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Review Article

In the eye of the storm: T cell behavior in the inflammatory microenvironment

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Abstract: Coordinated unfolding of innate and adaptive immunity is key to the development of protective immune responses. This functional integration occurs within the inflamed tissue, a microenvironment enriched with factors released by innate and subsequently adaptive immune cells and the injured tissue itself. T lymphocytes are key players in the ensuing adaptive immunity and their proper function is instrumental to a successful outcome of immune protection. The site of inflammation is a "harsh" environment in which T cells are exposed to numerous factors that might influence their behavior. Low pH and oxygen concentration, high lactate and organic acid content as well as free fatty acids and reactive oxygen species are found in the inflammatory microenvironment. All these components affect T cells as well as other immune cells during the immune response and impact on the development of chronic inflammation. We here overview the effects of a number of factors present in the inflammatory microenvironment on T cell function and migration and discuss the potential relevance of these components as targets for therapeutic intervention in autoimmune and chronic inflammatory diseases.

Keywords: T lymphocytes, inflammatory microenvironment, hypoxia, pH, fatty acids, lactate, reactive oxygen species

Introduction

In multicellular organisms inflammation is a characteristic reaction of the immune system to extrinsic or intrinsic danger stimuli that favors the reestablishment of tissue homeostasis. Acute inflammation on one hand is triggered by infection, tissue injury or malfunction and describes the short-term recruitment of immune cells into the affected site whose physiological purpose is pathogen clearance and tissue repair. Chronic inflammation on the other hand is a low-grade, constant and long-lasting tissue infiltration of immune cells, which often causes severe tissue damage and remodeling, a common feature of autoimmune and chronic inflammatory diseases, such as atherosclerosis, rheumatoid arthritis and diabetes [1].

T lymphocytes play a major role in the development of these inflammatory processes. Besides their direct cytotoxic activities, T cells orchestrate the unfolding immune response via the secretion of pro- and anti-inflammatory cytokines that influence the severity and outcome of the inflammatory reaction [2]. Both effector and regulatory T (Treg) cells are essential to establish and maintain an effective immune response or dampen an overshooting reaction to prevent autoimmune disease. Therefore, the resolution of the inflammatory process following pathogen clearance depends on the concerted localization of both these T cell subsets to reach an optimal ratio, which terminates effector immunity [3]. Several well-described stimuli direct the function and migration patterns of T lymphocytes, such as cytokines, chemo-attractants and -repellents or direct cell-cell interactions [4, 5].

In this context, some of the components of the inflammatory microenvironment are likely to impact on the dynamic progression of the inflammatory process. Low glucose, nutrient and oxygen concentration, high lactate and organic acid content, the presence of fatty acids and other metabolites make the inflammatory site a unique "milieu" that affects

