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A dynamical model of the regulatory network controlling lymphopoiesis

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ABSTRACT

Due to the large number of diseases associated to a malfunction of the hematopoietic system, there is an interest in knowing the molecular mechanisms controlling the differentiation of blood cell lineages. However, the structure and dynamical properties of the underlying regulatory network controlling this process is not well understood. This manuscript presents a regulatory network of 81 nodes, representing several types of molecules that regulate each other during the process of lymphopoiesis. The regulatory interactions were inferred mostly from published experimental data. However, 15 out of 159 regulatory interactions are predictions arising from the present study. The network is modelled as a continuous dynamical system, in the form of a set of differential equations. The dynamical behaviour of the model describes the differentiation process from the common lymphocyte precursor (CLP) to several mature B and T cell types; namely, plasma cell (PC), cytotoxic T lymphocyte (CTL), T helper 1 (Th1), Th2, Th17, and T regulatory (Treg) cells. The model qualitatively recapitulates key cellular differentiation events, being able to represent the directional and branched nature of lymphopoiesis, going from a multipotent progenitor to fully differentiated cell types.

1. Introduction

Blood cells originate in the bone marrow from hematopoietic stem cells (HSCs), which give origin to the myeloid and lymphoid lineages. Lymphopoiesis is the process of the generation of lymphocytes, which are the main effectors of the adaptive immune response (Bonilla and Oettgen, 2010). Lymphopoiesis starts with a multipotent cell known as the common lymphoid progenitor (CLP) that gives rise to NK, T, and B cells (Bryder and Sigvardsson, 2010). T lymphocytes promote and coordinate several responses by cells of the immune system, while B cells mediate the humoral response by the secretion of antibodies.

T cells precursors initiate as $CD4^-$ $CD8^-$ double negative (DN) cells, lacking CD4 and CD8 surface markers. After a process known as β-selection (Naito et al., 2011), thymocytes express the TCRα chain and the CD4 and CD8 co-receptors, thus becoming $CD4^+$ $CD8^+$ double positive (DP) cells. Then, DP cells commit to the exclusive expression of either CD4 or CD8, becoming either $CD4^+$ or $CD8^+$ single positive (SP) cells. Each of these lineages is characterized by distinct antigen specificities, $CD4^+$ cells are MHC II-restricted, whereas $CD8^+$ cells are MHC I-restricted (Xiong and Bosselut, 2012). $CD4^+$ cells can be divided into T helper 1 (Th1), Th2, Th17, and T regulatory (Treg) types. Th1 cells are characterized by the production of IFN-γ and T-bet (Szabo et al., 2003), and are involved in the protective immune response against intracellular viral and bacterial infections. Th2 cells express GATA3 and produce interleukin 4 (IL-4) (Murphy and Reiner, 2002), helping in the combat against extracellular pathogens. Th17 cells generate IL-17 and RORγt (Ivaylo et al., 2006), and are critical for the defense against extra-cellular pathogens. Treg cells, characterized for the expression of Foxp3 (Gavin et al., 2007), show an immunosuppressor activity. $CD8^+$ cells, in turn, give rise to effector cytotoxic (CTL) cells, which kill infected cells and are characterized by the production of perforin (Prf1), granzyme B (Gzmb), and IFN-γ (Morishima et al., 2010). Although other types of Th cells (Th9, Th22, Tfh) have been discovered (Crotty, 2014), the differentiation of these cell types is not studied in the present model. See, however (Martinez-Sanchez et al., 2015).

CLP cells differentiate towards the B cell lineage by means of Flt3 signalling and the stimulation of IL-7 (Bryder and Sigvardsson, 2010). At their early (pro-B) developmental stage, B cells generate an ample repertoire of antibodies, and later they generate a mature BCR with very high specificity (Johnson et al., 2005). Once B cells have completed their development in the bone marrow, they migrate to the bloodstream and into the secondary lymphatic

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organs, where they complete maturation. In these organs, naive B lymphocytes are activated by means of the recognition of free antigens or antigens presented in the surface of T lymphocytes. This event determines their differentiation towards effector plasma cells (PCs) (LeBien and Tedder, 2008), which secrete soluble antibodies.

