Immune Regulation of Herpes Simplex Virus type 2 Infection:

Special Emphasis on the Transcription Factor T-bet

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ABSTRACT

Genital herpes, caused by herpes simplex virus type 2 (HSV-2), is the most common genital ulcer disease worldwide. HSV-2 infection causes a variety of symptoms, ranging from subclinical/silent infection to severe and recurrent episodes of genital blisters and ulcers, and the virus can also in rare cases cause meningitis. In primary infection, the virus sequentially enters and replicates in epithelial cells followed by local sensory neurons. In the latter, a life-long infection is established via the induction of viral latency or dormancy. Latent virus is however continuously reactivated, also in those with a silent infection, which results in a low-grade viral replication and shedding into the vaginal lumen. This reactivation can in many individuals be amplified following e.g. stress, hormonal variations or immune suppression, which results in recurrent genital disease.

Cell-mediated immunity and interferons (IFN) are probably the most efficient immune mediators involved in combating HSV-2. In particular type I IFN (i.e. IFN-α/β) which block protein synthesis and thus viral multiplication, but also NK cells which destroy HSV-2-infected cells, play major roles in limiting the HSV-2 replication during the first days of infection. Then, once the sophisticated acquired immune response has had sufficient time to mature and expand, IFN-γ-producing and cytolytic CD4+ and CD8+ T cells appear, and these cells are instrumental in clearing the mucosal infection and probably also in later containment of latent virus.

The activation/induction of NK cells, T cells and IFN is tightly regulated and relies on an intricate network of membrane-bound and intracellular signaling systems. In this thesis I have investigated how a few selected signaling mediators or pathways affect HSV-2 immunity. One of these mediators, the transcription factor T-box expressed in T cells (T-bet), turned out to be particularly interesting as it influenced the function of both NK-cells and T-cells as well as the production of IFN. Because of its impact on such crucial components of the anti-HSV-2 innate and acquired immune response, I have chosen to focus my thesis framework on the transcription factor T-bet.

Key words: *Herpes simplex virus type 2, T-bet, Substance P, Neurokinin 1, IFN-α/β signaling, polymorphisms*

ORIGINAL PAPERS

This thesis is based on the following papers:

I. **Alexandra Svensson, Inger Nordström, Jia-Bin Sun, Kristina Eriksson.**
Protective immunity to HSV-2 infection is mediated by T-bet.
Journal of Immunology 2005 174:6266-6273

II. **Alexandra Svensson, Niklas Björkström, Ann-Marie Bergin, Gun-Britt Löwhagen, Petra Tunbäck, Lars Bellner, Peter Horal, Hans-Gustaf Ljunggren, Leonid Padyukov, Kristina Eriksson.**
A 3´-UTR polymorphism in TBX21 (T-bet) gene is a risk factor for genital HSV-2 infection in humans.
Submitted for publication

III. **Alexandra Svensson, Lars Bellner, Mattias Magnusson, Kristina Eriksson.**
Role of IFN-α/β signaling in the prevention of genital herpes virus type 2 infection.
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IV. **Alexandra Svensson, Joanna Kaim Carina Mallard, Annika Olsson, Ernst Brodin, Tomas Hökfelt, Kristina Eriksson.**
Neurokinin 1 receptor (NK1R) signaling affects the local innate immune defense against genital herpes virus infection.
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CONTENTS

INTRODUCTION

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T-BET IN THE IMMUNE SYSTEM

The expression of T-bet was first described in naïve CD4+ T cells (Szabo et al., 2000). T-bet is expressed early during the differentiation of CD4+ T cells into T helper (Th) 1 cells, and acts as a potent inducer of the hallmark Th1 cytokine interferon (IFN)-γ. The induction of T-bet can occur in several different ways, depending on the cell type and triggering factors. Even though T-bet is generally held to be the main regulator of Th1 differentiation, it is expressed in a wide range of immune cells. T-bet is important for the induction of IFN-γ both in plasmacytoid dendritic cells (pDCs) and in natural killer cells (NK) cells. NK cells also need T-bet for their maturation and effector functions (Robbins et al., 2005; Townsend et al., 2004; Lugo-Villarino et al., 2003; Szabo et al., 2002). CD8+ T cells and B cells also depend on T-bet for their effector functions. CD8+ T cells need T-bet for the production of IFN-γ and for cytotoxic responses, while B cells are dependent on T-bet for Th1-related antibody production. These cells are indispensable for innate and/or adaptive immunity, which makes T-bet a crucial factor in the host protection against viruses and other invading microorganisms.

CELLS THAT EXPRESS T-BET

T-bet is expressed in most cells of the immune system, including T cells, B cells, NK(T) cells, DCs, monocytes and macrophages, in which it regulates gene transcription and thereby the cellular responses.

CD4+ T cells

CD4+ T cells can differentiate into two main subtypes upon activation, Th1 and Th2 cells. Their ultimate functions are linked to the cytokines that they produce. Th2 cells mainly produce IL-4, while the hallmark cytokine of Th1 cells is IFN-γ.

The induction of IFN-γ was previously associated with IL-12 (O'Garra, 1998) in synergy with IL-18 (Takeda et al., 1998). However, six years ago, the transcription factor T-bet was discovered to be a very potent inducer of IFN-γ (Szabo et al., 2000) and it is now considered to be the main regulator of CD4+ T cell development into the IFN-γ-producing Th1 phenotype (Szabo et al., 2002). As the hallmark regulator of Th1 development, T-bet acts independently of both IL-12/Signal Transducer and Activator of Transcription (Stat) 4 and IL-18 (Mullen et al., 2001). The induction of T-bet is instead augmented by signals mediated through the T cell receptor (TCR) (Szabo et al., 2000) together with Stat1 (Afkarian et al., 2002). The expression of T-bet leads to IL-12Rβ2 expression and IL-12 activation via Stat4 and consequent remodeling of the IFN-γ gene locus leading to IFN-γ production (Mullen et al., 2001). T-bet may also act directly on the IFN-γ gene to initiate transcription. IFN-γ may exert a positive feedback to enhance T-bet expression via the IFN-γ receptor and Stat1, in autocrine and endocrine fashions, thereby creating an amplification loop, that stabilizes T-bet expression and the Th1 differentiation program (Grogan et al., 2001; Lighvani et al., 2001; Mullen et al., 2001; Szabo et al., 2000). Furthermore, ectopic expression of T-bet in human CD4+ T cells can induce IFN-γ gene expression and decrease Th2 cytokine production (Kitamura et al., 2005; Lametschwandtner et al., 2004; Sundrud et al., 2003). In addition to its direct activities in Th1 regulation, T-bet has the ability to redirect the Th2 response into a Th1 phenotype by inhibiting the production of Th2 cytokines and consequently Th2 differentiation. Thus, T-bet is a crucial determinant of Th1/Th2 differentiation status (Figure 1).

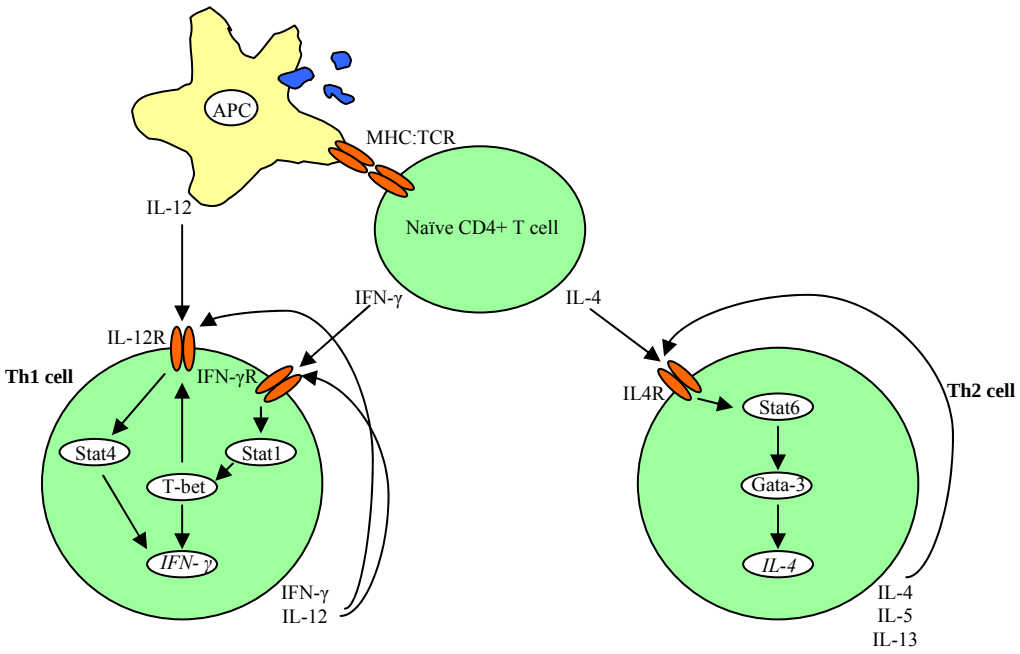

Figure 1. Overview of Th1 and Th2 cell differentiation in response to antigen stimulation.

