Hypoxia, Hypoxia-Inducible Factor-1α, and Innate Antileishmanial Immune Responses

Valentin Schatz, Patrick Neubert, Franz Rieger and Jonathan Jantsch*

Institute of Clinical Microbiology and Hygiene, University Hospital of Regensburg, University of Regensburg, Regensburg, Germany

Low oxygen environments and accumulation of hypoxia-inducible factors (HIFs) are features of infected and inflamed tissues. Here, we summarize our current knowledge on oxygen levels found in Leishmania-infected tissues and discuss which mechanisms potentially contribute to local tissue oxygenation in leishmanial lesions. Moreover, we review the role of hypoxia and HIF-1 on innate antileishmanial immune responses.

Keywords: hypoxia-inducible factor 1, leishmaniasis, macrophages, oxygen, Nos2, hypoxia

INTRODUCTION

Low oxygen (O₂) environments are a key feature of infected and inflamed tissue. Several lines of evidence demonstrate that oxygen levels of afflicted tissues are much lower than those currently used in standard cell culture experiments and, in general, correspond to values below 4% O₂ [reviewed in Ref. (1, 2)]. Low oxygen levels are able to incapacitate oxygen-dependent antimicrobial effector enzymes such as the phagocytes oxidase or inducible NO synthase which both require oxygen as cosubstrate in order to produce their antimicrobial reactive oxygen species (ROS) and reactive nitrogen species (RNS) [reviewed in Ref. (1, 2)]. Moreover, hypoxia is not only a state of reduced availability of oxygen but also in addition induces a transcriptional response, which is governed by the transcription factors (TFs) hypoxia-inducible factor (HIF)-1α and HIF-2α. Both TFs belong to the basic helix–loop–helix-PAS family of TF, consisting of HIF-1α or HIF-2α and its dimerization partner aryl hydrocarbon receptor nuclear translocator (ARNT) [reviewed in Ref. (3)]. Prolyl-hydroxylase domain (PHD) enzymes play a key role in the regulation of HIF-1α and HIF-2α since oxygen is a critical substrate for the PHD enzymes. Under conditions of ample oxygen, they hydroxylate HIF-α subunits that target HIF-α in a von Hippel-Lindau tumor suppressor-dependent manner to proteasomal degradation [reviewed in Ref. (4–6)].

Subsequent studies revealed that HIF-1α is not only involved in adaption of cells to low oxygen environments but that this TF is also stabilized upon infectious and inflammatory stimuli under conditions of ample oxygen as well. Furthermore, HIF-1α is required for inflammatory responses of innate immune cells in vitro and in vivo [reviewed in Ref. (1, 7–11)]. Mechanistically, normoxic, inflammatory HIF-1α stabilization is closely linked to nuclear factor (NF)-κB activation (12, 13), and involves transcriptional and posttranslational signaling events (14–16). Altogether, these findings demonstrate that hypoxic and inflammatory responses are intertwined.

Therefore, there is a growing interest to uncover the impact of hypoxia and HIF-1α in infectious diseases and its impact on host–pathogen interaction [reviewed in Ref. (1, 2, 17–21)]. In this review, we will summarize the evidence of hypoxia and the TF HIF-1α and its impact on innate immune responses directed against infection with Leishmania major, Leishmania amazonensis, and Leishmania donovani, which are able to cause cutaneous, mucocutaneous and systemic (visceral) diseases, respectively (Table 1).
OXYGEN LEVEL IN LEISHMANIAL LESIONS

In guinea-pigs, intravenous challenge experiments with guinea-pig adapted *Leishmania enrietti* demonstrate that skin tissue predisposes to the development of leishmanial lesions (22). Given that skin tissue is known to display low oxygen levels (23–25), these data suggest that low oxygen micro-environments might provide a safe haven for *Leishmania* parasites (22). Araújo et al. assessed lesional tissue oxygen levels with an immunohistochemical method in cutaneous *L. amazonensis* infection (26). For that purpose, they injected a 2-nitroimidazol derivative into *L. amazonensis* infected mice. Upon injection, these compounds are enriched in tissues with very low oxygen tensions and form adducts that can be visualized after staining with adduct-specific antibodies (27, 28). Using this technology, they found that in *L. amazonensis*-induced lesions very low oxygen tensions are present (26).

To the best of our knowledge, there are no data available on tissue oxygen levels after infection with *L. donovani* in liver tissue. Since it is known that steep oxygen gradients exist in the liver (27), it is very likely that *L. donovani*-infected liver tissues display low oxygen levels as well. Using 2-nitroimidazol based techniques to visualize hypoxic tissues, Hammami et al. revealed that in *L. donovani*-infected spleens, very low tissue oxygen prevail (29).

