bonding – produce a considerable number of oxygen radicals, i.e. oxygen atoms with additional unpaired electrons (reactive oxygen intermediates – ROI) [6, 7]. In a nutshell, although there is definitely less oxygen in an anoxic cell, ROI produced from the still present oxygen cause considerable damage to tissues affected by inflammation. Furthermore, the programmed cell death process (apoptosis) does not occur in immune cells due to hypoxia. Hypoxia inhibits apoptosis of neutrophils and macrophages in the first place, so overrepresentation of those cells can be observed in sites with low oxygen pressure [8].

The next phase is connected with the early revascularization process. It results in oxygen hypermetabolism due to an inflow of well-oxygenated blood [6].

An anoxic tissue is subject to many processes (decomposition of adenosine triphosphate [ATP] to adenosine, conversion of xanthine dehydrogenase [XD] into oxidase [XO]) which cause that oxygen reaching the cell is not fully reduced, and ROI are produced. That phenomenon is called the "oxygen paradox". Moreover, the reperfused tissue attracts great numbers of neutrophils which are the first-line defense by the immune system. Metabolism of the cells is based exclusively on anaerobic glycolysis, while a major mechanism of their toxic effect is the ability of converting oxygen into ROI. The cells are exceptionally effective in uptaking even scarce oxygen particles in order to produce ROI from it. With 100 times lower oxygen levels, the maximum ROI production by neutrophils decreases by about half only. The two phenomena – the "oxygen paradox" and the neutrophil conversion of oxygen into ROI – cause considerable impairment to energy metabolism in the area of an ongoing inflammatory process. The first two stages of inflammation can be called stages of constant disturbance of oxygen metabolism [6, 7, 9].

Only the third phase is the time for proper oxygen metabolism and beginning of relevant repair processes.

The above-mentioned phases are common for all types of inflammation, both local and generalized inflammation. In the case of local inflammation, immune cells (which cannot store much energy) gain energy necessary for their activity through destruction of the extracellular matrix (ECM): they produce metalloproteinases capable of degrading proline, hydroxyproline and glucuronic acid. Moreover, it has been observed that transcription of genes connected directly with the inflammatory process in those cells may be inhibited and replaced by activating genes connected with ECM degradation [10].

In chronic inflammation, the third phase (repair stage) with oxygen metabolism does not begin. It results from the fact that cells cannot draw energy "locally" from the already destroyed ECM while the "systemic" energy sources have run empty due to continued overac-
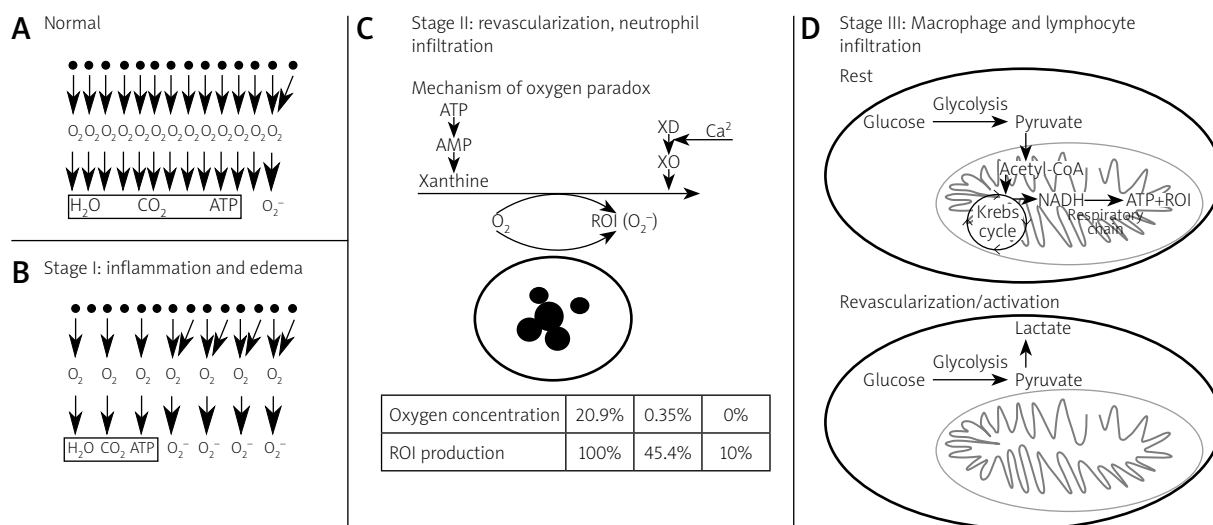
Fig. 1. Oxidative metabolism changes of inflammation in the 1st phase (edema) and 2nd phase (revascularization and infiltration of inflammatory cells). **A.** Normal conditions. With adequate oxygen levels, a small amount of ROI is generated in mitochondria, as a byproduct of oxygen metabolism. **B.** Hypoxia due to edema. Change in the oxygen-electrolytes ratio, despite the reduced absolute oxygen contents, a relative excess of toxic ROI is generated. **C.** Revascularization and first infiltration of inflammatory cells (neutrophils). Due to changes in the anoxic environment [ATP decomposition to adenosine, conversion of xanthine dehydrogenase (XD) to oxidase (XO), accumulation of large quantities of calcium] there are conditions conducive to conversion of inflowing oxygen to its active forms, ROI (oxygen paradox). Neutrophils inflowing with the blood (cells with anaerobic metabolism) convert even extremely low oxygen concentrations into ROI. **D.** Subsequent infiltration by lymphocytes and macrophages. In order to avoid damage due to excess oxygen metabolism, lymphocytes turn to anaerobic metabolism. In anoxic sites, macrophages convert to the M1 pro-inflammatory (anaerobic) phase, followed, in the acute inflammation setting, by the M2 anti-inflammatory (aerobic) phase that promotes reconstruction of tissues. In the chronic inflammatory setting, macrophage cells remain in the M1 anaerobic and pro-inflammatory phase.

