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Contents

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REVIEW

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Contents

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REVIEW

Follicular helper T lymphocytes in health and disease

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Abstract

A correct antibody response requires the participation of both B and T lymphocytes and antigen presenting cells. In this review we address the role of follicular helper T lymphocytes (TFH) in this reaction. We shall focus on the regulation of their development and function in health and disease. TFH can be characterized on the basis of their phenotype and the pattern of secretion of cytokines. This fact is useful to study their participation in the generation of antibody deficiency in primary immunodeficiency diseases such as common variable immunodeficiency, X-linked hyper IgM syndrome or X-linked lymphoproliferative disease. Increased numbers of TFH have been demonstrated in several autoimmune diseases and are thought to play a role in the development of autoantibodies. In chronic viral infections caused by the human immunodeficiency virus, hepatitis B or C virus, increased circulating TFH have been observed, but their role in the protective immune response to these agents is under discussion. Likewise, an important role of TFH in the control of some experimental protozoan infections has been proposed, and it will be important to assess their relevance in order to design effective vaccination strategies.

Key words: Follicular helper T (TFH) lymphocytes; TFH development; Chemokine (C-X-C motif) receptor 5; Interleukin-21; Programmed cell death-1/Programmed cell death ligand 1 (PDL-1) or PDL-2; Primary immunodeficiencies; Autoimmunity; Chronic viral infections; Protozoan infections

Core tip: Follicular helper T lymphocytes (TFH) are essential to establish a correct and protective humoral immune response. Correct regulation of their development and differentiation is necessary to achieve a normal antibody response. They can be characterized by their phenotype and function. It has been proposed that their role is important in the generation of immunodeficiency or autoimmunity, as well as in the control of chronic viral or protozoan infections. This review comments recent advances in human TFH research that may be useful in order to design adequate therapeutic or vaccination strategies.

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INTRODUCTION

The assembly of a correct antibody response requires the participation of B and T lymphocytes, as well as that of antigen presenting cells from the myeloid lineage. It involves a complex system of interactions and regulatory mechanisms. Failure of this equilibrium at any level disturbs and impairs the generation of an efficient, long term antibody response.

A subset of helper T cells, follicular helper T lymphocytes (TFH) is necessary to provide help to B lymphocytes in the process of antibody synthesis and maturation. TFH encompass a heterogeneous group of cells with distinct gene expression profile and function[1]. Without TFH the protective antibody responses are largely diminished. Primary immune deficient patients with genetic defects that affect the synthesis of molecules essential for TFH generation or function, such as the inducible costimulator (ICOS) or the signaling adaptor SLAM-associated protein (SAP), lack an efficient antibody response and may suffer recurrent infections that compromise their health and survival[2,3]. Excessive or dysregulated TFH can also result in the generation of autoantibodies and are associated to autoimmune diseases[4,5].

In this review we shall describe the nature and function of this T cell subset and we will focus on its role in the generation of immune deficiency or autoimmunity in humans. We will also address the importance of TFH in the assembly of an efficient humoral response for the control of chronic diseases caused by different infectious viral agents, *e.g.*, human immunodeficiency virus (HIV), hepatitis B virus (HBV) or C virus (HCV), as well as parasites or protozoa.

TFH PHENOTYPE AND FUNCTION

$CD4^+$ T helper (Th) cells present in B cell follicles have been recognized as an important subset of helper T lymphocytes necessary for the assembly of the antibody response involving T-B cooperation and B cell memory[1,6,7]. TFH have a typical phenotype, appropriate transcription factors and exhibit surface molecules essential for helper function. They secrete interleukins (ILs) that promote growth, differentiation and class switching of B cells (IL-4, IL-10 and IL-21). Plasticity is a main characteristic of TFH. Thus, TFH can also express many transcription factors thought to be master regulators of T helper cell lineages, as GATA binding protein 3 (GATA-3) and the T-box transcription factor (T-bet)[7].

Antigen presentation by dendritic cells (DC) is necessary to initiate TFH commitment[8-10]. As a consequence of this initial encounter, TFH express achaete-scute homologue 2 (Ascl2)[11], B cell lymphoma 6 (Bcl-6), chemokine (C-X-C motif) receptor 5 (CXCR5) and ICOS triggering the TFH differentiation program[10,12,13]. These events take place outside the B-cell follicle in the absence of B cells[10,13,14]. SAP-deficient $CD4^+$ T cells, which fail to sustain prolonged interaction with B cells, but interact normally with antigen-presenting DC, upregulate Bcl-6 and CXCR5 following activation[8,9,15]. Late cognate interactions with activated B cells are required to complete and sustain full differentiation of TFH[15]. However, B cell-mediated antigen presentation can be overcome when antigen in excess is presented by DC[8,9]. Apparently, when provision of antigen is limited, B cells are more efficient than DC to capture antigen through their high affinity antigen-B cell receptor[10]. Therefore, antigen availability would dictate the transition of initially DC-primed-TFH towards B-cell primed-TFH as the differentiation program progresses in the interfollicular zone (Figure 1). The importance of DC in the induction of a full TFH response relies both on their ability to migrate to the B cell follicles through the upregulation of CXCR5 and downregulation of the chemokine (C-C motif) receptor 7 (CCR7) (providing a favorable spatial location for DC-B cell-TFH interactions), but also on their ability to release DC-derived cytokines that are necessary for TFH development[15,16].

In addition to their presence in B cell follicles, TFH circulating counterparts have been identified in the blood stream[1] and share many of the phenotypic and functional characteristics of TFH residing in the follicles. The phenotypic hallmark of TFH is the surface expression of the chemokine receptor CXCR5, which enables their migration into B cell follicles, in response to the specific chemokine ligand CXCL13-rich follicular areas.

Deficiency of CXCR5 affects the antibody response. It impairs the germinal center (GC) response, reducing the frequency of GC B lymphocytes and isotype-switched antibody-secreting cells. ICOS is necessary for the induction of CXCR5 and for an efficient GC reaction[2]. In the absence of CXCR5, T cells cannot migrate to the follicles, but migration is not an absolute requirement for the formation of GC. CD40 ligand (CD40L), SAP and ICOS are other molecules expressed by TFH that are essential to ensure their ability to provide help to B cells[17].

An increased expression of CXCR5, ICOS, the inhibitory receptor programmed cell death-1 (PD-1) and SAP characterize the TFH phenotype, as well as the downregulation of CCR7 and the IL-7 receptor (CD127)[1,6].

The cytokine secreting profile of TFH includes the production of high amounts of IL-21. IL-10, IL-4 and IL-6 are also produced by TFH. All these cytokines are involved in the generation of an adequate antibody response by promoting growth differentiation and class switching of B cells. These characteristics of TFH have been demonstrated both in humans and in mice. Table 1 summarizes this information for human TFH.

As a group, TFH are heterogeneous. Despite the definition of a basic TFH profile, TFH have an inherent plasticity and they may convert to other cell subsets. Likewise, forkhead box P3 ($Foxp3^+$) regulatory T cells that express CXCR5 and Bcl-6 (TFR) and migrate to human tonsils or murine lymphoid tissue have been described[18]. They are closely related by their phenotype to classic TFH and derive from T regulatory (Treg) cells[19]. In humans $CXCR5^+$ $CD4^+$ T cells are a circulating pool of memory

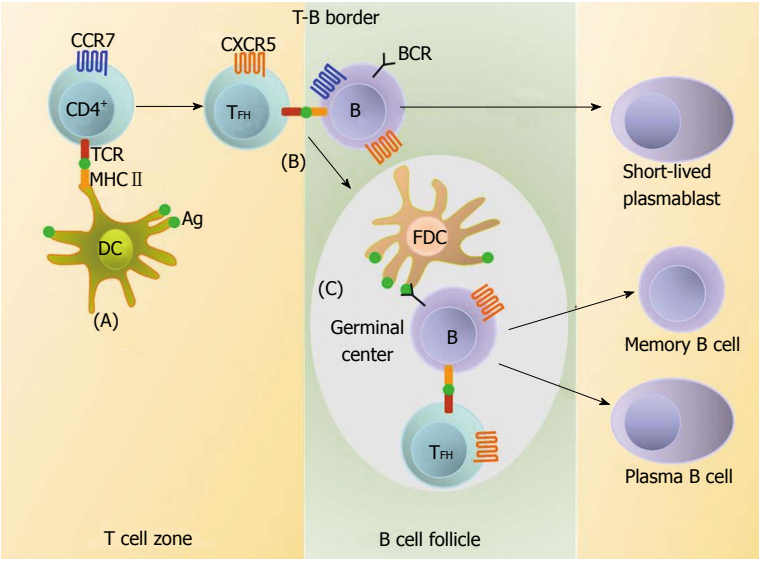

Figure 1 Follicular helper T cells and the differentiation program of B lymphocytes. A: Naïve CD4+ T cells are activated following recognition of antigen (Ag) presented by dendritic cells (DC) in T cell zones. Upon antigen activation and co-stimulation by DC, nascent TFH upregulate CXC-chemokine receptor 5 (CXCR5), downregulate CC-chemokine receptor 7 (CCR7) and migrate towards B cell follicles; B: At the T-B border TFH contact antigen-activated B cells that move to the T-cell zone after upregulating CCR7. TFH deliver help to B cells resulting in their differentiation into short-lived extrafollicular plasmablasts or their migration into B cell-follicles to form germinal centers (GCs); C: Within GC, TFH promotes the B cell differentiation into long-lived plasma cells and memory B cells. TFH: Follicular helper T lymphocytes; FDC: Follicle dendritic cell; BCR: B cell receptor; MHC: Major histocompatibility complex; TCR: T cell receptor.

Table 1 Follicular helper T lymphocytes markers

Marker	Human TFH		Naïve CD4+ T cell	Activated Non-TFH CD4+
	TFH	GC TFH		
CXCR5	+	++	-	-
Ascl2	?	++	?	?
Bcl-6	+	++	-	-
Blimp-1	-	-	+/-	++/variable
PD-1	+	++	-	Variable
ICOS	+	++	-	Variable
SAP	+	++	+	+
IL-21	+	++	-	Variable
IL-4	-/+	++	-	Th2+
CCR7	-/+	-	++	Variable

TFH: Follicular helper T lymphocytes; GC: Germinal center; CXCR5: Chemokine (C-X-C motif) receptor 5; Ascl2: Achaete-scute homologue 2; Bcl-6: B cell lymphoma 6; Blimp-1: B lymphocyte-induced maturation protein 1; PD-1: Programmed cell death-1; ICOS: Inducible costimulator; SAP: Signaling adaptor SLAM-associated protein; IL: Interleukin; CCR7: Chemokine (C-C motif) receptor 7; Th: T helper.

cells that comprises three CD4+ T helper subsets: Th1 TFH expressing CXCR5, CXCR3 and the transcription factor T-bet in the absence of CCR6; Th2 TFH expressing CXCR5 and the transcription factor GATA-3 in the absence of both CCR6 and CXCR3 and Th17 TFH expressing CXCR5, CCR6 and the transcription factor RORγt in the absence of CXCR3 (Table 2). These subsets of TFH have different helping abilities. While Th2 TFH and Th17 TFH can help naïve B cells to produce IgM, IgG and IgA, Th1 TFH cannot[1].

Furthermore, a subgroup of CXCR5+ CD4+ circulating lymphocytes with low CCR7 and high PD-1 expression have been identified as an early memory subset of TFH, which upon antigen exposure differentiates into mature TFH capable to provide a prompt protective antibody response[20].

REGULATION OF TFH DEVELOPMENT

TFH differentiation may be divided into two phases: the priming and the maintaining stages. Priming depends on antigen-presenting signaling of DC, while maintaining is related to sustained B cell-T cell interaction and the consequent signaling events. While most studies have pointed out the role of the transcription factor Bcl-6 as an initiator of the TFH differentiation program during the priming stage, recent work by Liu *et al*[11] demonstrated that Ascl2, another transcription factor, is crucial for TFH development and function. Ascl2 is a basic helix-loop-helix (bHLH) transcription factor[21]. It directly regulates TFH-related genes and inhibits Th1 and Th17 signature genes. Upregulation of Ascl2 precedes that of Bcl-6, indicating that Ascl2 and not Bcl-6 may be the initial trigger for the TFH differentiation program.

Large amounts of Bcl-6 expressed by TFH can be counterbalanced by the repressor B lymphocyte-induced maturation protein 1 (Blimp-1). While Bcl-6 favors the development of TFH *in vivo*, Blimp-1 regulates the function of Bcl-6 and inhibits the generation of TFH. Bcl-6 controls GC B cell differentiation by regulating cell cycle genes, regulating DNA damage response genes and suppressing a host of signaling pathways, including B cell receptor (BCR) signaling[22]. It is a member of the BTB-POZ (bric-a-bric, tramtrack, broad complex-poxvirus zinc finger) family of transcriptional repressors. These repressors directly bind to specific DNA sequences through their zinc-finger DNA binding domains with the BTB-POZ domain mediating transcriptional repression[23]. In both GC B cells and TFH, Bcl-6 controls TFH differentiation by regulating genes separate from those it controls in B cells [22]. Molecular crosstalk between GC B cells and TFH influences the survival, proliferation and differentiation of each cell type[24]. In addition to promoting the expression of TFH signature genes, Bcl-6 represses *Prdm1* (the gene encoding the transcriptional repressor Blimp-1). Bcl-6 antagonism of Blimp-1 is one of the key mechanisms by which Bcl-6 inhibits non-TFH differentiation. Bcl-6-dependent suppression of Blimp-1 activity (by removal of the Blimp-1 "brake") may favor the differen-

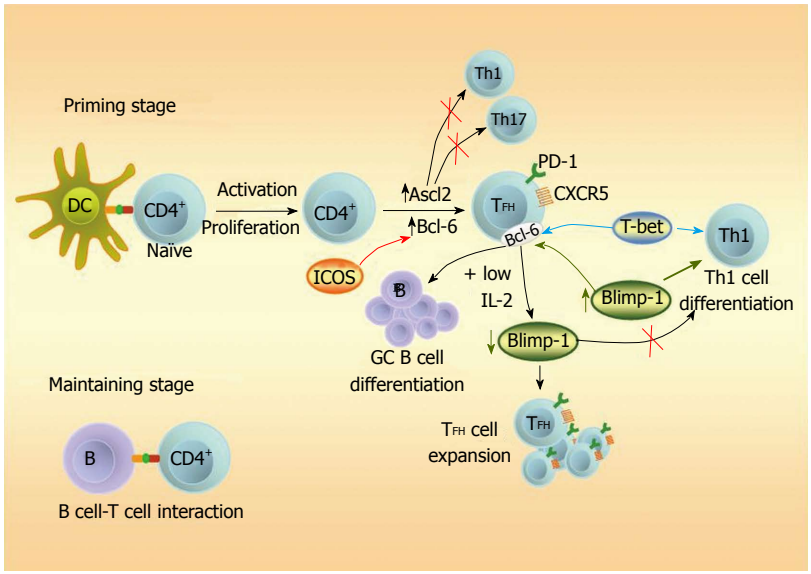

Figure 2 Regulation of Follicular helper T cell development. After antigen-presenting signaling of dendritic cells (DC) to CD4+ T cells during priming, achaete-scute homologue 2 (Ascl2) and B cell lymphoma 6 (Bcl-6) induced by the inducible costimulator (ICOS), trigger for TFH differentiation program and inhibit Th1 and Th17 genes. Bcl-6 also controls germinal center (GC) B cell differentiation. B lymphocyte-induced maturation protein 1 (Blimp-1) and the T-box transcription factor (T-bet) regulate the function of Bcl-6 and favor the induction of a Th1 profile. Under low interleukin 2 (IL-2) conditions, excess of Bcl-6 counteracts Blimp-1 allowing expansion of the TFH and reduction of the Th1 programs of differentiation. Initial priming is sufficient to acquire the TFH markers but cognate B cells are needed for the subsequent maintenance stage. TFH: Follicular helper T lymphocytes; CXCR5: Chemokine (C-X-C motif) receptor 5; Th: T helper.

