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Hypoxia and B cells

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ABSTRACT

The ability of cells to sense and adapt to changes in oxygen is mediated by hypoxia-inducible factor (HIF). Immune cells function in physiologically complex and varying environments whereby oxygen, pH, nutrients, metabolites and cytokines are continuously fluctuating. HIF is well known to play an important role in coordinating the adaptation and function of both innate immune cells and T cells in these complex environments. This review summarises recent discoveries concerning how hypoxia and HIF control B cell behaviour, and regulate antibody quality and decisions concerning tolerance. Hypoxia and HIF activation may provide an important context; coordinating metabolism with variable demands for quiescence, rapid proliferation, and differentiation. Understanding when and how HIF is activated during B cell development and response is important as drugs targeting HIF could influence antibody responses, providing novel therapeutic opportunities for vaccine adjuvants and in treating autoimmunity.

1. Introduction

The hypoxia-inducible factors (HIFs) are transcription factors stabilised under low oxygen (hypoxia), enabling cellular adaptation. Hypoxia and HIF play important roles in innate immunity and inflammation. HIF acts as a prosurvival signal in neutrophils and promotes migration, invasion and bactericidal activity of macrophages. In adaptive immunity, HIF has differing effects on T cell differentiation and function, depending on T cell subset and local stimuli [1]. There is clear evidence that HIF is modulated in B cell malignancies (that include non-Hodgkin's lymphomas, some leukaemias, and myelomas), but the role for hypoxia and HIF in normal B cell physiology has received much less attention. B cells are exposed to a range of oxygen tensions as they develop, migrate and differentiate. Yet the hypoxic control of B cell fate is not well understood. B cell development and function depend on stage-specific proliferative bursts, rapid growth and differentiation, which requires metabolic reprogramming and cell-cycle regulation. HIF is emerging as an integral part of this. This review discusses how hypoxia and HIF shape B cell function, regulating antibody quality and tolerance. Hypoxia and HIF could provide an important context as to whether antigens that activate B cells are from pathogens or self, and in coordinating metabolism with variable demands for quiescence, proliferation, and variable efficiency of oxidative metabolism. Furthermore, hypoxia and HIF may modulate immunoglobulin (Ig) class preference and affinity by affecting DNA repair. Understanding when and how HIF is activated during B cell development and response is of interest as the HIF pathway is pharmacologically tractable; HIF activators or inhibitors may influence antibody responses, providing novel therapeutic implications for vaccine adjuvants and in treating autoimmunity.

2. Immunity

Host defences are grouped under two arms of immunity; innate and adaptive. Innate immunity provides the first line of defence against infection. It is rapid, non-specific and blocks pathogen entry and eliminates pathogens and toxins. Innate immune cells include natural killer cells, neutrophils, monocytes, macrophages and dendritic cells. Adaptive immunity develops more slowly and involves the expansion and differentiation of antigen-specific lymphocytes. It is characterised by the production of high affinity, class-switched antibodies and immunological memory. High affinity antibodies are produced by affinity maturation. This process involves somatic hypermutation (SHM) of variable regions of Ig genes altering affinity to antigen. Affinity increases in response to prolonged and repeated antigen exposure, with selective survival of high affinity B cells. B cells also change their Ig isotype by class-switch recombination (CSR), producing antibodies with different effector functions. Memory cells generated can undergo rapid expansion on subsequent pathogen encounters providing long-term protection. The random recombination and high mutation frequency that occurs during SHM, and in other gene editing events during B cell development, also yield self-reactive B cells. Under

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these circumstances, a sophisticated selection mechanism exists to eliminate or modify these B cells, reducing the risk of attacking self, whilst permitting effective defence. This is a delicate balance, which when tipped may lead to autoimmunity, a condition underlying many diseases.

3. The life of a B cell

B cells consist of two lineages, B1 and B2. B1 cells (located in the peritoneum, spleen and at mucosal sites) are principally generated in the foetal liver and are sustained by self-renewal. B2 cells are generated in the bone marrow (BM) throughout life, forming the majority of the B cell pool. Haematopoietic progenitors proliferate, generating large numbers of proB cells that develop into preB cells by undergoing VDJ recombination at the Ig heavy-chain locus. The resulting Ig μ heavy-chain protein binds to surrogate light-chains and, along with Igα/Igβ signalling proteins, forms the membrane-associated pre-B cell receptor (preBCR). The preBCR triggers rearrangement at the light-chain locus and the resulting protein associates with the μ heavy-chain yielding the complete BCR on the surface of an immature B cell. Immature B cells egress from the BM as transitional B cells that migrate via the circulation to the spleen to complete maturation. There are two mature B2 subsets; follicular (FO) and marginal zone (MZ). FO B cells are recirculating and found in the spleen and lymph node (LN) follicles, whilst MZ B cells reside at the margins of splenic follicles. Maintenance of the naïve mature B cell pool depends on survival cytokines such as B-cell activating factor (BAFF) and A proliferation-inducing ligand (APRIL), recognition of self-antigen and BCR signalling strength [2,3].

Checkpoints occur throughout B cell development to eliminate self-reactive B cells. In the BM, B cells binding self-antigen change their receptor specificity (receptor editing), become anergic or are deleted by apoptosis, in a process called central tolerance. Similarly, autoreactive B cells in the periphery may apoptose or inhibitory receptors may engage, preventing activation. B cells recognising antigen in the absence of T cell help become anergic. Peripheral tolerance is more flexible as it also depends on interclonal competition of BCR signalling strength and on cytokine signals [2,3].