Although staining of hypoxic areas with 2-nitroimidazol-derivatives allows for identification of severely hypoxic tissue, this method does not provide quantitative data and is not suitable for continuous recording of lesional tissue oxygen levels (25). Alternative methodologies to quantitatively assess tissue oxygen levels rely on polarographic oxygen sensors or imaging of tissue oxygenation by using oxygen-dependent luminescence quenching (25). For instance, non-invasive luminescence-based oxygen imaging using sensor foils can be used to quantitatively assess tissue oxygenation in a non-invasive manner over time (25). Using this technology in a mouse model of self-healing cutaneous leishmaniasis, Mahnke et al. observed that oxygen tension in leishmanial skin lesions displayed oxygen levels around ~2.8% O₂ when the lesions reached their maximum size (30). Resolution and healing of the lesion was paralleled by an increase of tissue oxygenation (30). Altogether, these findings indicate that low oxygen levels prevail in *Leishmania*-infected tissue.

| Leishmania species | Type of disease                     | Tissue tropism               | Role of HIF-1α in mononuclear phagocytes |
|--------------------|-------------------------------------|------------------------------|------------------------------------------|
|                    |                                     |                              | In vitro                                 |
| *L. major*         | Cutaneous leishmaniasis             | (Local) skin                 | Parasite control                        |
|                    |                                     |                              | Induction of Nos2                       |
|                    |                                     |                              | Cutaneous control of parasites          |
| *L. amazonensis*   | Mucocutaneous leishmaniasis         | Skin with diffuse chronic progression | Parasite survival                      |
|                    |                                     |                              | Unknown                                  |
| *L. donovani*      | Visceral leishmaniasis              | Spleen, bone marrow, liver   | Parasite survival                       |
|                    |                                     |                              | Induction of MDSC                       |
|                    |                                     |                              | Propagation of parasites                |

Potential Factors Regulating Tissue Oxygen Levels in *Leishmania*-Infected Tissues

The mechanisms that drive low oxygen levels in *Leishmania* infected tissue are, however, largely unknown. Recent studies demonstrate that influx of granulocytes results in increased O₂ demand which ultimately results in low tissue oxygen levels (31). Low tissue oxygenation in *L. major*-infected tissue might result from either (i) infection-induced disturbance of local tissue perfusion or (ii) an increased local O₂ consumption by the infectious agent and/or infiltrating immune cells (Figure 1). However, the contribution of infiltrating granulocytes, monocytes, NK cells, and T cells on tissue oxygen levels in *Leishmania* infected tissues is unexplored. Moreover, *Leishmania* might interfere with inflammation induced angiogenesis. For instance, *Leishmania* is able to scavenge angiogenic factors such as VEGF-A. Thereby *Leishmania* might interfere with inflammation-associated angiogenesis and disturb local oxygen supply (32). Nevertheless infection with *L. major* eventually triggers a vascular endothelial growth factor (VEGF)-A/VEGFR receptor (VEGFR)-2-dependent proliferation of endothelial cells (33). Underscoring the potential important role of VEGF-A/VEGFR-2 signaling, Araújo and Giorgio suggest that VEGF-A levels upon infection might predict the outcome of *L. amazonensis*-infection. They recorded higher VEGF-A levels in healer mice compared to non-healer mice (34). Since VEGF-A is a known HIF-1α target (35) and HIF-1α is present in *L. amazonensis* and *L. major*-infected lesions in humans and in preclinical models (26, 33, 34, 36), it is possible that HIF-1α plays a role in infection-induced proliferation of the endothelial cells. However, to the best of our knowledge, this has not been tested in *L. major* and *L. amazonensis* mouse models yet. In addition to maintaining oxygenation of infected tissue, endothelial cell proliferation might curtail *L. major* induced-inflammatory responses (37). The activity of endothelial NO synthase plays an important role in endothelial cell mediated anti-inflammatory activity (38–40). Endothelial cell mediated anti-inflammatory activity might reduce the influx of immune cells and/or their O₂-consumption, thereby increase tissue oxygen levels and facilitate subsequent resolution of disease. Since endothelial NO synthase expression is linked to HIF-1α-accumulation (41), endothelial HIF-1α might promote anti-inflammatory properties in endothelial cells as well. Further studies, however, are required to investigate this issue. Earlier findings demonstrate that the “myeloid cell differentiation antigen carcinoembryonic antigen-related cell adhesion molecule 1”
Infiltration of immune cells might drive low tissue oxygenation in Leishmania major-infected skin. Upon L. major-infection, infiltration of granulocytes [polymorphonuclear granulocyte (PMN)], monocytes (Mono), dendritic cells (DC), NK cells, and T cells might induce increased local consumption of O2 resulting in hypoxic tissue O2 levels.

(CEACAM1) is involved in angiogenesis and antileishmanial control (42). Whether CEACAM1-dependent signaling affects VEGF-A/VEGFR and/or HIF-1α signaling in this state of affair is unknown. Further investigations are required to understand the interplay of inflammation-triggered angiogenesis, enhanced O2 demand in inflamed tissues, and its impact on overall tissue oxygenation in leishmaniasis.

**IMPACT OF HYPOXIA ON HOST–PATHOGEN INTERACTION**

**Internalization of Leishmania and Hypoxia**

After transmission of Leishmania by its vector, myeloid cells are the main host cell [reviewed in Ref. (43–45)]. Hypoxic conditions are known to modulate the phagocytic/endocytic uptake of mononuclear phagocytes (46, 47). However, to the best of our knowledge, the impact of oxygen levels below 4% O2 on Leishmania uptake or internalization is unexplored.