Table 2 Heterogeneity of follicular helper T lymphocytes in relation to other T helper cells

	Markers				
	CD4	CXCR5	CXCR3	CCR6	Foxp3
Th1 TFH	+	+	+	-	-
Th2 TFH	+	+	-	-	-
Th17 TFH	+	+	-	+	-
TFR TFH	+	+	-	-	+

TFH: Follicular helper T lymphocytes; Th: T helper; CXCR5: Chemokine (C-X-C motif) receptor 5; CXCR3: Chemokine (C-X-C motif) receptor 3; CCR6: Chemokine (C-C motif) receptor 6; Foxp3: Forkhead box P3; TFR: Foxp3+ CXCR5+ Bcl-6+ regulatory T cells.

tiation program of Th cells towards the induction of TFH effectors[25].

As Ascl2[11], Bcl-6 is responsible for the repression of a subgroup of signature genes in effector Th1 cells. It has been shown that Bcl-6 can interact with T-bet[26], which is required for establishment of a Th1 gene expression profile[27]. Under low IL-2 conditions the Bcl-6/T-bet ratio increases and excess Bcl-6 represses *Prdm1* and counteracts Blimp-1-mediated inhibition of the TFH signature genes, allowing for expansion of the TFH and reduction of the Th1 programs of differentiation[26]. At the priming stage Bcl-6 expression is induced in CD4+ T cells independent of CD40 or SAP signaling, while ICOS provides a critical early signal to induce Bcl-6 transcription[15].

Both Ascl2[11] and Bcl-6 upregulate CXCR5 expression on T cells during priming and this facilitates their entry to the T/B border. This initial DC integrin-dependent priming is sufficient to acquire the TFH markers (CXCR5, PD-1, high levels of Bcl-6), but cognate B cells are needed for the subsequent maintenance and survival stage[28] (Figure 2).

THE ROLE OF CYTOKINES IN TFH DEVELOPMENT AND FUNCTION

IL-21 has been recognized as an essential factor determining the maintenance of TFH. It is secreted by TFH and has an autocrine effect on them. Through interaction with the IL-21 receptor expressed on B lymphocytes, it promotes survival and proliferation of GC B cells. It has also direct effects on CD4+ non-TFH T cells (Th17)[29] and may induce Bcl-6 in TFH[30]. However, IL-21 requirement does not exclusively determine TFH differentiation, as IL-21-/- and IL-21R-/- mice can develop TFH[31]. The combined absence of both IL-21 and IL-6 abrogates TFH [6]. IL-6 and IL-21 redundantly contribute to TFH differentiation, but in the absence of other triggers as ICOS, these cytokine signals are insufficient for the instruction of TFH differentiation[6].

In addition, IL-21 has been shown to prime human naïve B cells to respond to IL-2 by enhancing their differentiation into plasmablasts. This mechanism operates through STAT3 (signal transducer and activator of transcription 3) signaling and provides an adjunctive role to IL-21-induced B cell differentiation[32].

PD-1 AND ITS LIGANDS HAVE A CRITICAL ROLE IN THE ASSEMBLY OF THE HUMORAL RESPONSE

PD-1, a member of the CD28 family of costimulatory molecules, is highly expressed in TFH, while most human B cells do not express it[33]. In general, engagement of PD-1 by its ligands (Programmed cell death ligand 1 -PD-L1- or Programmed cell death 1 ligand 2 -PD-L2-, belonging to the B7 family) inhibits T cell proliferation and cytokine induction and leads to downregulation of T cell responses[34].

The role of the PD-1/PD-L1 or PD-L2 axis in the generation of an adequate antibody response has been highlighted by Good-Jacobson *et al*[35]. Though PD-1 is commonly thought as a marker of "exhaustion", TFH cannot be considered as exhausted because they secrete abundant IL-21 and other cytokines during humoral response. In the absence of an operative PD-1/PD-L1

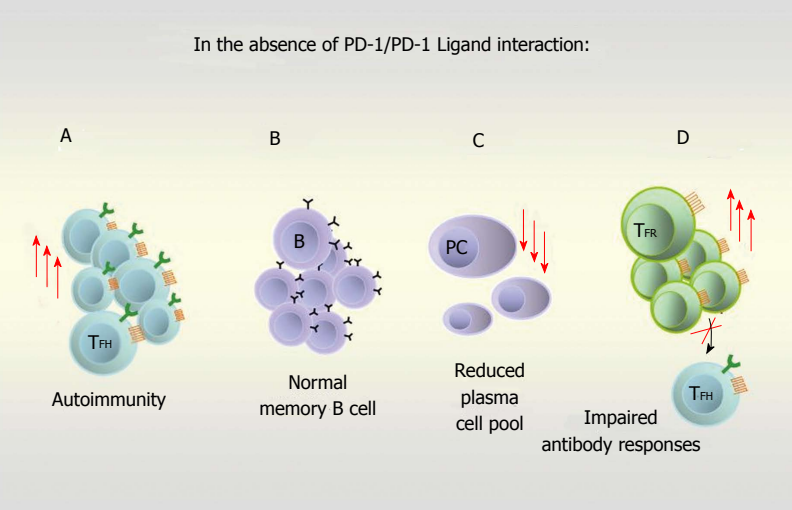

Figure 3 Inhibitory receptor programmed cell death 1, its ligands and their role in humoral response. In the absence of an operative programmed cell death 1 (PD-1)/PD-1 Ligand axis, follicular helper T lymphocytes (TFH) increase and autoimmunity may develop (A); memory B cells are formed normally (B); reduced plasma cells (PC) are found (C); Foxp3$^+$ CXCR5$^+$ Bcl-6$^+$ regulatory T cells (TFR) increase and have higher suppressive ability on TFH function leading to impaired antibody responses (D). PD-1: Programmed cell death-1; CXCR5: Chemokine (C-X-C motif) receptor; Bcl: B cell lymphoma.

Table 3 Follicular helper T lymphocytes in autoimmune diseases

Disease	TFH	Ref.
SLE	Increased CXCR5 and function	[46,47,48]
Myasthenia gravis	Increased CXCR5 and function	[49]
RA	Increased CXCR5 and function	[1,50]
Juvenile Dermatomyositis	Increased CXCR5 and function Mainly TFH with Th17 and Th2-like profile	[1]
ATD	Increased CXCR5, IL-21 high	[51]
Multiple Sclerosis	Increased CXCR5 IL-21 and IL-21R in neurons	[52,53]
Sjögren's syndrome	Increased CXCR5 TFH with Th17 and Th2-like profile IL-17?	[54,55]

TFH: Follicular helper T lymphocytes; SLE: Systemic lupus erythematosus; RA: Rheumatoid arthritis; ATD: Autoimmune thyroid disease; CXCR5: Chemokine (C-X-C motif) receptor 5; IL: Interleukin; Th: T helper.

or PD-L2 axis, TFH numbers increase and autoimmunity may develop. Memory B cells are formed normally but the plasma cell pool that depends on the late stage of the GC response is reduced[36]. There are conflicting reports about the function of the PD-1 pathway in controlling the humoral response. While some studies report attenuated antibody responses in conditions where the PD-1/PD-L1 and PD-L2 interactions were prevented[35,37], others observed heightened humoral responses[38]. Recent work by Sage *et al*[19], in which the contributions of TFH devoid of contaminating TFR could be analyzed[19] has clarified this question. In the absence of PD-1 and its ligand PD-L1, TFR were increased and had higher suppressive ability on TFH function, leading to impaired antibody responses. Thus, there is a dynamic control of antibody production by the balance between TFH and TFR cells and this equilibrium is tuned by PD-1/PD-L1 and PD-L2 interactions (Figure 3).

TFH AND IMMUNODEFICIENCY

Defects in humoral immune response lead to immunodeficiencies, such as common variable immunodeficiency (CVID), X-linked hyper IgM syndrome (HIGM) or X-linked lymphoproliferative disease (XLP)[39]. Since ICOS, CD40L and SAP are highly expressed in TFH, their role in the development of the humoral defects that characterize these diseases has been explored. In ICOS deficiency, which is a very rare condition, ICOS mutations are associated with CVID[40]. ICOS deficiency leads to a reduction of CXCR5$^+$ T cells (including TFH and TFR)[2]. However, most CVID patients do not have ICOS mutations and in these patients circulating CXCR5$^+$ CD4$^+$ T cells are not reduced[41]. In HIGM patients, lack of CD40L causes generalized dysfunction of CD4$^+$ T cells and inability to induce immunoglobulin switching[42]. It had been shown that peripheral CXCR5$^+$ T cells from XLP patients were unable to support immunoglobulin synthesis *in vitro*[43,44] and this led to the suggestion that TFH were not functional in XLP. In fact, absence of SAP affects the stability of the TFH B cell conjugates, necessary for the completion of an effective GC reaction and T-B cell cooperation[45]. However circulating TFH in XLP patients could be induced to express ICOS, CD40L, IL-4, IL-10 and IL-21 upon stimulation, although the kinetics of expression was different to that of normal TFH[46]. Nevertheless, the humoral response was impaired and the number of memory B lymphocytes was reduced in these patients[47], leading to persistent hypogammaglobulinemia.

TFH AND AUTOIMMUNITY

TFH emit instructive signals to B cells that favor the formation and maintenance of GC. Unwanted antibody responses may come together with the normal defensive antibody response against infectious agents, and in this scenario TFH may play a crucial role. Several studies have addressed the contribution of TFH to the generation of autoimmune diseases both in murine models and in humans[39,48]. Evidence involving TFH in the generation of an autoantibody response has accumulated, in particular in systemic lupus erythematosus (SLE), both in humans and in mouse models (sanroque mice) as well as in other

autoimmune conditions. A deficit in the process of selection of GC B cells has been pointed out in SLE patients. GC are abundant in secondary lymphoid organs in SLE mice[49]. In human SLE, GC are overactive and it has been reported that expansion of TFH is causally related to the abundance of GC that form in the absence of foreign antigen, to the production of anti-double-stranded DNA antibodies and to end organ disease[49]. Although circulating TFH are expanded in sanroque mice and in SLE patients, their abundance appears to be a stable phenotype and not a marker of disease activity. A summary of reports on TFH activity or TFH role associated to autoimmune diseases is shown in Table 3. Increased numbers of circulating TFH have been reported associated to increased autoantibody titers in patients with SLE[49-51], myasthenia gravis[52,53], rheumatoid arthritis and juvenile dermatomyositis[1,54], autoimmune thyroid disease[55], multiple sclerosis[56,57] or Sjögren's syndrome patients[58,59]. TFH numbers increase correlating with titers of autoantibodies and the severity of end-organ involvement.

Autoimmune manifestations are encountered in many patients with CVID. In contrast to other patients with autoimmune manifestations, and no CVID, circulating immunoglobulin levels and plasma antibody titers were low in these patients, but co-existed with elevated circulating TFH[41]. Expansion of TFH may play a key role in breakdown of the peripheral tolerance of autoreactive B cells. These cells, which are normally deleted during the GC reaction, may escape from the tolerance checkpoints due to the abundance of the survival help signals provided by excessive TFH[60].

TFH IN VIRAL DISEASES

The role of TFH in HIV infection is not completely clear. Despite profound depletion of $CD4^+$ T cells during HIV infection, both the bulk TFH and HIV-specific TFH populations are expanded in HIV-infected patients[61]. This expansion correlates with changes observed in the B cell compartment, such as the increased frequencies of GC B cells and plasma cells and the decreased frequency of memory B cells[61,62].

Furthemore, the increase of TFH associates with hypergammaglobulinemia in HIV-infected patients. However, the majority of these antibodies are not able to neutralize the virus. Even though there is an expansion of TFH in HIV-infected individuals, it seems that these cells are unable to provide appropriate B cell help[62]. On the other hand, a resting peripheral blood memory population of $CXCR5^+$ $PD\text{-}1^+$ $CXCR3^-$ $CD4^+$ T cells has been identified in rare HIV individuals that are able to generate broadly neutralizing antibodies. It has also been demonstrated that the frequency of this cell population correlates with the development of broadly neutralizing antibodies[63]. Lastly, it has been proven that TFH can be infected by HIV. Furthermore, it was suggested that these cells are a major reservoir that contributes to viral persistence[64].

