Transcriptional control of cytotoxic lymphocytes
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CHAPTER 8

General Discussion
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Discussion

Human CMV infection generate a lifelong specific T cell response with strong cytotoxic potential [1]. However, not much is known on how these cells are maintained in a quiescent and nonproliferative state, whilst maintaining a large reserve of cytotoxic granules that enable immediate effector function. Transcriptome analysis of CMV [2] specific CD8 T cells has identified Hobit as one of the most differentially expressed genes in these effector cells compared to naive and other non-cytolytic immune populations. Indeed, we have demonstrated that Hobit is a transcription factor, of which expression in the human immune system is restricted to long lived effector CD8 T cells, cytotoxic CD4 T cells and NK cells. Hobit regulates effector functions such as IFN-y and granzyme B production. Hobit mediates maintenance of effector lymphocytes as a metabolic suppressor and regulator of survival under cellular stress. In addition, Hobit plays a role in NK cell development and controls the expression of the surface receptor GPR56, which suppresses NK cell effector functions. Here, I would like to discuss the impact of these findings on the effector functions and maintenance of Hobit+ CD4 T cells, CD8 T cells and NK cells.

Hobit suppresses glycolysis and induces effector functions

Hobit induces the production of effector molecules such as IFN-y (Chapter 2) and granzyme B (Chapter 5). IFN-y exerts both antiviral and immunostimulatory effects [3]–[5], while granzymes can directly destroy infected cells [6]–[8] virus related proteins [9]. It is interesting that both IFN-y and granzyme B are induced by Hobit, as the regulatory mechanisms of these molecules vary largely. IFN-y mRNA but not protein can be detected in resting cells [10], while granzyme B is expressed in large amounts at both the mRNA and protein level in resting effector lymphocytes (Chapter 5) [1],[2],[11]. We have shown that Hobit regulates IFN-y expression at both the mRNA level and the protein level (Chapter 2) in line with a role of Hobit as a transcriptional regulator of IFN-y expression. The regulation of IFN-y expression is a multistep process that involves both transcriptional [12] and post-transcriptional [13],[14] regulatory mechanisms. Glycolysis contributes to the regulation of IFN-y production by inducing IFN-y protein production from pre-formed mRNA molecules [15],[16],[15],[16]. We have shown that Hobit has the potential to suppress glycolysis (Chapter 4)[15],[16]. We hypothesize that the interaction between Hobit, glycolysis and IFN-y is a complex process that effectively maintains these highly effective killer cells in check in the absence of an inflammatory milieu. The low glycolytic status of resting CD45RA+ effector (EMRA) cells (Chapter 4) might operate as a safety mechanism, guaranteeing that IFN-y is not produced until these cells encounter antigen; despite the high levels of Hobit that upregulate IFN-y expression. The mechanism of interaction between metabolic pathways and the regulation of cytotoxicity in EMRA cells is unknown. EMRA cells store pre-formed granzyme B protein inside cytolytic granules [17]–[19]. Murine memory cells that express high granzyme B mRNA transcripts but no granzyme B protein [20],[21]. However, EMRA cells lack spare respiratory capacity (SRC), while murine memory cells have SRC (Chapter 4), suggesting that EMRA cells are less capable of providing energy for protein synthesis [22]. As proteomic analysis of activated cytotoxic lymphocytes revealed
that the majority of proteins in these cells are granzymes, and protein synthesis is a high energy demanding process [23]–[25] (Chapter 4), an important question is how EMRA cells maintain high levels of pre-formed granzyme B protein. It is unlikely that EMRA cells can maintain continuous granzyme B production. An alternative for maintaining high levels of protein stocks without high energy expenditure is through the development of efficient storage mechanisms. Similar to pickles, granzyme molecules are stored in an acidic environment [26], suggesting that these granules have been developed to provide stable and energy-efficient long term protein storage. Thus, we hypothesize that EMRA cells have evolved mechanisms to maintain a high cytotoxic potential at minimal metabolic cost.

**Hobit and the extended lifespan of EMRA cells**

A hallmark of HCMV infection is memory inflation [27]–[29]. The impact of HCMV infection and memory inflation to the immune system is not entirely understood [30]–[38]. One of the main unanswered questions in the field relates to the molecular mechanisms behind memory inflation. EMRA CD8 T cells have a suppressed metabolic state with low glycolysis and low oxygen consumption (Chapter 4). It is interesting to note that dietary restriction which reduces metabolism extends the lifespan of organisms [39]–[42]. Metabolic suppression resulting in long-term survival might be the key to population expansion, despite the low basal replication rate of these cells [27]–[29],[43]. Considering that Hobit is expressed in EMRA cells during the expansion and maintenance phase (Chapters 2, 5 and 6), an important question is whether Hobit plays a role in memory inflation. During persistent infection with HCMV, Hobit may be involved in maintaining cytotoxic EMRA cells at a low metabolic state, thereby extending their lifespan and reducing the high energetic demand of constant proliferation. Alternatively, Hobit may be primarily expressed to induce a cytotoxic phenotype and metabolic suppression leading to life extension might arise as a byproduct. This is a difficult question to address in the human immune system, but murine Hobit might give us insight into how Hobit regulates the long term maintenance of CD8 T cells.