ROI – reactive oxygen intermediates, ATP – adenosine triphosphate, AMP – adenosine monophosphate, XD – xanthine dehydrogenase, XO – xanthine oxidase

tivation of the SNS. Changes in the metabolism of cells which infiltrate the inflammation-affected tissues later on, such as lymphocytes or macrophages, contribute to further continuous intensification of energy metabolism disorders (see sections below) (Fig. 1).

Cells participating in inflammation – disciplined soldiers or deserters?

> "Even Siamese brothers can be egoists"
> Anonym

Neutrophils

Neutrophils are the first cells to appear at sites affected by an inflammatory process. Their energy metabolism is limited to glucose, and the role of mitochondria is associated with cell suicide (apoptosis) only [11].

Energy processes taking place in neutrophils may indirectly intensify inflammation. Activity of two major glycolytic enzymes (glyceraldehyde 3-phosphate dehydrogenase, and lactate dehydrogenase) significantly increases in articular tissues in rheumatoid arthritis (AR) patients. Thus, glycolysis can play an important role in AR development because, firstly, its products – lactate and pyruvate – maintain excess proliferation of cells, angiogenesis and pannus formation, and secondly, because glycolytic pathway enzymes, such as glucose-6-phosphatase isomerase, enolase, aldolase and triosephosphate isomerase, act like autoantigens [12, 13].

Participation of neutrophils in the conversion of oxygen residues in anoxic tissues to ROI has already been discussed previously.

Lymphocytes

In contrast to neutrophils, non-activated lymphocytes are dependent on oxidative phosphorylation only. When activated during inflammation, they switch their metabolism to anaerobic glycolysis. It is a result of re-

duced oxygen concentration at the site of inflammation, which substantially affects the behavior of lymphocytes. Additionally, the microcapillary blockade caused by inflammatory cells (lymphocytes and neutrophils) increases hypoxia (non-reflow phenomenon), and thus further switching of inflammatory cells to anaerobic pathways, on a feedback basis [9].

Non-stimulated lymphocyte progenitor cells produce as much as 88% of their necessary energy in the form of ATP in the process of oxidative decomposition of glucose, while activated and proliferating cells switch to the anaerobic pathway, and derive as much as 86% of ATP from anaerobic glycolytic degradation to lactate, with only 14% derived from oxidation to CO_2 and water.

Although aerobic respiration is more effective in energy generation, it is just the anaerobic glycolysis that produces substrates for the structure of newly formed cells. Moreover, the final product of glycolysis, pyruvate, is an effective antioxidant. That is not however the reason for switching to anaerobic metabolism [14].

It turned out that unlike non-activated (aerobic) cells, the activated (anaerobic) ones produce virtually no toxic ROI, which are byproducts of energy-generating processes in mitochondria. More intense oxidative phosphorylation in activated lymphocytes would increase oxidative stress, and thus serious damage to cells. It has been established that minimization of oxidative stress, by decreasing ROI production during cellular activation, is a cell-protecting strategy. The ultimate effect is their longer life at the expense of reduced ability to regulate the inflammatory process [14].

Macrophages

Macrophages play a vital role in inflammation, and primarily in initiating repair processes. In response to signals, both from pathogens and from the body itself, monocytes/macrophages can undergo fast reprogramming.

Non-activated macrophages derive energy from oxidative phosphorylation (oxygen-dependent and taking place in mitochondria). Once activated in an inflammatory site, they convert to "proinflammatory/bacteriocidal" macrophages of M1 type, which derive energy mainly from anaerobic glycolysis; at the stage of inflammation abatement and repair of injured tissues, M1 macrophages convert to M2 macrophages, with anti-inflammatory properties and repair functions, which is accompanied by resuming oxygen-dependent energy metabolism (oxidative phosphorylation, and oxidation of free fatty acids).

It has been proven now that in chronic inflammation, the main subtype of macrophages present in the inflammation-affected tissues is M1 subtype. It is still unclear why M1 macrophages (pro-inflammatory, with anaerobic metabolism) do not convert into M2 subtype (anti-inflammatory, with oxygen metabolism) [15–17].