CD8+ T cells

CD8+ T cells recognize and kill infected cells and produce IFN-γ in response to antigen stimulation. T-bet is expressed in CD8+ T cells, although its role in these cells is somewhat controversial. T-bet is involved in the cytotoxic response of CD8+ T cells through the induction of perforin and granzyme B, however T-bet is not able to induce these factors by itself but instead acts together with eomesodermin, which is another T-box factor (Intlekofer et al., 2005; Pearce et al., 2003). How these factors interact to regulate the effector functions of these cells is however not clear. Eomesodermin appears to be the main factor in the CD8+ T cell response, and it has been shown that ectopic expression of eomesodermin is sufficient for the production of IFN-γ, perforin, and granzyme B (Pearce et al., 2003). However, T-bet is a potent regulator of CD8+ T cell differentiation into the type 1 phenotype (Szabo et al., 2000), and should therefore not be disregarded in the regulation of CD8+ T cell functions. Cooperation of T-bet with eomesodermin leads to the upregulation of CD122 (IL-2βR) and the regulation of IL-15 responsiveness, which indicates that these two factors also play roles in CD8+ T cell memory (Intlekofer et al., 2005).

The IFN-γ responses and cytotoxic effects of CD8+ T cells do not require T-bet after non-antigen stimulation with plate-bound anti-CD3 and anti-CD28 (Szabo et al., 2002) (Pearce et al., 2003). Similar effects are seen in HSV-2 infection, during which the CD8+ T cell response remains unaffected despite T-bet deficiency. Surprisingly, mice that lack T-bet can mount even higher cytotoxic responses than wild-type mice (Svensson et al., 2005). One possible explanation is that CD8+ T cells are dependent on IL-2 for their cytotoxic responses. In the absence of T-bet, CD8+ T cells may produce higher amounts of the cytokine, since T-bet is not able to inhibit IL-2 production, which may, at least to some extent, be the case in wild-type cells. Moreover, the role of CD8+ T cells may be of minor importance during HSV-2 infection, since mice depleted of CD8+ T cells are still able to clear the infection after vaccination (Harandi et al., 2001), which may also explain the loss of function of T-bet in HSV-2-specific CD8+ T cells. In contrast to HSV-2 infection, T-bet is necessary for the antigen-driven development of CD8+ T cells into effector cells, and for a sufficient cytotoxic response together with optimal IFN-γ production in response to lymphocytic choriomeningitis virus (Sullivan et al., 2003).
Taken together, these findings suggests that the two T-box factors cooperate to ensure CD8+ T cell function, and that T-bet assists eomesodermin in fine-tuning the regulation of the effector functions and memory responses of these cells.

NK cells and NKT cells

NK cells and NKT cells are crucial components of the innate immunity in that they rapidly recognize and kill infected cells. T-bet plays a crucial role in both the development and effector functions of these cell types.

NK cells that lack T-bet show decreased levels of IFN-γ and impaired cytotoxic capacities (Szabo et al., 2002), as well as reduced numbers of peripheral NK cells (Townsend et al., 2004). The effector functions of NK cells are closely related to their lineage maturation (Kim et al., 2002), although it is still unclear whether the impaired functions seen in the absence of T-bet are due to a defect in the maturation of NK cells (Robbins et al., 2005; Townsend et al., 2004) or that T-bet has a direct role in their

effector functions. In addition to IFN-γ, the perforin and granzyme B genes are targets for T-bet. These genes are also regulated by eomesodermin, which is unaffected by T-bet deficiency. This suggests a compensatory relationship between T-bet and eomesodermin in the regulation of the effector functions of NK cells, similar to what is seen for CD8+ T cells (Intlekofer et al., 2005). This underlines the central role of these transcription factors in the maturation of NK cells.

The importance of T-bet appears to be more clear-cut in NKT cells, where it is involved in the regulation of maturation, migration, survival and effector functions of these cells (Matsuda et al., 2006). The most pronounced effects of T-bet are seen in the terminal maturation of NKT cells and for the IFN-γ response. T-bet-deficient NKT cells are blocked in their terminal maturation and completely lack IFN-γ production, a deficiency that may be linked to the absence of eomesodermin as a coregulator in these cells (Townsend et al., 2004).

Dendritic Cells

Dendritic cells (DCs) are important for the early antiviral immune response as producers of antiviral cytokines and for their antigen-presenting functions. T-bet expression in DCs is necessary for the activation of Th1 cells during antigen presentation and for IFN-γ secretion in the DCs themselves (Lugo-Villarino et al., 2003). Under the control of IFN-γ, DCs express very high levels of T-bet (Lighvani et al., 2001) in a positive feed-back loop, similar to what is seen in T cells (Lugo-Villarino et al., 2003). T-bet expression in human myeloid DCs (mDCs) is induced by bryostatin-1 via the extracellular signal-regulated kinase (ERK) -1/2 pathway (Li et al., 2006). In murine pDCs, T-bet expression leads to the production of IFN-α via the binding of CpG motif-containing DNA sequences to TLR9, which is also under the control of IFN-γ albeit via a yet unknown pathway (Lugo-Villarino et al., 2005). HSV-2 can also bind to TLR9 and induce IFN-α production (Lund et al., 2003). This suggests that T-bet might have an important role in the early IFN-α-mediated immune response against HSV-2. Even though T-bet has a role in the functional

effects of pDCs, it does not seem to be needed for the differentiation or activation of pDCs or mDCs (Lugo-Villarino et al., 2003).

B cells

T-bet plays a significant role in B cells, in which its main function is to induce and maintain the Th1-related responses. The expression of T-bet in B cells is induced by the direct binding of Stat1 to the T-bet promoter (Xu and Zhang, 2005). The induction and maintenance of IFN-γ production is independent of Stat1 and is totally dependent on T-bet (Harris et al., 2005). T-bet-deficient B cells fail to undergo class switch recombination (CSR) into immunoglobulin (Ig) G2a in response to IFN-γ, and they consequently fail to develop into Th1-inducing effector cells (Gerth et al., 2003; Harris et al., 2005; Peng et al., 2002). T-bet-deficient B cells overproduce the Th2-related isotypes IgG1 and IgE. CSR into IgG2a can also be induced by the binding of CpG motif-containing DNA sequences to TLR9, which is totally dependent on the expression of T-bet (Peng et al., 2003). However, the suppression of IgG1 and IgE does not involve T-bet (Liu et al., 2003). In the HSV-2-mediated antibody response, T-bet-deficient B cells are impaired in terms of IgG2a production and surprisingly, are also impaired in their IgG1 antibody responses, which suggests an overall impairment of the HSV-2-specific immune response in T-bet-/- mice (Svensson et al., 2005). This may explain the dubious role of antibody-mediated protection in HSV-2 infection, since high titers of HSV-2 specific antibodies do not necessarily protect against infection, at least not in humans (Corey et al., 1999).

Non-immune cells

Aside from the important roles of T-bet in various lymphocytes and DCs, it has been detected in epithelial cells of the reproductive tract, with the highest T-bet expression in vaginal epithelial cells. T-bet expression is enhanced by IFN-γ, although these cells are not able to produce IFN-γ themselves. In endometrial epithelial cells, the expression of T-bet is regulated by the balance between estrogen and progesterone. However, the steroid hormones do not affect T-bet expression in vaginal epithelial cells (Kawana et al., 2005).

The high T-bet expression in vaginal epithelial cells may act to protect against invading pathogens in the genital tract, and may therefore play an important role in immune protection against genital HSV-2 infection.

REGULATION OF T-BET

INDUCTION AND ACTIVATION

The regulation and induction of T-bet involves complex sequences of events and most of the factors involved are only partially defined. The most well-described signaling pathway of T-bet induction is the IFN-γ pathway mediated by Stat1 signaling. In addition to IFN-γ, several other cytokines and signaling mediators in the immune system have been shown to activate T-bet in both Stat1-dependent and -independent fashions.