High frequency of peripheral blood TFH is also found in HBV-infected individuals[65,66]. It has been reported that treatment with adefovir dipivoxil reduces the frequency of TFH and the concentrations of hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg), but increases the concentrations of serum anti-HBsAg and "e" antigen antibodies (HBsAb, HBeAb), IL-2 and IFN-γ in drug-responding patients, although the precise relationship between the frequency of peripheral blood $CD4^+$ $CXCR5^+$ TFH and these parameters requires further investigation[66].

Peripheral blood TFH have also been associated with hypergammaglobulinemia in HBV-infected patients[67].

HCV-infected patients also show an increased percentage of peripheral blood TFH. This high percentage of TFH was associated with low levels of HCV viremia [68].

Even more, a study shows that liver TFH cells can be useful to predict the success of virological response following interferon-based treatment in HCV-infected patients. Tripodo *et al*[69] reported that the absolute number of liver TFH is lower in non-responders, intermediate in responding-relapsed patients and achieves a maximum in sustained virological response patients.

TFH IN PROTOZOAN DISEASES

Reports about the involvement of TFH within human infections caused by obligate intracellular parasites are still required. We will focus on the findings achieved using experimental protozoan infection in mice models to study the function of different factors, receptors and cytokines involved in pathways related to TFH.

It is well known that experimental infections with *Toxoplasma (T.) gondii* display a model of Th1 cell response induction[70]. This model was useful to evaluate if TFH represented a temporary "state" of differentiation rather than a distinct lineage parallel to other subsets[71]. Also, to confirm the action of T-bet as a suppressor of both TFH and humoral responses *in vivo*[71]. The generation of parasite-induced Th1 responses by *T. gondii*, also served to understand the association of the TFH marker ICOS with T helper cytokine production *in vivo*. $ICOS^+$ $CD4^+$ T helper cells produce a variety of different effector cytokines and their pattern depends on the infection challenge. Infection with *T. gondii* leads to IFN-γ production, while $ICOS^+$ $CD4^+$ T cells from the nematode Schistosoma mansoni (an inducer of Th2 responses) is associated with IL-10 secretion[72].

According to these findings, experimental models using *Leishmania (L.) major* also demonstrated that ICOS is a critical regulator of both Th1 and Th2 immune responses *in vivo*[73]. Chronic infection with *L. major*, a model of prominent T-B cell interaction, was also used to evaluate the contribution of IRF-4 (member of IFN-regulatory factor family) to the interaction of TFH and GC B cells. Bolling *et al*[74] demonstrated the implication of this factor, since $IRF4^{-/-}$ mice lacked GC formation, differentiation of GC B cells and lymph node $CD4^+$ T cells from these mice expressed reduced amounts of the TFH-related molecules ICOS, IL-21 and Bcl-6. *L. major*

infection model also helped to demonstrate the relation of TFH and IL-4. All the IL-4 secreting cells in lymph nodes during infection with this parasite were TFH and these cells were distinct from conventional Th2 cells based on phenotype, location and function[75].

Besides, analysis of the consequences of *in vivo* blockade of T cell inhibitory receptors indirectly associated with TFH were performed using blood-stage *Plasmodium* (*P.*) *yoelii* infection in mice. Butler *et al*[76] demonstrated that blockade of *PD-L1* and *LAG-3* (lymphocyte-activation gene 3) receptors led to improved parasite control associated with enhanced TFH numbers and substantial induction of plasma cell differentiation.

Experimental models in which mice were co-infected with *L. major* and *L. amazonensis* demonstrated that those mice that healed the lesions had more GC, more isotype switched GC B cells and more memory B cells than those who did not. A productive B cell response was required for healing a co-infection with these protozoans in this model[77].

The development of TFH was also assessed in order to find strategies to enhance the efficacy of recombinant protein subunit vaccines using lipid-based nanoparticles (NPs). In this context, Moon *et al*[78] studied the impact of NP delivery on immune responses elicited by a candidate *P. vivax* subunit vaccine. They found that prolonged antigen presentation by this vaccine contributed to expand TFH and promote GC induction. The $CD4^+$ TFH subset provided critical cytokines and signals required to initiate somatic hypermutation and affinity maturation of B cells[79], achieving broad antibody responses.

This information indicates that there is an association between protozoan infections, TFH and their related cytokines, receptors and B responses in the context of experimental mice models. Leishmaniasis, malaria, toxoplasmosis and other parasitic infections seriously affect humans. Reports about the implication of TFH and humoral responses are needed to better understand mechanisms involved in the progression and outcome of these diseases.

CONCLUSION

Clearly, our research on TFH demonstrates that they are essential for the generation of a long-lasting humoral response. Their role in the assembly of the GC reaction explains why their dysfunction or their inability to interact correctly with B cells leads to immunodeficiency, to autoimmunity or to inefficient management of infectious diseases. It will be necessary to understand how the regulation of their function may be modified or restored in order to revert TFH deficiency or over activity, as well as to design adequate strategies for antibody production in vaccination programs.

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REFERENCES

1 **Morita R**, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, Foucat E, Dullaers M, Oh S, Sabzghabaei N, Lavecchio EM, Punaro M, Pascual V, Banchereau J, Ueno H. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 2011; **34**: 108-121 [PMID: 21215658 DOI: 10.1016/j.immuni.2010.12.012]

2 **Bossaller L**, Burger J, Draeger R, Grimbacher B, Knoth R, Plebani A, Durandy A, Baumann U, Schlesier M, Welcher AA, Peter HH, Warnatz K. ICOS deficiency is associated with a severe reduction of CXCR5+CD4 germinal center Th cells. *J Immunol* 2006; **177**: 4927-4932 [PMID: 16982935]

3 **Cannons JL**, Yu LJ, Jankovic D, Crotty S, Horai R, Kirby M, Anderson S, Cheever AW, Sher A, Schwartzberg PL. SAP regulates T cell-mediated help for humoral immunity by a mechanism distinct from cytokine regulation. *J Exp Med* 2006; **203**: 1551-1565 [PMID: 16754717]

4 **Craft JE**. Follicular helper T cells in immunity and systemic autoimmunity. *Nat Rev Rheumatol* 2012; **8**: 337-347 [PMID: 22549246 DOI: 10.1038/nrrheum.2012.58]

5 **Yu D**, Vinuesa CG. Multiple checkpoints keep follicular helper T cells under control to prevent autoimmunity. *Cell Mol Immunol* 2010; **7**: 198-203 [PMID: 20364160 DOI: 10.1038/cmi.2010.18]

6 **Crotty S**. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol* 2011; **29**: 621-663 [PMID: 21314428 DOI: 10.1146/annurev-immunol-031210-101400]

7 **Cannons JL**, Lu KT, Schwartzberg PL. T follicular helper cell diversity and plasticity. *Trends Immunol* 2013; **34**: 200-207 [PMID: 23395212 DOI: 10.1016/j.it.2013.01.001]

8 **Deenick EK**, Chan A, Ma CS, Gatto D, Schwartzberg PL, Brink R, Tangye SG. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity* 2010; **33**: 241-253 [PMID: 20691615 DOI: 10.1016/j.immuni.2010.07.015]

9 **Goenka R**, Barnett LG, Silver JS, O'Neill PJ, Hunter CA, Cancro MP, Laufer TM. Cutting edge: dendritic cell-restricted antigen presentation initiates the follicular helper T cell program but cannot complete ultimate effector differentiation. *J Immunol* 2011; **187**: 1091-1095 [PMID: 21715693 DOI: 10.4049/jimmunol.1100853]

10 **Ballesteros-Tato A**, Randall TD. Priming of T follicular helper cells by dendritic cells. *Immunol Cell Biol* 2014; **92**: 22-27 [PMID: 24145854 DOI: 10.1038/icb.2013.62]

11 **Liu X**, Chen X, Zhong B, Wang A, Wang X, Chu F, Nurieva RI, Yan X, Chen P, van der Flier LG, Nakatsukasa H, Neelapu SS, Chen W, Clevers H, Tian Q, Qi H, Wei L, Dong C. Transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development. *Nature* 2014; **507**: 513-518 [PMID: 24463518 DOI: 10.1038/nature12910]

12 **Poholek AC**, Hansen K, Hernandez SG, Eto D, Chandele A, Weinstein JS, Dong X, Odegard JM, Kaech SM, Dent AL, Crotty S, Craft J. In vivo regulation of Bcl6 and T follicular helper cell development. *J Immunol* 2010; **185**: 313-326 [PMID: 20519643 DOI: 10.4049/jimmunol.0904023]

13 **Baumjohann D**, Okada T, Ansel KM. Cutting Edge: Distinct waves of BCL6 expression during T follicular helper cell development. *J Immunol* 2011; **187**: 2089-2092 [PMID: 21804014 DOI: 10.4049/jimmunol.1101393]

14 **Kerfoot SM**, Yaari G, Patel JR, Johnson KL, Gonzalez DG, Kleinstein SH, Haberman AM. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. *Immunity* 2011; **34**: 947-960 [PMID: 21636295 DOI: 10.1016/j.immuni.2011.03.024]

15 **Choi YS**, Kageyama R, Eto D, Escobar TC, Johnston RJ, Monticelli L, Lao C, Crotty S. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity* 2011; **34**: 932-946 [PMID: 21636296 DOI: 10.1016/j.immuni.2011.03.023]

16 **Nurieva RI**, Podd A, Chen Y, Alekseev AM, Yu M, Qi X, Huang H, Wen R, Wang J, Li HS, Watowich SS, Qi H, Dong C, Wang D. STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. *J Biol Chem* 2012; **287**: 11234-11239 [PMID: 22318729 DOI: 10.1074/jbc.M111.324046]

17 **Chen M**, Guo Z, Ju W, Ryffel B, He X, Zheng SG. The development and function of follicular helper T cells in immune responses. *Cell Mol Immunol* 2012; **9**: 375-379 [PMID: 22659733 DOI: 10.1038/cmi.2012.18]

18 **Chung Y**, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, Wang YH, Lim H, Reynolds JM, Zhou XH, Fan HM, Liu ZM, Neelapu SS, Dong C. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat Med* 2011; **17**: 983-988 [PMID: 21785430 DOI: 10.1038/nm.2426]

19 **Sage PT**, Francisco LM, Carman CV, Sharpe AH. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. *Nat Immunol* 2013; **14**: 152-161 [PMID: 23242415 DOI: 10.1038/ni.2496]

20 **He J**, Tsai LM, Leong YA, Hu X, Ma CS, Chevalier N, Sun X, Vandenberg K, Rockman S, Ding Y, Zhu L, Wei W, Wang C, Karnowski A, Belz GT, Ghali JR, Cook MC, Riminton DS, Veillette A, Schwartzberg PL, Mackay F, Brink R, Tangye SG, Vinuesa CG, Mackay CR, Li Z, Yu D. Circulating precursor CCR7(lo)PD-1(hi) CXCR5$^+$ CD4$^+$ T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity* 2013; **39**: 770-781 [PMID: 24138884 DOI: 10.1016/j.immuni.2013.09.007]

21 **van der Flier LG**, van Gijn ME, Hatzis P, Kujala P, Haegebarth A, Stange DE, Begthel H, van den Born M, Guryev V, Oving I, van Es JH, Barker N, Peters PJ, van de Wetering M, Clevers H. Transcription factor achaete scute-like 2 controls intestinal stem cell fate. *Cell* 2009; **136**: 903-912 [PMID: 19269367 DOI: 10.1016/j.cell.2009.01.031]

22 **Crotty S**, Johnston RJ, Schoenberger SP. Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. *Nat Immunol* 2010; **11**: 114-120 [PMID: 20084069 DOI: 10.1038/ni.1837]

23 **Basso K**, Dalla-Favera R. BCL6: master regulator of the germinal center reaction and key oncogene in B cell lymphomagenesis. *Adv Immunol* 2010; **105**: 193-210 [PMID: 20510734 DOI: 10.1016/S0065-2776(10)05007-8]

24 **Nutt SL**, Tarlinton DM. Germinal center B and follicular helper T cells: siblings, cousins or just good friends? *Nat Immunol* 2011; **12**: 472-477 [PMID: 21739669]

25 **Johnston RJ**, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B, Dent AL, Craft J, Crotty S. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 2009; **325**: 1006-1010 [PMID: 19608860 DOI: 10.1126/science.1175870]

26 **Oestreich KJ**, Mohn SE, Weinmann AS. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TFH-like gene profile. *Nat Immunol* 2012; **13**: 405-411 [PMID: 22406686 DOI: 10.1038/ni.2242]

27 **Szabo SJ**, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; **100**: 655-669 [PMID: 10761931]

28 **Cannons JL**, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J, Wakeland EK, Germain RN, Schwartzberg PL. Optimal germinal center responses require a multistage T cell: B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* 2010; **32**: 253-265 [PMID: 20153220 DOI: 10.1016/j.immuni.2010.01.010]

29 **Nurieva R**, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, Dong C. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 2007; **448**: 480-483 [PMID: 17581589]

30 **Nurieva RI**, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, Wang YH, Dong C. Bcl6 mediates the development of T follicular helper cells. *Science* 2009; **325**: 1001-1005 [PMID: 19628815 DOI: 10.1126/science.1176676]

31 **Linterman MA**, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, Verma NK, Smyth MJ, Rigby RJ, Vinuesa CG. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J Exp Med* 2010; **207**: 353-363 [PMID: 20142429 DOI: 10.1084/jem.20091738]

32 **Berglund LJ**, Avery DT, Ma CS, Moens L, Deenick EK, Bustamante J, Boisson-Dupuis S, Wong M, Adelstein S, Arkwright PD, Bacchetta R, Bezrodnik L, Dadi H, Roifman CM, Fulcher DA, Ziegler JB, Smart JM, Kobayashi M, Picard C, Durandy A, Cook MC, Casanova JL, Uzel G, Tangye SG. IL-21 signalling via STAT3 primes human naive B cells to respond to IL-2 to enhance their differentiation into plasmablasts. *Blood* 2013; **122**: 3940-3950 [PMID: 24159173 DOI: 10.1182/blood-2013-06-506865]

33 **Gunn MD**, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci USA* 1998; **95**: 258-263 [PMID: 9419363]

34 **Freeman GJ**, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000; **192**: 1027-1034 [PMID: 11015443]

35 **Good-Jacobson KL**, Szumilas CG, Chen L, Sharpe AH, Tomayko MM, Shlomchik MJ. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat Immunol* 2010; **11**: 535-542 [PMID: 20453843 DOI: 10.1038/ni.1877]