B1 and MZ B cells respond to T cell-independent (Ti) antigens, whilst FO B cells mainly respond to T cell-dependent (Td) antigens. Td responses occur in two steps, providing immediate and persistent protection. First, B cells undergo class-switch recombination (CSR) and differentiate into short-lived plasmablasts that secrete low-affinity antibodies (extrafollicular response). Second, activated B cells proliferate extensively under the influence of T follicular helper (Tfh) cells forming a germinal centre (GC). GCs are dynamic anatomic sites where B cells undergo diversification of their antigen-receptors by somatic hypermutation (SHM) and selection, producing long-lived plasma cells (PCs) that secrete high-affinity antibodies, and memory B cells [4]. Tolerance mechanisms similarly exist in GCs to eliminate self-reactive B cells produced following SHM [2].

4. Hypoxia-inducible factor (HIF)

There is a continuously operating oxygen-sensing system present in all metazoan cells that enables adaptation to changes in oxygenation. The system centres around Hypoxia-inducible factor (HIF), of which there are two isoforms; HIF-1 and HIF-2. HIFs are heterodimeric transcription factors consisting of a constitutively expressed HIF-1β subunit and an oxygen-dependent subunit (HIF-1α or HIF-2α). Under normoxia, HIF-α is hydroxylated on two proline residues by one of three prolyl hydroxylase domain enzymes (PHD1-3) [5]. The PHDs use oxygen and 2-oxoglutarate as co-substrates, acting as the oxygen sensors to HIF. Upon hydroxylation, the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex binds HIF-α leading to ubiquitination and proteosomal degradation [6]. HIF-α is also hydroxylated on an asparagine residue in the C-terminal transactivation domain by Factor inhibiting HIF-1 (FIH-1), another oxygen-dependent dioxygenase. This blocks interactions with the transcriptional co-activators CREB-binding protein (CBP)-p300 [7]. Hypoxia reduces hydroxylation leading to stabilisation and activation of HIF, which modulates the expression of hundreds of genes [8]. The hydroxylation rates of PHDs and FIH are sensitive to oxygen concentrations throughout the physiological range [9]. As a result HIF contributes to many physiologic (development, cell metabolism, apoptosis, inflammation and erythropoiesis) and pathologic conditions (ischemia, anaemia, atherosclerosis and cancer).

HIF can also be stabilised by O_2-independent mechanisms as a result of genetic mutations in *VHL* [10], *PHDs* [11] and *HIF2A* [12]. A complex bidirectional relationship exists between HIF and other signalling pathways. Of particular relevance to the immune system are the Phosphoinositide-3-kinase (PI3K), Mitogen-activated protein kinase (MAPK) and Nuclear factor-κB (NF-κB) pathways. The PI3K and MAPK pathways increase translation of HIF-α, and HIF transactivation through phosphorylation of HIF-α [13]. In turn HIF inhibits PI3K signalling by upregulating REDD1, which inhibits mechanistic target of rapamycin complex 1 (mTORC1) via activation of the tuberous sclerosis 1(TSC1)-TSC2 complex [14]. During inflammation, Tumour necrosis factor-α (TNF-α) activates NF-κB that increases transcription of HIF-1α, leading to stabilisation under normoxia [15]. HIF has similarly been reported to increase NF-κB activation in anoxic neutrophils [16].

5. Oxygen gradients in lymphoid tissues

B cells circulate between high oxygen levels in the alveolar capillaries (~13.2%), to lower oxygen tensions (pO_2) in mixed venous blood (~5.3%) and the microvasculature in lymphoid tissues, where pO_2 depends on the amount of oxygen extracted [17]. Tissue oxygenation is determined by vessel size and density, distance from the nearest vessel, and density and metabolic demand of resident cells. These structural features differ between lymphoid organs, setting a varied hypoxic landscape between lymphoid tissues. Elegant studies using non-invasive imaging techniques of the BM in live mice provide direct evidence for the existence of hypoxic niches and gradients (0.6–2.8% oxygen in extravascular regions). The lowest average pO2 (1.3%) was located deep within the peri-sinusoidal regions, > 40 μm from the well-vascularised endosteum [18]. Oxygen gradients exist in murine spleens and may range from 0.5% to 4.5% oxygen (mean 2.3%), with oxygenation being highest near the splenic artery and decreasing with distance [19,20]. The pO_2 of human neck LNs containing metastatic cells ranges from 0.1% to 3.6%. Although these LNs are pathological, it is possible that normal LNs may be similarly hypoxic [21]. These studies support that local hypoxia and oxygen gradients occur in lymphoid tissues, and that the oxygenation differs between lymphoid tissues. This is likely to influence both the level and duration of HIF activation in B cells. The extent to which B cells respond and adapt to changes in oxygenation as they encounter different lymphoid compartments is not well understood.

GCs are hypoxic and enriched for HIF-1α in lymphoid organs of mice and humans [22–25]. As cells become progressively further away from vessels, oxygenation falls and cells located > 30 μm away experience < 2% pO_2 [18,26]. Anatomically, the majority of GCs are located ≥40 μm away from blood vessels in murine spleens [24] and human GCs are also poorly vascularised [22]. An intriguing possibility is that hypoxic gradients could set the location for GC formation and thereby modulate anatomical colocalisation of immune cells and antigens, which is critical for mounting an effective immune response.

Thus over their life cycle, B cells are exposed to a wide range of oxygen tensions across the physiological range as they egress from the hypoxic BM into a well oxygenated circulation, and then encounter hypoxic microenvironments within lymphoid tissues, including GCs