Reasons for disturbed conversion of M1 macrophages into M2 macrophages are not clear. However, in chronic inflammation conditions, the adaptive phase (abatement, reconstruction) may sometimes occur, but it is ineffective [18].

Summary

Reasons why the acute inflammatory phase does convert into the reconstruction process but becomes chronic inflammation instead are complex, and they include the following:

1. Impaired energy flow to the immune system, leading consequently to disturbed energy homeostasis.
2. Depletion of available energy stores.
3. The inflammatory process stops at the stage of disturbed oxygen metabolism, and the phase of natural oxygenation and repair initiation does not begin.
4. Activity of neutrophils – anaerobic metabolism cells that can produce ROI in the setting of reduced oxygen concentration, thus intensifying the inflammatory process.
5. Activation of lymphocytes, connected with conversion to anaerobic metabolism, which prolongs their life while decreasing the overall metabolism, and thus reduces the possible impact on the course of inflammation.
6. Maintenance of macrophages at the anaerobic pro-inflammatory level of M1 type.
7. Intermediate metabolites produced in anaerobic glycolysis of cells infiltrating inflammation-affected tissues promote development of pannus, and enzymes of that cycle act as antigens.

Possibilities of comprehensive treatment (electromagnetic field) of above-discussed processes connected with oxygen metabolism of cells involved in inflammation will be discussed in the third part of this publication cycle.

References

1. Straub RH. Evolutionary medicine and chronic inflammatory state-known and new concepts in pathophysiology. J Mol Med (Berl) 2012; 90: 523-534.
2. Hansen JC, Gilman AP, Odland JØ. Is thermogenesis a significant causal factor in preventing the "globesity" epidemic? Med Hypotheses 2010; 75: 250-256.

3. Aller MA, Arias N, Fuentes-Julian S, et al. Coupling inflammation with evo-devo. Med Hypotheses 2012; 78: 721-731.

4. Straub RH, Cutolo M, Buttgereit F, Pongratz G. Energy regulation and neutroendocrine-immune control in chronic inflammatory diseases. J Intern Med 2010; 267: 543-560.

5. Straub RH, Besedovsky HO. Integrated evolutionary, immunological, and neuroendocrine framework for the pathogenesis of chronic disabling inflammatory diseases. FASEB J 2003; 17: 2176-2183.

6. Aller MA, Arias JL, Arias J. Post-traumatic inflammatory response: perhaps a succession of phases with nutritional purpose. Med Hypotheses 2004; 63: 42-46.

7. Aller MA, Arias JI, Alonso-Poza A, Arias J. A review of metabolic staging in severely injured patients. Scand J Trauma Resusc Emerg Med 2010; 18: 27.

8. Hannah S, Mecklenburgh K, Rahman I, et al. Hypoxia prolonges neutrophil survival in vitro. FEBS Lett 1995; 372: 233-237.

9. Delmastro-Greenwood MM, Piganelli JD. Changing the energy of an immune response. Am J Clin Exp Immunol 2013; 2: 30-54.

10. Higami Y, Barger JL, Page GP, et al. Energy restriction lowers the expression of genes linked to inflammation, the cytoskeleton, the extracellular matrix, and angiogenesis in mouse adipose tissue. J Nutr 2006; 136: 343-352.

11. Healy DA, Watson RW, Newsholme P. Glucose, but not glutamine, protects against spontaneous and anti-Fas antibody-induced apoptosis in human neutrophils. Clin Sci (Lond) 2002; 103: 179-189.

12. Spies CM, Straub RH, Buttgereit F. Energy metabolism and rheumatic diseases: from cell to organism. Arthritis Res Ther 2012; 14: 216.

13. Chang X, Wei C. Glycolysis and rheumatoid arthritis. Int J Rheum Dis 2011; 14: 217-222.

14. Brand KA, Hermfisse U. Aerobic glycolysis by proliferating cells: a protective strategy against oxygen species. FASEB J 1997; 11: 388-395.

15. Roiniotis J, Dinh H, Masendycz P, et al. Hypoxia prolongs monocyte/macrophage survival and enhanced glycolysis is associated with their maturation under aerobic conditions. J Immunol 2009; 182: 7974-7981.

16. Hristodorov D, Mladenov R, Huhn M, et al. Macrophage-targeted therapy: CD64-based immunotoxins for treatment of chronic inflammatory diseases. Toxins (Basel) 2012; 4: 676-694.

17. Hall CJ, Sanderson LE, Crosier KE, Crosier PS. Mitochondrial metabolism, reactive oxygen species, and macrophage function-fishing for insights. J Mol Med (Berl) 2014; doi: 10.007/s00109-014-1186-6.

18. Liu TF, Brown CM, El Gazzar M, et al. Fueling the flame; bioenergy couples metabolism and inflammation. J Leukoc Biol 2012; 92: 499-507.