Cytokine-mediated induction

In addition to IFN-γ, T-Bet is induced by IFN-α, IL-12, IL-15, IL-21, and IL-27. IFN-α is a central cytokine in innate immunity and its ability to activate cells of the acquired immune system makes it an important link between the innate and acquired immune systems. T-bet expression is induced by IFN-α via the expression of Stat1, independently of IFN-γ, leading to the expression of IL-12Rβ2 and a Th1 response (Hibbert et al., 2003). Together with signals from the TCR, IFN-α may enhance the Th1 response by further upregulating the expression of T-bet (Shibuya and Hirohata, 2005; Ylikoski et al., 2005). Thus, IFN-α acts in a manner similar to IFN-γ. In addition, the γ chain-related cytokines IL-15 and IL-21 can induce T-bet expression in NK cells and Th1 cells. In NK cells, IL-15 is a more potent inducer of T-bet than IL-21, while the opposite pattern is observed in Th1 cells (Strengell et al., 2002).

IL-12 is an important cytokine in the Th1 cell response because it enhances IFN-γ production and stabilizes the Th1 phenotype. In murine cells, IL-12 signaling acts

downstream of T-bet and IFN-γ to increase the expression and production of IFN-γ by signaling that is mediated through Stat4. However, in human CD4+ T cells, IL-12 may act independently of IFN-γ, together with signals from CD3/CD28 activation, to induce T-bet expression (Ylikoski et al., 2005).

IL-27 can induce T-bet expression in CD4+ T cells, CD8+ T cells, B cells and NK cells, although it is not essential for T-bet expression in any of these cells (Morishima et al., 2005). In developing CD4+ T cells, IL-27 induces T-bet expression (Hibbert et al., 2003; Kamiya et al., 2004; Lucas et al., 2003) via interactions with the cytokine receptor WSX-1 (Takeda et al., 2003) or via the activation of Stat1 under the control of IFN-γ (Lucas et al., 2003). IL-27 by itself is not sufficient to drive Th1 differentiation and may instead facilitate the expression of T-bet and Th1 differentiation by controlling IL-12R expression and thus IL-12 responsiveness (Lucas et al., 2003). However, the role of IL-27 in T-bet mediated Th1 differentiation is controversial, since IL-27 can induce Th1 differentiation independently of T-bet by the induction of intracellular adhesion molecule (ICAM)-1 (Owaki et al., 2005). Furthermore, IL-27-mediated cytotoxicity of CD8+ T cells and NK cells remains unaffected by T-bet deficiency (Lucas et al., 2003; Morishima et al., 2005). B cells are stimulated to undergo class-switching into the IgG2a isotype in response to T-bet induced IL-27 (Yoshimoto et al., 2004).

Induction through costimulatory factors

Costimulatory receptors are expressed on the cell surface to promote the differentiation and effector functions of immune cells. Several costimulatory factors expressed on CD4+ T cells stimulate and enhance Th1 differentiation and T-bet expression, including Notch1, signaling lymphocytic activation molecule (SLAM), glucocorticoid-induced TNFR family-related protein (GITR), leukocyte function-associated antigen-1 (LFA-1), and semaphorin (Sema) 4a.

Notch proteins are transcriptional activators that are expressed in a wide range of immune cells. In mammals four different Notch proteins have been identified (Notch1-4), that

bind five different ligands (Dallman et al., 2005). Following interaction with either of its ligands, the Notch/ligand complex is cleaved first by a metalloprotease and then by γ-secretase. These processes generate the active intracellular portion of notch (NotchIC), which translocates to the nucleus and interacts with C-promotor-binding-factor (CSL) to initiate the transcription of its target genes (Fortini, 2002; Fryer et al., 2002; Mumm and Kopan, 2000). The Notch signaling pathway regulates T-bet expression by acting directly on the T-bet promoter in a Stat1-independent manner in the early stages of Th1 differentiation. If the γ-secretase activity in the Notch pathway is blocked, no active NotchIC is generated and T-bet mediated IFN-γ production by Th1 cells is impaired (Minter et al., 2005).

LFA-1 is expressed on Th cells and is activated by interactions with ICAM-1 on DCs, which leads to Th1 polarization and a rapid increase in T-bet expression, which occurs independently of IL-12 (Smits et al., 2002). SLAM, which is a surface receptor expressed on lymphocytes and DCs, can induce IFN-γ production and proliferation in T cells by direct upregulation of T-bet. In addition to T-bet, SLAM can induce expression of Stat1 and NF-κB (Quiroga et al., 2004), i.e., transcription factors that may also play a role in the activation of the IFN-γ gene. Furthermore, T-bet is induced by the co-stimulatory receptors GITR (Patel et al., 2005) and Sema4a (Kumanogoh et al., 2005), as well as by the signal transducer p38 Mitogen-Activated Protein (p38MAP) kinase (Jones et al., 2003), of which all enhance Th1 cell differentiation through yet unknown signaling pathways. Inducers of T-bet are summarized in Figure 2.

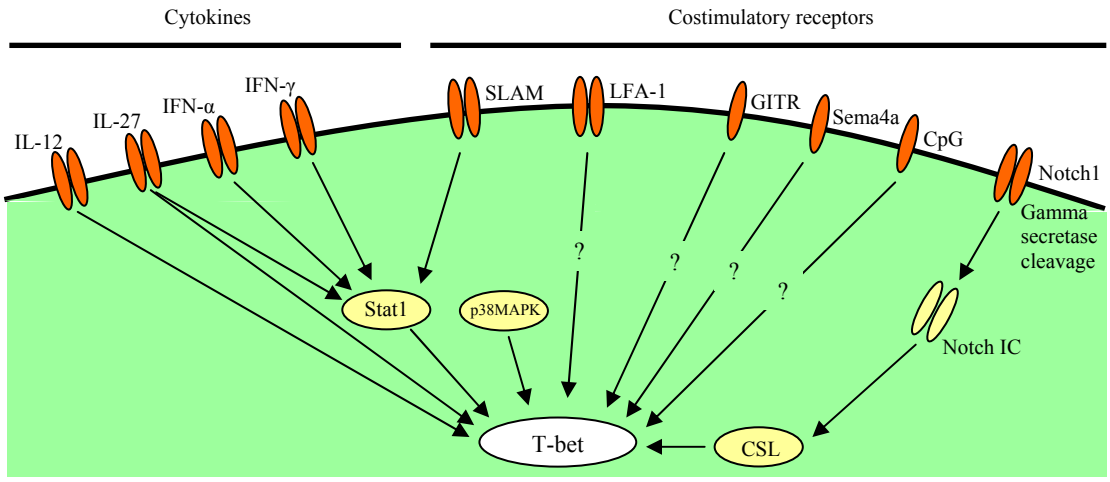

Figure 2. T-bet induction in CD4+ T cells. Abbreviations: SLAM: Signaling lymphocytic activation molecule; LFA-1: Leukocyte function-associated antigen-1; GITR: Glucocorticoid-induced TNFR family-related protein; Sema4a: Semaphorin 4a; CSL: C-promotor-binding-factor; p38MAPK: p38 Mitogen-Activated Protein kinase.

NEGATIVE REGULATION OF T-BET

The expression and functions of T-bet are negatively regulated by several factors that in different ways inhibit the T-bet expression.

Transforming growth factor (TGF)-β is involved in the development of regulatory T cells and is a potent inhibitor of T-bet. TGF-β blocks T-bet expression via signals mediated by hemopoietic protein tyrosine phosphatase (PTP), Src homology region 2 domain-containing phosphatase-1 (Shp-1) (Park et al., 2005), and small mad-related protein (SMAD) 2, 3 and 4 (Yu et al., 2006) early during Th1 priming, which leads to inhibition of Th1 development (Gorelik et al., 2002; Lin et al., 2005; Ylikoski et al., 2005). In addition to its inhibitory effects on T-bet, TGF-β also inhibits Stat4, leading to a decrease in IFN-γ production and further impairment of Th1 development (Lin et al., 2005). Other potent regulators of T-bet include peroxisome proliferators-activated receptor α (PPARα), the oncogene Vav1, inducible T cell kinase (Itk) and heat shock protein 60 (HSP60). PPARα and Vav1 inhibit the activation of p38 MAP kinase and thereby suppress T-bet expression (Jones et al., 2003; Tanaka et al., 2005). Itk acts as a Th2-promoting regulator to inhibit T-bet expression and Th1 differentiation downstream of the TCR (Miller et al.,

2004). The inhibition of T-bet by HSP60 leads to decreased IFN-γ and TNF-α production, and thus, there is no Th1 response (Zanin-Zhorov et al., 2005).
Negative regulators of T-bet are summarized in Figure 3.