**Polymorphonuclear Granulocytes (PMN), Hypoxia, and Leishmania**

Polymorphonuclear granulocytes are quickly attracted to the site of infection. Dependent on the Leishmania species and the host’s genetic background, they play a rather detrimental role in cutaneous leishmaniasis. PMN are inefficient in killing promastigotes and undergo apoptosis which hampers the proper activation of recruited dendritic cell (DC) [reviewed in Ref. (44, 48)]. In general, PMN are relatively short-lived cells, whose survival, however, is prolonged in NF-κB- and HIF-1α-dependent manner under hypoxic conditions. Moreover, hypoxia induces the production of macrophage inflammatory protein-1β which further facilitates neutrophil survival (49). In line with this, Monceaux et al. demonstrated recently that anoxic conditions prolong PMN survival (50). Therefore, hypoxia most likely favors influx and lifespan of PMN in leishmaniasis as well. Since these cells are not able to eliminate Leishmania, this might ultimately prolong and/or exacerbate the disease. However, to the best of our knowledge, there is no detailed analysis on the specific role of hypoxia on PMN–Leishmania interaction.

**DC, Hypoxia, and Leishmania**

Upon Leishmania exposure, DC phagocytose the parasite, become activated and establish an antileishmanial T cell response and thereby critically contribute to the resolution of disease [reviewed in Ref. (51)]. For instance, priming and induction of Leishmania-specific Th1 response results in secretion of IFN-γ which is required for antileishmanial immunity [reviewed in Ref. (44)]. In addition, even after resolution of disease, DC still contain viable parasites which are able to maintain antigen-specific T cell responses against L. major. This contributes to the long term protection against the parasites [reviewed in Ref. (51)]. Although, there is evidence that oxygen levels impact on DC functions, there are to the best of our knowledge only
limited reports on the contribution of hypoxia on DC-mediated establishment of antileishmanial T cell immunity. Hypoxia might impact on DC viability (52), migratory capacity (53), maturation, and antigen presentation (54–56).

**NK Cells, Hypoxia, and Leishmania**

In early phase of *Leishmania* infection, NK cells are activated by IL-12 and IL-18. These activated NK cells contribute to parasite control through production of IFN-γ [reviewed in Ref. (57)]. NK cells respond to hypoxia by downregulation of surface markers NKp46, NKp30, NKp44, NKG2D, and CD16, decreased cytotoxic and antiviral activity, and reduced IFN-γ expression (58–60). In contrast to these findings, Krzywinska et al. observed no mitigated IFN-γ secretion by NK cells under hypoxia (61). However, the impact of hypoxia on NK-mediated antileishmanial defense is unexplored and requires further studies.

**Monocytes/Macrophages, Hypoxia, and Leishmania**

A few days after *L. major* and *L. donovani* infection, recruited monocytes are highly abundant in afflicted tissues (62, 63). Inflammatory monocytes are able to upregulate their ROS production in the early stage of infection with *L. major* and *L. donovani* (64, 65). However, in inflammatory monocytes infected with *L. major*, ROS production does eliminate the parasites (63). Moreover, *L. donovani*, for instance, has the ability to overcome the ROS-mediated apoptosis of host macrophages via induction of suppressor of cytokine signaling proteins (66). It is established that IFN-γ and TNF are two key cytokines that empower macrophages (67–69) and monocytes (63) to clear *Leishmania*. In the mouse system this results in a robust induction of the antileishmanial effector enzyme, the inducible or type 2 NO synthase (iNOS or NOS2). A high level of NO production is prerequisite of the cutaneous defense against *Leishmania major* [reviewed in Ref. (70, 71)]. Moreover, in mouse models of visceral leishmaniasis, Nos2 expression is important for control of *L. donovani* in the liver (72), and contributes to the containment of *L. donovani* (73) in the spleen.

For induction of Nos2 and production of high level of leishmanicidal NO, costimulation of macrophages with TNF plus IFN-γ is required (63, 68, 72, 74–78). Low oxygen conditions, however, impair NO production of activated macrophages because the inducible NO synthase activity critically hinges on the availability of O2 as a substrate (79–81). Accordingly, under oxygen conditions below 4% O2 NO production of activated macrophages is diminished (30). *L. major* possesses a globin-coupled heme containing oxygen sensor soluble adenylate cyclase which allows for its adaption to hypoxic conditions (82). Hence, while *L. major* is able to adjust to hypoxia, low oxygen conditions impair the NO-dependent antileishmanial potential of macrophages resulting in impaired clearance and survival of *L. major* in activated macrophages under low oxygen conditions. However, leishmanicidal activity is restored when the cells are reoxygenated (30). These data demonstrate that local tissue oxygen levels—at least transiently—do not match the macrophages’ O2 demand to fight against *L. major*.