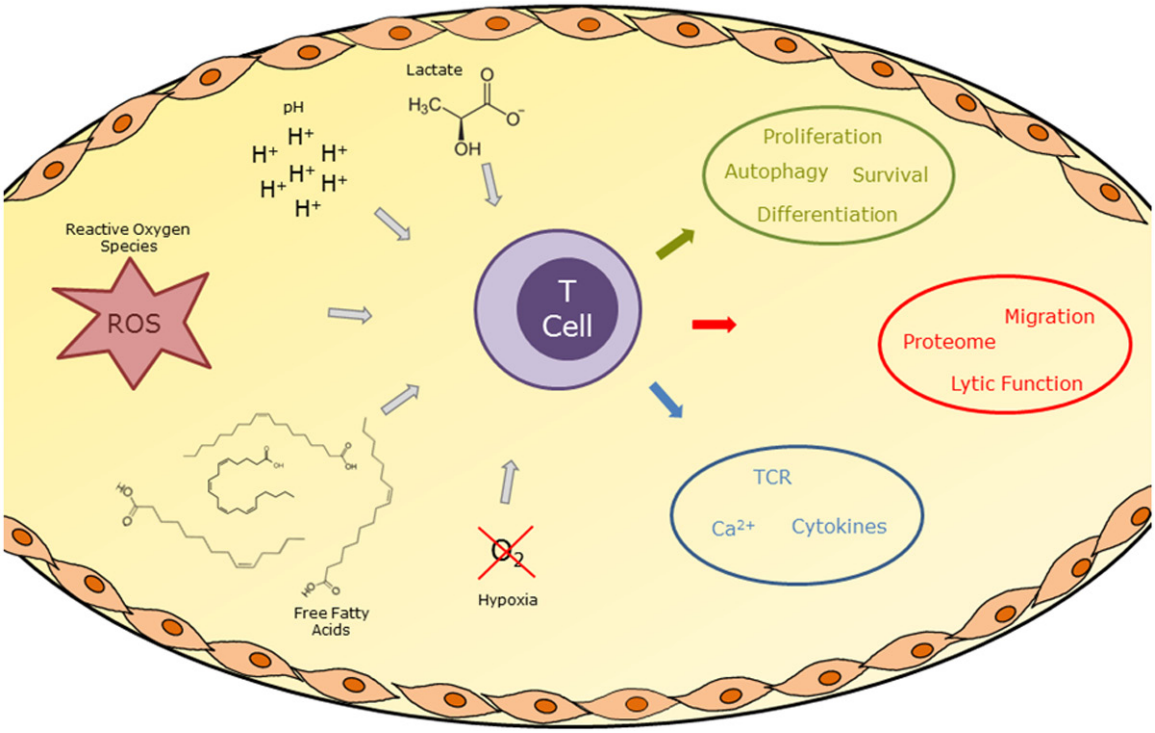

Figure 1. Factors in the inflammatory microenvironment. Several factors in the inflammatory microenvironment (e.g., oxygen concentration, pH, lactate, fatty acids and ROS) can influence the function of T cells and other immune cells on a number of levels and determine the outcomes of the inflammatory process.

immune cells in a yet underappreciated manner. Substantial evidence for the metabolic control of T cell function and their response to environmental changes has recently emerged. The discovery that T cell subsets rely upon different energy sources and respond to diverse signaling cues opens up novel ways of therapeutically interfering with immune cell function in chronic inflammatory disorders [6-11].

This review focuses on the effect of some of the essential factors present in the inflammatory microenvironment, including oxygen concentration, pH, fatty acids, lactate and reactive oxygen species (ROS), on immune cell function, particularly that of T cells, within the inflammatory site.

Components of the inflammatory "milieu"

The inflammatory site is a hostile and harsh environment for T cells to function and successfully fulfill their tasks. Although its molecular composition slightly differs from disease to disease, some similarities exist that could be considered for effective therapeutic targeting.

The effect on T cells of some of the factors present in the inflammatory microenvironment, particularly those commonly present in chronic inflammation, including oxygen concentration, pH, lactate, fatty acids and ROS will be discussed (**Figure 1**).

As the role of cytokines, chemokines and danger-associated molecular patterns (DAMPs) as major signaling molecules that regulate T cell function and correct tissue localization during an immune response has been extensively examined in recent excellent articles [12-15], we will not discuss their role in detail.

Hypoxia defines the inflammatory environment

Inflamed tissues are often characterized by decreased oxygen availability (<2%) [16], generally known as hypoxia. Inflammation-associated tissue hypoxia is due to an imbalance in oxygen demand and supply in inflammatory sites. On one hand hypoxia is a consequence of increased oxygen consumption by resident cells such as epithelia or vascular endothelia,

and also by recruited inflammatory cells, such as macrophages, neutrophils and T cells [17], which are metabolically extremely active. On the other hand oxygen supply in the inflammatory site is often decreased due to vascular occlusion or thrombosis [18]. Furthermore, not only inflamed tissues often become hypoxic, but hypoxia promotes inflammation due to the increased release of inflammatory cytokines by the hypoxic tissue and subsequent recruitment of inflammatory cells. Therefore, inflammation and hypoxia are intertwined in a forward feedback loop that is key to the establishment of chronic inflammation [18].