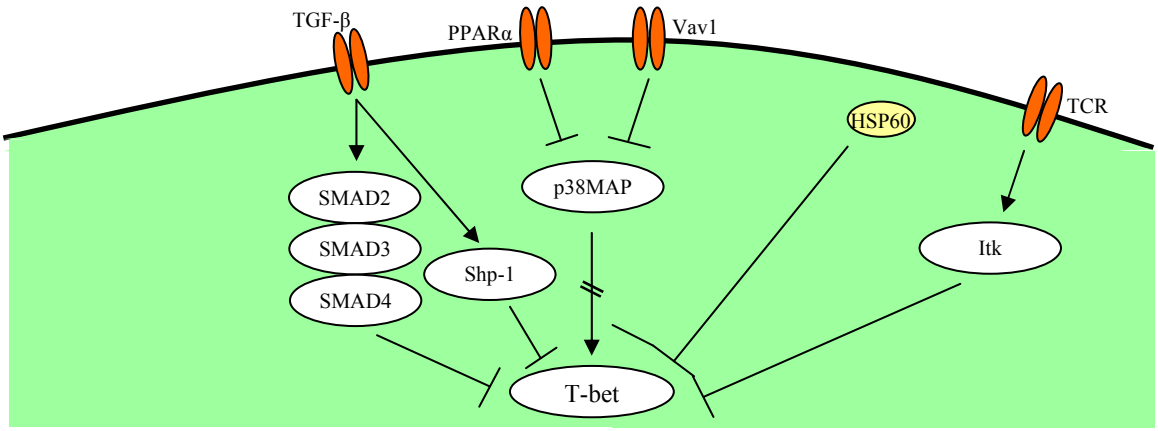

Figure 3. Inhibition of T-bet expression. Abbreviations: SMAD: small mad related protein; PPARα: peroxisome proliferators-activated receptor α; Vav1: Vav 1 oncogene; Shp-1: hemopoetic protein tyrosine phosphatase (PTP); Shp-1: Src homology region 2 domain-containing phosphatase-1; Itk: Inducible T cell kinase; HSP60: heat shock protein 60

In addition to Th1 and Th2 cells, CD4+ T cells can differentiate into Th1β cells. These cells arise independently of the Th1 and Th2 hallmark cytokines IFN-γ and IL-4. The inhibition of T-bet by TGF-β allows these cells to differentiate into a distinct IL-17-producing cell lineage that plays important role in autoimmune conditions (Harrington et al., 2005; Veldhoen et al., 2006). T-bet is involved in the decision to embark Th1 versus Th1β differentiation, since ectopic expression of T-bet inhibits the differentiation of Th1β cells by blocking IL-17 secretion, which promotes IFN-γ production and redirects Th1β cells into a Th1 phenotype (Mathur et al., 2006). This suggests that T-bet is a critical determinant in the Th1 versus Th1β cell fate.

THE T-BET / GATA-3 BALANCE

In contrast to T-bet-driven Th1 development, the Th2 differentiation program is driven by the transcription factor GATA-3 (Zheng and Flavell, 1997). Th2 cells produce IL-4, IL-5,

and IL-13. These cytokines are important for the cell-mediated immune response to extracellular pathogens, although they also play important roles in driving the inflammation processes in asthma and allergy. GATA-3 is induced by IL-4 via the expression of Stat6, most likely in some kind of feedback loop, since ectopic expression of GATA-3 leads to the production of IL-4 as well as IL-5 and IL-13 (Agnello et al., 2003). GATA-3 is repressed by T-bet during TCR-mediated Th1 differentiation, leading to the inhibition of Th2 cytokines at the transcriptional level (Hwang et al., 2005b). Furthermore, T-bet expression in developing Th2 cells can repress IL-4 and IL-5 production, and thereby redirect developing Th2 cells into a Th1 phenotype (Szabo et al., 2000). The shift of Th2 cells into a Th1 phenotype can also occur when fully Th2 polarized cells are restimulated with IL-12, which leads to elevated levels of T-bet and decreased GATA-3 expression (Smits et al., 2001), indicating a direct role for IL-12 in the activation of T-bet. Consequently, it has been suggested that inhibition of GATA-3, rather than positive regulation of the IFN-γ gene is the main function of T-bet (Usui et al., 2006). Although, the exact mechanism through which T-bet regulates the Th1/Th2 balance is not fully known, it possibly enhances Th1 differentiation by the regulation of both IFN-γ and GATA-3. T-bet acts directly by inducing Th1 activities as well as indirectly by the suppression of GATA-3, rather than acting more or less independently on each of the genes.

EFFECTOR FUNCTIONS OF T-BET

TH1 DIFFERENTIATION

The main effector function of T-bet is as a transcriptional activator of the IFN-γ gene and the Th1 response. Upon induction of IFN-γ gene expression, T-bet binds monomeric brachyury consensus sites in the IFN-γ promoter (Cho et al., 2003) and initiates chromatin remodeling of the IFN-γ gene locus during the first cell division of the developing Th1 cells (Mullen et al., 2002). T-bet initiates the transcription of the IFN-γ

gene by the establishment of long-range histone hyperacetylation across the IFN-γ gene region (Chang and Aune, 2005; Shnyreva et al., 2004) and by inhibiting the IFN-γ gene repressor, mSin3a (Tong et al., 2005). The binding of T-bet alone is sufficient for IFN-γ expression, although the effect can be enhanced by interactions with other transcription factors. T-bet induces expression of the transcription factor H2.0-like homeobox (Hlx), and thereafter interacts with Hlx to induce higher levels of IFN-γ (Martins et al., 2005; Mullen et al., 2002). To enhance further IFN-γ production, T-bet interacts with the transcription factor E26 transformation-specific (Ets)-1 in fully differentiated Th1 cells, and this interaction further stabilizes the Th1 phenotype. Even though the IFN-γ-promoting effect of T-bet is severely impaired in the absence of Ets-1, neither Ets-1 nor Hlx is necessary for INF-γ gene induction (Grenningloh et al., 2005).

In addition to the activation of IFN-γ gene expression and GATA-3 suppression, T-bet promotes Th1 differentiation by controlling IL-2 and IL-21 production during Th cell development (Szabo et al., 2000; Szabo et al., 2002). T-bet inhibits the binding of the IL-2-activating transcription factor v-rel reticuloendotheliosis viral oncogene homolog A (RelA) to the IL-2 promoter, and thereby prevents transcriptional activation of the IL-2 gene (Hwang et al., 2005a). By binding to the nuclear factor of activated T cells (NFATc2), T-bet prevents the activation of the IL-21 promoter, leading to the inhibition of IL-21 production, which further enhances the Th1 phenotype (Mehta et al., 2005).

T-bet may also activate the expression of non-cytokine genes during the development of Th1 cells. Osteopontin is involved in several processes in the body, including immune responses and has been suggested to affect Th1 responses by regulating IL-12 and IL-10. T-bet can bind directly to the promoter region of the osteoponin-coding gene *Opn* in developing CD4+ and CD8+ T cells, thereby directing them into a Th1 phenotype (Shinohara et al., 2005).

MIGRATION

The migration of immune cells into an infected tissue is a crucial event in the functioning of the immune system. Migration of T cells into the target tissue is mediated by the binding of P-selectin glycoprotein ligand-1 (PSGL-1) to P-selectin and E-selectin on the inflamed endothelium, as well as by the expression of cytokine and chemokine receptors on the migrating cells. Interestingly, T-bet can influence the capacity of inflammatory immune cells to migrate to target tissues, by affecting both the expression of homing receptors and the cytokine and chemokine receptor expression on CD4+ T cells. In the absence of T-bet, CD4+ T cells have a reduced migration capacity due to impaired binding to P-selectin, which is caused by a reduction in tyrosine sulfation of PSGL-1 (Lord et al., 2005). T-bet controls CD4+ T cell migration directly by regulating the levels of PSGL-1 through direct activation of the encoding genes, or indirectly via the control of IL-12Rβ2 expression (Underhill et al., 2005). T-bet binds directly to the promoter region of chemokine (C-X-C motif) receptor 3 (CXCR3) (Beima et al., 2006) which induces CXCR3 expression in naïve human cells, CD4+ central memory T cells and Th2 cells (Sundrud et al., 2003; Lametschwandtner et al., 2004). This is further emphasized by the finding that T-bet deficiency in mice is associated with substantially diminished expression of CXCR3 (Lord et al., 2005).

The effector functions of T-bet are summarized in Figure 4.