In visceral leishmaniasis, parasites express high tissue tropism toward monocytes in the spleen and bone marrow. *L. donovani*, e.g., induces expansion of hematopoietic stem cell-like mononuclear cells from bone marrow which serve as host cells for the parasite (62). Compared to the role of Nos2 in the defense against visceral leishmaniasis in the liver (72), the contribution of Nos2 in the control of parasite load in the spleen in visceral leishmaniasis is less pronounced. While there was no induction of Nos2 in spleen macrophages upon infection of hamsters with *L. donovani* (83), Nos2 expression, nevertheless, contributed to the parasite-containment in the spleen of *L. donovani* and *L. infantum*-infected mice (73, 84). In preclinical models of visceral leishmaniasis, the impact of local oxygen levels on Nos2-mediated antileishmanial defense and NO production in the spleen and liver is, however, to the best of our knowledge unexplored. It is tempting to speculate that low oxygen levels found in *L. donovani*-infected spleen tissue (29) incapacitate the Nos2-dependent antileishmanial defense in the spleen and, thus provide a safe niche for persistence and proliferation of *L. donovani*.

In contrast to *L. major*, exposure of *L. amazonensis*-infected macrophages to O2 levels of 5% and 1% O2 resulted in enhanced clearance of intracellular parasites (85, 86). These findings suggest that either (1) NO-independent mechanisms are involved in control of *L. amazonensis* under hypoxic conditions and/or (2) *L. amazonensis* is not able to adjust to low oxygen conditions. For instance, the divergent effect of hypoxia on the survival of members of the genus *Leishmania* in mononuclear phagocytes under hypoxia might be due to different metabolic requirements and O2-demands of the respective *Leishmania* species investigated. While it is known that *L. major* and *L. donovani* are able to adjust to hypoxic conditions by increasing their rate of glycolysis (87, 88), to the best of our knowledge detailed investigations on the intermediary metabolism of *L. amazonensis* upon exposure to low oxygen conditions are lacking.

**MONONUCLEAR PHAGOCYTES, HIF-1α, AND ANTI LEISHMANIAL IMMUNITY**

In addition to low oxygen conditions, various pathogens and pathogen-derived molecules are able to induce HIF-1α even in the presence of ample oxygen [reviewed in Ref. (1, 8–17)]. Inflammatory HIF-1α accumulation under normoxic conditions critically hinges on NF-kB activation (12, 13). HIF-1α transactivation potential, however, is highly contextual. For instance, hypoxic and inflammatory HIF-1α activation, both, result in activation of glycolytic HIF-1α-target genes while only inflammatory HIF-1α activation results in robust induction of inflammatory target genes such as Nos2 (89).

**Leishmania and Stabilization of HIF-1α in the Presence of Ample Oxygen**

Currently, there is no consensus on whether *Leishmania* on its own is able to induce HIF-1α accumulation under normoxic conditions (Table 1). Infection of mouse macrophages in vitro with *L. amazonensis* and *L. donovani* triggers HIF-1α accumulation under normoxic conditions without requiring any additional
inflammatory signals (90, 91). Blockade of this Leishmania-induced HIF-1α accumulation with cadmium chloride or RNAi results in impaired Leishmania recovery under normoxic conditions (90–93). In contrast to these findings and in contrast to stimulation with Toll-like receptor ligands [reviewed in Ref. (1, 8, 9)] or infection with various pathogens such as S. pyogenes (35, 94), M. tuberculosis (95), Histoplasma capsulatum (96), infection of mouse macrophages with L. major does not induce HIF-1α accumulation on its own but requires exogenous stimulation by IFN-γ and/or LPS (36). In line with this, Hammami et al. demonstrated that upon L. donovani-infection HIF-1α accumulation in CD11c+ mouse mononuclear phagocytes in vivo requires interferon regulatory factor-5-dependent inflammatory signaling (97). This suggests that L. donovani infection on its own is not sufficient to induce HIF-1α accumulation in mouse mononuclear phagocytes in vivo as well (97).

The different potential of various Leishmania species to induce HIF-1α accumulation in mononuclear phagocytes is unclear. HIF-1α accumulation in mononuclear cells might, for instance, help Leishmania to fuel its metabolic needs (98, 99). Mechanistically, the differential ability of certain Leishmania species to interfere with NF-κB activation (100–102) might underlie the divergent ability of different Leishmania species to block infection-associated inflammatory HIF-1α accumulation under normoxic conditions. However, further studies are required to assess this issue.

Role of HIF-1α Expression in Mononuclear Phagocytes

Earlier findings demonstrate a requirement of HIF-1α for Nos2-induction (89, 94, 103–105). In line with this, LPS/INFγ-coactivated HIF-1α-deficient macrophages displayed a diminished Nos2-dependent production of NO under normoxic conditions and impaired killing of L. major in vitro. Conditional targeting of HIF-1α in macrophages revealed that HIF-1α-activation is required for antileishmanial defense against cutaneous L. major-infection under normoxic conditions (36). This was paralleled by a blunted induction of Nos2 in lesional myeloid cells (36). However, it remains unclear whether in addition to Nos2 other HIF-1α-regulated targets are involved in cutaneous containment of Leishmania as well.