36 **Good-Jacobson KL**, Shlomchik MJ. Plasticity and heterogeneity in the generation of memory B cells and long-lived plasma cells: the influence of germinal center interactions and dynamics. *J Immunol* 2010; **185**: 3117-3125 [PMID: 20814029 DOI: 10.4049/jimmunol.1001155]

37 **Hamel KM**, Cao Y, Wang Y, Rodeghero R, Kobezda T, Chen L, Finnegan A. B7-H1 expression on non-B and non-T cells promotes distinct effects on T- and B-cell responses in autoimmune arthritis. *Eur J Immunol* 2010; **40**: 3117-3127 [PMID: 21061440 DOI: 10.1002/eji.201040690]

38 **Hams E**, McCarron MJ, Amu S, Yagita H, Azuma M, Chen L, Fallon PG. Blockade of B7-H1 (programmed death ligand 1) enhances humoral immunity by positively regulating the generation of T follicular helper cells. *J Immunol* 2011; **186**: 5648-5655 [PMID: 21490158 DOI: 10.4049/jimmunol.1003161]

39 **Shekhar S**, Yang X. The darker side of follicular helper T cells: from autoimmunity to immunodeficiency. *Cell Mol Immunol* 2012; **9**: 380-385 [PMID: 22885524 DOI: 10.1038/cmi.2012.26]

40 **Warnatz K**, Bossaller L, Salzer U, Skrabl-Baumgartner A, Schwinger W, van der Burg M, van Dongen JJ, Orlowska-Volk M, Knoth R, Durandy A, Draeger R, Schlesier M, Peter HH, Grimbacher B. Human ICOS deficiency abrogates the germinal center reaction and provides a monogenic model for common variable immunodeficiency. *Blood* 2006; **107**: 3045-3052 [PMID: 16384931]

41 **Coraglia A**. B immunological memory in X-linked lymphoproliferative disease (XLP) and in common variable immunodeficiency (CVID) [Doctoral Thesis]. Argentina: University of Buenos Aires, 2012

42 **Korthäuer U**, Graf D, Mages HW, Brière F, Padayachee M, Malcolm S, Ugazio AG, Notarangelo LD, Levinsky RJ, Kroczek RA. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature* 1993; **361**: 539-541 [PMID: 7679206]

43 **Ma CS**, Hare NJ, Nichols KE, Dupré L, Andolfi G, Roncarolo MG, Adelstein S, Hodgkin PD, Tangye SG. Impaired hu-

moral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4+ T cells. *J Clin Invest* 2005; **115**: 1049-1059 [PMID: 15761493]
44 **Ma CS**, Deenick EK. Human T follicular helper (Tfh) cells and disease. *Immunol Cell Biol* 2014; **92**: 64-71 [PMID: 24145858 DOI: 10.1038/icb.2013.55]
45 **Qi H**, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature* 2008; **455**: 764-769 [PMID: 18843362 DOI: 10.1038/nature07345]
46 **Coraglia A**, Felippo M, Schierloh P, Malbran A, de Bracco MM. CD4+ T Lymphocytes with follicular helper phenotype (T(FH)) in patients with SH2D1A deficiency (XLP). *Clin Immunol* 2011; **141**: 357-364 [PMID: 21996454 DOI: 10.1016/j.clim.2011.09.007]
47 **Malbran A**, Belmonte L, Ruibal-Ares B, Baré P, Massud I, Parodi C, Felippo M, Hodinka R, Haines K, Nichols KE, de Bracco MM. Loss of circulating CD27+ memory B cells and CCR4+ T cells occurring in association with elevated EBV loads in XLP patients surviving primary EBV infection. *Blood* 2004; **103**: 1625-1631 [PMID: 14604960]
48 **Vinuesa CG**, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM, Yu D, Domaschenz H, Whittle B, Lambe T, Roberts IS, Copley RR, Bell JI, Cornall RJ, Goodnow CC. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 2005; **435**: 452-458 [PMID: 15917799]
49 **Simpson N**, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, Manku H, Vyse TJ, Roncador G, Huttley GA, Goodnow CC, Vinuesa CG, Cook MC. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum* 2010; **62**: 234-244 [PMID: 20039395 DOI: 10.1002/art.25032]
50 **Grammer AC**, Slota R, Fischer R, Gur H, Girschick H, Yarboro C, Illei GG, Lipsky PE. Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154-CD40 interactions. *J Clin Invest* 2003; **112**: 1506-1520 [PMID: 14617752]
51 **Terrier B**, Costedoat-Chalumeau N, Garrido M, Geri G, Rosenzwajg M, Musset L, Klatzmann D, Saadoun D, Cacoub P. Interleukin 21 correlates with T cell and B cell subset alterations in systemic lupus erythematosus. *J Rheumatol* 2012; **39**: 1819-1828 [PMID: 22859347 DOI: 10.3899/jrheum.120468]
52 **Drachman DB**. Myasthenia gravis. *N Engl J Med* 1994; **330**: 1797-1810 [PMID: 8190158]
53 **Luo C**, Li Y, Liu W, Feng H, Wang H, Huang X, Qiu L, Ouyang J. Expansion of circulating counterparts of follicular helper T cells in patients with myasthenia gravis. *J Neuroimmunol* 2013; **256**: 55-61 [PMID: 23305745 DOI: 10.1016/j.jneuroim.2012.12.001]
54 **Ma J**, Zhu C, Ma B, Tian J, Baidoo SE, Mao C, Wu W, Chen J, Tong J, Yang M, Jiao Z, Xu H, Lu L, Wang S. Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. *Clin Dev Immunol* 2012; **2012**: 827480 [PMID: 22649468 DOI: 10.1155/2012/827480]
55 **Zhu C**, Ma J, Liu Y, Tong J, Tian J, Chen J, Tang X, Xu H, Lu L, Wang S. Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease. *J Clin Endocrinol Metab* 2012; **97**: 943-950 [PMID: 22188745 DOI: 10.1210/jc.2011-2003]
56 **Romme Christensen J**, Börnsen L, Ratzer R, Piehl F, Khademi M, Olsson T, Sørensen PS, Sellebjerg F. Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression. *PLoS One* 2013; **8**: e57820 [PMID: 23469245 DOI: 10.1371/journal.pone.0057820]
57 **Tzartos JS**, Craner MJ, Friese MA, Jakobsen KB, Newcombe J, Esiri MM, Fugger L. IL-21 and IL-21 receptor expression in lymphocytes and neurons in multiple sclerosis brain. *Am J Pathol* 2011; **178**: 794-802 [PMID: 21281812 DOI: 10.1016/j.ajpath.2010.10.043]
58 **Li XY**, Wu ZB, Ding J, Zheng ZH, Li XY, Chen LN, Zhu P. Role of the frequency of blood CD4(+) CXCR5(+) CCR6(+) T cells in autoimmunity in patients with Sjögren's syndrome. *Biochem Biophys Res Commun* 2012; **422**: 238-244 [PMID: 22575453 DOI: 10.1016/j.bbrc.2012.04.133]
59 **Szabo K**, Papp G, Barath S, Gyimesi E, Szanto A, Zeher M. Follicular helper T cells may play an important role in the severity of primary Sjögren's syndrome. *Clin Immunol* 2013; **147**: 95-104 [PMID: 23578551 DOI: 10.1016/j.clim.2013.02.024]
60 **King C**, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu Rev Immunol* 2008; **26**: 741-766 [PMID: 18173374 DOI: 10.1146/annurev.immunol.26.021607.090344]
61 **Lindqvist M**, van Lunzen J, Soghoian DZ, Kuhl BD, Ranasinghe S, Kranias G, Flanders MD, Cutler S, Yudanin N, Muller MI, Davis I, Farber D, Hartjen P, Haag F, Alter G, Schulze zur Wiesch J, Streeck H. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. *J Clin Invest* 2012; **122**: 3271-3280 [PMID: 22922259 DOI: 10.1172/JCI64314]
62 **Cubas RA**, Mudd JC, Savoye AL, Perreau M, van Grevenynghe J, Metcalf T, Connick E, Meditz A, Freeman GJ, Abesada-Terk G, Jacobson JM, Brooks AD, Crotty S, Estes JD, Pantaleo G, Lederman MM, Haddad EK. Inadequate T follicular cell help impairs B cell immunity during HIV infection. *Nat Med* 2013; **19**: 494-499 [PMID: 23475201 DOI: 10.1038/nm.3109]
63 **Locci M**, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, Su LF, Cubas R, Davis MM, Sette A, Haddad EK, Poignard P, Crotty S. Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. *Immunity* 2013; **39**: 758-769 [PMID: 24035365 DOI: 10.1016/j.immuni.2013.08.031]
64 **Perreau M**, Savoye AL, De Crignis E, Corpataux JM, Cubas R, Haddad EK, De Leval L, Graziosi C, Pantaleo G. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J Exp Med* 2013; **210**: 143-156 [PMID: 23254284 DOI: 10.1084/jem.20121932]
65 **Hu TT**, Song XF, Lei Y, Hu HD, Ren H, Hu P. Expansion of circulating TFH cells and their associated molecules: involvement in the immune landscape in patients with chronic HBV infection. *Virol J* 2014; **11**: 54 [PMID: 24655429 DOI: 10.1186/1743-422X-11-54]
66 **Feng J**, Lu L, Hua C, Qin L, Zhao P, Wang J, Wang Y, Li W, Shi X, Jiang Y. High frequency of CD4+ CXCR5+ TFH cells in patients with immune-active chronic hepatitis B. *PLoS One* 2011; **6**: e21698 [PMID: 21750724 DOI: 10.1371/journal.pone.0021698]
67 **Ma Z**, Xie Y, Wang Y, Ma L, He Y, Zhang Y, Lian J, Guo Y, Jia Z. Peripheral blood $CD4^+$; $CXCR5^+$; follicular helper T cells are related to hyperglobulinemia of patients with chronic hepatitis B. *Xibao Yufenzi Mianyixue Zazhi* 2013; **29**: 515-518;521 [PMID: 23643274]
68 **Feng J**, Hu X, Guo H, Sun X, Wang J, Xu L, Jiang Z, Xu B, Niu J, Jiang Y. Patients with chronic hepatitis C express a high percentage of CD4(+)CXCR5(+) T follicular helper cells. *J Gastroenterol* 2012; **47**: 1048-1056 [PMID: 22426636 DOI: 10.1007/s00535-012-0568-1]
69 **Tripodo C**, Petta S, Guarnotta C, Pipitone R, Cabibi D, Colombo MP, Craxì A. Liver follicular helper T-cells predict the achievement of virological response following interferon-based treatment in HCV-infected patients. *Antivir Ther* 2012; **17**: 111-118 [PMID: 22267475 DOI: 10.3851/IMP1957]
70 **Jankovic D**, Kullberg MC, Feng CG, Goldszmid RS, Collazo CM, Wilson M, Wynn TA, Kamanaka M, Flavell RA, Sher A. Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *J Exp Med* 2007; **204**: 273-283 [PMID: 17283209]
71 **Nakayamada S**, Kanno Y, Takahashi H, Jankovic D, Lu

KT, Johnson TA, Sun HW, Vahedi G, Hakim O, Handon R, Schwartzberg PL, Hager GL, O'Shea JJ. Early Th1 cell differentiation is marked by a Tfh cell-like transition. *Immunity* 2011; **35**: 919-931 [PMID: 22195747 DOI: 10.1016/j.immuni.2011.11.012]

72 **Bonhagen K**, Liesenfeld O, Stadecker MJ, Hutloff A, Erb K, Coyle AJ, Lipp M, Kroczek RA, Kamradt T. ICOS+ Th cells produce distinct cytokines in different mucosal immune responses. *Eur J Immunol* 2003; **33**: 392-401 [PMID: 12645936]

73 **Greenwald RJ**, McAdam AJ, Van der Woude D, Satoskar AR, Sharpe AH. Cutting edge: inducible costimulator protein regulates both Th1 and Th2 responses to cutaneous leishmaniasis. *J Immunol* 2002; **168**: 991-995 [PMID: 11801630]

74 **Bollig N**, Brüstle A, Kellner K, Ackermann W, Abass E, Raifer H, Camara B, Brendel C, Giel G, Bothur E, Huber M, Paul C, Elli A, Kroczek RA, Nurieva R, Dong C, Jacob R, Mak TW, Lohoff M. Transcription factor IRF4 determines germinal center formation through follicular T-helper cell differentiation. *Proc Natl Acad Sci USA* 2012; **109**: 8664-8669 [PMID: 22552227 DOI: 10.1073/pnas.1205834109]

75 **Reinhardt RL**, Liang HE, Locksley RM. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat Immunol* 2009; **10**: 385-393 [PMID: 19252490 DOI: 10.1038/ni.1715]

76 **Butler NS**, Moebius J, Pewe LL, Traore B, Doumbo OK, Tygrett LT, Waldschmidt TJ, Crompton PD, Harty JT. Therapeutic blockade of PD-L1 and LAG-3 rapidly clears established blood-stage Plasmodium infection. *Nat Immunol* 2012; **13**: 188-195 [PMID: 22157630 DOI: 10.1038/ni.2180]

77 **Gibson-Corley KN**, Boggiatto PM, Bockenstedt MM, Petersen CA, Waldschmidt TJ, Jones DE. Promotion of a functional B cell germinal center response after Leishmania species co-infection is associated with lesion resolution. *Am J Pathol* 2012; **180**: 2009-2017 [PMID: 22429963 DOI: 10.1016/j.ajpath.2012.01.012]

78 **Moon JJ**, Suh H, Li AV, Ockenhouse CF, Yadava A, Irvine DJ. Enhancing humoral responses to a malaria antigen with nanoparticle vaccines that expand Tfh cells and promote germinal center induction. *Proc Natl Acad Sci USA* 2012; **109**: 1080-1085 [PMID: 22247289 DOI: 10.1073/pnas.1112648109]

79 **Breitfeld D**, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, Förster R. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* 2000; **192**: 1545-1552 [PMID: 11104797]

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REVIEW

Granulysin and its clinical significance as a biomarker of immune response and NK cell related neoplasms

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Abstract

Granulysin is a cytotoxic granular protein that was identified from human T cells by using the gene subtraction method in 1987. Based on its amino acid homology, granulysin belongs to the saposin-like protein family. The bioactive 9-kDa form of granulysin is processed from the 15-kDa pro-product in the cytoplasmic granules. It is expressed in CD8-positive αβT cells 5 d after mitogenic stimulation and constitutively in natural killer (NK) cells and γδT cells, although regulation of its expression has not yet been precisely determined. The 9-kDa granulysin form has anti-microbial activity against microorganisms such as bacteria, fungi, mycobacteria and parasites, as well as tumoricidal activity against some tumors at 1-10 μmol/L concentrations. Granulysin is secreted in both Ca-dependent and -independent manners. In sera, only the 15-kDa form is detectable and is expected to be a biomarker for immune potency, acute viral infection, anti-tumor immune reaction, acute graft *vs* host disease, and NK cell associated neoplasm.