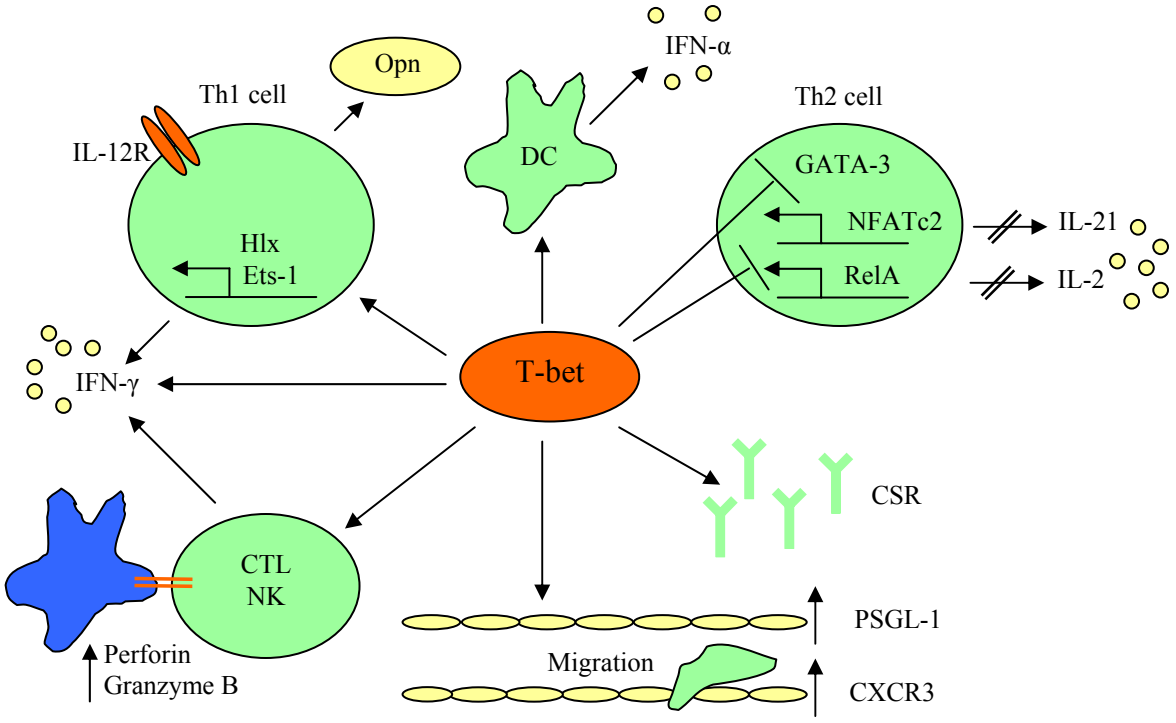

Figure 4. Effector functions of T-bet. Abbreviations: Hlx: H2.0-like homeobox; Ets-1: E26 transformation-specific; Opn: osteoponin; NFATc2: nuclear-factor of activated T cells; RelA: v-rel reticuloendotheliosis viral oncogene homolog A; CSR: class-switch recombination; PSGL-1: P-selektin glycoprotein ligand 1. CXCR3: chemokine (C-X-C motif) receptor 3

TBX21 AND POLYMORPHISMS

T-bet is encoded by the *TBX21* gene and belongs to the family of DNA-binding T-box proteins. The *TBX21* gene consists of 6 exons and 5 introns and is located on chromosome 17 in humans and on chromosome 11 in mice. The T-bet protein is 530 amino acids (aa) long, with a 189-aa T-box DNA-binding domain. The human and murine proteins share 87% homology and within the T-box domain, the level of homology is 98%, with only a 3-aa mismatch (Szabo et al., 2000; Zhang and Yang, 2000).

Genetic variations in the human genome can sometimes affect disease susceptibility and/or severity, although most of the variations have no effect on the outcome of the phenotype. Many of the basal functions in the body are genetically determined and remain unaffected by the influence of environmental factors. It has recently been discovered that the Th1 effector function is genetically inherited. In monozygotic twins, variability in T-bet and IFN-γ gene expression is very low compared to that in dizygotic twins, which indicates that Th1 fate is genetically determined. However, Th2 regulatory factors, such as GATA-3, NFAT and NF-κB are not genetically determined (Hohler et al., 2005). Genetic variations in *TBX21* have been associated with a few different diseases, including asthma, diabetes, and HSV-2 infection. Susceptibility to asthma and airway hyper-responsiveness in asthmatic children has been associated with upstream SNPs in the *TBX21* gene (Raby et al., 2006), and an SNP in the promoter region has been associated with aspirin-induced asthma in Japanese asthmatics. The latter SNP, with involves a substitution of a tyrosine with a cystein, leads to increased transcriptional activity of *TBX21* (Akahoshi et al., 2005). Polymorphisms in *TBX21* may be of importance in the treatment of asthma, since an SNP in *TBX21* has been shown to enhance the effects of inhaled corticosteroid treatment in asthmatic children (Tantisira et al., 2004). The prevalence of Type 1 diabetes has also been correlated with variations in *TBX21*, and this has been linked to increased transcriptional activity of the IFN-γ gene (Sasaki et al., 2004). In HSV-2 infection, an SNP in the non-coding region at the 3´-end of *TBX21* is strongly associated with the incidence of genital HSV-2 infection (Svensson, submitted for publication).

Diseases associated with polymorphisms in *TBX21* are summarized in Table 1 and Figure 5.

Table 1. Polymorphisms in *TBX21* that are associated with disease.

Disease	Type	Region in T-bet	Position	Function
HSV-2 infection	SNP	Non-coding 3´-region	rs17244587	Unknown
Asthma	SNP	Promoter region	rs4794067	Increased transcription
	SNP	Upstream of *TBX21*	rs9910408	Unknown
AHR	SNP	Non-coding 3´-region	rs9910408	Unknown
Diabetes	SNP	Coding region 5´-end	rs2240017	Increased IFN-γ transcription

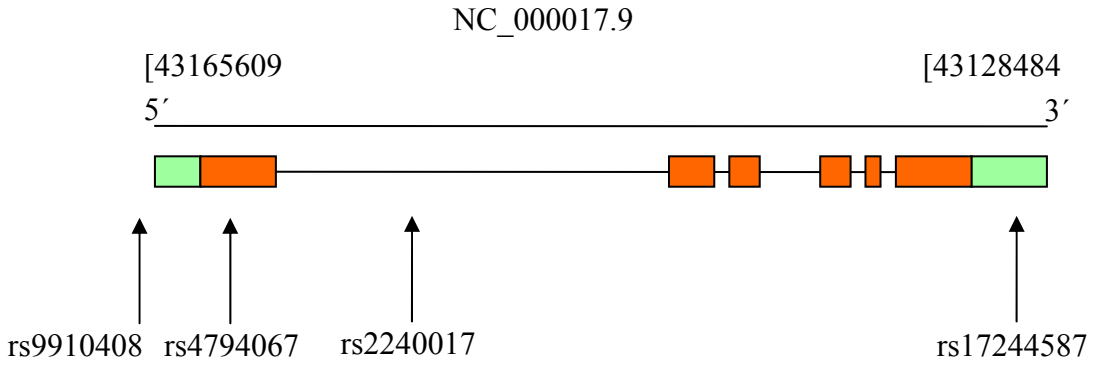

Figure 5. Genomic structure of *TBX21*. Disease-associated loci are marked with arrows: rs9910408 is associated with asthma and AHR; rs4794067 is associated with asthma; rs2240017 is associated with diabetes; and rs17244587 is associated with HSV-2 infection.

T-BET AND HUMAN DISEASES

VIRAL INFECTIONS

Antiviral immunity relies on several immune cell-types. One of the most important cells in antiviral immunity is the IFN-γ-producing Th1 cell. Therefore, T-bet is a key factor in the immune response against viral infections, not only because of its role as a master regulator of the IFN-γ response but also because it regulates the effector functions of the immune cells. The effect of T-bet varies depending on the antigen that triggers the immune response as well as the cell types involved.

Immune responses to several viral infections have been shown to be dependent on T-bet for full functionality. T-bet plays a major role in both the innate and acquired immune responses to HSV-2 infection. Mice deficient for T-bet have severe problems in mounting an innate immune response to HSV-2, owing to an impaired NK cell response, in terms of both IFN-γ production and cytotoxicity. Furthermore, the acquired immune response to HSV-2 is dependent upon T-bet. T-bet-deficient mice vaccinated with an attenuated strain of HSV-2 cannot generate protective immunity to the infection due to impaired CD4+ T cell and IFN-γ responses, whereas wild-type mice survive this infection without any clinical symptoms or signs of disease (Svensson et al., 2005). Moreover, the incidence of HSV-2 infection is strongly associated with an SNP in *TBX21*, for which the AA genotype is found in HSV-2-infected individuals and not in healthy controls. However, this SNP does not affect the severity of disease and does not affect the CD4+ T cell responses or NK cell phenotype. A functional effect of this polymorphism should however not be ruled out, since T-bet affects several other factors in addition to those in NK cells and CD4+ T cells. A possible role for the *TBX21* SNP may be in the epithelial cells of the vaginal tract, since these cells are targets of HSV-2 and express high levels of T-bet. Therefore, it is of interest to correlate the SNP found in HSV-2 patients with the levels and functions of T-bet in these cells with regard to both HSV-2 susceptibility and disease severity (Svensson, submitted for publication).