While HIF-1α in mononuclear phagocytes is protective in L. major-induced cutaneous leishmaniasis, HIF-1α expression in these cells emerged as detrimental factor in visceral leishmaniasis. Hammami et al. used CD11c Cre-deleter mice to target HIF-1α in Cd11c+ mononuclear phagocytes and to test the contribution of HIF-1α in a L. donovani-induced preclinical model of visceral leishmaniasis (29). In line with an inhibitory role of HIF-1α in DC-mediated T cell responses (106), Hammami et al. provide evidence that in L. donovani-infected mice HIF-1α in CD11c+ cells limits the expansion of short lived effector CD8+ T cells and thereby exacerbates disease at early time points after infections (29). In a recently published follow-up study, they demonstrate that HIF-1α in CD11c+ cells limits the frequency and numbers of granulocyte–monocyte progenitors and inflammatory monocytes. Moreover, HIF-1α in CD11c+ cells enhances the inhibitory function of splenic mononuclear phagocytes while this TF is not involved in tissue neovascularization and splenomegaly (29). Of note, Hammami et al. demonstrate that HIF-1α negatively impacts on the expression of Nos2 in L. donovani-infected mononuclear phagocytes. Discrepancies of this finding to the published literature on the requirement of HIF-1α on Nos2-expression and NO production might be caused by the different context of HIF-1α stabilization (29). This issue, however, demands further detailed investigation. Overall, the data suggest that HIF-1α in CD11c+ cells favors a myeloid-derived suppressor cell-like (MDSC) behavior of these cells. These findings were paralleled by an impaired antileishmanial control in the bone marrow of L. donovani-infected mice at late time points (29).

Taken together, these findings demonstrate that HIF-1α expression in CD11c+ mononuclear phagocytes favors development of visceral leishmaniasis in spleen and bone marrow, while HIF-1α in LysM+ myeloid cells supports defense against cutaneous L. major infection (Table 1). These findings underscore that the role of HIF-1α in myeloid cells is highly dependent on the respective context. HIF-1α is known to promote proinflammatory function of mononuclear phagocytes (35, 36, 94, 95, 105). However, the very same TF is able to induce regulatory and immunosuppressive functions in mononuclear phagocytes as well (107–109). Therefore, it is very likely that the differential context of HIF-1α stabilization in visceral and cutaneous leishmaniasis impacts on the functional consequence of HIF-1α activation. Further studies are needed to uncover the distinct signals that ultimately result in differential HIF-1α-dependent innate immune cell function.

AUTHOR CONTRIBUTIONS

VS, PN, and FR: contributed to writing of the manuscript. PN: provided the figure. JJ: conception of the manuscript, together with VS, PN, and FR writing of the article.

FUNDING

Jonathan Jantsch receives support from the Deutsche Forschungsgemeinschaft and from the Bayerisches Staatsministerium für Wirtschaft und Medien, Energie und Technologie.

REFERENCES

1. Jantsch J, Schödel J. Hypoxia and hypoxia-inducible factors in myeloid cell-driven host defense and tissue homeostasis. Immunobiology (2015) 220:305–14. doi:10.1016/j.imbio.2014.09.009
2. Arena ET, Tinevez JY, Nigro G, Sansonetti PJ, Marteyn BS. The infectious hypoxia: occurrence and causes during Shigella infection. Microbes Infect (2017) 19:157–65. doi:10.1016/j.micinf.2016.10.011
3. Semenza GL. A compendium of proteins that interact with HIF-1alpha. Exp Cell Res (2017) 356:128–35. doi:10.1016/j.yexcr.2017.03.041
22. Kanan MW. Mucocutaneous leishmaniasis in guinea-pigs inoculated intra-

20. Friedrich D, Fecher RA, Rupp J, Deepe GS Jr. Impact of HIF-1alpha and

23. Eickhoff J, Ishihara S, Jacobsen E. PaO2 by skin electrode.

13. Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, et al. 

25. Hofmann J, Meier RJ, Mahnke A, Schatz V, Brackmann F, Trollmann R, 

5. Semenza GL. Hypoxia-inducible factors in physiology and medicine. 

8. Dehne N, Brune B. HIF-1 in the inflammatory microenvironment.

7. Sitkovsky M, Lukashev D. Regulation of immune cells by local-tissue oxygen 

6. Bishop T, Ratcliffe PJ. Signaling hypoxia by hypoxia-inducible factor protein 

hydrolases: a historical overview and future perspectives. Hypoxia (Auckl) (2014) 2:197–213. doi:10.1474/HP.47598

26. Araújo AP, Arrais-Silva WW, Giorgio S. Infection by Leishmania amazonensis in mice: a potential model for chronic hypoxia. Acta Histochem (2012) 114:797–804. doi:10.1016/j.acthis.2012.01.007

27. Arteel GE, Thurman RG, YATES JM, Raleigh JA. Evidence that hypoxia mark-

28. Gross MW, Karbach U, Groebe K, Franko AJ, Muellerkieser W. Calibration of 

misonidazole labeling by simultaneous measurement of oxygen-tension and 

labeling density in multicellular spheroids. Int J Cancer (1995) 61:567–73. 