Key words: Granulysin; Saposin-like protein family; Natural killer cell; Cytotoxic T lymphocyte

Core tip: Granulysin is a cytotoxic granular protein expressed in cytotoxic T cells, natural killer (NK) cells and γδT cells, and has anti-microbial activity against microorganisms such as bacteria, fungi, mycobacteria and parasites, as well as tumoricidal activity against some tumors. It is secreted constitutively and in a trigger-dependent manner. Clinically, serum granulysin is a unique biomarker for immune response, immune capacity and NK cell related neoplasms.

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INTRODUCTION

Granulysin is a cytotoxic granular protein that was identified in human T cells by using the gene subtraction method in 1987[1]. While granulysin is not expressed in resting αβT cells, it is constitutively expressed in natural killer (NK) cells and γδT cells. In contrast to other cytotoxic granular proteins, such as perforin and granzyme, granulysin is expressed in αβT cells later following antigenic stimulation (Figure 1). In this review, we summarize the structure, the *in vivo* and *in vitro* functions, and the regulation of expression of granulysin. Furthermore, we

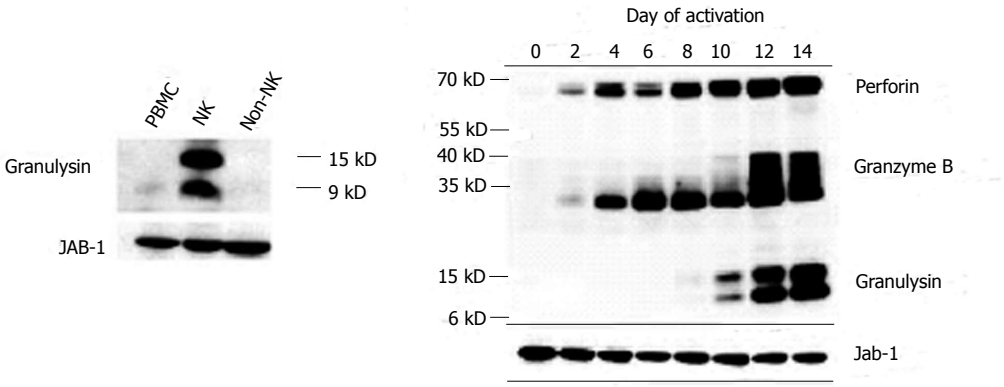


Figure 1 Expression of granulysin after T cell activation analyzed by western blotting. Granulysin is expressed later compared to perforin and granzyme B after T cell activation. In natural killer (NK) cells, granulysin is constitutively expressed. Jab-1 (c-Jun activation domain-binding protein-1) is used as internal control. PBMC: Peripheral blood mononuclear cell.

Table 1 Saposin-like protein family members and their proposed functions

Family member	Function	Identity to 9-kDa granulysin (amino acid)[1]	Similarity to 9-kDa granulysin (amino acid)[2]
Saposin A	Sphingolipid hydrolase activator	21	46
Saposin B	Sphingolipid hydrolase activator	19	50
Saposin C	Sphingolipid hydrolase activator	19	53
Saposin D	Sphingolipid hydrolase activator	20	46
Pulmonary surfactant protein B	Lipid organization in pulmonary surfactant	19	53
Acyloxyacyl hydrolase	Phagocytic cell lipase	22	50
Acyloxyacyl hydrolase	Sphingolipid hydrolase	13	42
Amoebapore A	Pore-forming Entamoeba histolytica granule protein	16	47
Amoebapore B	Pore-forming Entamoeba histolytica granule protein	13	42
Amoebapore C	Pore-forming Entamoeba histolytica granule protein	18	47
NK-lysin	Lytic porcine T cell and NK cell granule protein	35	66
Granulysin	Lytic human T cell and NK cell granule protein	100	100

[1]Identity denotes the percentage of identical amino acids; [2]Similarity denotes amino acids that share chemical properties, for example, charge or hydrophobicity. NK: Natural killer.

present results examining granulysin as a biomarker and discuss future investigations with granulysin.

STRUCTURE AND FUNCTION

Two isoforms of granulysin with molecular weights of 15-kDa and 9-kDa, respectively, have been identified and the biologically active 9-kDa isoform is derived from the 15-kDa isoform by intracellular processing (Figure 1). Based on amino acid sequence homology, the 9-kDa granulysin protein belongs to the saposin-like protein (SAPLIP) family containing the sphingolipid hydrolase activators of the central nervous system (Table 1)[2,3]. The gene is located at chromosome 2p11.2 in humans and homologues have been identified in pig, horse and cow. The absence of a homologous gene in rodents (mice) makes it difficult to investigate its physiological significance using rodent models[4,5]. Recently, Huang *et al*[6] and Liu *et al*[7] characterized a mouse model in which the human granulysin gene was introduced. This chimeric mouse model may be useful for the advanced functional analysis of granulysin in the future. Granulysin has cytotoxic activity similar to other SAPLIP family proteins such as amoebapore A, B, C (*Entamoeba histolytica* pore-forming protein) and NK-lysin (a porcine lytic granule protein)[8].

Crystal structure analysis of granulysin suggests that it consists of five α-helices (Figure 2). Although a physiological cell surface receptor for granulysin has not yet been identified, it is speculated that granulysin folds into a structure in which the positively charged active site interacts with negatively charged sites on bacterial or tumor cells and exhibits its cytotoxic activity. It is hypothesized that granulysin molecules aggregate on the target cell surface in an electric charge energy-dependent manner, and they rotate in the direction from α-helix1 to α-helix2 to α-helix3, pierce the cell membrane, and enter the cell[9,10]. Whereas synthetic peptides consisting of α-helix2 and α-helix3 kill bacterial and tumor cells, peptides consisting of α-helix3 alone kill bacterial, but not tumor cells. Substitution of cysteine residues in α-helix2 and α-helix3 with serine residues deprives the synthetic peptides of cytotoxic activity for human tumor cells, and replacing arginine residues with glutamine residues also abolishes its activity. When the cysteine residue is in the non-reduced state, the cytotoxic activity for tumor cells is lost while the cytotoxic activity for bacteria remains unaffected[10,11] (Figure 2B), suggesting that the reduced cysteine residue is essential for the cytotoxic activity for tumor cells. Substitution of D-amino acids 32-42 with L-amino acids maintains the same cytotoxic activity but induces resis-

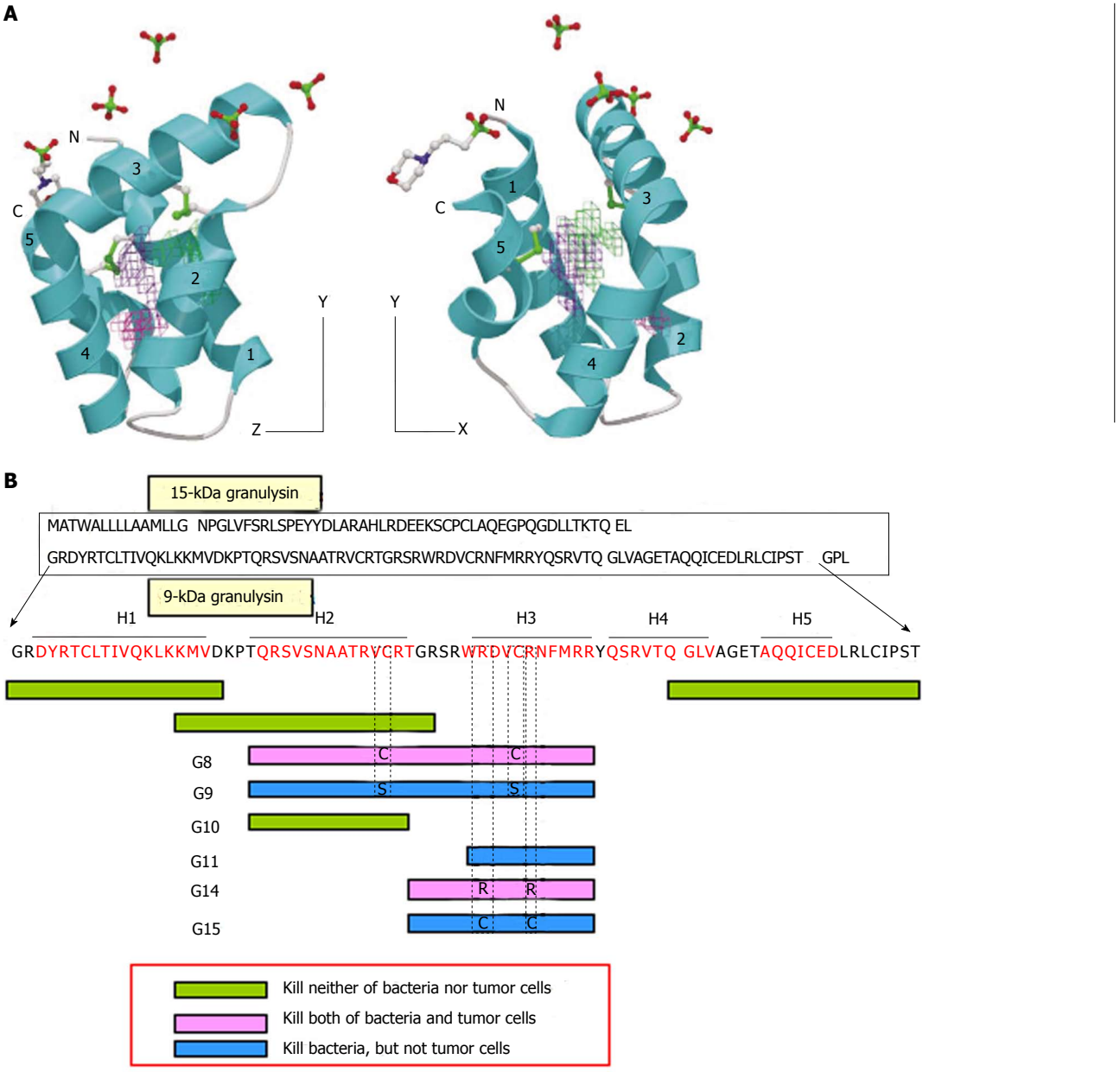


Figure 2 Granulysin. A: 3-D structure model of 9-kDa granulysin. Granulysin consists of five a-helices. Cytotoxic active site ranges between helix-2 and helix-3, in which positive electric charges are located[9]; B: Scheme of cytotoxic active site in granulysin. Amino acid sequence of granulysin and its biologically active site are illustrated. See STRUCTURE AND FUNCTION in the text for detailed explanation.

tance to inactivation by trypsin and the serum. These observations raise the possibility for the development of new synthetic peptides with cytotoxic activity, specifically for bacteria or for the development of biologically active peptides that can act for a long time *in vivo*[13].

EXPRESSION AND CYTOTOXIC ACTIVITY

Granulysin is expressed by activated cytotoxic T lymphocytes (CTL), mainly by CD8-positive T lymphocytes and some CD4-positive T lymphocytes[1,14]; it is also expressed by NK cells and γδT cells constitutively[15,16]. B cells and granular leukocytes do not express granulysin, but monocytes may express granulysin when activated. There is also a report indicating that granulysin was expressed in a megakaryocyte cell line, but whether it is expressed in platelets remains unclear[17].

Granulysin is synthesized as 15-kDa protein in the cytoplasm. The N-terminal amino acid sequence is thought to contain a transportation signal that directs granulysin to a cell granule. Some of the amino acids at the N- and C-termini are removed by unknown mechanisms within the cell granule to produce the active 9-kDa protein[14]. When the pH within the cytosomal granules is increased due to the presence of the H^+-ATPase inhibitor concanamycin A, processing to the 9-kDa protein is inhibited. Furthermore, against artificial cell membranes, the membrane injury activity of the 9-kDa granulysin is markedly reduced at pH 6.4 or lower. This most likely

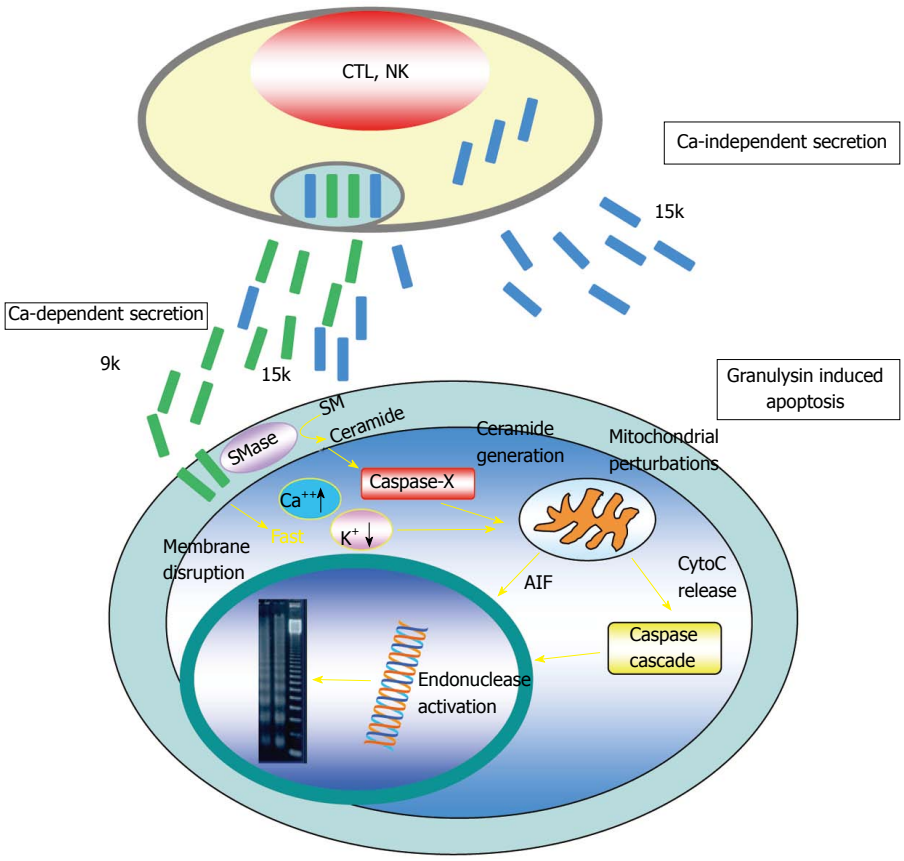


Figure 3 Schematic model of how granulysin kills target cell. (Cited from ref.[48] revised by author). NK: Natural killer; AIF: Apoptosis-inducing factor; CTL: Cytotoxic T lymphocytes.

explains why active 9-kDa granulysin does not cause autolysis in cytotoxic granules[18-20]. The CTL and NK cell granules have similar amounts of both the 15-kDa and the 9-kDa molecules, but while the 9-kDa molecules stay within the cytotoxic granules, the 15-kDa molecules are secreted constantly (Figure 3). Most of the 15-kDa molecules are thought to be secreted *via* an alternative pathway without entering the cytotoxic granules. Since the 15-kDa molecule does not have cytotoxic activity, its physiological role is currently not understood. Recently, it has been reported that 15-kDa granulysin induces differentiation of monocytes to dendritic cells and may modulate the immune response[21]. However, the 15-kDa molecule is detectable in serum and its potential significance as a biomarker has been recently reported.