In addition to HSV-2 infection T-bet has been shown to be important in the immune defense against other viral pathogens. Mice deficient for T-bet cannot mount a sufficient immune response to vaccinia virus infection so they succumb to infection due to impaired NK cell functions and decreased IFN-γ production, similar to the case involving HSV-2 infection (Matsui et al., 2005). In an *in vitro* study of human mononuclear cells from adults and newborns, it has been shown that Varicella zoster virus (VZV) rapidly induces a Th1 response in adult cells with high-level expression of T-bet and downregulation of GATA-3 together with high production of IFN-γ (Yu et al., 2003). Th1 cell responses are not observed in cord blood-derived mononuclear cells due to their naïve status. This implies an important role for T-bet in the memory responses of CD4+ T cells, since most of the adult population (>95%) is/has been infected with VZV, and can therefore mount a rapid immune response to the virus. During the establishment of HIV-1 infection, the Th1 response is induced early during the establishment of the virus in the host. The binding of the HIV-1 transcription activator Tat to CD4+ T cells induces vigorous proliferation of CD4+ T cells as well as IFN-γ production and T-bet expression, which lead to the activation of other cells that can limit viral replication (Kulkarni et al., 2005). A protective role for T-bet has also been suggested in Dengue fever, whereby patients with hemorrhagic dengue fever have significantly lower levels of T-bet and IFN-γ than patients who lack hemorrhagic symptoms, suggesting an important role for T-bet in the regulation of disease severity (Chen et al., 2005). In patients with human T lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP), increased levels of T-bet have been observed in comparison to HTLV-1-uninfected individuals, which suggests a pathogenic role for T-bet that contrasts with its roles in other viral infections (Nishiura et al., 2004).

BACTERIAL AND PARASITIC INFECTIONS

Besides its protective role in viral infections, T-bet is involved in several bacterial and parasitic infections. This was first demonstrated by Szabo and colleagues, who showed that T-bet is necessary for the Th1-mediated immune response against *Leishmania major*. Mice deficient in T-bet fail to resolve the infection due to diminished IFN-γ production

and the subsequent high parasitic loads (Szabo et al., 2002). The same trend was observed in a model of *Leishmania donovani* infection, where T-bet deficient mice had high parasitic loads and failed to develop a Th1 response (Rosas et al., 2006). Similar to *Leishmania* infection, T-bet-/- mice are unable to control infection with *Staphylococcus aureus*, due to impaired CD4+ T cell responses and decreased IFN-γ production (Hultgren et al., 2004). In addition, in infection with *Mycobacterium tuberculosis*, mice that lack T-bet have elevated levels of bacteria and succumb significantly earlier to infection than the control mice; in this case the inability to control infection was probably due to a reduced number of CD8+ T cells in the lung (Sullivan et al., 2005). Similarly, T-bet-deficient mice cannot control *Salmonella* infection and succumb to infection, due to impaired *Salmonella*-specific IFN-γ and IgG2a production (Ravindran et al., 2005). In addition to these bacterial infections, T-bet expression has been shown to govern the Th1 responses to lymphatic filariasis infection (Babu et al., 2005). In contrast to most other infections, T-bet is not required for innate or acquired immunity to *Listeria monocytogenes*, such that T-bet deficient mice are able to resolve the infection, since they are able to produce IFN-γ in spite of T-bet deficiency (Way and Wilson, 2004). These data indicate an alternative T-bet-independent pathway for route to IFN-γ induction, perhaps involving the IL-12/Stat4 pathway.

AUTOIMMUNE DISEASES

In contrast to the beneficial effects of T-bet expression during pathogenic infections, the activation of T-bet and the Th1 response may lead to inflammatory diseases. Several studies of autoimmune diseases have shown that loss of T-bet has a protective influence on disease progression, while others have shown opposite effects. Thus, the role of T-bet in autoimmune diseases remains to be clarified.

Multiple sclerosis

A growing body of evidence suggests that T-bet has a pathogenic role in multiple sclerosis (MS). T-bet-deficient mice are protected against this disease (Nath et al., 2006), and *in vivo* suppression of T-bet during antigen-specific T cell differentiation with T-bet-

specific oligonucleotides or siRNA can inhibit disease development (Lovett-Racke et al., 2004). Furthermore, MS patients have elevated levels of T-bet-expressing CD3+ cells in brain and spinal cord lesions (Skundric et al., 2006), as well as in blood-derived PBMCs (Frisullo et al., 2006). However, low-level T-bet expression does not necessarily correlate with reduced severity of MS (Koguchi et al., 2006).

Rheumatoid arthritis

The role of T-bet in rheumatoid arthritis (RA) is controversial. RA lesions are characterized by the infiltration of both cells of the innate and acquired immune systems, including DCs, NK cells, and T cells, into the joints. In a model of collagen-induced arthritis, T-bet-deficient mice have shown substantially reduced jointinflammation, which has been associated with compromised ability to prime T cells and decreased proinflammatory responses of DCs (Wang et al., 2006). The pathogenic role of T-bet is further evidenced in RA patients who have a predominance of CD3+ T cells in their joints; a large proportion of these cells express T-bet (Wang et al., 2006). In contrast, low levels of T-bet mRNA expression and IFN-γ have been associated with severe symptoms of RA (Kawashima and Miossec, 2005), which raises questions as to the exact role of T-bet in this disease.

Systemic Lupus Erythematosus

T-bet seems to have a pathogenic role in Systemic Lupus Erythematosus (SLE). T-bet is required for the development of SLE in mice. T-bet generates autoreactive antibodies and mediates both CSR and the affinity maturation of autoreactive B cells (Peng et al., 2002). Furthermore, patients with SLE show elevated levels of T-bet mRNA and T-bet protein in the urinary sediment and kidney tissues (Chan et al., 2006b; Chan et al., 2006a).

Diabetes

The incidence of Type 1 diabetes is reduced by the loss of T-bet, at least in the transgenic mouse model of Type 1 diabetes, due to impaired CD8+ T cell responses, including a

reduction in the number of cells, decreased IFN-γ production, and elevated IL-2 levels (Juedes et al., 2004). This suggests a therapeutic opportunity for T-bet as a candidate target in the treatment of Type 1 diabetes.

Autoimmune myocarditis

Unlike the autoimmune diseases described above, T-bet seems to have a protective role autoimmune myocarditis. In a mouse model, T-bet act as a negative regulator of the autoimmune response by controlling non-specific CD8+ T cell bystander functions in the heart. T-bet-deficient mice develop severe experimental autoimmune myocarditis (EAM), which is correlated with increased production of IL-17 by lymphocytes, that infiltrate the heart (Rangachari et al., 2006). This suggests a role for Th1β cells in the regulation of autoimmune myocarditis.

Gastrointestinal diseases

T-bet plays a key role in the regulation of mucosal T cell activation in inflammatory bowel diseases (IBD), and this is manifested in different ways depending on the syndrome. The levels of T-bet and IFN-γ are increased in CD4+ T cells from patients with Crohn´s disease, whereas patients with ulcerative colitis express low levels of T-bet, that are comparable to those in healthy controls (Matsuoka et al., 2004). In a Th1-related adoptive transfer model of IBD, T-bet-deficient cells transferred into severe combined immunodeficient (SCID) mice failed to induce Th1-mediated colitis, while the transfer of cells that overexpressed T-bet gave an earlier onset of severe Th1-mediated IBD (Neurath et al., 2002).

ASTHMA

A role for T-bet in asthma has been shown both in humans and in mice. Patients with asthma have low levels of T-bet, which is due to low-level expression of TLR9, leading directly to the downregulation of Th1 cytokines (Dong et al., 2006). Similar to asthma patients, T-bet-deficient mice undergo airway remodeling under the regulation of T-bet

and GATA-3 (Kiwamoto et al., 2006). This airway remodeling is associated with the overproduction of Th2-related cytokines in the absence of T-bet (Finotto et al., 2005) and has been suggested to develop spontaneously in T-bet-/- mice (Finotto et al., 2002). However, the spontaneously developed asthma symptoms are explained by *Mycoplasma pulmonis* colonization of the airways of T-bet-deficient mice, and is associated with high bacterial loads and decreased IFN-γ responses, which triggers a Th2 response with asthma associated-symptoms (Bakshi et al., 2006).

CANCER

T-bet is frequently expressed in both normal lymphocytes and tumor cells. Therefore, T-bet may have a role in different lymphomas. T-bet expression has been found in B cell lymphoproliferative disorders, in neoplastic T cells from patients with peripheral T cell lymphomas and in Hodgkins lymphoma. Although the role of T-bet in these disorders is not completely clear, it has been suggested that T-bet is involved in the development and growth of these neoplastic cells, which makes T-bet a good marker for lymphoproliferative disorders (Dorfman et al., 2003; 2004; 2005). However, in cutaneous T cell lymphoma, T-bet expression is down-regulated (Hahtola et al., 2006), which indicates different roles for T-bet in different types of cancers.