doi:10.1002/ijc.291601422

29. Hammami A, Abidin BM, Charpentier T, Fabie A, Duguay AP, Heinonen KM, 

et al. HIF-1alpha is a key regulator in potentiating suppressor activity 

and limiting the microbicidal capacity of MDCS-like cells during visceral 

leishmaniasis. PLoS Pathog (2017) 13:e1006616. doi:10.1371/journal. 

ppat.1006616

30. Mahnke A, Meier RJ, Schatz V, Hofmann J, Castiglione K, Schleicher U, et al. 

Hypoxia in Leishmania major skin lesions impairs the NO-dependent 

leishmanicidal activity of macrophages. J Invest Dermatol (2014) 134: 

2339–46. doi:10.1038/jid.2014.121

31. Campbell EL, Bruyninckx WJ, Kelly CJ, Glover LE, McNamee EN, Bowers BE, 

et al. Transmigrating neutrophils shape the mucosal microenvironment 

through localized oxygen depletion to influence resolution of inflammation. 

Immunity (2014) 40:66–77. doi:10.1016/j.immuni.2013.11.020

32. Fritzsche C, Schleicher U, Bogdan C. Endothelial nitric oxide synthase 

limits the inflammatory response in mouse cutaneous leishmaniasis. Cell (2003) 112:645–57. doi:10.1016/S0092-8674(03)00154-5

33. Weinkopf T, Konradt C, Christian DA, Discher DE, Hunter CA, Scott P. 

Leishmania major infection-induced VEGF-A/VEGFR-2 signaling promotes 

lymphangiogenesis that controls disease. J Immunol (2016) 197:1823–31. 

doi:10.4049/jimmunol.1600717

34. Araújo AP, Giorgio S. Immunohistochemical evidence of stress and 

inflammatory markers in mouse models of cutaneous leishmaniasis. Arch 

Dermatol Res (2015) 307:671–82. doi:10.1007/s00004-015-1564-0

35. Cramer T, Yamanishi Y, Clausen BE, Bastian F, Pflatrong F, Ricardo Blum S. 

Large-scale investigation of Leishmania interaction networks with host 

extracellular matrix by surface plasmon resonance imaging. Infect Immun (2014) 82:594–606. doi:10.1128/IAI.01164-13

36. Schatz V, Strussmann Y, Mahnke A, Schley G, Waldner M, Ritter U, et al. 

Myeloid cell-derived HIF-1alpha promotes control of Leishmania major. 

J Immunol (2016) 197:4034–41. doi:10.4049/jimmunol.1601080

37. Fritzsche C, Schleicher U, Bogdan C. Endothelial nitric oxide synthase 

limits the inflammatory response in mouse cutaneous leishmaniasis. 

Immunobiology (2010) 215:826–32. doi:10.1007/jmbo.2010.05.022

38. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of 

leukocyte adhesion. Proc Natl Acad Sci U S A (1991) 88:4651–5. doi:10.1073/ 

pnas.88.11.4651

39. Lefer DJ, Jones SP, Girod WG, Baines A, Grisham MB, Cockrell AS, et al. 

Succinate is an inflammatory signal that induces IL-1beta production by 

myeloid cells in the presence of ample oxygen. Microbes Infect (2015) 309:42–9. doi:10.1016/j.microbi.2016.06.002

40. Cramer T, Yamanishi Y, Clausen BE, Bastian F, Pflatrong F, Ricardo Blum S. 

Large-scale investigation of Leishmania interaction networks with host 

extracellular matrix by surface plasmon resonance imaging. Infect Immun (2014) 82:594–606. doi:10.1128/IAI.01164-13

41. Rodriguez-Miguelez P, Lima-Cabello E, Martinez-Florez S, Almar M, 

et al. Reactive oxygen species and nitric oxide in cutaneous leishmaniasis. 

Arch Dermatol Res (2017) 309:42–9. doi:10.1016/j.cellimm.2016.06.002

42. Dietz I, Jerchel S, Szaszak M, Shima K, Rupp J. When oxygen runs short: the 

microenvironment drives host-pathogen interactions. Microbes Infect (2012) 

14:311–6. doi:10.1016/j.micinf.2011.11.003

43. Charpentier T, Hammami A, Stager S. Hypoxia inducible factor alphas: a 

critical factor for the immune response to pathogens and Leishmania. 