In diabetes and obesity oxygen consumption rises due to the increase of adipose mass. As a consequence, the adipose tissue becomes hypoxic. A study showed that both wild type mice on high fat diet and leptin-deficient (ob/ob) mice have a decreased partial oxygen pressure in their white adipose tissue [19]. In the hypoxic environment adipocytes increase the expression of the pro-inflammatory cytokines TNFα, IL-1, IL-6 and TGFβ and the chemokine MIF, which in turn are responsible for macrophage and T cell recruitment [19]. Furthermore, the expression of the extracellular enzyme matrix metalloproteinase-9 (MMP-9) is elevated during hypoxia and this has been shown to correlate with early neutrophil infiltration in the inflammatory site [20]. In a recent study, Fujisaka et al. demonstrated that hypoxia in the adipose tissue is responsible for the induction of inflammatory M1 polarity of macrophages in a HIF1α-dependent and -independent manner [21].

As the above-mentioned studies show, hypoxia and inflammation are tightly interconnected and tissue hypoxia can be found in a variety of other chronic inflammatory diseases. Atherosclerosis [22], rheumatoid arthritis [23], inflammatory bowel disease [24], sepsis [25], ischemic stroke [26] and fast growing tumors [27] have all been associated with severe shortage of oxygen in the inflamed tissues. Cells exposed to an environment with low oxygen concentration up-regulate several survival mechanisms, which are under the control of the oxygen sensitive transcription factor, HIF1α. Under normoxic conditions (2-3%) [16], HIF1α is hydroxylated by prolyl-hydroxy-domain containing enzymes (PHDs), which target HIF1α for ubiquitinylation-dependent degradation by the proteasome. However, hypoxia-induced inactivation of PHDs triggers HIF1α translocation to the nucleus where it promotes the transcription of up to 200 target genes. Many of these genes are important for cell survival [28]. Nevertheless, despite the activation of survival pathways, some highly proliferative cells, such as T lymphocytes, cannot cope with the depletion of oxygen. In a recent study, Gaber and colleagues observed that $CD4^+$ T cells have a reduced rate of proliferation and survival in a model of phytohemagglutinin-stimulated T cell growth in hypoxic conditions [16].

In addition to the induction of obvious survival pathways in hypoxic environments, $CD4^+$ T cells simultaneously decrease cytokine production. This decline in T cell effector function has been proposed to be a protective mechanism against prolonged tissue damage caused by hyper reactive T cells in inflammatory conditions [29]. However, the lytic function of cytotoxic $CD8^+$ T lymphocytes (CTLs) has been shown to be unaffected or even enhanced by low oxygen pressure [30].

In conclusion, oxygen levels in inflamed tissues are generally lower than in healthy tissues, making hypoxia a main feature of the inflammatory microenvironment and an important factor in the development and progression of inflammation.

Low pH inhibits cellular function

The effect of intra- and extracellular pH variations on cell function has long been recognized. A decrease in pH generally inhibits most cellular functions including enzyme activities, ion transport, DNA and protein synthesis, and influences cAMP and calcium levels [31]. The inflammatory microenvironment is characterized by a decrease in pH. Measurements of pH in the synovial fluid of healthy (7.768±0.044), traumatic (7.559±0.031) or osteoarthritic (7.549±0.040) patients, for instance, showed a decrease of about 0.2 pH units in an array of patients [32]. An even steeper decrease of pH was found in atherosclerotic plaques in humans (7.15) [33]. The tumor environment can have pH values below 6, which has been suggested to contribute to the establishment of immune escape mechanisms [31].

The low pH in inflamed tissues often originates from increased metabolism of parenchymal

and recruited inflammatory cells in conjunction with decreased oxygen availability [31]. Most cells rely upon oxidative phosphorylation for ATP production in the presence of oxygen. During hypoxia, cells switch their energy metabolism to anaerobic glycolysis, which leads to increased production of lactate. Increased lactate in turn is coupled, even if only indirectly, with the secretion of protons that causes a drop in pH [34].

Although most studies that focus on the effect of extracellular pH on cell function show generally a decrease, different effects can occur in distinct cell types. Trevani et al. showed that lowering the extracellular pH to 6.5 enhances the activation of neutrophils, which might lead to an intensification of the innate immune response [35]. The acidic pH causes an intracellular accumulation of calcium that leads to an elevated production of H_2O_2 [35]. Another group has shown that acidic pH drives activation of integrin $\alpha v\beta_3$, a receptor for vitronectin [36]. This increase in integrin affinity and avidity leads to a strengthening of cell-cell connections, which in turn slows down the migration of neutrophils [37]. Unlike neutrophils, T cells exposed to low pH display a decrease in their immune function. As reported by Redegeld et al., cytotoxic T lymphocytes lose their lytic function in a low pH environment [38]. Furthermore, it is known that low pH causes reduced interactions of lymphocytes with the extracellular matrix, which in turn leads to reduced migration. The specific molecular mechanisms of these pH-afforded effects on lymphocytes migration, however, remain elusive [31].