In addition to the beneficial roles of T-bet in several diseases, it may also have a protective role in cancer. T-bet has been shown to regulate the progression of prostate cancer by suppressing tumor growth and the development of metastatic capacity (Peng et al., 2004). This is perhaps attributable to a regulatory function of T-bet on the anti-tumor activity of IL-27 (Hisada et al., 2004).
Diseases that are affected by T-bet are summarized in Table 2.

Table 2. Diseases affected by T-bet expression.

+: Beneficial effect of T-bet; -: Nonbeneficial effect of T-bet; ?: Unknown/uncertain effect of T-bet.

Infectious diseases	**Model**	**Role in disease**	**References**
HSV-2	Human/Mouse	+	Svensson, 2005
Vaccinia virus	Mouse	+	Matsui, 2005
HIV-1	Human	+	Kulkarni, 2005
Dengue	Human	+	Chen, 2005
VZV	Human	+/?	Yu, 2003
Leishmania	Mouse	+	Szabo, 2002; Rosas, 2006
Tuberculosis	Mouse	+	Sullivan, 2005
Salmonella	Mouse	+	Ravingran, 2005
Listeria	Mouse	+	Way, 2004
Mycoplasma	Mouse	+	Bakshi, 2006
HTLV-1	Human	-	Nishiura, 2004
Filariasis	Human	+	Babu, 2005
Non infectious diseases	**Model**	**Role in disease**	**References**
MS	Human/Mouse	-	Nath, 2006; Lovett-Racke, 2004; Skundric, 2006; Frisullo, 2006; Koguchi, 2006
RA	Human/Mouse	-	Wang, 2006; Kawashima, 2005
Type 1 Diabetes	Human/Mouse	-	Juedes, 2004
IBD	Human/Mouse	+/-	Matsuoka, 2004; Neurath, 2002
SLE	Human/Mouse	-	Peng, 2002; Chan, 2006
Myocarditis	Mouse	+	Rangachari, 2006
Atherosclerosis	Mouse	-	Buono, 2005
Behcet´s disease	Human	-	Li, 2003
Liver disease	Mouse	-	Sibler, 2003
Anemia	Human	-	Solomou, 2006
Asthma	Human/Mouse	+	Dong, 2006; Kiwamoto, 2006; Finotto, 2002; 2005
Cancer	Human	+/?	Dorfman, 2003; 2004; 2005; Hahtola, 2006; Peng, 2004; Hishada, 2004

CONCLUSIONS

T-bet is a key regulator of the Th1 immune response and of Type 1-related immunity, playing important roles in the effector functions of T cells, NK cells, DCs, and B cells. T-bet is required for immunity to a wide range of infections and is especially active in Th1 mediated immune responses. Research on T-bet and its role in infection has increased in the last few years, and the data obtained to date indicate that T-bet exerts a protective function against most infectious diseases, with only a few exceptions. Most of the studies have been performed in mouse models. The role of T-bet in human infections is less well studied. An SNP in the T-bet gene has been correlated with the incidence of HSV-2 infection, and high levels of T-bet have been observed in response to HIV-1 infection. The major role of T-bet during infection is to induce IFN-γ production. T-bet may therefore serve as a potent therapeutic target against many viral infections. However, high-level expression of inflammatory cytokines and factors that mediate T-bet should be approached with caution, since several autoimmune conditions have been correlated with high levels of T-bet and associated Th1 cytokines.

Nonetheless, the accumulating data strongly indicate a protective role for T-bet in the pathogenesis of infectious diseases. Further research should lead to new insights and further understanding of the relationship between T-bet, cytokines, and infectious diseases, which may lead to the development of new therapeutic strategies for infectious diseases, autoimmune conditions, and other diseases.

SUMMARY OF THE THESIS

PAPER I

In this paper, we evaluated the role of T-bet in the innate and acquired immune response to HSV-2 infection in mice. T-bet-deficient mice failed to mount a protective immune response against HSV-2. Mice that lacked the gene for T-bet had elevated viral titers, both in vaginal tissue and in the CNS, resulting in rapid disease development and mortality. This was associated with impaired NK cell function, in terms of both IFN-γ production and in cytotoxicity. Vaccinated T-bet-/- mice could not clear HSV-2 infection and succumbed to the infection, due to a diminished IFN-γ response from T-bet-/- CD4+ T cells. In contrast, all of the HSV-2 infected wild-type mice cleared the infection without any signs of disease. Therefore, T-bet is necessary for protective immunity against HSV-2 infection in mice.

PAPER II

In this paper, we show that a single nucleotide polymorphism (SNP) in the T-bet gene is strongly associated with the incidence of genital HSV-2 infection. An SNP that involved the substitution of an adenine for a guanine residue in a non-coding site of the *TBX21* gene was significantly more common in HSV-2 infected individuals than in age- and sex-matched healthy controls. Furthermore, the homozygous variant of this allele was exclusively found in HSV-2-infected individuals. This polymorphism did not affect the severity of disease, nor did it have any functional effects on NK cell phenotype or CD4+ T cell responses. These results indicate that inherited variations in the T-bet gene may affect susceptibility to genital herpes infection in humans.

PAPER III

In this paper, we evaluated the role of IFN-α/β signaling in the prevention of genital HSV-2 infection. Viral replication and disease progression were enhanced in mice that lacked the IFN-α/β receptor (IFN-α/β R-/-). However, IFN-α/βR-/- mice were still able to mount a normal acquired immune response to HSV-2 after vaccination. By administering

synthetic antiviral TLR ligands to IFN-α/βR-/- mice and to wild-type mice we are able to show that the antiviral effects of these ligands are strongly dependent upon IFN-α/β signaling, and that overall IFN-α/β signaling is important for protection against HSV-2 infection.

PAPER IV

In this paper, we show that the local innate immune response to HSV-2 is mediated by Substance P signaling via the neurokinin 1 receptor (NK1R). Mice that lack NK1R showed increased viral replication in vaginal tissues and in the CNS, and died due to infection in a shorter time than wild-type mice. This difference is most likely due to impaired NK cell responses in the vaginal tract in NK1R-deficient mice and a direct antiviral effect of Substance P. The acquired immune response was not affected by NK1R deficiency. Thus, Substance P signaling through its receptor NK1R contributes to the innate immune response to genital HSV-2 infection in mice.

CONCLUDING REMARKS

Genital herpes infection caused by HSV-2 is today one of the most common sexually transmitted diseases worldwide. HSV-2 infects the genital mucosa and the surrounding sensory neurons. The development of immunity to HSV-2 infection involves a wide spectrum of components of the immune system. This thesis evaluates the individual contributions of the various innate and acquired immune mediators in regard to the incidence, severity, prevention and treatment of HSV-2 infection.

The innate immune response to HSV-2 encompasses a range of antiviral factors, whereby NK cells and type 1 interferons (IFN) play crucial roles. The type 1 IFNs include IFN-α and IFN-β, which are induced rapidly in response to interactions between viral factors and pattern recognition receptors (PRR), such as TLRs, mannose receptors and cytosolic receptors, to restrict viral replication. This thesis describes how IFN-α/β is dependent upon the IFN-α/β receptor signaling pathway to ensure sufficient innate immunity to HSV-2, since mice that lack this receptor have increased viral loads and succumb early to infection in comparison to wild-type mice. Furthermore, mice treated with antiviral synthetic TLR ligands were to different extents protected against disease in an IFN-α/β-dependent manner.

It is firmly established that NK cells are essential factors in the immunity to HSV-2, since they are the main producer of IFN-γ in the innate immunity and have the capacity to recognize and kill virus-infected cells in the absence of MHC-I. NK cells are dependent upon the transcription factor T-bet for their maturation and function. Thus, T-bet is an important factor for NK cell responses in HSV-2 infection. Mice that lack T-bet have severely impaired NK cell responses, in terms of both IFN-γ production and cytotoxicity. However, it is not clear if this is due to the reduced number of NK cells or to a functional defect in T-bet-/- NK cells. The role of T-bet in NK cells is not known and it remains to be elucidated whether the impaired NK cell responses in these mice depend on a failure

in cell maturation or if it is a direct lapse in effector function. Nevertheless, T-bet is an essential component of innate HSV-2 immunity.