Cell Immunol (2016) 309:42–9. doi:10.1016/j.cellimm.2016.06.002

44. Scott P, Novais FO. Cutaneous leishmaniasis: immune responses in protec-

tive and pathogenesis. Nat Rev Immunol (2016) 16:581–92. doi:10.1038/nri.2016.72
54. Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG, et al.
55. Spirat L, Djibril SB, Bramme BF, Dubowski T, et al. Hypoxia causes an increase in phagocytosis by macrophages in a HIF-1α-dependent manner. J Leukoc Biol (2007) 82:1257–65. doi:10.1189/jlb.0307195
56. Elia AR, Cappello P, Puppo M, Fraone T, Vanni C, Eva A, et al. Human dendritic cells differentiated in hypoxia down-modulate antigen uptake and change their chemokine expression profile. J Leukoc Biol (2008) 84:1472–82. doi:10.1189/jlb.0208082
57. Laskay T, Vandenberghe G, Solbach W. Neutrophil granulocytes – Trojan horses for Leishmania major and other intracellular microbes? Trends Microbiol (2003) 11:210–4. doi:10.1016/S0928-4839(03)00075-1
58. Walmsley SR, Print C, Farahi N, Peysoonnaux C, Johnson RS, Cramer T, et al. Hypoxia-induced neutrophil survival is mediated by HIF-1α-dependent NF-kappaB activity. J Exp Med (2005) 201:105–15. doi:10.1084/jem.20040624
59. Moreaux V, Chiche-Lapierre C, Chaput C, Witko-Sarsat V, Prevost MC, et al. Hypoxia, HIF-1α, and lipopolysaccharide. J Cell Physiol (2012) 227:587–95. doi:10.1002/jcp.22761
60. Xu Q, Yang MX, Kong BH, Qi L, Lam YL, Yan S, et al. Hypoxia inhibits the migratory capacity of human monocyte-derived dendritic cells. Immunol Cell Biol (2005) 83:668–73. doi:10.1111.j.1440-1711.2005.01383.x
61. Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG, et al. Hypoxia and hypoxia-inducible factor-1α modulate lipopolysaccharide-induced dendritic cell activation and function. J Immunol (2008) 180: 4697–705. doi:10.4049/jimmunol.180.7.4697
62. Spirig R, Djaferzadeh S, Regueira T, Shaw SG, Von Garnier C, Takala J, et al. Effects of TLR agonists on the hypoxia-regulated transcription factor HIF-1α and dendritic cell maturation under normoxic conditions. PLoS One (2010) 5.e1001983. doi:10.1371/journal.pone.0100198
63. Kohler T, Reizis B, Johnson RS, Weighardt H, Forster I. Influence of hypoxia-inducible factor-1α on dendritic cell differentiation and migration. Eur J Immunol (2012) 42:1226–36. doi:10.1002/eji.201142053
64. Bogdan C. Natural killer cells in experimental and human leishmaniasis. Front Cell Infect Microbiol (2017). doi:10.3389/fcimb.2017.00002
65. Naldini A, Morena E,ucci P, Miglietta D, Riboldi E, Sozanni S, et al. Hypoxia affects dendritic cell survival: role of the hypoxia-inducible factor-1α and lipopolysaccharide. J Cell Physiol (2012) 227:587–95. doi:10.1002/jcp.22761
66. Vone Stet U, Tenzor S. Cutsaneous leishmaniasis: distinct functions of dendritic cells and macrophages in the interaction of the host immune system with Leishmania major. Int J Med Microbiol (2017). doi:10.1016/j.ijmm.2017.09.002
67. Naldini A, Morena E,ucci P, Miglietta D, Riboldi E, Sozanni S, et al. Hypoxia affects dendritic cell survival: role of the hypoxia-inducible factor-1α and lipopolysaccharide. J Cell Physiol (2012) 227:587–95. doi:10.1002/jcp.22761
68. Vone Stet U, Tenzor S. Cutsaneous leishmaniasis: distinct functions of dendritic cells and macrophages in the interaction of the host immune system with Leishmania major. Int J Med Microbiol (2017). doi:10.1016/j.ijmm.2017.09.002
69. Naldini A, Morena E,ucci P, Miglietta D, Riboldi E, Sozanni S, et al. Hypoxia affects dendritic cell survival: role of the hypoxia-inducible factor-1α and lipopolysaccharide. J Cell Physiol (2012) 227:587–95. doi:10.1002/jcp.22761
70. Xu Q, Yang MX, Kong BH, Qi L, Lam YL, Yan S, et al. Hypoxia inhibits the migratory capacity of human monocyte-derived dendritic cells. Immunol Cell Biol (2005) 83:668–73. doi:10.1111/j.1440-1711.2005.01383.x
71. Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG, et al. Hypoxia and hypoxia-inducible factor-1α modulate lipopolysaccharide-induced dendritic cell activation and function. J Immunol (2008) 180: 4697–705. doi:10.4049/jimmunol.180.7.4697
72. Diefenbach A, Schindler H, Donhausen N, Lorenz E, Laskay T, Macmicking J, et al. Type I interferon (IFNalpha/beta) and type 2 nitric oxide synthase does not require functional inducible nitric oxide synthase or phagocyte oxidative activity. J Leukoc Biol (2005) 77:311–20. doi:10.1189/jlb.0904545
73. Ding AH, Nathan CE, Steuer DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. J Immunol (1988) 141:2407–12.
74. Green SJ, Meltzer MS, Hibbs JB Jr, Nacy CA. Activated macrophages destroy intracellular visceral Leishmania donovani. J Exp Med (1999) 189:741–6. doi:10.1084/jem.189.4.7471
75. White JK, Mastroeni P, Popoff JF, Evans CA, Blackwell JM. Slc11a1-mediated reaction to Salmonella enterica serovar Typhimurium and Leishmania donovani infections does not require functional inducible nitric oxide synthase or phagocyte oxidative activity. J Leukoc Biol (2005) 77:311–20. doi:10.1189/jlb.0904545
76. Diefenbach A, Schindler H, Donhausen N, Lorenz E, Laskay T, Macmicking J, et al. Type I interferon (IFNalpha/beta) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. Immunity (1998) 8:77–87. doi:10.1016/S1074-7613(00)00460-4
77. Diefenbach A, Schindler H, Rollinghoff M, Yokoyama WM, Bogdan C. Requirement for type 2 NO synthesis for IL-12 signaling in innate immunity. Science (1999) 284:951–5. doi:10.1126/science.284.5416.951
78. Olekhnovitch R, Ryffel B, Mousset A, Bousso P. Collective nitric oxide production provides tissue-wide immunity during Leishmania infection. J Clin Invest (2011) 124:1711–22. doi:10.1172/JCI72505
79. Albina JE, Henry WL Jr, Mastroferruccio B, Martin BA, Reichner JS. Macrophage activation by culture in an anoxic environment. J Immunol (1995) 155:4391–6.
80. Robinson MA, Baumgardner JE, Otto CM. Oxygen-dependent regulation of nitric oxide production by inducible nitric oxide synthase. Free Radic Biol Med (2011) 51:1952–65. doi:10.1016/j.freeradbiomed.2011.08.034
81. Wiese M, Gerlach RG, Popp I, Matuszak J, Mahapatro M, Castiglione K, et al. Hypoxia-mediated impairment of the mitochondrial respiratory chain inhibits the bacterial activity of macrophages. Infect Immun (2012) 80(4):1435–66. doi:10.1128/IAI.00972-11
82. Alm G, Sun Santora S, Roy J, Makherjee S, Bose M, Raha S, Adak S. Globin-coupled heme containing oxygen sensor soluble adenylate cyclase in Leishmania prevents cell death during hypoxia. Proc Natl Acad Sci U S A (2013) 110:16790–5. doi:10.1073/pnas.1304145110
8. Kong F, Saldarriaga OA, Spratt H, Osorio EY, Travi BL, Luxon BA, et al. Transcriptional profiling in experimental visceral leishmaniasis reveals a broad oligenic inflammatory environment that conditions macrophages toward a disease-promoting phenotype. *PLoS Pathog* (2017) 13:e1006165. doi:10.1371/journal.ppat.1006165.