**Hobit: A tale of mice and men**

Murine and human Hobit may regulate similar molecules, but the expression pattern of Hobit in both species is quite dissimilar; as circulating murine, in contrast to human CD8 T cells and NK cells, do not express Hobit [44],[45]. Murine Hobit expression is restricted to NKT cells [45], Tissue Resident Memory (TRM) T cells and Tissue Resident NK cells [46]. In these lymphocyte populations, murine Hobit regulates effector functions such as granzyme B production (Chapter 5) [45],[46] as well as development and/or maintenance in non lymphoid tissues [45],[46]. TRM cells have high effector potential [47] and are known to reside within non lymphoid tissues for extended periods of time in a quiescent state [48]–[50]. These characteristics are remarkably similar to human EMRA cells. Hobit expression is essential for the formation of TRM populations in mice [46], suggesting that murine and human Hobit both regulate lymphocyte maintenance and effector function in a similar fashion (Chapters 2 and 5) [44],[46].
Compared to Blimp-1 [51], the gene sequences of Hobit orthologues are not well conserved among vertebrates [52]. Despite the low overall homology, murine and human Hobit have a high degree of similarity within their zinc finger region [53], which encodes its transcriptional activity (Chapter 2) [44]. The shared transcriptional activity of murine and human Hobit [44]–[46] suggests that evolutionary pressures selectively retain the Zinc Finger region. In contrast, environmental forces between mice and humans may have driven genetic differences underlying the distinct expression pattern of murine and human Hobit. The most simple explanation for the divergence in Hobit cell type expression between mice and men is co-evolution between host and virus [54]. Murine CMV and human CMV might have shaped the immune system of their hosts in different ways, inducing differences including the expression pattern of Hobit. Another possibility relates directly to different experimental conditions. The laboratory environment in which mice are maintained does not recapitulate the diversity of microorganisms and pathogenic challenges that humans face on a day to day basis. A recent paper has shown that changes in the environment, in which mice are maintained can lead to a closer recapitulation of the human T cell compartment [55]. Feral mice and conventional lab mice co-housed with feral mice contain a Krg1+GranzymeB+CD27-CD8 T cell population that resembles the phenotype of human EMRA cells. It would be interesting to check whether the murine “EMRA” population expresses Hobit. Thus, despite the differential pattern of Hobit expression between mice and humans, Hobit regulates similar pathways in both species, suggesting experiments in both systems are complementary and can provide better understanding on the functions and mechanisms of Hobit-driven transcriptional regulation.

On the maintenance of natural killer cells.

The transcriptional machinery behind human NK cell development is not entirely understood. The high expression of Hobit within the NK cell lineage suggests that Hobit might also play a role in NK cell development and/or maintenance. Our preliminary results suggest that Hobit is involved in the final stages of NK cell development from CD34+ progenitor cells (Chapter 6). In order to understand this data, analogies with its parologue Blimp-1 (PRDM1) are informative. In human NK cells, Blimp-1 acts as tumor suppressor that inhibits proliferation [56]–[58]. Dysregulation of Blimp-1 expression can lead to very aggressive NK cell lymphomas. Considering the extent of shared functions and transcriptional targets between Hobit and Blimp-1 (Chapter 2 and 5) [46], it is reasonable to assume that Hobit is also capable of suppressing proliferation in NK cells. Thus, our data indicate that Hobit regulates terminal NK cell differentiation that may include instruction of a cytotoxic program (Chapters 2, 4, 6 and 7).

IL-15 can induce Hobit mRNA expression in activated murine CD8 T cells, in a T-bet dependent manner [46]. Interestingly, we have shown that IL-15 in the presence of IL-7 and Flt-3 also induces Hobit expression during human NK cell development from CD34+ progenitor cells (Chapter 6). IL-15 is absolutely essential for NK cell development [59],[60] and this might be one of the mechanisms underlying high Hobit expression in the human NK cell lineage. Circulating murine NK cells differ largely from CD56dim human NK cells. Despite the absolute necessity of IL-15 to their de-
velopment [59], murine NK cells do not express Hobit and do not express pre formed granzyme B cytotoxic granules [61]. Thus, it appears that the expression regulation of Hobit is a complex process yet to be fully elucidated, as IL-15 is necessary, but not sufficient to induce Hobit expression.

**One ring to rule them all: Concluding remarks**

In 2013 cancer immunotherapy was selected by the magazine Science as the breakthrough of the year [62] and the therapeutic use of T cells [63]–[67] and NK cells [68]–[70] experience continuous and steady growth. Achievement of the ultimate balance between effector function and expansion potential is essential for successful cell therapy [71]. Our current understanding of Hobit suggests that it acts as a master regulator of terminally differentiated lymphocytes across multiple lineages. These findings suggest that a better understanding of the mechanisms and roles of Hobit in the immune system might offer opportunities to improve current strategies of cell therapy.
References


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