9-kDa granulysin is released when co-cultured with target K562 cell and its release is prohibited by depletion of calcium, indicating Ca-dependent and trigger-dependent excretion of 9-kDa granulysin (see GRANULYSIN AS A BIOMARKER).

The 9-kDa molecule can kill gram-negative and gram-positive bacteria, fungi, parasitic worms, acid-fast bacilli and malarial parasites directly, but not intracellular parasites in the absence of perforin. Some studies also suggest that granulysin cannot enter the cytoplasm of the parasite in the absence of perforin[22].

Hata *et al*[23] reported that granulysin inhibits the growth of the varicella virus and induces apoptosis in infected cells. Granulysin-expressing CD4-positive T lymphocytes also kill *Cryptococcus neoformans*. Recently, Ochoa *et al*[24] reported that CD4-positive T lymphocytes infiltrating the lesions in leprosy patients express granulysin and are associated with control of the leprosy bacillus. Granulysin also has been reported to possess cytotoxic activity against some tumor cells[25]. The cytotoxic effects of granulysin against Jurkat cells are mediated by the entry of extracellular calcium into the cell after cell membrane destruction by granulysin, thereby inducing the release of intracellular calcium. Intracellular potassium (K) is reduced by a calcium-dependent K pump. This results in injury to the mitochondria and inhibits oxidative phosphorylation. With the release of cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria, caspases are activated within several minutes and apoptosis is induced. This model of apoptosis induction by granulysin is evidenced by the fact that inhibition of the calcium-dependent K pump *via* suppression of intracellular calcium release inhibits apoptosis induction. In addition, granulysin also induces late caspase activation through an alternative pathway by activating membrane sphingomyelinase and inducing ceramide formation[9,10,26,27] (Figure 3).

The 9-kDa granulysin also has pro-inflammatory functions similar to defensins and acts as a chemotactic factor for CD-4 positive and CD8-positive T lymphocytes and monocytes. This chemotactic activity is affected at 10 nM concentrations of granulysin, which is much lower than that required for its cytotoxic activity (1-10 μmol/L). It is speculated that granulysin acts through a

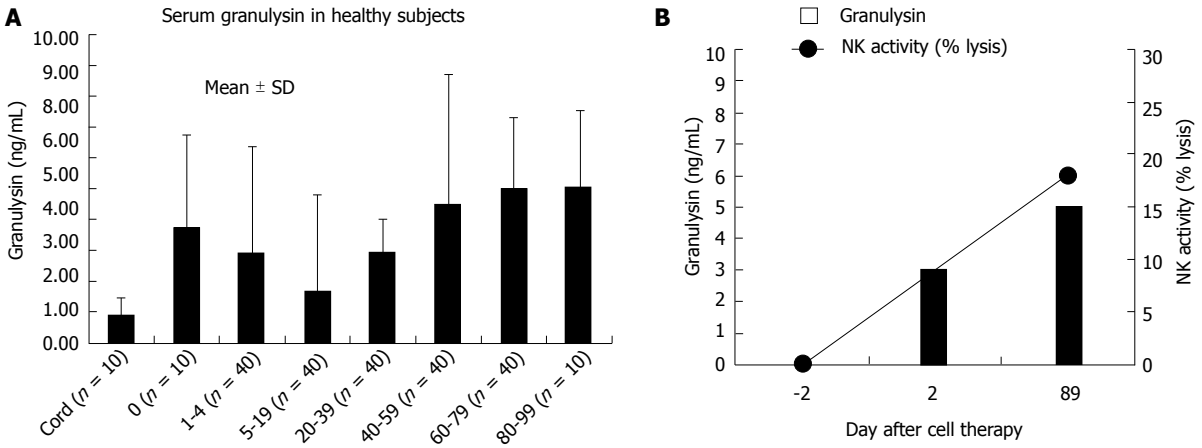


Figure 4 Serum granulysin in healthy subjects (A, see GRANULYSIN AS A BIOMARKER in the text) and relationship with natural killer cell activity (B). Serum granulysin is increased along with the recovery of natural killer (NK) cell activity in infant with combined immunodeficiency after cell therapy.

G protein conjugate receptor because the chemotaxis can be inhibited with pertussis toxin, but the details of the receptor are as yet unknown. The 9-kDa granulysin acts on monocytes and a cell line with monocytic-lineage (U937), and induces RANTES, monocyte chemotactic protein (MCP) 1, MCP-3, Macrophage inflammatory protein-1α, Interleukin (IL)-10, IL-1, IL-6 and interferon (IFN)-α[28].

REGULATION OF GRANULYSIN EXPRESSION

In comparison to its physiological functions, the regulation of granulysin expression remains to be elucidated.

The binding sites for activator protein-1 (AP-1), CCAAT/enhancer binding protein β (C/EBPβ) and nuclear factor kappa B (NF-κB) have been identified in the promoter region of the granulysin gene. Using the reporter assay system in which the monocyte-lineage cell line THP-1 is stimulated with *Acholeplasma laidlawii* (*A. laidlawii*) (mycoplasma), Kida *et al*[29,30] reported that two AP-1 binding sites (from -277 to -271 bp and from -96 to -86 bp) and the C/EBPβ binding site (from -1003 to -990 bp) are important for regulation of transcription, and that the former acts positively while the latter acts negatively. In the system described above, although *A. laidlawii* stimulation activated NF-κB through toll-like receptor 2 (TLR2) and the p50 homodimer bound to the NF-κB region, there was no influence on granulysin transcription[30].

NK cells express granulysin and IL-2 receptor β and γ chain constitutively. The expression of granulysin mRNA and protein was not altered after stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin, IL-2 or IFN-α[31]. Expression of granulysin was increased in CD8-positive T lymphocytes five days after antigen stimulation as mentioned above. Endsley *et al*[32] reported that CD4-positive T lymphocytes did not express granulysin even after PMA and ionomycin stimulation, whereas Zheng *et al*[33] reported that CD4-positive T lymphocytes expressed granulysin in the presence of IL-2 through PI3K and STAT5 activation, although anti-CD3 stimulation alone did not induce granulysin expression[33]. Transient activation of STAT5 occurred 30 to 60 min after IL-2 stimulation, following which a reactivation of STAT5 was observed after 3 d that induced IL-2 receptor β expression. Consequent interaction of IL-2 with IL-2 receptor β activated PI3K and induced granulysin[34]. Granulysin expression is inhibited by the anti-IL-2 receptor β antibody but not by the anti-IL-2 receptor α antibody, indicating the importance of IL-2 receptor α in inducing granulysin expression. Evidence for STAT5-controlled expression of granulysin also comes from the observation that patients with HIV infection have an increased susceptibility to *Cryptococcus neoformans*, which is probably due to insufficient activation of STAT5 and PI3K in CD4-positive T lymphocytes, resulting in reduced expression of granulysin[33].

Scherer *et al*[35] examined the expression of granulysin mRNA after stimulation with tuberculin purified protein derivative (PPD) in lymphocytes from bovine immunized with Bacille de Calmette et Guerin (BCG)[35]. Compared to non-immunized bovine controls, granulysin mRNA was increased more than 50 times in CD8-positive T lymphocytes 12 h after immunization and 48 h after immunization in CD4-positive T lymphocytes. Furthermore, whereas the mRNAs of perforin, IFN-γ and Fas-ligand in CD4-positive T lymphocytes increased after PMA + ionomycin stimulation, as well as after PPD stimulation, granulysin mRNA was not enhanced after PMA + ionomycin stimulation, corroborating the previous observation by Endsley *et al*[32].

GRANULYSIN AS A BIOMARKER

As mentioned above, the 15-kDa and 9-kDa granulysin forms exist at approximately a 1:1 ratio in cells. The precise mechanism of this conversion and its regulation is unknown. The non-active 15-kDa precursor of granulysin is secreted constantly, but the active 9-kDa form is released in a calcium-dependent manner. Based on the observation that the 9-kDa form is not detected in the culture medium even after *in vitro* stimulation, it

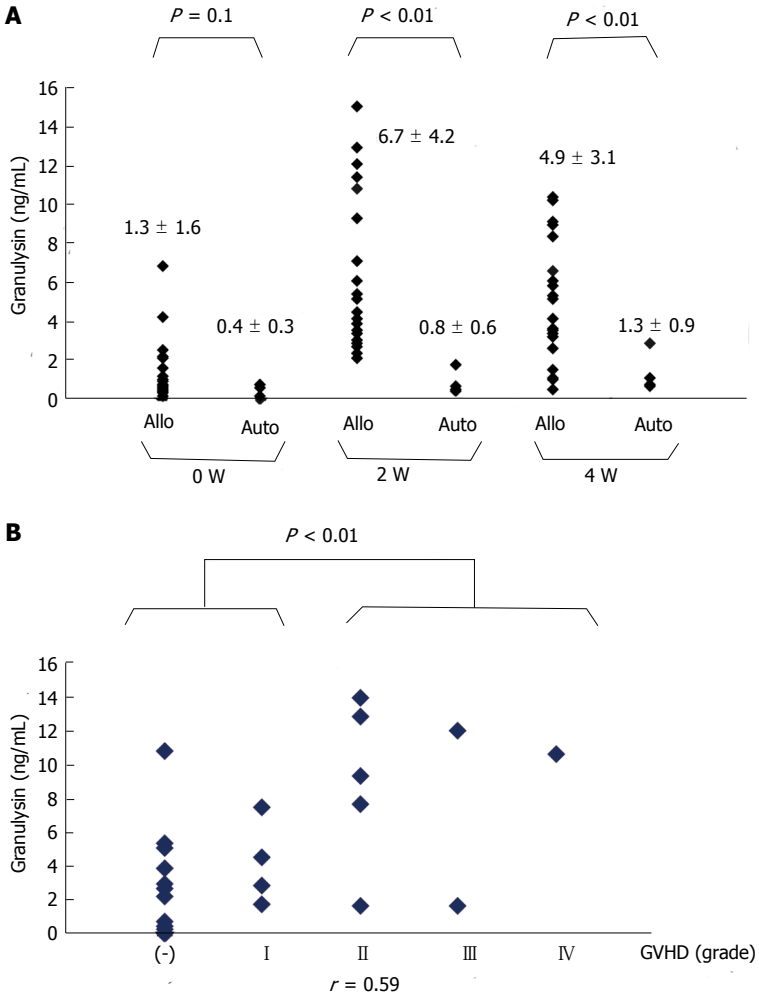


Figure 5 Trend of serum granulysin. A: In patients with allo-hematopoietic stem cell transplantation (HSCT) (n = 21) and auto-HSCT (n = 5). Serum granulysin is elevated 2 wk after allogeneic hematopoietic stem cell transplantation, but not autologous hematopoietic stem cell transplantation; B: With the grade of graft-versus-host disease (GVHD). Serum granulysin and the grade of GVHD were plotted and their correlation coefficient was calculated (r = 0.59). The serum granulysin level of patients with grade 2 or more is significantly higher than that of patients with grade 1 or no GVHD.

is possible that the active form is immediately adsorbed, consumed or destroyed. By contrast, the 15-kDa form is easily detected in the culture medium and serum and is increased after *in vitro* stimulation[36]. This indicates that the 9-kDa and 15-kDa forms are released together after stimulation, but only the 15-kDa form is detected. Any increase in the release of the 9-kDa form is therefore estimated to arise indirectly from the increased amount of the 15-kDa form, since inhibition of cellular secretion using Brefeldin A increased the intracellular levels of granulysin in CTL and NK cells but did not affect intracellular perforin and granzyme levels[36].

Granulysin as a biomarker in cell-mediated immunity

To estimate the levels of serum granulysin in healthy subjects, a novel, highly sensitive Enzyme Linked Immuno-Sorbent Assay method was used (Figure 4A). Levels of granulysin gradually increase with aging and are extremely low in umbilical cord blood. These levels reflect the levels of constitutively secreted granulysin and can be correlated either with NK cell activity or the number of NK cells and γδT cells, which constitutively express granulysin[36]. It is well known that NK activity increases with ageing until the age of 40 and decreases thereafter. The discrepancy between granulysin level and NK activity after the age of 40-50 is not well explained. One possibility is that the ratio of conversion from 15-kDa to 9-kDa changes after the age of 40. We have no data concerning this issue, which remains to be investigated.