9. Nascimento MS, Carregaro V, Lima-Junior DS, Costa DL, Ryffel B, Duthie MS, et al. Interleukin 17A acts synergistically with interferon gamma to promote protection against *Leishmania infantum* infection. *J Infect Dis* (2015) 211:1015–26. doi:10.1093/infdis/jiu531.

10. Cöllnne MC, Arrais-Silva WW, Picoli C, Giorgio S. Effect of hypoxia on macrophage infection by *Leishmania amazonensis*. *J Parasitol* (2004) 90:510–5. doi:10.1645/GE-3286.

11. Degrossoli A, Giorgio S. Functional alterations in macrophages after hypoxia selection. *Exp Biol Med (Maywood)* (2007) 232:88–95. doi:10.3181/003797-207-2320088.

12. Walsh MJ, Blum J. Effects of hypoxia and acute osmotic stress on intermediary metabolism in *Leishmania* promastigotes. *Mol Biochem Parasitol* (1992) 50:205–14. doi:10.1016/0166-6851(92)90271-8.

13. Blum J. Energy metabolism in *Leishmania*. *J Bioenerg Biomembr* (1994) 26:147–55. doi:10.1007/BF00763063.

14. Jantsch J, Wiese M, Schodel J, Castiglione K, Glasner J, Kolbe S, et al. Fecher RA, Horwath MC, Friedrich D, Rupp J, Deepe GS Jr. Inverse correlation between IL-10 and HIF-1alpha in macrophages infected with *Histoplasma capsulatum*. *J Immunol* (2016) 197:1287–97. doi:10.4049/jimmunol.1600266.

15. Fecher RA, Horwath MC, Friedrich D, Rupp J, Deepe GS Jr. Inverse correlation between IL-10 and HIF-1alpha in macrophages infected with *Histoplasma capsulatum*. *J Immunol* (2016) 197:565–79. doi:10.4049/jimmunol.1600342.

16. Hamnami A, Charpentier T, Smans M, Stager S. IFN-5-mediated inflammation limits CD8+ T cell expansion by inducing HIF-1alpha and impairing dendritic cell functions during *Leishmania* infection. *PLoS Pathog* (2015) 11:e1004938. doi:10.1371/journal.ppat.1004938.

17. Naderer T, Ellis MA, Semee ME, De Souza DP, Curtis J, Handman E, et al. Virulence of *Leishmania major* in macrophages and mice requires the gluconeogenic enzyme fructose-1,6-bisphosphatase. *Proc Natl Acad Sci U S A* (2006) 103:5502–7. doi:10.1073/pnas.0509196103.

18. Rodrigues-Contreras D, Ahsan H, Feng X, Tran K, Yates PA, Kamhawi S, et al. Regulation and biological function of a flagellar glucose transporter in *Leishmania mexicana*: a potential glucose sensor. *FASEB J* (2015) 29:11–24. doi:10.1096/fj.14-251991.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Schatz, Neubert, Rieger and Jantsch. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.