To sum up, low pH is commonly found in the inflammatory microenvironment and affects the migration and effector function of lymphocytes and other immune cells, thereby contributing to the progression of the inflammatory process.

'Fat' signals

The presence of free fatty acids (FFAs) in the inflammatory environment is yet to be proven to be a general occurrence. In obesity though, free fatty acids are abundant in the fat mass due to the fat breakdown in mature adipocytes [39]. As obesity is a major risk factor for atherosclerosis, FFAs have also been found to be highly enriched in the arterial walls [40]. Together with cholesterol, FFAs are the forerunners of plaque formation, as they accumulate at the level of the arterial wall and trigger resident macrophages to become foam cells [41].

In addition to macrophages, it is becoming increasingly evident that T cells are recruited in the very early stages of atherosclerotic plaque formation and play a major role in the inflammatory process driving the development of atherosclerosis [40]. $CD4^+$ T_{H1} cells are responsible for the amplification of the local immune response in atherosclerotic plaques via the secretion of the pro-inflammatory cytokines INFγ, IL-2 and IL-3 as well as TNFα and β [42]. $CD4^+$ T cells have also been shown to polarize to a T_{H1} phenotype with increased IFNγ expression in obese children [43].

Considering the crucial role of T cells in the pathogenesis of lipid-enriched inflammatory diseases, it is important to understand the possible effects that lipids might exert on T cell function. Based on the geometry of the double bond, unsaturated fatty acids exist in two isoforms: *cis*-isomers and *trans*-isomers. In *cis*-isomers, adjacent hydrogen atoms are on the same side of the double bond, whereas in *trans*-isomers adjacent hydrogen atoms are on opposite sides of the double bond. With *cis*-isomers the rigidity of the double bond freezes their conformation and causes the chain to bend and restricts the conformational freedom of the fatty acid. In *trans*-isomers, instead, double bonds are more flexible, therefore they do not cause the chain to bend much and their shape is similar to straight saturated fatty acids. These two isoforms have different effects on T cells. Stulnig et al. reported decreased calcium-signaling responses in cultured Jurkat T cells, when treated with long chain *cis*-unsaturated FFAs. In contrast, *trans*-unsaturated or saturated FFAs had no effect. Moreover, they observed that primary T cells isolated from blood of patients given an elevated dose of *cis*-unsaturated FFAs also showed diminished calcium signaling after in-vitro activation [44].

Zeyda et al. went on to identify the specific steps of the TCR downstream signaling pathway that are affected by polyunsaturated fatty acids (PUFAs) [45]. A selective inhibition of mitogen activated protein kinase (MAPK), Janus Kinase (JNK), was observed, with no effect on other MAP kinases. Although JNK was inhibited, they were unable to detect any change in

AP-1 (c-JUN) activity, the main downstream transcription factor of JNK. They found instead a selective inhibition of NF-AT transcription factor, which is possibly due to diminished calcium signaling. As NF-AT activates IL-2 transcription [46], the observed decrease in IL-2 expression upon PUFAs treatment was somehow expected. Notably, also the T_{H2} type cytokine IL-13 was suppressed by the same treatment, suggesting an additional mechanism of action of PUFAs downstream of TCR [45].

Geyeregger et al. demonstrated yet another mechanism of PUFAs action in T cells following activation with the superantigen (SAg) staphylococcus enterotoxin E. Herein, the SAg induces a non-specific T cell activation by peptide-independent ligation of MHC molecules with the TCR that results in polyclonal T cell expansion and massive cytokine release. In this model, the formation of the immunological synapse was inhibited following treatment with the n-3 PUFA, eicosapentaenoic acid (EPA). Upon EPA treatment, cytoskeletal rearrangements, necessary for the formation of the synapse were blunted, probably due to reduced Vav-phosphorylation [47], which acts as a promoter of the synapse formation via linking the actin cytoskeleton to TCR signaling [48].