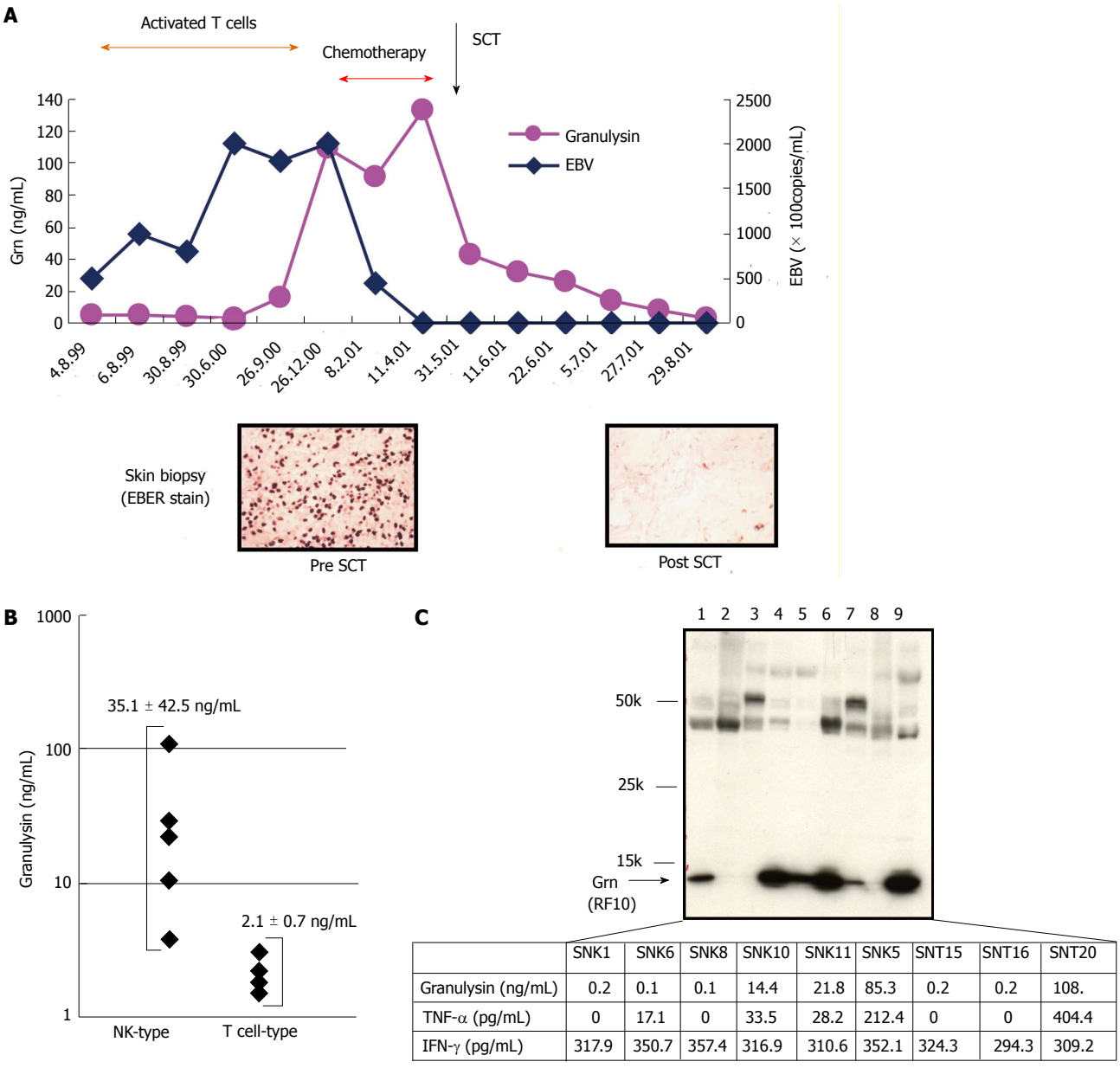
In infants with severe immunodeficiency without NK cells, serum granulysin was undetectable and became measurable when a cell-mediated immunity function was restored by hematopoietic stem cell transplantation (unpublished data). After transfusion of autologous *in vitro*-activated T cells back into a patient with incompetent cell-mediated immunity, levels of serum granulysin were increased along with the recovery of NK activity (Figure 4B)[36]. These observations indicate that serum granulysin is useful as a new biomarker for evaluation of cell-mediated immunity.

Granulysin as a biomarker in acute virus infection

Infectious mononucleosis is an acute disease resulting from primary Epstein-Barr (EB) virus infection, in which activated CD8-positive CTLs are increased in the peripheral blood. Increased CD8-positive CTLs are reactive and cytotoxic against EBV-infected B lymphocytes. Serum granulysin is markedly increased during an acute phase of infectious mononucleosis and becomes normalized in convalescence[36].

Granulysin as a biomarker of hemophagocytic lymphohistiocytosis

Hemophagocytic lymphohistiocytosis is a histiocytosis-



	SNK1	SNK6	SNK8	SNK10	SNK11	SNK5	SNT15	SNT16	SNT20
Granulysin (ng/mL)	0.2	0.1	0.1	14.4	21.8	85.3	0.2	0.2	108.
TNF-α (pg/mL)	0	17.1	0	33.5	28.2	212.4	0	0	404.4
IFN-γ (pg/mL)	317.9	350.7	357.4	316.9	310.6	352.1	324.3	294.3	309.2

Figure 6 Serum granulysin. A: Clinical course and trend of serum granulysin in a natural killer (NK) cell type chronic active EB virus infection (CAEBV) patient. For detailed explanation, see GRANULYSIN AS A BIOMARKER, 6: Granulysin in NK cell-related tumors or neoplasm in the text; B: Serum granulysin in patients with NK type and T cell type CAEBV, serum granulysin in patients with NK type (n = 5) and T cell type (n = 4) chronic active EB virus infection. Only in NK type CAEBV, serum granulysin is significantly elevated. Serum granulysin in patients with NK type (n = 5) and T cell type (n = 4) chronic active EB virus infection. Only in NK type CAEBV, serum granulysin is significantly elevated. C: Expression of granulysin and cytokine production in EB virus infected cell lines (SNK1,5,6,10,11: NK cell type, SNT8,15,16,20: γδT cell type). Western blotting was performed by using a monoclonal antibody, RF10 which reacts with 15-kDa but not 9-kDa granulysin. TNF-a and IFN-g in the culture supernatant were assayed by ELISA method. TNE: Tumor necrosis factor; INF: Interferon.

related disease characterized clinically by fever, pancytopenia, hepatosplenomegaly and hyperlipidemia. T cells are strongly activated during the acute phase of hemophagocytic lymphohistiocytosis (HLH) and levels of Th1 cytokines, such as IL-12, IL-18 and IFN-γ, are abnormally high, which leads to the abnormal activation of macrophages. Serum ferritin and soluble IL-2 receptor (sIL2R) have been reported as clinical markers of HLH. The treatment of HLH includes immunosuppressive therapy, anti-cancer drug chemotherapy and hematopoietic stem cell transplantation in severe cases. We measured serum granulysin in 24 HLH patients prior to treatment and reported that levels of granulysin were extremely high during the acute phase of HLH. Since serum granulysin levels decreased in parallel with disease regression following therapy, granulysin seems to be useful as a novel biomarker of HLH[37].

Granulysin as a biomarker of tumor immunity

Kishi *et al*[38] examined intracellular levels of granulysin and perforin in NK cells of cancer-bearing patients and healthy subjects by flow cytometry. Levels of intracellular granulysin were significantly decreased in cancer-bearing patients, while those of intracellular perforin were not

changed compared to healthy subjects[38]. Spontaneous regression of neuroblastoma has been observed frequently in infants younger than one year old. We previously reported a case study of an infant with neuroblastoma IVS who showed dramatic spontaneous regression. During the regression, serum granulysin and IFN-γ levels were transiently and markedly elevated[39]. Although interpretation of these observations is difficult, it seems that serum granulysin is related to tumor immunity and could be a novel biomarker of tumor immunity.

Granulysin as a biomarker in acute graft-versus-host disease

Elevated granulysin mRNA levels have been reported in infiltrating cells of acutely rejected kidneys from renal transplant patients[40]. To examine whether serum granulysin is a marker of acute graft-*vs*-host disease (GVHD) in hematopoietic stem cell transplantation (HSCT), we first isolated alloantigen-specific CTLs and confirmed that serum granulysin was released in an allospecific manner *in vitro*. Next, we examined serum granulysin in autologous and allogeneic hematopoietic stem cell transplantation cases. Serum granulysin was significantly and transiently increased in allogeneic HSCT 2 wk after SCT (6.7 ± 4.2 ng/mL), but not in autologous HSCT (0.8 ± 0.6 ng/mL) (Figure 5A). We also examined and found a significant correlation in the severity of acute GVHD and levels of granulysin (Figure 5B). Efficacy of soluble IL-2 receptor (sIL2R) has been reported as a biomarker of acute GVHD[41]. However, there were cases in which the change of sIL-2R levels and the symptoms of GVHD did not correlate in clinical settings. As per our observations, sIL-2R correlated well with serum granulysin during the first two months after HSCT, but serum granulysin reflected GVHD symptoms much better than sIL-2R thereafter. This discrepancy seems interesting in understanding the complicated pathology of GVHD and highlights the utility of serum granulysin as a biomarker that is distinct from sIL-2R for acute GVHD.

Granulysin in NK cell-related tumors or neoplasms

αβT cells express granulysin only after being activated and/or on maturation to CTLs. However, as mentioned above, granulysin is expressed constitutively in NK cells and γδT cells. Based on the foregoing observations, we examined the possibility of evaluating granulysin as a marker for NK-related tumors. Chronic active EB virus infection (CAEBV) is a disease with poor prognosis, presenting with fever, mosquito hypersensitivity, lymphadenopathy and hepatosplenomegaly, in which T cells or NK cells infected with EB virus are detected in the peripheral blood, and is usually classified as either the NK cell type or the T cell type. Interestingly, CD4-positive αβT cells are infected with the T cell type of EB virus. NK cell type CAEBV has been named hydroa vacciniforme because it is characterized clinically by varicelliform eruptions characterized histologically by infiltrating EB virus-positive cells. CAEBV frequently progresses to hemophagocytic syndrome or malignant lymphoma after a chronic clinical course. Figure 6A shows the levels of serum granulysin and blood EB viral genome in a patient with NK cell type CAEBV during a long-term clinical course. Serum granulysin and blood EB viral genome increased with progress of the disease. While blood EB viral genome decreased in response to chemotherapy, serum granulysin levels normalized only after allogeneic hematopoietic stem cell transplantation. A comparison of serum granulysin levels in NK cell type and T cell type CAEBV patients indicated that serum granulysin was significantly increased only in NK cell type patients (Figure 6B). Expression of granulysin was also confirmed by analyzing NK cell and γδT cell lines established from CAEBV patients[42]. CD4-positive αβT cell lines have not yet been established, but examination of a tumor tissue from a patient who presented with an EB virus-positive, CD4-positive lymphoma over the course of CAEBV[43], did not reveal any expression of granulysin (unpublished observations). Interestingly, cell lines with granulysin expression also showed enhanced TNF-α production, although the levels of INF-γ production were the same (Figure 6C). Culturing in the presence of the NF-κB inhibitor did not affect the expression of granulysin in these cell lines (unpublished observation). Sekiguchi *et al*[44] reported that serum granulysin was significantly increased in patients with aggressive NK cell leukemia[44]. Granulysin has also been implicated in the cell death of keratinocytes in Stevens-Johnson syndrome and toxic epidermal necrolysis[45]. Iwai *et al*[46] reported that histological examination of granulysin expression is useful for distinguishing Stevens-Johnson syndrome/toxic epidermal necrolysis from erythema multiforme major.

FUTURE DIRECTIONS

CTL and NK cells secrete the 15-kDa precursor of granulysin constitutively, whereas they secrete both the 15-kDa precursor and the active 9-kDa granulysin forms when exerting cytotoxic activity. Only the 15-kDa form can be detected in sera or culture media, because the active 9-kDa form may be adsorbed, consumed or destroyed rapidly. This characteristic is quite different from that of other cytotoxic granular proteins such as perforin and granzyme, and makes granulysin a unique biomarker of cell-mediated immunity, tumor immunity, infection and GVHD. Structural analysis of granulysin provides the potential for the development of new innovative agents by designing novel analogous proteins using biomolecular technology. The effectiveness of a granulysin-DNA vaccine for tuberculosis in mice models has been recently reported[47]. While many unknowns remain concerning granulysin regulation and function, the combination of novel biotechnological methods will make it possible to develop novel immune, anti-cancer and anti-infection treatment strategies. One difficulty for granulysin research comes from the fact that there is no homologous gene for granulysin in mice. Although granulysin was discovered in 1987, a new report that granulysin is associated with the onset of Stevens-Johnson syndrome

has refreshed interest in granulysin research. The clinical analysis of granulysin as a biomarker has only just begun and it is expected that new findings will be obtained in the future through both basic and clinical studies.