While there is a large quantity of molecular information regarding the differentiation of lymphocytes, there is no consensus regarding the structure and dynamical behaviour of the underlying regulatory network. Regulatory networks are constituted by the regulatory interactions among nodes, which usually represent molecules or molecular complexes. These interactions may be either directly conveyed by means of a physical molecular interaction, or may be elicited via (possibly unknown) intermediaries (Albert and Thakar, 2014; Le Novère, 2015). The modelling of regulatory networks as dynamical systems has shown to be an adequate approach to understand the molecular mechanisms integrating several types of signals that control cell differentiation processes (Kestler et al., 2008). Dynamical models of small parts of the network controlling the differentiation of T cells have been published before (Abou-Jaoudé et al., 2015; Martínez-Sosa and Mendoza, 2013; Mendoza and Xenarios, 2006; Mendoza, 2006, 2013; Mendoza and Pardo, 2010; Naldi et al., 2010), with the aim of describing the expression patterns observed in naive, Th1, Th2, Th17, Treg, CTL cell lineages and their precursors, under wild type and mutant backgrounds. The present work introduces an expanded regulatory network that now incorporates for the first time the decision between the T and B cell lineages. The modelling of the regulatory network is able to simulate the expression patterns observed experimentally for CLP, pro-B, B naive, PC, DP, $CD4^+$ naive, Th1, Th2, Th17, Treg, $CD8^+$ naive, and CTL cells. This model recovers the branching process leading from the pluripotent CLP to the fully differentiated effector cells.

2. Methods

2.1. The molecular basis of the regulatory network

The regulatory network presented in this work is an extension of previous models (Martínez-Sosa and Mendoza, 2013; Mendoza and Xenarios, 2006; Mendoza, 2006, 2013; Mendoza and Pardo, 2010). Therefore, several interactions of the network shown in Fig. 1 have been explained in previous works. For brevity, the following paragraphs explain only the interactions not included in the previous versions of the model. Furthermore, Table A.1 contains a set of key references used to infer the regulatory network of Fig. 1.

Flt3L-deficient mice have significantly reduced numbers of CLPs and B-cell progenitors (McKenna et al., 2000; Nutt and Kee, 2007). Flt3L is expressed by bone marrow stroma (Lisovsky et al., 1996) and binds to its receptor, Flt3, which is expressed in CLPs (Sitnicka et al., 2002). Flt3 is upregulated by Hoxa9 (Gwin et al., 2010), and in turn, Flt3 activates ERK via phosphorylation (Åhsberg et al., 2010).

Notch signalling regulates mature T cell activation and differentiation (Amsen et al., 2004). It is known that Bcl11b (Li et al., 2010a,b), HEB (Wang et al., 2006) and TCF-1 (Germar et al., 2011; Weber et al., 2011) are positively regulated by Notch1 in thymic precursors. Also, Notch1 accelerates the degradation of JAK3 (Nie et al., 2008), and inhibits Runx1 (Giambra et al., 2012). Runx1 is a transcription factor that is also inhibited by Runx3 (Giambra et al., 2012) and TCR (Wong et al., 2011).

The process of T-cell specification is conformed by a succession of distinct regulatory states, which start with the activation of TCF-1 that causes the activation of GATA-3 and Bcl11b (Ma et al., 2013; Rothenberg, 2012). However, GATA3 is repressed by HEB factors during T-cell specification (Braunstein and Anderson, 2011), and repressed by Runx1 during Th1 cell differentiation (Wong et al., 2011).