Furthermore, the n-3 PUFAs, EPA and docosahexaenoic acid (DHA), were shown to affect T cell migration and expression of MMP-9 in Jurkat T cells. Both EPA and DHA significantly decreased the migration of T cells and reduced activity of the enzyme MMP-9, which has been associated with the disruption of the blood brain barrier and subsequent invasion of the central nervous system by inflammatory cells. Authors of this study proposed a possible treatment for patients with multiple sclerosis, whereby EPA / DHA supplied exogenously could benefit the disease prognosis [49]. In atherosclerosis or rheumatoid arthritis instead enrichment of n-3 PUFAs in the inflamed tissues could potentially be harmful due to the inhibition of the migratory capacity of inflammatory cells which would become unable to leave the inflammatory site timely, thereby favoring the development of the disease into a chronic state.

Lactate, a multi-faceted signaling molecule

The short chain monocarboxylate lactate has a long history in biochemistry, reaching from the first description of lactic acid in sour milk in 1780 and the presence in human blood in 1843 [50], to the discovery of lactate production by highly proliferative tumor cells [51] and the finding that lactate was enriched in several inflammatory diseases and the tumor microenvironment [52]. Generally, lactate is considered a metabolic 'waste product' and the result of high glycolytic activity in proliferating cells. This view on lactate has been changing considerably in recent times, as its role as an important signaling molecule is being recognized [53].

In the brain, lactate acts as a fuel source for oxidative energy metabolism in neurons. Thereby, high glutamate levels increase the glycolytic activity of specific glial cells known as astrocytes, which in turn produce lactate and transport it into neurons, which use it as an additional energy source [54]. Besides neurons, lactate has significant effects on other cell types as well. The migration potential of tumor cells, for instance, increases in the presence of lactate [55]. Furthermore, Baumann et al. showed that glioma cell migration is enhanced, as lactate induces a TGF-β2-dependent regulation of matrix metallopeptidase 2 (MMP-2) [56]. These findings could have important implications in the understanding of tumor progression and metastasis formation.

Lactate indirectly stimulates the migration of endothelial cells via enhanced VEGF secretion, which is a potential promoter of wound healing [57]. This effect is possibly explained by the uptake of extracellular lactate by endothelial cells [58] and the stimulation of HIF1α up-regulation [59], which has been shown to correlate with increased VEGF secretion [60].

Most importantly, lactate is locally enriched in inflamed tissues, such as the joints in rheumatoid arthritis, atherosclerotic plaques and tumors [52, 61, 62]. Moreover, elevated lactate levels in the blood have been associated with increased prevalence of atherosclerosis and type II diabetes [63]. Fischer et al. showed that human cytotoxic T cells are able to take up lactate and thereupon are inhibited in their proliferation potential. This effect was only observed in the presence of elevated H^+ ions, or low pH [64]. It is tempting to speculate that T cells entering the inflammatory site become exposed to elevated levels of lactate, between 10 and 30 mM, which disables their ability to emigrate and favor their retention. If proven correct, this hypothesis could provide an additional mecha-

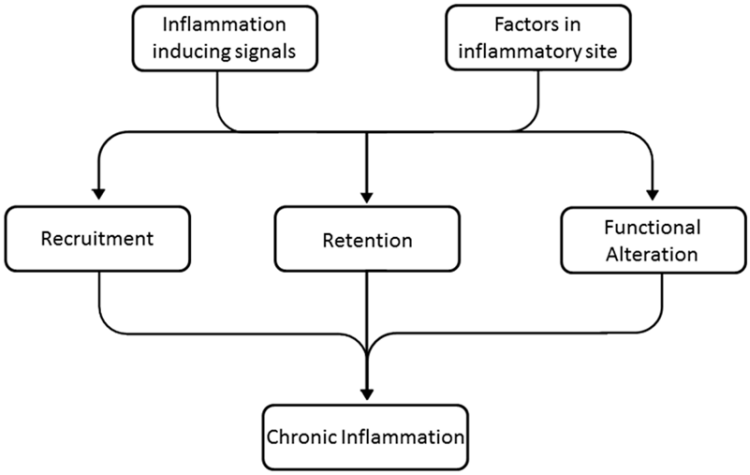

Figure 2. Effects of factors present in the inflammatory microenvironment on the establishment of chronic inflammation. Not only inflammation inducing signals (e.g., cytokines, chemokines, DAMPs) lead to the recruitment of immune cells to the site of inflammation. Additionally factors within the inflammatory microenvironment (depicted in Figure 1) affect the behavior of immune cells in terms of recruitment, retention and function, and contribute to the establishment of chronic inflammation.

nism to explain the issue of non-resolving inflammation and its progression to a chronic process [65, 66].