HSV-2 is a neurotropic virus that infects the dorsal root ganglia and in rare cases, spreads to the spinal cord and causes meningitis. The ganglia are rich in different neuropeptides, such as Substance P (SP). In addition to neurons, SP is expressed in several cells of the immune system. In this thesis I show that SP has direct antiviral activity and that signaling through its receptor neurokinin 1 (NK1R) leads to innate immune protection against HSV-2 infection. Moreover, NK1R and SP signaling have significant roles in the local innate immune response to HSV-2 but not in the systemic responses, since vaginal NK cells from NK1R-deficient mice were significantly impaired in their killing capacity, while spleen derived NK cells from these mice could kill target cells to the same extent as NK cells from the wild-type mice.
Taken together, these results show that the innate immunity to HSV-2 infection is dependent upon a wide range of factors, including signaling factors IFN-α/βR, T-bet, and SP/NK1R.

IFN-γ is a key cytokine in the immune response to HSV-2 infection, and is perhaps the most important factor in the HSV-2-induced immunity. The need for IFN-γ is evidenced in both mice and in humans. Mice that lack IFN-γ cannot mount protective immunity to HSV-2, and humans with high levels of IFN-γ are, at least to some extent, protected from severe and painful symptoms of HSV-2 infection. The prominent role of IFN-γ also makes T-bet an important player in the antiviral responses to HSV-2, in terms of both innate and acquired immunity. Vaccinated T-bet-deficient mice cannot mount protective immunity to HSV-2 and they succumb to the infection. This is due to an impaired CD4+ T cell response and in particular, to diminished IFN-γ production by these cells. T-bet deficiency also leads to decreased levels of both IgG2a and IgG1. However, it remains unclear whether antibodies have any role in protection against HSV-2 infection, since mice that lack B cells still generate protective immunity to HSV-2 infection. The role of T-bet in CD8+ T cell function is controversial. In our hands, T-bet deficiency did not

affect the effector functions of CD8+ T cells. However, this may be due to the minor role of CD8+ T cells in HSV-2 infection.

Neither IFN-α/βR nor NK1R deficiency affected the acquired immune responses to HSV-2 infection, since all vaccinated mice survived the infection without any signs of disease. This suggests a prominent role for T-bet, but not for IFN-α/β signaling or Substance P, in the HSV-2 specific protective immunity.

All biological events, including the immune responses discussed above, are controlled by regulatory networks that affect a vast array of genes. Variations in these genes lead to changes in the function of their protein products, which may lead (for example) to increased susceptibility to diseases or to protection against disease. I found a variation in the T-bet gene that is strongly associated with the incidence of HSV-2 infection, but not with the severity of disease. Functional consequences of this polymorphism are not related to NK cell numbers, NK cell phenotypes, T cells numbers or IFN-γ production by T cells. However, since the correlation between this polymorphism and HSV-2 incidence is very strong it will be interesting and important to evaluate other mechanisms that are involved in protection against HSV-2 infection.

Further research regarding the effects of genetic variations and the exact roles of signaling mechanisms could contribute to new insights and improved knowledge of immunity to HSV-2 infection, which may lead to new approaches to the development of novel preventive and therapeutic treatments against genital herpes infection.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Människan är ständigt utsatt för olika mikrober som kan orsaka infektion och sjukdom. För att skydda oss från infektioner finns immunförsvaret, ett avancerat nätverk av celler som oskadliggör främmande partiklar. Men även mikrober har utvecklat olika strategier för att etablera sig hos sin värd och undgå igenkänning av immunförsvaret, vilket försvårar för immunsystemet att skydda oss mot infektioner. Alla patogener har avancerade strategier för att etablera sig hos sin värd och vissa har utvecklat så avancerade strategier att de kan överleva i kroppen livet ut. Herpes simplex virus (HSV) är ett typiskt exempel på en sådan patogen. Det finns två typer av HSV, HSV-1 som orsakar vad som i dagligt tal kallas munherpes och HSV-2 som orsakar genitalherpes. Genitalherpes är idag en av de vanligaste sexuellt överförbara sjukdomarna i världen. Drygt 20 % av Sveriges vuxna befolkning är infekterade och det finns i dagsläget inget vaccin. Infektionen ger ett brett spektrum av symptom, framförallt i form av blåsor och sår, som hos vissa individer är mycket svåra och smärtsamma, medan andra helt saknar kliniska symptom, s.k. tyst infektion. För att kunna ta fram ett vaccin mot genital herpes är det av stor vikt att känna till de mekanismer som är involverade i immunförsvaret mot HSV-2 infektion.

Det mest effektiva immunförsvaret mot HSV-2 utgörs i huvudsak av NK celler, T celler och cytokiner. NK (Natural Killer) celler är oftast de första cellerna på plats vid en infektion, precis som namnet antyder har de förmågan att direkt känna igen och döda virusinfekterade celler. T celler har flera uppgifter men deras huvuduppgift vid HSV-2 infektion är att producera anti-virala cytokiner. Cytokiner är små proteiner som fungerar som signalsubstanser och regulatorer i immunsystemet. I HSV-2 infektion är det framförallt två cytokiner som är viktigare än andra, de kallas interferon (IFN)-γ och IFN-α. Celler så väl som cytokiner är beroende av en rad mekanismer inne i cellerna som styr dess funktioner. Jag har undersökt hur dessa signaleringsmekanismer påverkar sjukdomsförloppet vid genital herpes samt om de kan ha någon funktion vid utvecklandet av eventuella vaccin mot HSV-2 infektion.

För att ta reda på hur olika signaleringsmekanismer påverkar immuniteten mot HSV-2 använde jag möss, där jag studerade immunsystemets funktion efter HSV-2 infektion hos möss som saknar vissa specifika gener. Till skillnad från människor kan möss vaccineras mot HSV-2 infektion, vilket leder till total immunitet. Möss som inte vaccineras med ett försvagat virus blir mycket sjuka.

Alla händelser i kroppen är beroende av att gener aktiveras, för att detta ska ske krävs s.k. transkriptionsfaktorer. I de två första arbetena undersökte jag hur en sådan här transkriptionsfaktor, T-bet, påverkar immuniteten mot HSV-2. Det är sedan tidigare känt att T-bet är nödvändig för att det ska bildas IFN-γ, som i sin tur är nödvändigt i immunförsvaret mot HSV-2. Det visade sig att möss som saknar T-bet (T-bet-/- möss) insjuknar snabbare än vanliga möss. Detta var associerat med en försämrad NK cell funktion. NK celler från T-bet-/- möss var mycket sämre på döda virus infekterade celler. Möss som vaccineras mot HSV-2 smittas aldrig av HSV-2, så var inte fallet med de möss som saknar T-bet. Majoriteten av T-bet-/- djuren insjuknade och ett flertal dog after att de infekterats med HSV-2. Detta berodde till stor del på att deras T celler inte producerade tillräckligt med IFN-γ.

Många sjukdomar beror på förändringar och variationer i olika gener. För att ta reda på om variationer i genen för T-bet påverkade HSV-2 infektion studerade jag hur T-bet genen såg ut hos en grupp HSV-2 infekterade individer och jämförde med en grupp friska kontroll individer. Jag upptäckte att en genvariant bara fanns hos HSV-2 infekterade individer och inte hos de friska kontrollpersonerna. Denna genkandidat kan därför sägas vara associerad med incidensen av genital HSV-2 infektion. Däremot har jag ännu inte lyckats förstå hur denna genvariant påverkar graden av symptom eller funktionen hos NK eller T celler. Sammantaget betyder de här resultaten att T-bet är oerhört viktig för både det naturliga och det förvärvade immunförsvaret mot HSV-2 infektion och att variationer i T-bet genen kan påverka incidensen av genital HSV-2 infektion.

Som tidigare nämnt är IFN-α en viktig cytokin vid HSV-2 infektion. IFN-α stänger av proteinsyntesen i virus infekterade celler och förhindrar därför bildandet av nya

viruspartiklar. I tredje arbetet visar jag att möss som saknar receptorn för IFN-α får en svårare HSV-2 infektion än normala möss. Dessutom visar sig IFN-α vara den viktigaste faktorn för att ett flertal anti-virala behandlingar ska fungera.

Eftersom HSV-2 har förmågan att etablera sig i nerver och i vissa fall kan orsaka hjärnhinneinflammation studerade jag i sista arbetet hur signalsubstanser i nervsystemet påverkar HSV-2 infektion. Jag såg att HSV-2 infektion ökade mängden av en signalsubstans som kallas Substans P och att den kan förhindra att viruset förökar sig. Vidare såg jag att möss som är defekta signaleringsvägen för Substans P insjuknade snabbare av HSV-2 smitta än vanliga möss. Detta visar att även nervsystemets signaleringsvägar är viktiga för immunförsvarets funktion.

Sammanfattningsvis så visar den här avhandlingen hur en transkriptionsfaktor (T-bet), en cytokinfamilj (IFN-α/β) och en neuropeptid (Substans P) påverkar immunförsvaret vid en HSV-2 infektion. Alla tre faktorerna var avgörande för infektionskänsligheten, men av dessa tre var det endast T-bet som behövdes vid vaccinering.

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