Th17 cells are producers of TNF-α (Annunziato et al., 2013), and since RORγt is the master transcription factor of Th17 cells (Ivaylo et al., 2006), it seems reasonable to propose that RORγt directly or indirectly induces TNF-α. TNF-α binds directly to members of the TNFR family. Specifically, TNRF2 are present at high levels in T cells (Zhang et al., 2013). TNRF2 triggers the activation of Akt (So and Croft, 2013), which in turn inhibits the phosphorylation of SMAD3 (Roffe et al., 2010; Zhang et al., 2013), resulting in a reduction of the activation of Foxp3 (Zhang et al., 2013). Furthermore, SMAD3 is a signal transduction molecule of the TGF-β/TGF-βR pathway (Massagué and Xi, 2012). Natural Treg cells are producers of TGF-β (Zhang et al., 2014), thus there should exist a mechanism by which Foxp3, the master regulator of Treg cells (Fontenot et al., 2003), induce the expression of TGF-β.

Vitamin B9 acts through the folate receptor FR4, which is a marker of Treg cells (Kunisawa et al., 2012; Yamaguchi et al., 2007). Therefore, there should exist a mechanism for Foxp3 to promote the expression of FR4. FR4, in turn, affects the level of the inhibitor of apoptosis Bcl2 (Kunisawa et al., 2012), which is also upregulated by the IL-7R/JAK3/STAT5/ pathway (Jiang et al., 2005; Malin et al., 2010; Möröy and Khandanpour, 2011; Qin et al., 2001).

The transcription factor Ikaros has an important role during the early steps of fate decision leading to B-cell lineage. Ikaros induces Gfi1 expression (Möröy and Khandanpour, 2011; Spooner et al., 2009), which in turn inhibits PU.1 (Möröy and Khandanpour, 2011; Ramírez et al., 2010; Spooner et al., 2009).

Runx1 deficiency causes severe reduction of B cell progenitors because of the resulting lack of Ebf1 (Seo et al., 2012). The B cell lineage can be identified by the expression of CD19, which is regulated by the master regulator Pax5 (Nutt and Kee, 2007). Pax5 establishes a mutual inhibition circuit with Blimp1 (Kikuchi et al., 2012; Lin et al., 2002; Yasuda et al., 2012), and regulates Bach2 positively (Kallies and Nutt, 2010; Kikuchi et al., 2012) and Flt3 negatively (Holmes et al., 2006). In turn, it is known that Pax5 is upregulated by STAT5 and Ebf1 (Hirokawa et al., 2003; O'Riordan and Grosschedl, 1999), and downregulated by Irf4 (Decker et al., 2009; Nutt et al., 2011).

After its activation by antigen, BCR signalling initiates plasma cell differentiation, a step that involves the activation of the NF-κB (De Silva et al., 2012) and ERK1/2 (Yasuda et al., 2012) pathways. NF-κB, in turn, upregulates Irf4 (De Silva et al., 2012; Klein and Dalla-Favera, 2008) and Helios (Serre et al., 2011).

Plasma cell differentiation is inhibited by Bach2, by the direct repression of Blimp1 (Nutt et al., 2011; Ochiai et al., 2006), which in turn activates XBP1 (Kikuchi et al., 2012; Klein et al., 2003). Blimp1 is involved in a mutually activatory regulation with Irf4 (Kallies et al., 2004; De Silva et al., 2012), and a mutually inhibitory regulation with Bcl6 (Cimmino et al., 2008; Klein et al., 2003; Kusam et al., 2004; Tunyaplin et al., 2004). Maturation of plasma cells is accompanied by the abatement of Bcl6. Irf4 is a known repressor of Bcl6 (Alinikula et al., 2011; Saito et al., 2007), while Ebf1 and IL-21R augment its expression (Bouamar et al., 2013; Nutt et al., 2011; Yi et al., 2010). Then, Bcl6 is an activator of Bach2 (Alinikula et al., 2011). Finally, plasma cell differentiation is promoted by IL-21/IL-21R signalling acting through STAT3 (De Silva et al., 2012; Nutt et al., 2011).

The biological information presented within this section was translated into a set of logical expressions, presented in Table A.2.

2.2. Modelling the regulatory network as a continuous dynamical system

The regulatory network controlling lymphopoiesis was implemented as a continuous dynamical system with the use of the