As the above-mentioned studies demonstrate, lactate is a multi-faceted signaling molecule and its effects on cell function are likely to be more far-reaching than understood at present.

Reactive oxygen species

The effects of reactive oxigen species (ROS) on cells have historically been associated with physiological dysfunction and cellular damage. This view is about to be extended by recent findings suggesting roles for ROS in intracellular signaling events, induction of apoptotic pathways, cell activation and feedback mechanisms to metabolism [67]. Increased ROS production has been associated with a wide variety of diseases, including neurodegenerative disorders, diabetes, cancer and atherosclerosis [68-70]. ROS, such as superoxide (O_2^-), can be generated from several sources, which include NADPH oxidase (NOX), xanthine oxidase (XO), nitric oxide synthase (NOS), cytochrome P450 and the mitochondrial electron transport chain (ETC) [71]. Most ROS are generated via the electron reduction of the oxygen molecule, O_2, during oxidative phosphorylation in the mitochondria. Superoxide is then transformed into H_2O_2 by superoxide dismutase to prevent ROS from causing intracellular damage to the cell [67]. It is thought that 0.2-2% of the oxygen consumed from mitochondria is transformed to superoxide [72]. This mitochondrial ROS can have several effects on the cell, including induction of autophagy under starving conditions [73], regulation of differentiation and promotion of aging [74].

As most ROS are produced in the mitochondria, they exert their damaging function mainly inside the cell. ROS are nevertheless abundantly present in the sites of inflammation and thus affect immune cells in their function. Besides the superoxide release that happens during the respiratory burst of neutrophils, also phagocytes have the ability to produce and release superoxide by using a plasma membrane bound NADPH oxidase [75].

Although superoxide cannot permeate the membrane due to its negative charge, the main product of the superoxide dismutase, H_2O_2, is permeable and might contribute to the majority of the signaling function of ROS [76]. It has recently been shown that ROS production in mitochondria is required for the induction of NF-AT and subsequent IL-2 production in activated T cells [77]. In addition, it has been reported that redox signaling via H_2O_2 could alter the proteome of activated T cells both in a qualitative and quantitative manner, thereby directly affecting the function of T cells in inflammation [78]. Therefore, H_2O_2 appears to be an important factor in the inflammatory environment in which it affects immune cells activation and function.

Conclusions

In this review we describe a number of components of the inflammatory microenvironment that can have critical effects on the function of the immune cells recruited to the site of inflammation and might have an impact on the progression of inflammation into a chronic condi-

tion. With regard to T lymphocytes, these factors can promote long-term accumulation of activated T cells in the site of inflammation due to the increase of both recruitment and retention mechanisms. Furthermore, these factors can cause functional alteration of the immune cells present in the inflammatory microenvironment. All these aspects conceivably contribute to the development of chronic inflammation [1] (**Figure 2**).

At present, most studies have focused on effects elicited by a single component on a defined cell type, however the overall landscape of the inflammatory process is likely to be dynamically and qualitatively defined by the effects of multiple factors on a variety of immune cellular components. For this reason it will be essential to systematically study the effects of these factors on immune cells recruited to the inflammatory sites. Not only should they be studied alone but also in combination, in order to establish potential synergistic or neutralizing effects on the outcome of the inflammatory process.

Although inflammatory microenvironments may differ among diseases, they all exert effects on the recruited immune cells leading to the persistence of the inflammatory process. Therefore, an accurate description of inflammatory elements and comparison in different pathologies is of crucial importance. This would enable a better understanding of the inflammatory process leading to the development of chronic disease, and open up a variety of novel therapeutic interaction possibilities.

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