REFERENCES

1 **Jongstra J**, Schall TJ, Dyer BJ, Clayberger C, Jorgensen J, Davis MM, Krensky AM. The isolation and sequence of a novel gene from a human functional T cell line. *J Exp Med* 1987; **165**: 601-614 [PMID: 2434598 DOI: 10.1084/jem.165.3.601]

2 **Munford RS**, Sheppard PO, O'Hara PJ. Saposin-like proteins (SAPLIP) carry out diverse functions on a common backbone structure. *J Lipid Res* 1995; **36**: 1653-1663 [PMID: 7595087]

3 **Leippe M**. Ancient weapons: NK-lysin, is a mammalian homolog to pore-forming peptides of a protozoan parasite. *Cell* 1995; **83**: 17-18 [PMID: 7553868 DOI: 10.1016/0092-8674(95)90229-5]

4 **Andreu D**, Carreño C, Linde C, Boman HG, Andersson M. Identification of an anti-mycobacterial domain in NK-lysin and granulysin. *Biochem J* 1999; **344** Pt 3: 845-849 [PMID: 10585872]

5 **Davis EG**, Sang Y, Rush B, Zhang G, Blecha F. Molecular cloning and characterization of equine NK-lysin. *Vet Immunol Immunopathol* 2005; **105**: 163-169 [PMID: 15797485 DOI: 10.1016/j.vetimm.2004.12.007]

6 **Huang LP**, Lyu SC, Clayberger C, Krensky AM. Granulysin-mediated tumor rejection in transgenic mice. *J Immunol* 2007; **178**: 77-84 [PMID: 17182542]

7 **Liu B**, Liu S, Qu X, Liu J. Construction of a eukaryotic expression system for granulysin and its protective effect in mice infected with Mycobacterium tuberculosis. *J Med Microbiol* 2006; **55**: 1389-1393 [PMID: 17005788 DOI: 10.1099/jmm.0.46706-0]

8 **Andersson M**, Gunne H, Agerberth B, Boman A, Bergman T, Sillard R, Jörnvall H, Mutt V, Olsson B, Wigzell H. NK-lysin, a novel effector peptide of cytotoxic T and NK cells. Structure and cDNA cloning of the porcine form, induction by interleukin 2, antibacterial and antitumour activity. *EMBO J* 1995; **14**: 1615-1625 [PMID: 7737114]

9 **Anderson DH**, Sawaya MR, Cascio D, Ernst W, Modlin R, Krensky A, Eisenberg D. Granulysin crystal structure and a structure-derived lytic mechanism. *J Mol Biol* 2003; **325**: 355-365 [PMID: 12488100 DOI: 10.1016/S0022-2836(02)01234-2]

10 **Kaspar AA**, Okada S, Kumar J, Poulain FR, Drouvalakis KA, Kelekar A, Hanson DA, Kluck RM, Hitoshi Y, Johnson DE, Froelich CJ, Thompson CB, Newmeyer DD, Anel A, Clayberger C, Krensky AM. A distinct pathway of cell-mediated apoptosis initiated by granulysin. *J Immunol* 2001; **167**: 350-356 [PMID: 11418670]

11 **Wang Z**, Choice E, Kaspar A, Hanson D, Okada S, Lyu SC, Krensky AM, Clayberger C. Bactericidal and tumoricidal activities of synthetic peptides derived from granulysin. *J Immunol* 2000; **165**: 1486-1490 [PMID: 10903754]

12 **Ernst WA**, Thoma-Uszynski S, Teitelbaum R, Ko C, Hanson DA, Clayberger C, Krensky AM, Leippe M, Bloom BR, Ganz T, Modlin RL. Granulysin, a T cell product, kills bacteria by altering membrane permeability. *J Immunol* 2000; **165**: 7102-7108 [PMID: 11120840]

13 **Hamamoto K**, Kida Y, Zhang Y, Shimizu T, Kuwano K. Antimicrobial activity and stability to proteolysis of small linear cationic peptides with D-amino acid substitutions. *Microbiol Immunol* 2002; **46**: 741-749 [PMID: 12516770 DOI: 10.1111/j.1348-0421.2002.tb02759.x]

14 **Peña SV**, Hanson DA, Carr BA, Goralski TJ, Krensky AM. Processing, subcellular localization, and function of 519 (granulysin), a human late T cell activation molecule with homology to small, lytic, granule proteins. *J Immunol* 1997; **158**: 2680-2688 [PMID: 9058801]

15 **Obata-Onai A**, Hashimoto S, Onai N, Kurachi M, Nagai S, Shizuno K, Nagahata T, Matsushima K. Comprehensive gene expression analysis of human NK cells and CD8(+) T lymphocytes. *Int Immunol* 2002; **14**: 1085-1098 [PMID: 12356674 DOI: 10.1093/intimm/dxf086]

16 **Spada FM**, Grant EP, Peters PJ, Sugita M, Melián A, Leslie DS, Lee HK, van Donselaar E, Hanson DA, Krensky AM, Majdic O, Porcelli SA, Morita CT, Brenner MB. Self-recognition of CD1 by gamma/delta T cells: implications for innate immunity. *J Exp Med* 2000; **191**: 937-948 [PMID: 10727456 DOI: 10.1084/jem.191.6.937]

17 **Kitamura N**, Koshiba M, Horie O, Ryo R. Expression of granulysin mRNA in the human megakaryoblastic leukemia cell line CMK. *Acta Haematol* 2002; **108**: 13-18 [PMID: 12145461 DOI: 10.1159/000063061]

18 **Hanson DA**, Kaspar AA, Poulain FR, Krensky AM. Biosynthesis of granulysin, a novel cytolytic molecule. *Mol Immunol* 1999; **36**: 413-422 [PMID: 10449094 DOI: 10.1016/S0161-5890(99)00063-2]

19 **Kataoka T**, Shinohara N, Takayama H, Takaku K, Kondo S, Yonehara S, Nagai K. Concanamycin A, a powerful tool for characterization and estimation of contribution of perforin- and Fas-based lytic pathways in cell-mediated cytotoxicity. *J Immunol* 1996; **156**: 3678-3686 [PMID: 8621902]

20 **Uellner R**, Zvelebil MJ, Hopkins J, Jones J, MacDougall LK, Morgan BP, Podack E, Waterfield MD, Griffiths GM. Perforin is activated by a proteolytic cleavage during biosynthesis which reveals a phospholipid-binding C2 domain. *EMBO J* 1997; **16**: 7287-7296 [PMID: 9405358 DOI: 10.1093/emboj/16.24.7287]

21 **Clayberger C**, Finn MW, Wang T, Saini R, Wilson C, Barr VA, Sabatino M, Castiello L, Stroncek D, Krensky AM. 15 kDa granulysin causes differentiation of monocytes to dendritic cells but lacks cytotoxic activity. *J Immunol* 2012; **188**: 6119-6126 [PMID: 22586033]

22 **Stenger S**, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, Ganz T, Thoma-Uszynski S, Melián A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, Modlin RL. An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science* 1998; **282**: 121-125 [PMID: 9756476 DOI: 10.1126/science.282.5386.121]

23 **Hata A**, Zerboni L, Sommer M, Kaspar AA, Clayberger C, Krensky AM, Arvin AM. Granulysin blocks replication of varicella-zoster virus and triggers apoptosis of infected cells. *Viral Immunol* 2001; **14**: 125-133 [PMID: 11398808]

24 **Ochoa MT**, Stenger S, Sieling PA, Thoma-Uszynski S, Sabet S, Cho S, Krensky AM, Rollinghoff M, Nunes Sarno E, Burdick AE, Rea TH, Modlin RL. T-cell release of granulysin contributes to host defense in leprosy. *Nat Med* 2001; **7**: 174-179 [PMID: 11175847 DOI: 10.1038/84620]

25 **Peña SV**, Krensky AM. Granulysin, a new human cytolytic granule-associated protein with possible involvement in cell-mediated cytotoxicity. *Semin Immunol* 1997; **9**: 117-125 [PMID: 9194222 DOI: 10.1006/smim.1997.0061]

26 **Okada S**, Li Q, Whitin JC, Clayberger C, Krensky AM. Intracellular mediators of granulysin-induced cell death. *J Immunol* 2003; **171**: 2556-2562 [PMID: 12928406]

27 **Gamen S**, Hanson DA, Kaspar A, Naval J, Krensky AM, Anel A. Granulysin-induced apoptosis. I. Involvement of at least two distinct pathways. *J Immunol* 1998; **161**: 1758-1764 [PMID: 9712041]

28 **Deng A**, Chen S, Li Q, Lyu SC, Clayberger C, Krensky AM. Granulysin, a cytolytic molecule, is also a chemoattractant and proinflammatory activator. *J Immunol* 2005; **174**: 5243-5248 [PMID: 15843520]

29 **Kida Y**, Kuwano K, Zhang Y, Arai S. Acholeplasma laidlawii up-regulates granulysin gene expression via transcription factor activator protein-1 in a human monocytic cell line, THP-1. *Immunology* 2001; **104**: 324-332 [PMID:

11722647]
30 **Kida Y**, Shimizu T, Kuwano K. Opposing roles of activator protein-1 and CCAAT/enhancer binding protein beta in the regulation of inducible granulysin gene expression in a human monocytic cell line, THP-1. *Immunology* 2002; **107**: 507-516 [PMID: 12460196]
31 **Mori S**, Jewett A, Cavalcanti M, Murakami-Mori K, Nakamura S, Bonavida B. Differential regulation of human NK cell-associated gene expression following activation by IL-2, IFN-alpha and PMA/ionomycin. *Int J Oncol* 1998; **12**: 1165-1170 [PMID: 9538144]
32 **Endsley JJ**, Hogg A, Shell LJ, McAulay M, Coffey T, Howard C, Capinos Scherer CF, Waters WR, Nonnecke B, Estes DM, Villarreal-Ramos B. Mycobacterium bovis BCG vaccination induces memory CD4+ T cells characterized by effector biomarker expression and anti-mycobacterial activity. *Vaccine* 2007; **25**: 8384-8394 [PMID: 17996992 DOI: 10.1016/j.vaccine.2007.10.011]
33 **Zheng CF**, Ma LL, Jones GJ, Gill MJ, Krensky AM, Kubes P, Mody CH. Cytotoxic CD4+ T cells use granulysin to kill Cryptococcus neoformans, and activation of this pathway is defective in HIV patients. *Blood* 2007; **109**: 2049-2057 [PMID: 17038537 DOI: 10.1182/blood-2006-03-009720]
34 **Zheng CF**, Jones GJ, Shi M, Wiseman JC, Marr KJ, Berenger BM, Huston SM, Gill MJ, Krensky AM, Kubes P, Mody CH. Late expression of granulysin by microbicidal $CD4^+$ T cells requires PI3K- and STAT5-dependent expression of IL-2Rbeta that is defective in HIV-infected patients. *J Immunol* 2008; **180**: 7221-7229 [PMID: 18490721]
35 **Capinos Scherer CF**, Endsley JJ, de Aguiar JB, Jacobs WR, Larsen MH, Palmer MV, Nonnecke BJ, Ray Waters W, Mark Estes D. Evaluation of granulysin and perforin as candidate biomarkers for protection following vaccination with Mycobacterium bovis BCG or M. bovisDeltaRD1. *Transbound Emerg Dis* 2009; **56**: 228-239 [PMID: 19389081]
36 **Ogawa K**, Takamori Y, Suzuki K, Nagasawa M, Takano S, Kasahara Y, Nakamura Y, Kondo S, Sugamura K, Nakamura M, Nagata K. Granulysin in human serum as a marker of cell-mediated immunity. *Eur J Immunol* 2003; **33**: 1925-1933 [PMID: 12884856 DOI: 10.1002/eji.200323977]
37 **Nagasawa M**, Ogawa K, Imashuku S, Mizutani S. Serum granulysin is elevated in patients with hemophagocytic lymphohistiocytosis. *Int J Hematol* 2007; **86**: 470-473 [PMID: 18192122 DOI: 10.1007/BF02984011]
38 **Kishi A**, Takamori Y, Ogawa K, Takano S, Tomita S, Tanigawa M, Niman M, Kishida T, Fujita S. Differential expression of granulysin and perforin by NK cells in cancer patients and correlation of impaired granulysin expression with progression of cancer. *Cancer Immunol Immunother* 2002; **50**: 604-614 [PMID: 11807624 DOI: 10.1007/s002620100228]
39 **Nagasawa M**, Kawamoto H, Tsuji Y, Mizutani S. Transient increase of serum granulysin in a stage IVs neuroblastoma patient during spontaneous regression: case report. *Int J Hematol* 2005; **82**: 456-457 [PMID: 16533752 DOI: 10.1532/IJH97.05091]
40 **Sarwal MM**, Jani A, Chang S, Huie P, Wang Z, Salvatierra O, Clayberger C, Sibley R, Krensky AM, Pavlakis M. Granulysin expression is a marker for acute rejection and steroid resistance in human renal transplantation. *Hum Immunol* 2001; **62**: 21-31 [PMID: 11165712 DOI: 10.1016/S0198-8859(00)00228-7]
41 **Nagasawa M**, Isoda T, Itoh S, Kajiwara M, Morio T, Shimizu N, Ogawa K, Nagata K, Nakamura M, Mizutani S. Analysis of serum granulysin in patients with hematopoietic stem-cell transplantation: its usefulness as a marker of graft-versus-host reaction. *Am J Hematol* 2006; **81**: 340-348 [PMID: 16628730]
42 **Nagasawa M**, Ogawa K, Nagata K, Shimizu N. Serum granulysin as a possible biomarker of natural killer cell neoplasms. *Br J Haematol* 2010; **148**: 812-814 [PMID: 19912220]
43 **Nagasawa M**, Hirai K, Mizutani S, Okawa H, Yata J. EBV infection induced transformation of benign T lymphoproliferative state in patient with chronic active EBV infection into malignant lymphoma: implication of EBV infection as additive oncogenic factor in tumorigenesis. *Leuk Res* 1999; **23**: 1071-1078 [PMID: 10576513]
44 **Sekiguchi N**, Asano N, Ito T, Momose K, Momose M, Ishida F. Elevated serum granulysin and its clinical relevance in mature NK-cell neoplasms. *Int J Hematol* 2012; **96**: 461-468 [PMID: 22890551]
45 **Chung WH**, Hung SI, Yang JY, Su SC, Huang SP, Wei CY, Chin SW, Chiou CC, Chu SC, Ho HC, Yang CH, Lu CF, Wu JY, Liao YD, Chen YT. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Nat Med* 2008; **14**: 1343-1350 [PMID: 19029983]
46 **Iwai S**, Sueki H, Watanabe H, Sasaki Y, Suzuki T, Iijima M. Distinguishing between erythema multiforme major and Stevens-Johnson syndrome/toxic epidermal necrolysis immunopathologically. *J Dermatol* 2012; **39**: 781-786 [PMID: 22458564]
47 **Kita Y**, Hashimoto S, Nakajima T, Nakatani H, Nishimatsu S, Nishida Y, Kanamaru N, Kaneda Y, Takamori Y, McMurray D, Tan EV, Cang ML, Saunderson P, Dela Cruz EC, Okada M. Novel therapeutic vaccines [(HSP65 + IL-12)DNA-, granulysin- and Ksp37-vaccine] against tuberculosis and synergistic effects in the combination with chemotherapy. *Hum Vaccin Immunother* 2013; **9**: 526-533 [PMID: 23249609]
48 **Clayberger C**, Krensky AM. Granulysin. *Curr Opin Immunol* 2003; **15**: 560-565 [PMID: 14499265]

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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Both personal authors and an organization as author

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

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Conference paper

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Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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